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Precambrian transitions in oxygen and temperature:

Insights from mineralogy, paleobiology, and molecular evolution

A dissertation submitted in partial satisfaction of the

requirements for the degree of Doctor of Philosophy

in Geology

by

Amanda Kathryn Garcia

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ABSTRACT OF THE DISSERTATION

Precambrian transitions in oxygen and temperature: Insights from mineralogy, paleobiology, and molecular evolution

by

Amanda Kathryn Garcia Doctor of Philosophy in Geology University of California, Los Angeles, 2018 Professor James William Schopf, Chair

Though the Precambrian geologic record has to date elucidated numerous features of the ancient geobiological environment, several fundamental aspects of the Precambrian Earth remain enigmatic. This dissertation describes studies using tools from mineralogy, paleobiology, and molecular evolution to explore two of these aspects: (1) the history of atmospheric oxygenation through the Neoproterozoic-Cambrian transition, associated with the diversification of oxygen-requiring metazoans and (2) the long-term evolution of Earth surface temperature since the Archean (~3000 Ma).

It has long been postulated that the Neoproterozoic-Cambrian transition was correlated with an increase in environmental oxygen on the basis of minimum physiological requirements of metazoans through the Cambrian Explosion, as well as isotopic and redox-sensitive metal analyses of sediments. However, the history of oxygenation during this geobiologically significant interval is not sufficiently or quantitatively constrained. Experimental calibrations reported here of fluorescence signatures associated with an oxygen-dependent mechanism for Sm³⁺-substitution in apatite are used to devise a semi-quantitative apatite oxygen paleobarometer indicative of ambient O₂ concentrations during Sm³⁺ emplacement. These calibrations are used for the interpretation of comparable fluorescence signatures of microfossil-associated apatite specimens of the late Neoproterozoic Doushantuo and Early Cambrian Chulaktau Formation phosphorites, as well as biomineral apatite scale microfossils of the mid-Neoproterozoic Fifteenmile Group. The characterized fluorescence spectral features evidence generally low, but locally variable O₂ concentrations during apatite precipitation, and are consistent with moderate increased oxygenation of the shallow sediment environment of the Chulaktau relative to the Doushantuo phosphorite. The expanded use of this apatite oxygen paleobarometer approach can be expected to provide a more refined and quantitative understanding of Neoproterozoic-Cambrian oxygenation during the Cambrian Explosion.

The long-term evolution of Earth's surface temperature through the Precambrian is similarly unconstrained. Paleotemperatures inferred from isotopic compositions of marine cherts suggest Earth's oceans cooled from 70 ± 15 °C in the Archean. However, this interpretation has been met with skepticism due to uncertainties regarding post-depositional isotopic alterations of ancient samples, the isotopic composition of the Archean ocean, and the possibility of a local geothermal depositional environment. Thermostability analyses of ancestral enzymes, reconstructed by molecular evolutionary models applied to phylogenies of extant descendants, provide an independent method by which to assess this temperature history. Reported

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experimental thermostability measurements of ancestral kinases derived from modern photosynthetic taxa limit interpretations of ancient temperatures to the photic zone. Our results suggest that Earth's surface temperature has cooled from ~65-80 °C in the Archean, a finding consistent with previous isotope- and protein-reconstruction-based interpretations. Interdisciplinary studies such as this hold promise for providing new insight into the coevolution of life and the environment through Earth history. The dissertation of Amanda Kathryn Garcia is approved.

Daniele Bianchi

David K. Jacobs

Tina Irene Treude

James William Schopf, Committee Chair

University of California, Los Angeles

For my parents and Paulus

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- 2017 EPSSO Student Symposium (Los Angeles, USA)
- 2016 Paleoenvironment and ambient oxygen during the Precambrian-Cambrian transition

working group meeting (Los Angeles, USA)

CHAPTER 1:

Introduction

The Precambrian, the interval stretching from the Earth's formation at ~4500 Ma to the beginning of the Phanerozoic Eon at ~541 Ma, constitutes nearly the first 90% of geologic history. Despite this enormous length of time, the continual recycling processes of weathering, erosion, metamorphism, and subduction have left the present world with only ~20% Precambrian outcrops, of which only ~0.05% are Precambrian sedimentary rocks capable of preserving traces of ancient life (Blatt and Jones, 1975). The traces that have been retained and studied evidence an early Earth dominated by microbial life, and record large-scale physical, chemical, and biological transitions in planetary evolution that cannot be sufficiently captured by the breadth of time encompassed by the more recent Phanerozoic. Precambrian studies, despite being inherently data-poor relative to those of the Phanerozoic, are thus uniquely well-suited to address impactful questions regarding interactions between life and the physicochemical environment over immense geologic timescales. Insights from these studies have helped shape our understanding on the origins, early evolution, and proliferation of life within an everchanging planetary system, and guide the search for life elsewhere in the universe.

Over the past few decades, the morphological (referring to microfossils as well as microbially-mediated sedimentary structures) and chemical fossil record has confidently been extended to ~3500 Ma (Walter et al., 1980; Schopf and Walter, 1983; Walsh and Lowe, 1985; Schopf and Packer, 1987; Strauss and Moore, 1992; Hofmann, 2000; Ueno et al., 2001), and potential hints of life extend even further back to as old as ~4100 Ma (Mojzsis et al., 1996;

Ohmoto et al., 2014; Bell et al., 2015; Nutman et al., 2016; Dodd et al., 2017). The vast antiquity of life on Earth is even more intriguing given the geologic evidence that the Precambrian biosphere underwent a myriad of transitions in climate as well as atmospheric and ocean composition (perceived as "extreme" relative to the modern biosphere) through this time, clearly demonstrating the broad range of habitable conditions experienced through its evolution. Of additional interest in Precambrian studies is how the changing physical environment has affected the evolutionary trajectory of ancient life – and vice versa – as well as resolving the timing of such transitions. For this endeavor, the field has benefited from an interdisciplinary approach, drawing from geology, biology, chemistry, and atmospheric and planetary science, among others, and from deciphering records not only preserved in rocks, but in the biochemistry and genomic information present in modern organisms – inherited evolutionary adaptations of a bygone age. This research strategy that combines multiple lines of independently converging evidence is essential for the investigations of a sparsely evidenced ancient past.

1.1 – PURPOSE AND SIGNIFICANCE

In this dissertation, I will discuss the development and use of novel and independent approaches to understanding the co-evolution of life and the Precambrian environment, centered on two major questions: (1) Earth's oxygenation leading up to and through the close of the Precambrian during the Neoproterozoic-Cambrian transition and the Cambrian Explosion of Life, and (2) Earth surface temperature evolution since the Archean (~3000 Ma). Both these questions are far from understudied. However, though accumulating geological and biological evidence has served to broadly constrain these environmental aspects, quantifiable details

regarding timing and extent of Precambrian oxygenation and temperature trends are not sufficiently known.

In Chapter 2, I will discuss the experimental development of a semi-quantitative apatite oxygen paleobarometer based on an oxygen-dependent mechanism for Sm³⁺-substitution in apatite, detectable by fluorescence spectroscopy. These calibrations of fluorescence spectral characteristics produced by artificially substituted apatite under varying ambient O_2 concentrations are necessary for their comparison to similar signals detected in Neoproterozoic-Cambrian phosphorite and biomineral apatite specimens associated with the most globally widespread phosphogenic event in geologic history. Phosphogenesis is commonly understood to be tied to shifts in climate, tectonics, ocean circulation, carbon cycling, and, most significantly for the aims described here, to atmospheric and marine oxygenation (e.g., Cook and Shergold, 1986; Donnelly et al., 1990; Papineau et al., 2010). An apatite oxygen paleobarometer metric ("AOP), derived from relative peak characteristics experimentally associated with anoxic and oxic conditions, provides the capability to quantitatively constrain environmental O₂ concentrations during this phosphogenic episode, contemporaneous with and perhaps directly linked to the Cambrian Explosion of oxygen-requiring, hard-shelled metazoans (Cloud, 1976; Runnegar, 1982; Cook and Shergold, 1984; Schiffbauer et al., 2016).

Previous estimations of oxygenation for this time period, based primarily on minimum physiological needs of complex metazoans (Nursall 1959; Berkner & Marshall, 1965; Rhoads & Morse, 1971; Cloud, 1976; Runnegar, 1982; Mills et al., 2014), carbon isotope excursions (Fike et al., 2006; Grotzinger et al., 2011), and, increasingly, on redox-sensitive trace element abundances (e.g., Scott et al., 2008; Sahoo et al., 2012; Chen et al., 2015; Stolper and Keller, 2018) are generally limited to qualitative assessments of marine oxygenation or are quantitatively related to dissolved oxygen by mass-balance modeling rather than experimental calibration. Previously reported direct estimations of Neoproterozoic atmospheric oxygen are restricted to few measurements of gaseous inclusions in halite (Blamey et al., 2016), but are susceptible to uncertainty given the high chance of post-depositional recrystallization. By contrast, an apatite-based oxygen paleobarometer is independent of mass-balance assumptions and can be quantitatively related by experimental calibrations to local dissolved O₂ concentrations during apatite precipitation and Sm³⁺ incorporation in Neoproterozoic- and Cambrian-age specimens.

In Chapter 3, I describe the detection and characterization of Sm³⁺-attributed fluorescence signatures, comparable to those produced during experimental calibrations, from biomineral and microfossil-associated phosphoritic apatite associated with the Neoproterozoic-Cambrian phosphogenic event. The analyzed late Neoproterozoic Doushantuo and Early Cambrian Chulaktau phosphorite specimens are ideal for investigations of ancient local O₂ concentrations as they are (1) constrained to a shallow water setting, (2) associated with well-preserved microfossils that constrain the diagenetic timing of apatite mineralization, and (3) representative of a variety of modes for apatite crystallization, including microfossil-permineralization, infilling, and -replacement as well as concretionary ooid structures. Together, these specimens provide a broad but simultaneously targeted sampling of the apatite-precipitating environment. Fluorescence of mid-Neoproterozoic apatite scale microfossils, interpreted to be the earliest examples of eukaryotic biomineralization (Cohen et al., 2011; 2017), offer a particularly unique

window into the oxygenation of the water column environment inhabited by an ancient, potentially planktonic biomineralizing host organism.

By contrast to Chapters 2 and 3, which describe a geological approach to investigating aspects of the ancient physical environment, I describe in Chapter 4 a novel application of a genomics-based approach to elucidating the history of Earth surface temperature through the Precambrian (slightly modified from a published manuscript; Garcia et al., 2017). The presence of hyperthermophilic bacterial and archaeal taxa near the root of the tree of life (Woese, 1987) suggests a role for high environmental temperatures in the development of early life during the Precambrian. Similarly, the most complete paleotemperature trend for the Precambrian, given by oxygen and silicon isotopes measured from marine cherts, register ~55 to 85 °C ocean temperatures during the Archean (Knauth and Lowe, 1978; Knauth and Lowe, 2003; Robert and Chaussidon, 2006). However, these isotopic analyses have been subject to skepticism arising primarily from questions related to potential post-depositional alteration of isotopic signatures, original isotopic composition of the ancient ocean, or a localized geothermal depositional setting (e.g., hydrothermal vent), a debate which to date has not been settled (e.g., Degens and Epstein, 1962; Perry, 1967; Weis and Wasserburg, 1987; Kasting et al., 2006; Hren and Tice, 2009; Chakrabarti et al., 2012; de Wit and Furnes, 2016). Because of the inherent difficulty in accounting for these isotopic uncertainties, an independent approach is required.

The strategy followed here for inferring past temperatures from genomic information is rooted in the methods for ancestral sequence reconstruction (ASR), originally termed "paleogenetics" (Pauling and Zuckerlandl, 1963). ASR methods integrate observed extant biomolecular (nucleic acid or protein) sequences with estimated phylogenies describing the evolutionary relatedness of sampled organisms, to statistically infer the biomolecular sequences of common ancestors. Enzymes, possessing a required catalytic function dictated by their amino acid composition, must necessarily be adapted to the physical and chemical conditions of the surrounding environment. Therefore, the phenotypic characteristics measured from experimentally reconstructed ancestral enzymes, representative estimations of the "true" enzymatic predecessors, must reflect the ancient environmental conditions to which they had adapted.

ASR studies have been previously used to experimentally infer the thermostabilities of ancient proteins (e.g., Gaucher et al., 2008; Hobbs et al., 2012; Akanuma et al., 2013), and have additionally evidenced a gradually decreasing temperature trend for the Precambrian environment from hot ocean temperatures in the Archean (Gaucher et al., 2008). However, these interpretations may be limited to lineages that inhabited locally hot geothermal environments (e.g., hydrothermal vents) and extrapolation to global ocean temperatures may not be appropriate.

To restrict temperature interpretations to the ancient surface environment in the present study, ancestral kinase enzymes have only been reconstructed from extant photosynthetic taxa represented in the Precambrian fossil record. Robustness of the reconstruction methods, and thus for the measured thermostabilities of experimentally reconstructed enzymes, is given by the testing of multiple phylogenetic models as well as of ancestral enzymatic catalytic activity. This

integration of biomolecular techniques with the geological and paleobiological record provides insight into the evolution of Precambrian surface temperatures.

Together, these studies of Neoproterozoic-Cambrian oxygenation as evidenced by an apatite oxygen paleobarometer, and of Precambrian surface temperature evolution interpreted from ancestral enzyme thermostabilities offer new ways to approach fundamental, unsolved problems regarding the Precambrian geobiological environment. The continued investigation of these questions has direct implications for understanding the evolution of Precambrian life, and how life has shaped and itself been shaped by the history of physical and chemical environmental evolution.

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CHAPTER 2:

Experimental characterization of Sm³⁺-substituted apatite fluorescence and development of an apatite oxygen paleobarometer

2.1 – ABSTRACT

Global, massive apatite-bearing phosphorites spanning the Neoproterozoic-Cambrian transition represent a unique, biogeochemical period in geologic history related to major transitions in metazoan diversification and, possibly, of marine oxygenation. However, current understanding of this postulated increase in oxygenation is limited by qualitative and not uncommonly contrasting interpretations of the marine redox conditions evidenced by isotopic and trace element abundances, as well as by the oxygen requirements of early metazoan physiology. Fluorescence signatures detected in Sm³⁺-substituted, microfossil-associated apatite in early Cambrian shallow water phosphorites have been identified by previous experimental studies as evidence of an oxygen-dependent mechanism for Sm³⁺ incorporation in apatite. This mechanism involves the simultaneous substitution of Sm³⁺ for the Ca2 site in apatite and of O²⁻ for a neighboring anion site, potentially enabling the interpretation of oxygen concentrations during apatite formation and providing a novel approach to investigate the history of marine oxygenation during the Neoproterozoic-Cambrian phosphogenic event.

In the experiments described, heat-promoted substitution of Sm^{3+} in apatite powder pellets has been conducted under gaseous O₂ concentrations ranging from 0 to 20.9%. These methods provide a calibration for the comparison of resulting fluorescence signals to those observed in fossil-encrusting and -permineralizing apatite in naturally occurring phosphorites that should reflect the levels of oxygen in the immediate precipitating environment. The results reported suggest that a metric describing the relative spectral characteristics derived from endmember oxic and anoxic experiments, here described as an apatite oxygen paleobarometer (AOP), robustly correlates with experimental O₂ concentrations. The application of this semiquantitative metric to fossil-associated apatite in phosphorites spanning the Neoproterozoic-Cambrian transition is expected to provide a fundamentally new dataset by which to probe ancient marine redox conditions and improve current understanding of oxygenating during the geologically rapid diversification of early metazoans.

2.2 – INTRODUCTION

Apatite [Ca₅(PO₄)₃(F,OH,Cl)], well-described as an important accessory mineral in igneous and metamorphic settings and as a major vertebrate biomineral, also occurs in massive sedimentary phosphorite formations deposited during widespread episodic phosphogenic events in geologic history. Though the oldest known such phosphogenic event occurred at ~2.0 Ga during the Paleoproterozoic, the most extensive was a global episode extending from the Neoproterozoic into the early Cambrian, forming shallow marine phosphorites today reported from every continent except Antarctica (Notholt and Sheldon, 1986; also see Chapter 3, Figure 1). This event was contemporaneous with a postulated increase in atmospheric oxygen to near-modern levels across the Neoproterozoic-Cambrian boundary (reviewed in Lyons et al., 2014; Och and Shields-Zhou, 2012) and the major early diversification of metazoans (e.g., Schiffbauer et al., 2016). An increase in shallow water marine oxygenation may have resulted from the same increase in primary productivity and organic burial that would have promoted phosphogenesis

(Donnelly et al., 1990; Planavsky et al., 2011; Cui et al., 2016; Reinhard et al., 2017), though the latter was also likely affected by a complex interplay of climate, tectonics, and ocean circulation (e.g., Cook and Shergold, 1986). Globally distributed phosphorites such as those spanning the Neoproterozoic to Cambrian are not known from the more recent Phanerozoic, during which phosphate biomineralization became an increasingly significant component of the global phosphorous cycle (e.g., Cook and Shergold, 1984; Van Cappellen, 2003; Dornbos, 2011). Thus, the Precambrian apatite-bearing phosphorites perhaps evidence punctuated episodes of significant environmental oxygenation and biogeochemical change essentially unique to the pre-Phanerozoic environment (Papineau, 2010).

The structure and chemistry of apatite permits trace substitutions of redox-sensitive elements as well as rare-earth elements, "REEs" (e.g., Pan and Fleet, 2002), establishing it as a useful repository of environmental information for phosphorites. REE abundance and distribution patterns in phosphorites deposited during Paleoproterozoic (Khan et al., 2012) and Neoproterozoic-Cambrian (Ilyin, 1998; Mazumdar et al., 1999; Yang et al., 1999; Shields and Stille, 2001; Chen et al., 2003; Chunhua and Ruizhong, 2005; Jiang et al., 2007; Zhu et al., 2014) phosphogenic events have been used to determine ancient seawater geochemistry and rough approximations of redox conditions.

In a departure from these more traditional applications of phosphorite-related REEs, Schopf et al. (2015) used fluorescence spectroscopy to detect Ca-site-specific substitution of Sm^{3+} into Early Cambrian microfossil-encrusting and -permineralizing apatite. Based on evidence of an oxygen-dependent mechanism for Ca-site-specific substitution of Sm^{3+} (Gaft et



Figure 1. Apatite crystal lattice structure. (A) Unit cell structure of apatite, viewed down the c-axis, showing relative positioning of the Ca1 (red) and Ca2 (green) sites. Local structure and symmetry of the Ca1 and Ca2 sites illustrated by (B) and (C), respectively. The A site (purple) refers to F^- , Cl^- , or OH^- anions most commonly present in apatite. Modified after Hughes et al. (1989).

al., 1997; Chen et al., 2002a), these signatures were interpreted to reflect the oxygen conditions in the immediate apatite-precipitating microbe-inhabited environment.

Trivalent rare-earth elements (REE^{3+} s) are readily accommodated at either of two nonidentical Ca²⁺ sites within the apatite lattice, termed the Ca1 and Ca2 sites (e,g,, Hughes et al., 1989; Figure 1). These sites differ considerably in their stereochemistries: the Ca1 site is a tricapped trigonal prism coordinated with nine oxygen atoms (CaO₉) and the Ca2 site is an irregular polyhedron coordinated with six oxygen atoms and a F⁻, Cl⁻, or OH⁻ anion (CaO₆A). Because local charge balance in either case must be maintained in the substitution of a trivalent for a divalent cation, two possible mechanisms involve either the creation of a neighboring Ca²⁺site vacancy or the substitution of an additional O²⁻ anion (Fleet and Pan, 1994):

$$2REE^{3+} + \Box = 3Ca^{2+} \tag{1}$$

$$REE^{3+} + O^{2-} = Ca^{2+} + A^{-}$$
(2)

where \Box (open square symbol) represents a neighboring Ca²⁺ vacancy and A⁻ represents the native F⁻, OH⁻, or Cl⁻ anion within the apatite lattice.

Because empirical studies of synthetic and natural apatites generally demonstrate a firstorder decrease in REE³⁺ Ca2:Ca1 preference with decreasing REE³⁺ ionic radius (Hughes et al., 1991; Fleet and Pan, 1995; Fleet and Liu, 2000) – an unexpected pattern given the smaller Ca2 site compared with the Ca1 site – it is possible that substitution mechanism instead exerts important controls on REE³⁺ Ca-site preference (discussed by Fleet & Pan, 1995). Gaft et al.



Figure 2. Fluorescence spectra measured from artificially Sm³⁺-substituted natural apatite, activated in vacuum (red) and air (green). Modified after Gaft et al. (1997).

(1997) demonstrated that REE³⁺-bearing apatites produce distinctly differing fluorescence spectra when activated in vacuum or in air, interpreted to reflect REE³⁺ positioning at the Ca1 and Ca2 sites, respectively (Figure 2). This effect has been supported by Ca2-site substitution in REE₂O₃-doped apatite (Mackie and Young, 1973) and structural refinements of vacuum- and air-REE³⁺-substituted apatite, suggesting that REE³⁺ incorporation at the Ca1 site is controlled primarily by Equation 1 (Chen et al., 2002b), whereas incorporation at the Ca2 site is controlled by the oxygen-dependent reaction shown by Equation 2 (Chen et al., 2002a).

An oxygen-dependent mechanism for REE³⁺ substitution in apatite, detectable by fluorescence spectroscopy (Gaft et al., 1997; Schopf et al., 2015), has enormous potential for elucidating the nature and history of local marine oxygenation during phosphogenic events across the Neoproterozoic-Cambrian boundary. Current interpretations of oxygenation for this period are commonly qualitative and generally rely on coeval carbon isotopic excursions (Fike et al., 2006; Grotzinger et al., 2011), redox-sensitive trace element abundances (e.g., Scott et al., 2008; Sahoo et al., 2012; Sperling et al., 2015; Stolper and Keller, 2018), and minimum physiological oxygen requirements estimated for animals extant at that time (Nursall 1959; Berkner & Marshall, 1965; Rhoads & Morse, 1971; Runnegar, 1982; Mills et al., 2014). Other than a small number of somewhat uncertain oxygen measurements of gaseous inclusions in halite (Blamey et al., 2016), there do not currently exist direct, quantitative methods for inferring environmental oxygen concentrations during the Neoproterozoic-Cambrian transition. Such a tool would be provided by an experimentally well-calibrated "oxygen paleobarometer" based on the fluorescence spectra of REE³⁺-substituted apatite indicative of relative REE³⁺ Ca2:Ca1-sitepreference and therefore of relative oxygen levels during apatite precipitation. Its application to

assessment of oxygenation across the Neoproterozoic-Cambrian phosphogenic episode would hold promise for improved quantitative understanding of environmental oxygen concentrations.

Results are here reported from calibration experiments for the oxygen-dependent mechanism of Sm³⁺ substitution at the Ca2 site in apatite, the fluorescence spectra of which have been previously described in limited experimental analyses (Gaft et al., 1997) and detected in apatite of an Early Cambrian phosphorite (Schopf et al., 2015). By performing a series of Sm³⁺activation experiments under varying gaseous oxygen concentrations, we have developed a semiquantitative metric by which to assess the relative oxygenation evidenced by the fluorescence signatures of Sm³⁺-substituted apatite in Neoproterozoic-Cambrian phosphorites. The spectroscopic methods used here are non-destructive and therefore ideal for the analyses of fossiliferous geological samples. The application of this oxygen paleobarometer can be expected to contribute to a more complete understanding of the oxygen-dependent incorporation of REE³⁺s into apatite and of the interrelated history of environmental oxygen and the "Cambrian Explosion" of megascopic multicellular life.

2.3 – MATERIALS AND METHODS

Experimental heat-substitution of Sm³⁺ in hydroxyapatite

Sm³⁺-doped apatite powder pellets were made from mixtures of 95 wt.% hydroxyapatite, "HAp" [Ca₅(PO₄)₃OH], reagent (Sigma-Aldrich, synthetic) and 5 wt.% samarium oxide (Sm₂O₃; Sigma-Aldrich). Though natural and synthetic fluorapatite crystals have been used in previous studies of REE³⁺ substitution and luminescence (e.g., Gaft et al., 1997; Chen et al., 2002a; Chen et al., 2002b), HAp-powder reagent is readily obtainable and is similarly relevant to authigenic geological and modern phosphorite apatite formation (Crosby et al., 2014; Schulz and Schulz, 2005). Mixtures were homogenized by grinding in an alumina mortar and pestle, followed by further vortex mixing in vials for ~5 mins. In addition to HAp + Sm_2O_3 (5 wt.%) mixtures, HAp + Sm_2O_3 (2.5 wt.%) and HAp + $SmCl_3$ (10 wt.%, doubled to compensate for stoichiometrically less Sm^{3+} in $SmCl_3$; Sigma-Aldrich) mixtures were also prepared by these same methods for limited experimental analyses on the effects of Sm^{3+} concentration and reagent composition.

HAp + Sm₂O₃ (5 wt.%), HAp + Sm₂O₃ (2.5 wt.%), HAp + SmCl₃ (10 wt.%), and HAponly pellets were compressed in a pellet press with ~400 N applied to the lever to a final preheated weight of ~0.01 g (diameter \approx 3 mm). Pellets were placed into a quartz boat (1200 °C working temperature) such that the upward-facing surface in the press was the same upwardfacing surface in the boat. The pellet-containing boat was then inserted into an OTF-1200X high pressure/temperature tube furnace equipped with an oxidation-resistant Ni-based superalloy (GH747) tube (inner diameter = 52 mm, length = 1000 mm; MTI Corporation). Heating was maintained over a 44 cm zone to within ±1 °C by a PID temperature controller, with furnace temperatures during experiments not exceeding the 1000 °C maximum temperature for continuous heating specified by the manufacturer. To prevent leaks from the apparatus, flange bolts were tightened to a torque limit of 20 Nm.

The experimental heating trials conducted are summarized in Table 1. For all experimental trials, the furnace tube was first evacuated to $\sim 1 \times 10^2$ Pa by use of a backing pump
Pellet composition	Tube internal atmosphere	Initial internal total pressure (Pa)	Maximum temperature (°C)	Duration at max. temperature (hrs)	Number of trials
HAp + Sm ₂ O ₃ (5 wt.%)	0% O ₂ (vacuum)	1×10^{-3}	900	5	4
	0% O ₂ (argon)	1×10^5	900	5	3
	0.2% O ₂	1×10^5	900	5	3
	1% O ₂	1×10^5	900	5	4
	2% O ₂	1×10^5	900	5	4
	10% O ₂	1×10^5	900	5	3
	20.9% O ₂	1×10^5	900	5	3
$\begin{array}{r} HAp + \\ Sm_2O_3 \\ (2.5 \text{ wt.\%}) \end{array}$	2% O ₂	1×10^5	900	5	1
HAp + SmCl ₃ (10 wt.%)	0% O ₂ (vacuum)	1×10^{-3}	900	5	2
HAp only	0% O ₂ (argon)	1×10^5	900	5	1

Table 1. Experimental heating conditions of Sm³⁺-doped HAp and HAp-only pellets.

(Edwards) prior to heating. For vacuum trials, the tube was then further evacuated before sealing to $\sim 1 \times 10^{-3}$ Pa by use of a turbo-molecular pump (Edwards). Internal pressure was monitored by a vacuum pressure gauge (Edwards).

For all other trials, the evacuated furnace tube was filled to 1×10^5 Pa with either argon or the relevant percentage O₂ gas composition, mixed from gaseous nitrogen and varying concentrations of O₂ (0.2 to 20.9%, Praxair). The outflow furnace tube valve was fitted with a plastic hose connected to an O₂ sensor (Greisinger) and lead into a beaker of water to prevent gas backflow. Prior to each experiment, the inlet valve was opened and the relevant gas mixture was permitted to flow through the apparatus for ~30 mins to flush the furnace tube and ensure that the outflow gas was within ±1 % O₂ from the desired percentage O₂ composition, after which the system was sealed at 1×10^5 Pa.

Furnace tube temperature for all experimental trials was initially increased linearly to 900 °C (following Gaft et al., 1997, to ensure complete Sm³⁺ substitution) over a 1 hr period and was then maintained for 5 hrs before being allowed to cool undisturbed to room temperature. The resulting HAp pellets were then collected and sealed in vials for spectroscopic analysis.

To minimize the effects of accumulated oxygen contamination on its internal surfaces, the furnace tube was re-conditioned following each oxygen-containing experimental trial by heating at 1000 °C for 8 hrs under continuous vacuum pumping ($\sim 1 \times 10^{-3}$ Pa). Anoxic (0% O₂) experiments following either additional anoxic or oxic ($\geq 0.2\%$ O₂) experiments produced consistent results, demonstrating the efficacy of this contamination-prevention measure.

Raman and fluorescence spectroscopy

Raman and fluorescence spectroscopic analyses of experimentally Sm³⁺-substituted HAp pellets were performed at UCLA using a T64000 triple-stage confocal laser-Raman system equipped with a Coherent Innova argon ion laser source. Laser excitation at 457.9 nm and 150 mW power was used for both Raman and fluorescence analyses.

Raman and fluorescence point spectra at the pellet surface were acquired within either a ~ 200 to 3000 cm⁻¹ spectral window centered at ~ 1600 cm⁻¹ (for Raman analyses) or a ~ 590 to 660 nm window centered at 625 nm (for fluorescence analyses). Multi-window point spectra containing both Raman and fluorescence spectral features were acquired between ~ 465 and 700 nm. To account for spatial fluorescence heterogeneity, fluorescence spectra were also collected over a two-dimensional grid covering a 30×30 -point area (point width $\sim 1 \mu$ m) of the pellet surface and averaged. For two-dimensional measurements in which intense, spatially variable, and broad fluorescence bands in the ~ 596 to 616 nm and ~ 644 to 652 nm regions obscured the intensities of more interpretable fluorescence peaks (see following discussion), spatial subsets within which such fluorescence was less intense were instead averaged. Point spectra and two-dimensional analyses were processed in LabSpec v.5. Fluorescence spectra were first normalized to the intensity of the major peak within the 605 to 607 nm region before averaging over the total set of spectra acquired for a single experimental condition (Table 1).

Fluorescence spectral analysis and oxygen paleobarometer metric

Fluorescence peaks, including those of particular interest positioned at ~605, 607, 652, and 654 nm (simultaneously acquired within the higher resolution, ~590 to 660 nm narrow

window for each measurement), were fitted within spatially averaged pellet fluorescence spectra using PeakFit v.4.12 (following procedures similar to those detailed in Schopf et al., 2005). Spectral regions were first isolated within the 600 to 612 nm and 647 to 659 nm regions (for the analysis of 605 to 607 nm and 652 to 654 nm peaks, respectively) to reduce baseline fitting uncertainty and then smoothed with the automated fast Fourier transform filtering tool. Manual smoothing adjustments were made in few rare cases for which spectral output did not retain local maxima visually identifiable in the unsmoothed spectra. Peaks were identified by a standard deconvolution procedure that included automated "best fit" baseline subtraction and were subsequently manually adjusted for peak positioning and shape while allowing for variation between Gaussian and Lorentzian peak-character. The resulting peak fit was then further refined using an iterative graphical fit algorithm under default constraints until the fit returned r² values of >0.9970 (but typically >0.9990) and p < 0.00001, detailing the closeness of fit between the smoothed spectrum and that modeled by the summation of the fitted peaks.

An apatite oxygen paleobarometer metric (AOP) developed for this study is given by the following equation:

$$AOP_{\lambda(o)-\lambda(a)} = \frac{P_{\lambda(o)}}{P_{\lambda(o)}+P_{\lambda(a)}}$$
(3)

where $P_{\lambda(o)}$ is the area of the modeled major fluorescence peak observed from oxic experimental conditions (positioned at either 605 or 652 nm) and $P_{\lambda(a)}$ is the area of a neighboring modeled fluorescence peak (positioned at either 607 or 654 nm) observed from anoxic experimental

conditions. For example, the AOP value calculated from peaks within the 605 to 607 region is given by the following equation:

$$AOP_{605-607} = \frac{P_{605}}{P_{605} + P_{607}}$$
(4)

In essence, the AOP value is then a numerical metric between zero (indicating the presence of only the major 607 or 654 nm peaks observed from anoxic experimental conditions) and one (indicating the presence of only the major 605 and 652 nm peaks observed from oxic experimental conditions). The AOP values reported here were calculated independently both for the 605 to 607 nm and 652 to 654 nm regions of each fluorescence spectrum, such values for each region of each experiment being subsequently averaged to give a mean $\overline{\text{AOP}}$ value at its particular experimental percentage O₂ concentration.

2.4 – RESULTS

Sm³⁺-activated apatite fluorescence

The hydroxyapatite composition of the HAp + Sm_2O_3 (5 wt.%) pellets was confirmed by Raman spectroscopy before and after heating by identification of diagnostic vibrational peaks, notably the intense ~963 cm⁻¹ peak related to the PO₄ v₁ vibrational mode (e.g., Kravitz et al., 1968; O'Shea et al., 1974; Figure 3). HAp Raman peak positions did not shift substantially before and after heating, except within spectra measured for <1% O₂ experiments. The spectra resulting from these low O₂ trials exhibit two additional v₁ mode peaks at ~949 and 967 cm⁻¹.



Figure 3. Representative Raman spectra of unheated, 0%-O₂-heated, and 20.9%-O₂-heated HAp, HAp + Sm₂O₃ (5 wt.%), and HAp + SmCl₃ (10 wt.%) pellets. Note splitting of major 963 cm⁻¹ vibrational apatite peak into 947 cm⁻¹ and 967 cm⁻¹ peaks in 0%-O₂-heated HAp and HAp + Sm₂O₃ (5 wt.%) pellets, indicative of partial conversion to tricalcium phosphate (see text for further discussion).

The presence of these peaks is consistent with the partial conversion of HAp to tricalcium phosphate $[Ca_3(PO_4)_2]$ under anoxic conditions (de Aza et al., 1997), potentially due to the loss of OH⁻ at high temperatures as seems evident by the disappearance under these conditions of the OH⁻ stretch Raman peak at ~3574 cm⁻¹ (e.g., O'Shea et al., 1974; Liao et al., 1999; Ou et al., 2013; Figure 4), and the unavailability of other accommodating anions such as O²⁻ (Liao et al., 1999). Though these additional peaks were also observed in vacuum-heated HAp-only pellets, vacuum-heated HAp + SmCl₃ (10 wt.%) retained the ~963 cm⁻¹ Raman peak and exhibited fluorescence spectra nearly identical to those exhibited by vacuum-heated HAp + Sm₂O₃ pellets. On the basis of these considerations, we conclude that the probable compositional changes evidenced by the data acquired from the anoxic heating of apatite do not complicate the recorded fluorescence spectra in a manner that would obscure the incorporation of oxygen into the apatite studied.

Full fluorescence spectra (~495 to 700 nm window) of HAp + Sm_2O_3 (5 wt.%) pellets heated under end-member "anoxic" (0% O_2 , vacuum) and "oxic" (20.9% O_2) conditions are shown in Figure 4. Identified peak positions for both spectra are listed in Table 2 together with those attributed to the Sm^{3+} -substitution of apatite reported by other workers.

There is an overall consensus between the peaks observed in this study and those previously documented in artificially-Sm³⁺-doped synthetic and natural apatites, as well as for naturally Sm³⁺-bearing apatites, supporting the interpretation that the fluorescence spectra studied here do arise from successful Sm³⁺ incorporation into powdered HAp. This interpretation



Figure 4. Representative full fluorescence spectra measured from Sm^{3+} -substituted HAp + Sm_2O_3 (5 wt.%) pellets, heated in a 0% (vacuum) or 20.9% O₂ atmosphere. Arrows with dotted lines indicate shoulder features within the 20.9% O₂ spectrum observed at the same wavelength positions as peak features within the 0% O₂ spectrum. Note the presence of the OH⁻ vibrational Raman peak in the spectrum measured from a 20.9%-O₂-activated pellet (see text for further discussion). Identified peak wavelength positions are also listed in Table 2.

is further supported by the observed absence of a discernible fluorescence signal in heated non-Sm³⁺-substituted HAp-only pellets.

It does not appear possible to directly correlate all previously published Sm³⁺-bearing apatite fluorescence peak spectra with one or the other anoxic or oxic end-member spectra reported here. For example, peaks observed in naturally occurring Sm³⁺-bearing fluorapatites (Reisfeld et al.,1996) are also observed here in both anoxic and oxic spectra. Some of this lack of consonance arises from differences in the level of detail reported in such earlier analyses of Sm³⁺-attributed fluorescence. Nevertheless, there is a close correlation between the fluorescence spectrum reported by Gaft et al. (1997; Figure 2) of Sm³⁺-substitution into naturally occurring fluorapatite in an ambient oxic environment with that of the Sm³⁺-substitution into powdered HAp under oxic conditions reported here (Figure 4). This comparison suggests that discrepancies between fluorescence spectra previously observed in Sm³⁺-bearing apatites may be primarily attributed to differing O₂ availability during natural or artificial Sm³⁺ substitution, producing spectral discrepancies greater than those arising from crystallographic differences between apatite structural end-members (e.g., hydroxyapatite and fluorapatite) and between synthetic and natural samples.

To date, there have been only a limited number of studies reporting Sm^{2+} luminescence spectra in minerals (e.g., Gaft et al., 2001) and in apatite specifically (Gaft et al., 2005). In such work, luminescence in apatite from the substitution of Sm^{2+} is reported to be characterized by intense emission at 734 nm, a spectral band not observed in the samples studied here. We therefore conclude that the fluorescence spectral signal we report from anoxic experimental

"Anoxic" 0% O ₂	"Oxic" 20.9% O ₂	Gaft et al., 1997 (vacuum)	Gaft et al., 1997 (air)	Mitchell et al., 1997	Reisfeld et al., 1996	Czaja et al., 2010
doped powder hydroxyapatite	doped powder hydroxyapatite	doped natural fluorapatite	doped natural fluorapatite	doped synthetic fluorapatite	natural apatite, multiple samples	doped glass & natural apatite
λ _{ex} =457.9	λ _{ex} =457.9	λ _{ex} not specified	λ _{ex} not specified	cathodoluminescence	$\lambda_{ex}=308^{\dagger}, 360^{\ddagger}, 460^{\$}$	$\lambda_{ex}=444$
551	552					
	562	564		564	563 [§] , 564 [†]	
557						
				566		
570	569*, 570			571		
	574					
577	576*					
	596					
	598					599, 598
		599		599	599 [†]	
	600					
607	605*, 607		604, 606	607	607^{\ddagger}	
	610					
616	614*			617		
627	627					
636	637					
	644	645		645	646 [§]	
	648					
654	652*, 654		651	655	654 [‡]	
			656			
662	660*		661			

Table 2. Fluorescence and cathodoluminescence emission peak wavelengths (nm) assigned to Sm³⁺ activation of apatite, reported from the present study and by previous workers. Excitation wavelength (λ_{ex}) units in nm.

*Fluorescence peaks observed only in oxic spectra that appear downshifted by 1 to 2 nm from neighboring peaks prominent in anoxic spectra

conditions is indeed derived from incorporated Sm³⁺, rather than Sm²⁺-substitution resulting from Sm³⁺ reduction by heating under anoxic conditions (e.g., Nogami & Abe, 1996).

Sm³⁺ fluorescence spectrum variability with increasing oxygen

Figure 5 shows fluorescence spectra (590 to 660 nm region) of HAp + Sm_2O_3 pellets heated under a series of increasingly oxic conditions (0 to 20.9% O₂). Each such spectrum shows the results from a single analysis and its spatial average across a two-dimensional area of the pellet surface, a technique used to offset the effects of spatial fluorescence heterogeneity (as is discussed below).

Fluorescence spectra produced by Sm^{3+} substitution under $\leq 0.2\%$ O₂ conditions vary only minimally and have major peaks positioned at 570, 607, and 654 nm (Figures 4 and 5). Pellets heated in an anoxic 100% Ar atmosphere (not shown) produce nearly identical fluorescence spectra as those heated in vacuum.

For oxygen levels above 1% O_2 , our data shows the appearance of additional and often variably intense broad fluorescence bands in the ~596 to 616 nm and ~644 to 652 nm regions. The major 570, 607, and 654 nm peaks observed in anoxic spectra are less intense, observed only as shoulders on newly present major O_2 -related peaks at 569, 605, and 652 nm. The 1 to 2 nm peak downshift pattern (e.g., 607 to 605 nm) between anoxic and oxic fluorescence peaks is also evidenced by other minor bands within the full Sm³⁺ fluorescence spectrum (Figure 5 and Table 2). Though this 1 to 2 nm wavelength separation is relatively narrow, it is unlikely that the major



Figure 5. Average narrow fluorescence spectra measured from Sm^{3+} -substituted hydroxyapatite pellets heated under the full experimental range of percentage O₂ concentrations. "0% O₂" spectra acquired from pellets heated in vacuum. Individual spectral measurements (n) at each percentage O₂ concentration are themselves spatial averages across a two-dimensional section of the pellet surface, normalized to the intensity of either the 605 or 607 nm peak (see Section 3.3 for further detail). Grey shaded regions represent ± 1 standard deviation from the averaged spectrum (black). Major peak wavelength positions observed from oxic and anoxic experimental conditions and used in AOP calculations (see text) are indicated by green and red bars, respectively.

peaks observed in anoxic and oxic spectra have been misinterpreted. In fact, this separation can be confidently distinguished due to the measured 0.03 nm spectral resolution provided by the instrument used for these analyses. Moreover, in such oxic spectra, all major peaks are observed simultaneously (e.g., both 605 and 607 nm peaks), though with varying relative intensities.

We thus note that the major 570, 607, and 654 nm fluorescence peaks are produced by Sm^{3+} -substitution under all conditions, but the 569, 605, and 652 nm peaks are only produced under oxic ($\geq 1\%$ O₂) conditions. The data also demonstrate that this transition between anoxic and oxic spectral character proceeds gradually from 0% to 20.9% O₂, a finding illustrated at high resolution by, for example, the gradually changing relative intensities between 605 to 607 nm and 652 to 654 nm peaks shown in Figure 5.

It is unlikely that this evident spectral transition from anoxic to oxic conditions can be attributed to the presence of inert gases such as Ar or N₂ during these analyses. As noted above, heating in 100% Ar and in 0.2% O₂, balanced by 99.8% N₂, produce fluorescence spectra essentially identical to those produced by heating in vacuum. It is similarly unlikely that the presence of oxygen in the Sm_2O_3 reagent used in these studies contributes significantly to the observed spectral variability, an interpretation consistent with the unchanged weight percent of Sm_2O_3 used in all experimental trials (c.f., Mackie and Young, 1973).

The variability observed between individual spectra measured for $\geq 1\%$ O₂ conditions is contributed mostly by variations of band intensity within the ~596 to 616 nm and ~644 to 652 nm regions. Though these fluorescence bands are only observed for $\geq 1\%$ O₂ experiments, their relative intensities have significant spatial variability (i.e., across the pellet surface) and do not correlate well linearly with increasing O_2 concentration, as compared with, for example, the relative intensity of the 605 nm peak. Therefore, though the presence of these fluorescence bands is strongly linked with oxic conditions, their relative intensities are somewhat more ambiguous to interpret than those of the 569/570, 605/607, and 652/654 nm peaks.

During this study, significant spectral variability was also noted in the relative intensities between the 569/570, 605/607, and 652/654 nm peaks for experiments performed at 1-2% O₂ conditions, presumably resulting from slightly differing experimental conditions. This is potentially due to a sharp transition in spectral character within this percentage O₂ range, which perhaps cannot be resolved to the degree to which our methods can control for O₂ concentration during heating. Nevertheless, the average spectra from multiple trials retain the correlation between 569, 605, and 652 nm peak relative intensities and O₂ concentration. This ambiguity is significantly diminished in spectra measured for ≥ 10 % O₂ experiments, in which 569, 605, and 652 nm peaks have consistently high intensities relative to 570, 607, and 654 nm peaks.

Oxygen-dependence of Sm³⁺ substitution at the Ca2 site

An oxygen-dependent mechanism for REE^{3+} substitution in apatite (e.g., Fleet and Pan (1994) has been experimentally supported specifically for substitution at the Ca2 site by Mackie & Young (1973), Gaft et al. (1997), and Chen et al. (2002a). The incorporation of Sm^{3+} at either the Ca1 or Ca2 sites must maintain local charge balance; therefore, it is reasonable that the double substitution of Sm^{3+} for Ca²⁺ and O²⁻ for OH⁻ in HAp as specified by Equation 2 (above, section 3.1) would apply primarily to the Ca2 site, coordinated to the anion site at which O²⁻

would likely be incorporated (Figure 1). Chen et al. (2002a) discussed that O²⁻ incorporation would produce local distortion and expansion of the Ca2 site, perhaps explaining the observed greater preferential incorporation of relatively large REEs, including Sm³⁺, at the typically smaller Ca2 site (Fleet and Pan, 1995; Fleet and Liu, 2000).

The gradual spectral transition observed for Sm^{3+} -incorporation under increasingly oxic conditions is then most plausibly explained by the increasing addition of Sm^{3+} into the Ca2 site of HAp. The differences in local structure and symmetry between the two Ca sites can be expected to contribute to differences in fluorescence spectra produced by Sm^{3+} , as well as by other REE³⁺s including Eu³⁺ and Pr³⁺ (Gaft et al., 1997; Gaft et al., 2005). It is also possible that the broad bands within the ~596 to 616 nm and ~644 to 652 nm spectral regions arise by additional, relatively disordered positioning of Sm^{3+} within the apatite lattice. This interpretation, though currently a rather speculative hypothesis, is supported by similar broad fluorescence bands identified from Sm^{3+} -doped amorphous phosphate glasses (Czaja et al., 2010).

In addition to the REE³⁺ substitution mechanisms related to Ca vacancies (Equation 1) and oxygen incorporation (Equation 2), other possible mechanisms involving the incorporation of either Si⁴⁺ or Na⁺ have also been proposed (Fleet and Pan, 1994). Such mechanisms, however, are not relevant to our study because neither Si⁴⁺ nor Na⁺ was present in any significant amount in the analyzed experimentally heated HAp pellets. It is similarly implausible that the high temperature-stable quartz boat, used consistently in all experiments, underlying the pellets during heating contributed to spectral variability, as is evidenced by the absence of discernible spectral differences of pellet surfaces whether in contact or out of contact with the boat. Results of these experiments indicate that the ratio between the peak intensities exhibited by the HAp pellets processed under oxic and anoxic experimental conditions remains relatively constant above 2% O_2 . If it is assumed that these relative intensities are representative of the relative abundances of substituted Ca1 and Ca2 sites – as the evidence strongly suggests – this observation may indicate a saturation of Ca2 substitution by Sm³⁺ via an oxygen-dependent mechanism for conditions as low as 2% O_2 . And because similar peak intensity ratios were observed from heating of HAp + Sm₂O₃ (2.5 wt.%) in 2% O_2 , conditions incorporating both lower Sm³⁺ and O_2 concentrations, this saturation effect seems more likely to be intrinsically associated with a limit to the structural deformation of the apatite lattice resulting from increasing incorporation of O^{2-} (Chen et al., 2002a) that prevents further incorporation of Sm³⁺ into the Ca2 site relative to the Ca1 site. Accordingly, it is notable that in no case did the results indicate that Sm³⁺ was completely accommodated by the Ca2 site (resulting in the presence of only the oxic peaks), whereas the resulting spectra indicate that complete accommodation of Sm3+ at the Ca1 site occurred under anoxic conditions.

Apatite oxygen paleobarometer metric

The relative intensities of oxic and anoxic Sm^{3+} -attributed fluorescence peaks are demonstrably correlated with varying O₂ concentrations during heating of HAp pellets. The quantitative metric derived from this oxygen-dependent spectral variability – the apatite oxygen paleobarometer (AOP) discussed in Section 3.3. – has therefore been devised to enable the interpretation of similar signatures in apatite naturally occurring in phosphorites. AOP values (see Equation 3, above) are listed in Table 3 and plotted in Figure 6, values that are representative of relative modeled areas of anoxic (607, 654 nm) and oxic (605, 652 nm) peaks at increasing O_2 concentrations, such values ranging from zero (only 607 and 654 nm peaks present) to one (only 605 and 652 nm peaks present).

As is shown in Figure 6, the experimentally based AOP values increase steeply over the 0 to 2% O_2 range and appear to saturate above 10% O_2 at values of ~0.8 to 0.9. Some of the observed trend is evidently affected by two fluorescence measurement outliers that yield AOP values of zero at both 1 and 2% O_2 . The inclusion of these outlier measurements significantly increases the standard deviation of the trend and pushes the mean to a lower AOP value, giving the impression of a more gradual transition across the relatively lower percentage O_2 levels. As discussed above, the data dispersion observed at O_2 concentrations may result from methodological challenges encountered in controlling the relevant experimental conditions, a difficulty that prevented the accurate resolution of the rapid transition in spectral character. Were these two outlier measurements to be omitted, the significant first-order trend of increasing AOP with increasing percentage O_2 would be retained (though the slope in the 0 to 2% O_2 range would be steeper).

With the exception of these anomalous outlier measurements, the relatively tight clustering of AOP values for each experimental condition reflects the robustness of the correlation between the analyzed relative peak areas and O₂ concentration. This semiquantitative metric thus provides significant promise for its application to naturally occurring

Table 3. AOP values calculated from modeled peak areas. Uncertainty expressed as standard error (SE, derived from uncertainty in the peak fitting procedure) or standard deviation for $\overline{\text{AOP}}$ (SD, reflective of the dispersion in calculated AOP values for each experimental condition).

Oxygen Level	$AOP_{605-607}\pm SE$	$\overline{AOP}_{605-607} \pm SD$	$AOP_{652-654}\pm SE$	$\overline{AOP}_{652\text{-}654}\pm SD$	$\overline{AOP}_{total} \pm SD$	r ² of	r ² of
						modeled	modeled
						spectrum	spectrum
						(600-612	(647-659
						nm)	nm)
0% O ₂ (vacuum)	0*	0.0303 ± 0.0607	0*	0.0820 ± 0.0134	0.0562 ± 0.1178	0.9982	0.9978
	0*		0*			0.9977	0.9973
	0*		0*			0.9988	0.9977
	0.1213 ± 0.0087		0.3282 ± 0.0534			0.9992	0.9988
0.2% O ₂	0.0228 ± 0.0055	0.0482 ± 0.0231	0*	0.0169 ± 0.0293	0.0325 ± 0.0291	0.9994	0.9994
	0.0537 ± 0.0048		0*			0.9998	0.9991
	0.0681 ± 0.0196		0.0507 ± 0.0101			0.9983	0.9988
1% O ₂	0*	0.4917 ± 0.3476	0*	0.4316 ± 0.2897	0.4616 ± 0.2980	0.9993	0.9995
	0.5342 ± 0.0599		0.5561 ± 0.0481			0.9989	0.9993
	0.6212 ± 0.0330		0.5473 ± 0.0487			0.9995	0.9994
	0.8112 ± 0.0466		0.6229 ± 0.0505			0.9993	0.9999
2% O ₂	0*	0.6144 ± 0.4198	0*	0.6171 ± 0.4147	0.6157 ± 0.3876	0.9991	0.9994
	0.6891 ± 0.0589		0.7227 ± 0.0110			0.9995	0.9996
	0.8743 ± 0.0588		0.8712 ± 0.0172			0.9986	0.9994
	0.8940 ± 0.0349		0.8744 ± 0.0328			0.9996	0.9998
10% O ₂	0.7227 ± 0.0461	0.7859 ± 0.0582	0.8274 ± 0.0302	0.8510 ± 0.0213	0.8185 ± 0.0530	0.9995	0.9994
	0.7978 ± 0.0060		0.8687 ± 0.0117			0.9995	0.9998
	0.8373 ± 0.0375		0.8570 ± 0.0302			0.9990	0.9998
20.9% O ₂	0.8708 ± 0.0511	0.8812 ± 0.0129	0.8954 ± 0.0186	0.8568 ± 0.0373	0.8690 ± 0.0207	0.9990	0.9996
	0.8771 ± 0.0467		0.8209 ± 0.0403			0.9997	1.000
	0.8956 ± 0.0631		0.8541 ± 0.0688			0.9985	0.9992

*Zero values indicate complete absence of a modeled 605 or 652 nm peak; therefore, uncertainty values cannot be given.

apatite samples and for more precisely quantifying the oxygen-dependent mechanism of REE³⁺-substitution into the apatite lattice.

Applications of the apatite oxygen paleobarometer for Neoproterozoic-Cambrian phosphorites

Fluorescence-based analysis of the oxygen-dependent incorporation of Sm³⁺ into apatite can be expected to provide significant insight into relative marine oxygen concentrations during the Neoproterozoic-Cambrian phosphogenic event. Sm³⁺ substitution in microfossil-encrusting and permineralizing apatite previously reported from Early Cambrian phosphorites of Kazakhstan (Schopf et al., 2015), an association that directly indicates the setting of apatite formation, illustrates the potential applicability of the AOP metric to geological samples with such analyses of these and other globally widespread Neoproterozoic- to Cambrian-age shallow water fossil-associated phosphorites being envisioned to ultimately provide a quantitative and relatively detailed understanding of marine oxygenation during this interval (for relevant data illustrating the application of this approach to the Kazakhstan phosphorites, see Chapter 4 of this dissertation).

For appropriate application of the AOP metric to naturally occurring phosphoritic apatite samples, mechanistic differences between Sm³⁺ substitution studied experimentally and the processes that occur during the natural incorporation of Sm³⁺ during apatite crystallization must be considered. Solid state heat-promoted substitution of Sm³⁺ into the apatite lattice over short hour-long timescales differs significantly from the much lower temperatures and longer



Figure 6. Calculated AOP values from individual fluorescence spectra of Sm^{3+} -substituted HAp pellets, plotted relative to O₂ concentration during experimental heating. Individual AOP data points calculated both from relative modeled peak areas within the 605 to 607 nm region (orange) and 652 to 654 nm region (blue). Data point error bars represent ± standard error generated from the peak fitting algorithm. The grey shaded region represents ± 1 standard deviation from the total mean (black).

timescales involved in such substitution during authigenic precipitation in biologically productive, marine shallow waters. Nevertheless, because the oxygen-dependent substitution mechanism should be identical in the synthetic and natural systems – its main determinant being the apatite lattice structure – and because the correlation established here between AOP and percentage O_2 is robust, the AOP can be expected to be at least semi-quantitatively applicable to naturally occurring authigenic apatite formation. Future studies that result in further quantitative refinement of the fluorescence-based analyses and of the associated AOP metric, combined with experimental studies of Sm³⁺ substitution into apatite chemically precipitated at ambient temperature under controlled aqueous O₂ concentrations (attempted but found not to be feasible for this study at the present time) should fully establish the efficacy of the heat-promoted experimental approach used here.

The ability to quantitatively or semi-quantitatively assess shallow marine oxygen concentrations across the Neoproterozoic-Cambrian transition would provide a novel and independent approach by which to better understand global oxygenation immediately preceding and coinciding with the "Cambrian Explosion" of life, the major diversification event of invertebrate metazoan lineages and one of the most significant events in the history of life.

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CHAPTER 3:

An apatite oxygen paleobarometer across the Neoproterozoic-Cambrian transition

3.1 – ABSTRACT

Levels of Earth atmospheric and marine oxygenation are postulated to have increased substantially through the Neoproterozoic-Cambrian transition, marking the end of the Precambrian and the beginning of the fossil-evidenced diversification of hard-shelled, oxygen-requiring metazoans during the "Cambrian Explosion" of life. However, currently established redox proxies applied to this interval, including chemostratigraphic variations in stable isotopes and redox-sensitive trace element abundances, do not quantitatively reflect ancient levels of oxygen in the immediate marine environment and do not uncommonly result in contrasting interpretations. Fluorescence signatures of Sm³⁺-substitution in apatite, experimentally shown to correlate with oxygen availability during Sm³⁺ emplacement and detected in Early Cambrian microfossil-associated apatite deposited during a globally extensive Neoproterozoic-Cambrian phosphogenic episode, provide a promising new strategy for an improved understanding of environmental oxygenation during the early evolution of megascopic life.

Fluorescence spectroscopic analyses presented here of apatite-encrusted and permineralized microfossils and apatitic ooids preserved within the late Neoproterozoic Doushantuo (South China) and Early Cambrian Chulaktau (South Kazakhstan) Formation phosphorites, as well as biomineral apatite scale fossils of the mid-Neoproterozoic Fifteenmile Group (Alaska, USA-Yukon, Canada border), have been conducted for their comparison with experimentally verified fluorescence signatures indicative of oxygen-dependent Sm³⁺-

substitution in apatite. The intensity variability between Sm³⁺-attributed fluorescence peaks, associated with anoxic or oxic conditions, are here reported between phosphatized specimen types and from whole phosphorite formations and are interpreted to reflect varying dissolved oxygen concentrations in the immediate apatite-precipitating environment. This variability is expressed in terms of the semi-quantitative apatite oxygen paleobarometer ("AOP") metric devised in Chapter 2 of this dissertation. These results are consistent with low (<10% O₂) oxygen levels in both the Doushantuo and Chulaktau local depositional environments but reflect modest increased oxygenation in the latter. Low oxygen AOP values calculated for mid-Neoproterozoic apatite scales are consistent with previous estimations of Fifteenmile basin oxygenation. Together, these analyses lay the groundwork for the application of the AOP metric to numerous other shallow water, microfossiliferous apatite-bearing phosphorite deposits associated with the Neoproterozoic-Cambrian phosphogenic event and quantitative investigations of the history of marine oxygenation during the Cambrian Explosion.

3.2 – INTRODUCTION

The rise of atmospheric oxygenation over Earth's 4.5-billion-year history constitutes one of the most significant geobiological transitions evidenced by the geologic record. Though the present-day atmosphere is composed of approximately 21% O₂, there existed virtually no free ambient oxygen during the first two billion years following the formation of the planet. Earliest oxygen "whiffs" at ~2.9 Ga (Planavsky et al., 2014) and ~2.5 Ga (Anbar et al., 2007) preceded an appreciable rise at ~2.3 to 2.1 Ga (termed the Great Oxidation Event, or "GOE"; Holland, 2002). However, it was potentially not until at least ~0.8 to 0.4 Ga that atmospheric oxygen

levels reached near-modern concentrations, coincident with the geologically rapid diversification of oxygen-requiring metazoans during the "Cambrian Explosion" (e.g., Schiffbauer et al., 2016).

Though this postulated second increase in atmospheric oxygen concentrations was more recent, details regarding the exact nature and timing of this increase are currently incompletely known (reviewed in Lyons et al. 2004; Och and Shields-Zhou, 2012). Evidence for a Neoproterozoic oxygenation event include perturbations to the marine carbon cycle evidenced by negative sedimentary carbon isotopic excursions (Fike et al., 2006; Grotzinger et al., 2011), variations in stable isotope and redox-sensitive trace element abundances (e.g., Scott et al., 2008; Sahoo et al., 2012; Chen et al., 2015; Stolper and Keller, 2018), and minimum physiological O₂ requirements for basal metazoans extant at that time (Nursall 1959; Berkner & Marshall, 1965; Rhoads & Morse, 1971; Cloud, 1976; Runnegar, 1982; Mills et al., 2014). Though a synthesis of these finding is broadly suggestive of increased Neoproterozoic-Cambrian oxygenation, the resulting interpretations are generally qualitative (i.e., oftentimes inferring "anoxic," "euxinic," or "oxic" conditions) and/or dependent on assumptions associated with mass-balance modeling. Still other studies suggest that extensive marine O₂ ventilation did not occur until well into the Cambrian (Canfield et al., 2008; Sperling et al., 2015). Measurements reported from Neoproterozoic-age gaseous inclusions in halite are suggestive of direct quantitative assessments of atmospheric O₂ concentrations (Blamey et al., 2016), but are somewhat problematic due to the susceptibility of halite to post-depositional recrystallization and contamination of measured inclusions.

This interval is also contemporaneous with the most globally widespread phosphogenic event in geologic history that produced Neoproterozoic- and Cambrian-age, shallow marine, commonly microfossiliferous phosphorites studied from all continents except Antarctica, though concentrated in central and eastern Asia (Notholt and Sheldon, 1986; Figure 1). The biogeochemical conditions known to favor phosphate deposition, including extensive organic carbon burial, are themselves both sensitive to marine redox conditions and could potentially have given rise to the expansion of the free oxygen reservoir (Donnelly et al., 1990; Cui et al., 2016). Neoproterozoic and Cambrian cycling of phosphorous and oxygenation must therefore have been inextricably linked (Planavsky et al., 2011; Reinhard et al., 2017).

Previously reported fluorescence spectra of microfossil-permineralizing and -encrusting apatite of the Early Cambrian Chulaktau Formation phosphorite (South Kazakhstan), comparable to experimentally calibrated fluorescence signatures of oxygen-dependent Sm³⁺-substitution in apatite, suggest that spectroscopic analyses of phosphorite mineralogy may provide a unique window into local marine O₂ concentrations through the Neoproterozoic-Cambrian transition (Schopf et al., 2015). The ubiquitous phosphorite mineral apatite incorporates significant abundances of rare earth elements (REEs) due to its crystal-chemical makeup (e.g., Pan and Fleet, 2002). Whereas abundances and distributions of REEs in Neoproterozoic-Cambrian phosphorites have traditionally been used to qualitatively interpret marine redox conditions during deposition (Ilyin, 1998; Mazumdar et al., 1999; Yang et al., 2007; Zhu et al., 2014), naturally occurring apatite fluorescence signatures may instead be quantitatively or semi-



Figure 1. Geographic and age distribution of Neoproterozoic- and Cambrian-age phosphorites. Modified after Cook and Shergold, 1984 and references therein.

quantitatively related to experimental calibrations of such signatures for the interpretation of dissolved O_2 in the immediate apatite-precipitating environment.

The present study characterizes Sm³⁺-attributed apatite fluorescence signatures from three geologic settings across the Neoproterozoic-Cambrian boundary: apatitic microfossil and ooid specimens from the late Neoproterozoic Doushantuo Formation (South China) and the Early Cambrian Chulaktau Formation (South Kazakhstan), as well as apatite scale microfossils from the mid-Neoproterozoic Fifteenmile Group (Alaska, USA-Yukon, Canada border) interpreted as the earliest evidence of eukaryote biomineralization (Cohen et al., 2011; 2017). Comparison of these fluorescence signatures with those produced by previous experimental calibrations of heatpromoted Sm³⁺-substitution in apatite under varying O₂ concentrations (Chapter 2 of this dissertation) permits the assessment of local redox conditions during the shallow water, early diagenetic stages of phosphatization represented by the studied fossil-associated apatite specimens. The spectral features experimentally determined to be characteristic of anoxic or oxic conditions are quantitatively described by the apatite oxygen paleobarometer metric, or "AOP," devised in Chapter 2 and related to percentage O₂. This approach demonstrates a promising new avenue by which to interrogate the history of marine oxygenation recorded by Neoproterozoic-Cambrian phosphorites during an interval of significant geochemical and evolutionary transition.

Neoproterozoic-Cambrian phosphogenesis

The suite of environmental factors during the Neoproterozoic-Cambrian transition that produced the most extensive phosphogenic episode in geologic history can generally be understood by analogy with modern phosphate depositional processes (reviewed in Föllmi,

1996). Though phosphogenesis can occur in a variety of marine and terrestrial environments, large-scale modern authigenic phosphorite formation is best studied in regions of upwelling situated along western continental margins, such as the coast of Peru (Veeh et al., 1973; Froelich et al., 1988). In these settings, phosphate-rich deep ocean waters upwell into shallower shelf environments, boosting primary productivity and the burial of organic matter (Kasakov, 1937; Cook and Shergold, 1986). Degradation of organic material in shallow sediment depths concentrates phosphate in low pH pore waters such that saturation is achieved with respect to apatite (commonly carbonate-bearing fluorapatite, or "francolite," though hydroxyapatite is also known to form authigenically as well; Schulz and Schulz, 2005) (Krajweski et al., 1994). Other mediating factors involved with phosphogenesis include adsorption to iron oxyhydroxides that shuttle phosphate from the oxygenated water column to the more reducing sediment interface (Berner, 1973), as well as microbial activity that can form sediment microenvironments conducive to apatite precipitation (Krajweski et al., 1994). Modern upwelling-related phosphorites typically form as phosphatic concretions and crusts, which can be reworked and concentrated by sea currents (Föllmi, 1996).

Phosphogenesis during the Neoproterozoic-Cambrian event was likely similarly generated by phosphate delivery to shallows by upwelling currents and redox- or microbemediated concentration of phosphate in sediments beneath productive waters, though these ancient phosphorites were formed at shallower depths than modern upwelling-driven deposits (Cook and Shergold, 1984). Large-scale climactic and tectonic factors may have additionally promoted extensive phosphogenesis; Cyrogenian-age glaciations, evidenced by tillites commonly underlying phosphorite deposits, may have accelerated continental weathering and

the transport of phosphorous to the marine environment, and paleogeographic reconstructions of the broadly contemporaneous rifting of Rodinia place numerous epicontinental platforms and restricted basins at low, productive paleolatitudes that would have concentrated phosphates in regions corresponding to present-day localities of phosphorites (reviewed in Cook and Shergold, 1986; Papineau et al., 2010). Finally, a potentially stratified redox structure of the marine system characterized by anoxic bottom waters, typically not observed for the majority of the Phanerozoic, may have accelerated organic burial beyond that which might be expected from upwelling-driven primary productivity alone, resulting both in phosphogenesis and increased environmental oxygenation (Donnelly et al., 1990).

These conducive environmental conditions may help to explain several unique aspects of Neoproterozoic-Cambrian phosphogenesis, including its global expansiveness and richness in phosphate-preserved, shallow water microbiota. The "Doushantuo-type" taphonomic window – exemplified by exquisitely and three-dimensionally preserved metazoan embryo fossils from the Neoproterozoic Doushantuo Formation (Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2000) – is restricted to the Neoproterozoic and Cambrian and is defined by early-diagenetic phosphatization and preservation of labile cellular structures and soft tissues (Butterfield, 2003). Though phosphatized fossils are known from the later Phanerozoic, preservation in these more recent instances is more commonly observed for recalcitrant organic materials, shells, and originally phosphate-rich skeletal and coprolite debris, reflective of limiting availabilities of phosphatization characteristic of Doushantuo-type microfossils demonstrates the immense

favorability of phosphate deposition during the Neoproterozoic-Cambrian transition, a biogeochemical phenomenon not observed in the more recent Phanerozoic geologic record.

Though the numerous postulated environmental factors involved with producing such large-scale Neoproterozoic-Cambrian phosphogenesis warrant further investigation, the various lines of evidence discussed above illustrate the unique biogeochemical environment linking phosphogenesis and oxygenation of the marine system (e.g., Papineau, 2010).

Geologic setting

The Doushantuo and Chulaktau phosphorites studied here are two examples of shallow water, peloidal and microfossiliferous deposits typical of the Neoproterozoic-Cambrian phosphogenic event (Notholt and Sheldon, 1986). Doushantuo deposits preserve phosphatic carbonate facies formed on shallow shelf environments of the South China Yangtze platform following its rift from Gondwana during the Neoproterozoic (Metcalfe, 1996; Li et al., 1996). The Weng'an locality in central Guizhou Province consists of two phosphorite sequences separated by dolomite beds (Xiao et al., 2000). The upper phosphorite represents the shallower environment of the two, deposited above fair-weather wave base, and is composed of grainstone and dolomite-cemented intraclastic phosphorite. Phosphatized algal thalli and embryos occur as intraclasts, likely having been concentrated with other phosphatic fragments by periodic winnowing and redeposition in a relatively high energy environment. The age of the Neoproterozoic Doushantuo Formation is constrained by radiometric U-Pb zircon dates of underlying and overlying ash beds, and yield a conservative estimate of between ~630 and 550 Ma (Condon et al., 2005). If the separation between upper and lower phosphorite sequences can

be interpreted as subaerial exposure resulting from sea-level fluctuation contemporaneous with the Gaskiers glaciation, the upper, embryo-bearing phosphorite studied here can be further constrained to no older than ~580 Ma.

Deposits of the Early Cambrian Chulaktau Formation (South Kazakhstan) include peloidal and interbedded siliceous phosphorites. As in the Doushantuo Formation, the depositional environment of the Chulaktau phosphorite is interpreted as an exceedingly shallow (evidenced by desiccation cracks and oolitic grainstones; Schopf et al., 2015), partly restricted basin setting that would have promoted the concentration and sedimentation of siliceous and phosphatic oozes (Eganov et al., 1986). Phosphatic intraclasts and ooids are evidenced to have been periodically reworked and concentrated, along with phosphatized mat-forming cyanobacteria, acritarchs, and small shelly fossils indicative of a shallow water biota. The maximum age of the Chulaktau Formation estimated to be ~770 Ma on the basis of U-Pb zircon dates of underlying volcanic tuffs (Sovietov, 2008; Levashova et al., 2011). However, biostratigraphic correlations with faunal and microbial fossil taxa place the Chulaktau firmly in the Early Cambrian (reviewed in Schopf et al., 2015).

Apatite scale microfossils recovered from the Fifteenmile Group (Alaska, USA-Yukon, Canada border) are not representative of global or regional phosphogenesis, but rather may precede the oldest Neoproterozoic-Cambrian phosphorites by up to ~100 Ma. Age estimates for fossil-bearing deposits lie between ~810-720 Ma on the basis of radiometric dating and chemostratigraphic correlation (Macdonald et al., 2010a; 2010b; 2011). They importantly are not evidenced to have been authigenically precipitated by phosphate-rich pore waters, but are rather
interpreted as biologically controlled apatite biomineralization of mid-Neoproterozoic-age protists of speculative green algal taxonomic affinity (Cohen et al., 2011). Evidence for biologically controlled precipitation is based upon organized crystallographic patterning of the apatite microstructure, as well as the absence of diagenetic deformation and phosphatization of co-occurring sedimentological features and microfossils (Cohen et al., 2011; 2017). Though taxonomic affinity remains uncertain, primarily from the lack of both ancient and modern examples of protistan apatite biomineralization, the morphological and compositional similarity of the fossil scales to those produced by a green alga (Domozych et al., 1991) suggests that the apatite scale microfossils may have been formed by a planktonic organism inhabiting surface waters of the Fifteenmile basin. Therefore, unlike those measured from microfossil-associated apatite of the Doushantuo and Chulaktau phosphorites, oxygen-related fluorescence signatures of the Fifteenmile apatite scale microfossils should in these studies provide a unique sampling of the water column environment rather than that of the sediment or sediment-water interface.

Oxygen-dependent Sm³⁺ substitution of apatite

Together, microfossil-permineralizing, -infilling, and -encrusting apatite and phosphatic ooids of the Chulaktau and Doushantuo phosphorites and biomineralized scale fossils of the Fifteenmile Group provide a diverse suite of specimens with which to characterize apatite fluorescence related to oxygen-dependent Sm³⁺-substitution. The incorporation of trivalent rare earth elements (REE³⁺s) within the apatite [Ca₅(PO₄)₃(F,OH,Cl)] structure can occur at either of two non-identical Ca²⁺ sites, the high symmetry Ca1 site (structure CaO₉) and the low symmetry Ca2 site (structure CaO₆A, where A = F⁻, OH⁻, or Cl⁻) (e,g,, Hughes et al., 1989; also see Chapter

3, Figure 1). Possible substitution mechanisms that compensate for local charge difference between the substituting REE^{3+} and Ca^{2+} include (Fleet and Pan, 1994):

$$2REE^{3+} + \Box = 3Ca^{2+}$$
(1)

$$REE^{3+} + O^{2-} = Ca^{2+} + A^{-}$$
(2)

where \Box (open square symbol) represents a neighboring Ca²⁺ vacancy and A⁻ represents the relevant F⁻, Cl⁻, or OH⁻ anion within the apatite lattice.

Artificial REE³⁺ substitution of synthetic apatites in vacuum and in air have demonstrated that Ca-site preference depends on ambient oxygen availability during incorporation. Experiments conducted in vacuum result in REE³⁺ substitution at the Ca1 site by the mechanism described by Equation 1 (Chen et al., 2002b), whereas experiments conducted in air result in REE³⁺ substitution at the Ca2 site by the oxygen-dependent mechanism described by Equation 2 (Chen et al., 2002a). Fluorescence spectroscopy of apatite substituted with REE³⁺s (including Sm³⁺) reveals distinctly differing peaks in spectra measured from either vacuum- or air-activated apatite, suggestive of differentially substituted Ca-sites under either anoxic and oxic experimental conditions (Gaft et al., 1997; also see Chapter 3, Figure 2).

Because Sm³⁺-attributed fluorescence spectra comparable to those observed in experiments has been detected in Early Cambrian phosphoritic apatite (Schopf et al., 2015), a well-calibrated fluorescence spectroscopic strategy for the detection of oxygen-dependent Sm³⁺substitution signatures holds great potential for elucidating ancient marine oxygen concentrations during Neoproterozoic-Cambrian apatite formation. The results described in Chapter 3 demonstrate that calibration experiments (1) confirm an oxygen-dependent substitution mechanism for Sm³⁺ at the apatite Ca2 site, (2) document the relatively gradual spectral change under progressively oxygenated experimental conditions, and (3) semi-quantitatively relate spectral character, as represented by the relative intensities of particularly the 605 and 607 nm fluorescence peaks, to oxygen concentrations during heat-promoted Sm³⁺ emplacement. These experimental refinements provide the basis for the interpretation of phosphorite and biomineral apatite fluorescence signatures.

3.3 – MATERIALS AND METHODS

Geologic samples

Apatite-permineralized and -infilled embryo and apatite-permineralized algal fragment fossil specimens were identified and selected within a geologic thin section (WSB-60-120-2) prepared from a dolomitic phosphorite sample collected from the upper Phosphorite Bed "A" at the Weng'an county Chuan Yan Dong Mine locality of the Doushantuo Formation (central Guizhou Province, south-central China).

Selected apatite-associated microfossil specimens from the Chulaktau Formation phosphorites for analysis include permineralized helical cyanobacterial trichomes, infilled cyanobacterial sheaths, and apatite-replaced multiradiate sponge spicules (thin section CHUL-4681-273-D), as well as permineralized straight cyanobacterial filaments (thin section PPRG-P 1476 1-A). Apatitic ooids, which occur in thin laminae, were also selected for analyses (thin section PPRG-P 1476 1-B). Phosphatic bedded black chert samples used for the preparation of the CHUL-4681-273-D and PPRG-P 1476 thin sections were collected from the Kurtlybulakt and Au-Sakan section localities, respectively, of the Chulaktau Formation (Maly Karatau Range, southern Kazakhstan).

Chert thin sections containing apatite scale microfossils (UT1-7a, UT1-7b) were prepared from samples collected at the Fifteenmile Group exposure straddling the Alaska-Yukon border (approximate map coordinates N65°16', W140°0'), generously provided by Professor Nicholas J. Butterfield of Cambridge University, England.

Optical and confocal laser scanning microscopy

Optical images of specimens within phosphorite and chert thin sections were acquired at the University of California, Los Angeles (UCLA) with a Leitz Orthoplan (#654016659) microscope (Leitz, Wetzlar, Germany) equipped with an Olympus DP12 Microscope Digital Camera (Olympus, Melville, NY). Thin section samples were coated with a thin veneer of fluorescence-free microscopy immersion oil prior to optical photomicrography.

Two- and three-dimensional confocal laser scanning micrographs of specimens were acquired at UCLA using an Olympus Fluoview 300 confocal laser scanning biological microscope system. Argon ion laser excitation at 488 nm and 20 mW power was provided by an equipped Melles Griot laser source, and filters in the light-path removed wavelengths <510 nm from the fluorescence emitted by the analyzed specimens. Thin section samples were coated with

a thin veneer of fluorescence-free microscopy immersion oil prior to image acquisition. Threedimensional data was acquired by rendering stacked two-dimensional fluorescence image series in Volview v.3.4.

Raman and fluorescence spectroscopy

Raman and fluorescence spectroscopic analyses of thin section apatite specimens were conducted at UCLA using a T64000 triple-stage confocal laser-Raman system equipped with a Coherent Innova argon ion laser source. Laser excitation at 457.9 nm and ~150 mW power was used for both Raman and fluorescence analyses. Thin sections were coated with a thin veneer of fluorescence-free microscopy immersion oil, previously demonstrated to have no effect on the spectral features of interest (Schopf et al., 2002).

Raman and fluorescence point spectra and images displaying the two-dimensional spatial distribution of organic and mineralogical components were acquired within either a \sim 200 to \sim 3000 cm⁻¹ spectral window centered at \sim 1600 cm⁻¹ (for Raman analyses) or a \sim 590 to 660 nm window centered at 625 nm (for fluorescence analyses). Multi-window point spectra were also acquired between 465 nm and 750 nm, containing both Raman and fluorescence spectral features.

Point spectra and images were processed using Labspec v.5. including spectral etaloning effect removal and automatic polynomial baseline correction. Etaloning effects were removed by normalizing to a dark spectrum acquired under the same measurement parameters as that for the specimen fluorescence spectrum. Noise reduction of the Raman images was achieved by the

Fourier transform smoothing function in LabSpec. Pixel intensities in Raman images correspond to the peak intensities at specified wavenumbers or wavelengths associated with organic or mineralogical components of interest.

Three-dimensional fluorescence data were acquired by manually acquiring series of twodimensional fluorescence images at 1- μ m-spaced focal depths through each specimen. Stacked two-dimensional images processed in LabSpec were aligned and rendered in ImageJ v.1.50i, permitting fluorescence image manipulation in three dimensions.

Fluorescence spectral peak analysis

Following methods detailed in Chapter 3 of this dissertation, Sm³⁺-attributed apatite fluorescence peaks positioned at ~605 and 607 nm acquired within a ~590 to 660 nm spectral window were fitted using PeakFit v.4.12. Though the spectral analysis of apatite pellets described in Chapter 3 includes fitting of 652 and 654 nm peaks, the intensity of such peaks in phosphorite and biomineral apatite specimens was not sufficient for reliable fitting. The 605 and 607 nm peaks were first isolated within a 600 to 612 nm spectral region to reduce baseline fitting uncertainty and subsequently smoothed by the Savitsky-Golay algorithm. Smoothing levels were manually adjusted to best capture visually distinguishable peaks not reliably resolved by the automatic smoothing tool in PeakFit. Peaks within the smoothed spectrum were then identified by a standard deconvolution procedure that included automated "best fit" baseline subtraction, and subsequently manually adjusted for peak position and shape while allowing for variation between Gaussian and Lorentzian peak-character. The modeled peak fit was then refined using an iterative graphical fit algorithm under default constraints through at least 1000 iterations and

until the fit returned r^2 values >0.9990 and p < 0.00001, detailing the closeness of fit between the smoothed spectrum and that modeled by the summation of the fitted peaks.

The method for apatite oxygen paleobarometer (AOP) value calculation described in Chapter 3 was used to calculate AOP values from the fluorescence spectra acquired from phosphorite and biomineral apatite specimens analyzed here. As mentioned above, only the 605 and 607 nm peaks were of sufficient intensity for analysis, and used to calculate AOP according to the following equation:

$$AOP_{605-607} = \frac{P_{605}}{P_{605}+P_{607}}$$
(3)

where P is the area of either the 605 or 607 nm peaks, the relative intensities of which have been experimentally demonstrated to correspond to ambient oxygen concentration during Sm³⁺- substitution of apatite. The AOP used here is then a numerical metric between zero (indicating only the presence of the 607 nm peak) and one (indicating the presence of only the 605 nm peak).



Figure 2. Optical (A, E-F, I), CLSM (B), kerogen Raman (C, G), and apatite Raman (D, H) images of apatite-permineralized and infilled algal and embryo microfossils of the Doushantuo Formation. (A-E) Embryo fossils exhibiting isopachous and botryoidal apatite mineralization of encapsulating envelopes and internal organic components. (F-H) Permineralized fragments of algal thalli. Note finely preserved cellular structure visible in optical images. The square-shaped region with diminished fluorescence indicated by the red arrow in (B) is a consequence of previous Raman imaging and is not relevant to compositional interpretation. Kerogen Raman images display the relative spatial distribution of the ~1610 cm⁻¹ band, and apatite Raman images, the relative spatial distribution of the ~964 cm⁻¹ band.

3.4 – RESULTS

Phosphorite apatite mineralization

In the studied Doushantuo thin section, apatite occurs as fragments of permineralized and fragmented algal thalli, infilled embryos, and unidentified, apatite- or quartz-inclusion-rich intraclasts within a dolomite matrix (Figure 2). Apatite permineralization of algal fragments, identified by the intense Raman band at ~964 cm⁻¹, preserves cellular morphology and organic, carbonaceous composition, visually demonstrated by optical (Figure 2F, 2I) and Raman imagery of the kerogen band intensity at ~1610 cm⁻¹ (Figure 2G). Phosphatization of embryos occurs as external and internal coatings of isopachous apatite of encasing carbonaceous membrane envelopes, as well as fine permineralization and spherule encrustation on internal bodies (similarly described in Xiao et al., 1999). Partially mineralized embryos retain hollow cavities, though some are secondarily infilled with coarse crystalline apatite. Growth laminae of apatite coatings are readily apparent in CLSM images (Figure 2B).

Phosphatization in the Chulaktau Formation specimens occurs as the permineralization of cellularly preserved, mat-forming cyanobacteria, including filamentous and coccoidal forms described in detail in Schopf et al. (2015), encrusted and infilled cyanobacterial sheaths devoid of internal cellular trichomes, and mineral replacement of biomineral fragments, including numerous multiradiate spicules (Figures 3-4). Apatite mineralization is evidently tightly associated with kerogen both in microfossils and in abiogenically formed phosphatic ooids that presumably incorporated particulate organic material during apatite concretion.



Figure 3. Optical (A-C, E-G), two-dimensional (D) and three-dimensional (J-K) CLSM, kerogen Raman (H), and apatite Raman (I) images of phosphatized ooids and microfossils of the Chulaktau Formation. (A-D) Phosphatic, kerogen-rich ooids displaying concretionary mineral growth laminae. (E-K) Multiradiate, apatite-replaced spicule specimens. Raman images were acquired within a spatial subset indicated by the red box in (G), and display the spatial variability of the ~1610 cm⁻¹ (blue, kerogen) and ~964 cm⁻¹ (red, apatite) Raman bands. The z-axis shown in (J) of the rotated, three-dimensional CLSM image also shown in (K) is parallel to the vertical orientation in thin section.

Euhedral encrusting apatite crystals, similarly identified in Schopf et al. (2015), are, of the studied specimens, unique to the Chulaktau Formation phosphorites (Figure 5). These crystals range from submicron to >100 μ m in size, and are readily identifiable by typically hexagonal and equant or tabular crystal habit, though some appear elongate and prismatic (Figure 5B). Encrusting crystals are observed to have nucleated on a variety of substrates, though all have been observed on phosphatized microfossil or otherwise unidentified or abiogenic intraclasts. "Free-floating" euhedral crystals, unattached to phosphatized substrates within the encompassing chert matrix, were likely dislodged prior to lithification, as they are typically proximal to other encrusted intraclasts or crystalline debris, potentially indicative of periodic agitation of phosphatized substrate clasts by wave action.

Sm³⁺ apatite fluorescence

Sm³⁺-attributed fluorescence point spectra were measured from several representative specimens of phosphatized microfossils and ooids selected in the Doushantuo and Chulaktau phosphorites as well as biomineral apatite scale microfossils from the Fifteenmile Group. These include Doushantuo permineralized algal thalli and infilled embryos, as well as Chulaktau cellularly preserved helically-coiled and straight cyanobacterial filaments, vacant cyanobacterial sheaths, apatite-replaced spicules, phosphatic ooids, and single, euhedral encrusting crystals. Together, these samples provide a broad sampling of authigenic and biomineral apatite fluorescence signatures.

Single, representative fluorescence spectra acquired within a broad spectral range from the Doushantuo and Chulaktau Formations, as well as averaged spectra within a narrow, higher



Figure 4. Optical (A, C-D, H, I, K-L, P, R), CLSM (B, E, J), kerogen Raman (F, M, O), and apatite Raman (G, N, Q) images of apatite-permineralized, helically-coiled and straight cyanobacterial fossils and infilled cyanobacterial sheaths of the Chulaktau Formation. (A-C, F-H) Apatite-permineralized helical cyanobacterial filaments. Note large, euhedral encrusting apatite crystals

nucleated on the left edge of the specimen in (C). (D-E, M-N, P, R) Cyanobacterial sheaths infilled with isopachous and spherulitic apatite growth. Note crystalline growth laminae visible in fluorescence imagery (E). (I-L, O, Q) Apatite-permineralized, straight cyanobacterial trichomes. The encapsulating organic sheath, not readily apparent in optical images, is detected by fluorescence (J) and kerogen Raman imagery (O). Red box in (A) refers to imaged region in (F-G), red box in (D) refers to imaged region in (M-N), and red box in (I) refers to imaged region in (O, Q). Kerogen Raman images display spatial intensity distribution of the ~1610 cm⁻¹ band, and apatite Raman images display that of the ~964 cm⁻¹ band.

resolution spectral range from each geologic setting are shown in Figure 6. Fluorescence peaks assigned to Sm³⁺-substitution within apatite within this narrow spectral window include those at 597, 598, 605, 607, 623, 645, and 654 nm, though there is observed spectral variability between individual measurements, notably between the relative intensities of the 605 and 607 nm peaks of particular interest here due to their utility in distinguishing between Ca1- and Ca2-site substitution. Peak assignments are based on comparisons to previously published fluorescence spectra of natural or artificial Sm³⁺-substitution of naturally occurring or synthetic apatite crystals (e.g., Reisfeld et al., 1996; Gaft et al., 1997; Mitchell et al., 1997; Gaft et al., 2005), as well as artificially Sm³⁺-substituted phosphate glasses (Czaja et al., 2010) and the apatite powders discussed in Chapter 2 of this dissertation (also see Chapter 2, Table 2).

Distinguishing Sm^{3+} fluorescence peaks (particularly the 605 and 607 nm peaks) from those potentially produced by other REEs or transitional metals is moderately simplified by the fact that many do not have assigned peaks within the ~600 to 650 nm region. However, there is some overlap with Dy^{3+} , Eu^{3+} , Pr^{3+} , Tb^{3+} , and Mn^{2+} fluorescence in this region. Dy^{3+} , Eu^{3+} , and Tb^{3+} do not likely contribute greatly to the Sm^{3+} -attributed spectral features of interest because they do not produce discrete fluorescence peaks in the 605 to 607 nm region (Gaft et al., 1997; 2005).

Pr³⁺-substitution of crystalline apatite and phosphate glasses has been previously documented to produce fluorescence features in the 605 to 607 nm region (Gaft et al., 1997; Czaja et al., 2010). However, Pr³⁺-substitution is not commonly associated with a fluorescence peak at 654 nm. In addition, the broadly decreasing fluorescence intensity across the 597 to 598,



Figure 5. Optical (A-C, D, J), two-dimensional Raman (E, K), two-dimensional fluorescence (F, L), and three-dimensional fluorescence images (G-I, M-O) of euhedral, encrusting apatite crystals of the Chulaktau Formation. Encrusting crystals noted by black arrows. Red boxes in (D) and (J) refer to imaged regions in (E-I) and (K-O), respectively. Raman images show layered intensity distributions of kerogen (blue, ~1610 cm⁻¹) and apatite (red, ~964 cm⁻¹). Fluorescence images show layered intensity distributions of the 619 nm (red) and 605 nm (green) fluorescence peaks, associated with Sm³⁺ substitution at the Ca1 and Ca2 sites, respectively. The 619 nm peak was selected here in lieu of the 607 nm peak used in further analyses because both intensities are strongly correlated and the 619 nm peak produced a nearly identical, but higher contrast fluorescence image. Blue edges of boxes in three-dimensionally manipulated fluorescence images (G-I, M-O) are parallel with the top plane of the thin section. Dotted edges in (I) and (O) indicate virtually sliced surfaces corresponding to the dotted cross section lines in (F) and (L), respectively, to permit viewing of three-dimensional fluorescence patterns within the interiors of the imaged crystals.

605 to 607, and 654 nm bands observed in our specimens is more closely matched by spectral patterns of Sm³⁺-substituted natural apatite and phosphate glasses. Furthermore, the similarity between fluorescence peak positions observed in phosphorite and biomineral apatite and those observed in artificially Sm³⁺-substituted apatite powders (Chapter 2) is particularly important due to their spectroscopic measurement with the same excitation wavelength (457.9 nm), a methodological variable which is known to impart variability in emission spectra of apatite (e.g., Gaft et al., 2005) and may account for differences in fluorescence spectra reported by other workers. A fluorescence peak at 619 nm, only observed in Chulaktau encrusting crystals (Figure 7), is admittedly not commonly observe in Sm³⁺-substituted apatite and could potentially be assigned to other substituting elements. However, the intensity of the 619 nm peak is strongly correlated with the intensity of the 597 and 654 nm peaks, both of which have been previously associated with Sm³⁺, and therefore can be speculatively assigned to Sm³⁺ as well. In sum, the fluorescence spectral features observed in phosphorite and biomineral apatite specimens is most parsimoniously attributed to the presence of substituting Sm³⁺.

Fluorescence features produced by Mn^{2+} -substitution in apatite are known to occur within the ~600 to 650 nm region, but are notably broad (Gaft et al., 1997). We cannot rule out Mn^{2+} as potentially contributor to the broad background fluorescence centered at ~570 nm observed in the studied apatite specimens (Figure 6), though this may also be attributed to kerogen fluorescence in the same region. The intensity of the background fluorescence does increase in darker colored regions of phosphatized specimens, indicative of higher carbonaceous content, and has been observed to obscure discrete peaks assigned to Sm^{3+} (but does not alter the relative



Figure 6. Individual representative full fluorescence spectra (top panel) and higher resolution, averaged fluorescence spectra (bottom panel) of phosphatized microfossil and ooid specimens. Doushantuo full fluorescence spectrum acquired from an embryo specimen (#17, see Appendix A) and Chulaktau full fluorescence spectrum acquired from a spicule specimen (#56, see Appendix A). Spectra in the bottom panel are averaged from individual measurements from each geologic setting, normalized to the intensity of the 607 nm peak. Red and green vertical bands indicate peak positions associated with Sm³⁺ substitution at the Ca1 and Ca2 sites of apatite, respectively, experimentally determined in Chapter 2 of this dissertation. Though the 652 and 654 nm bands are generally too weak for detection, peak positions are highlighted for comparison with experimentally substituted apatite fluorescence (see Fig. 5 in Chapter 2). Correction for etaloning effects was not made for Fifteenmile spectra, resulting in noise at longer wavelength regions but which does not obscure the 605 to 607 nm peak region of particular interest. Grey shaded regions represent ± one standard deviation from the mean spectrum.

intensities of the 605 and 607 nm peaks as modeled by the peak fitting algorithm described below). The fluorescence point spectra reported here are therefore acquired from light-colored regions of the studied apatite specimens, minimally obscured by Mn²⁺- or kerogen-attributed background fluorescence.

There is visually distinguishable fluorescence spectral variability, particularly within the 605 to 607 nm spectral region, both between specimen types and between averaged spectra representative of each geologic setting (Figure 6). Fluorescence spectra acquired from apatitic algal and embryo specimens of the Doushantuo Formation generally exhibit lower 605:607 nm intensity ratios than those acquired from cyanobacterial, spicule, and ooid specimens of the Chulaktau formation. Spectra from Chulaktau phosphatic ooids exhibit the greatest 607:605 nm intensity ratios. However, because such visual estimations of relative peak contributions can be affected by background fluorescence and instrument noise, a more rigorous and quantitative peak fitting method for the calculation of relative peak areas is described in the following subsection.

Sm³⁺-attributed fluorescence spectra of euhedral, encrusting apatite crystals are typically more intense and refined than those measured from cryptocrystalline, phosphatized microfossils and ooids. The greater signal intensity permits two- and three-dimensional fluorescence imaging, which shows the spatial distinction between areas characterized by either high 605 or 607 nm intensity (Figure 5). In the studied crystals (selected for consistent orientation such that the crystal c-axis is parallel to the instrument laser beam), the 605 nm peak intensity is greatest in the interior regions closest to the nucleating substrate, but gradually diminishes in relation to increasing 607 nm intensity towards the crystal exterior (Figure 7). This patterning evident in

two-dimensional fluorescence images is also demonstrated by three-dimensional fluorescence imaging to be consistent throughout the volume of the crystal and is indicative of concentric zonation resulting from changing chemical conditions during crystal growth.

Apatite oxygen paleobarometer calculation

Relative intensities of the 605 and 607 nm peaks within fluorescence spectra acquired from phosphorite and biomineral apatite specimens have been quantified by the apatite oxygen paleobarometer (AOP) metric developed in Chapter 2. Peaks positioned at 652 and 654 nm are not incorporated into AOP calculations here due to their relatively low measured intensities and the resulting uncertainty in estimating analytical areas by a peak fitting algorithm. Mean AOP values for each specimen type and for each encompassing geologic setting are listed in Table 1 and plotted in Figure 8, alongside cross-sectional AOP values acquired from a euhedral encrusting Chulaktau crystal. The mean AOP values demonstrate a significant distinction between the data distribution of total Doushantuo (0.2459 ± 0.0522 , n = 19) and Chulaktau (0.3945 ± 0.1167 , n = 48, dataset excluding euhedral crystal specimens) specimens. The significance of this distinction has been determined statistically by an unpaired, nonparametric Mann-Whitney *U* test (*U* = 45, two-tailed, $\alpha = 0.05$) and rejects the null hypothesis that the Doushantuo and Chulaktau AOP datasets have the same distribution. Fifteenmile apatite scale fossils have a mean AOP value of 0.3513 ± 0.1018 . Finally, AOP values gradually decrease from



Figure 7. Fluorescence spectra acquired across a cross-section through the width of a Chulaktau euhedral, encrusting apatite crystal (also shown in Figure 4D). Measurement points A-G extend from the interior to the exterior of the crystal. Red and green vertical bands indicate peak positions associated with Sm³⁺ substitution at the Ca1 and Ca2 sites of apatite, respectively, as determined experimentally in Chapter 2 of this dissertation.

the interior (~0.8) to exterior (~0.3) regions of encrusting euhedral Chulaktau crystals. Because the AOP metric has been experimentally verified to correlate with ambient O_2 concentration during Sm³⁺ emplacement in apatite (Chapter 2), the variability of AOP values calculated from phosphorite and biomineral apatite specimens are interpreted to reflect variable aqueous O_2 concentrations in the immediate apatite-precipitating environment.

3.5 – DISCUSSION

Phosphatization timing and setting

Apatite mineralization of microfossils and ooids of the Doushantuo and Chulaktau Formation phosphorites, and indeed most likely for all shallow water, microfossiliferous phosphorites associated with the Neoproterozoic-Cambrian phosphogenic episode, must have been an exceedingly early diagenetic phenomenon. As previously discussed by Xiao et al. (1999; 2000) and Schopf et al., (2015) regarding phosphatized microfossils from the Doushantuo and Chulaktau Formations, respectively, rapid mineralization of labile, cellular structures must have been necessary to preserve fine microfossil structures to such high fidelity. Though post-mortem organic degradation by microbial activity may have been protracted in reducing conditions (Martin et al., 2005; Raff et al., 2006; 2008), early mineralization of three-dimensionally preserved microfossils in the uppermost few millimeters of sediment (Dornbos et al., 2006) would have been needed to prevent compaction in the relatively high energy, shallow subtidal environment of Doushantuo and Chulaktau deposition. Experimental phosphatization of soft

Geologic setting	Specimen type	Apatite mineralization type	# of specimens	# of measured point spectra (n)	Mean AOP ± SD*	Thin section
Fifteenmile			2	10	0.3513 ± 0.1018	
	Scale	Biomineralization	2	10	0.3513 ± 0.1018	UT1-7a, UT1-7b
Doushantuo			6	19	0.2459 ± 0.0522	
	Embryo	Infilling	3	10	0.2069 ± 0.0317	WSB-60-120-2
	Algae	Permineralization	3	9	0.2875 ± 0.0370	WSB-60-120-2
Chulaktau			15	48	0.3945 ± 0.1167	
	Cyano Sheath	Infilling	3	9	0.3104 ± 0.0446	CHUL-4681-273-D
	Cyano helical filament	Permineralization	3	9	0.3623 ± 0.0792	CHUL-4681-273-D
	Spicule	Replacement	3	9	0.4315 ± 0.0830	CHUL-4681-273-D
	Cyano straight filament	permineralization	3	9	0.4351 ± 0.0938	PPRG-P 1476 1-A
	Ooid	Concretion	3	12	0.4506 ± 0.1534	PPRG-P 1476 1-B

Table 1. Mean AOP values by formation and apatite-mineralized specimen type. Mean values for each geologic setting are averaged across equally weighted specimen type averages. SD refers to standard deviation.

tissues and invertebrate eggs have demonstrated that partial to complete authigenic mineralization can occur on the order of days to weeks (Briggs & Kear, 1993; Martin et al., 2003), suggesting that these studied Doushantuo and Chulaktau apatite specimens may have mineralized immediately relative to typical geologic timescales.

Though apatite formation in both the Doushantuo and Chulaktau phosphorites likely occurred quite soon after deposition, diagenetic phases of phosphatization can be distinguished by sedimentological relations. Of the studied specimens, apatite mineralization of microfossils likely occurred earliest in the diagenetic process as evidenced by their lack of compaction and degradation. In contrast, phosphatic ooids preserve a more prolonged diagenetic process through gradual concretion from interior to rim. Finally, in the Chulaktau Formation, both microfossil and ooid phosphatization would generally have preceded encrustation of euhedral apatite crystals. This is evidenced by nucleation of said crystals on microfossils and ooids, which most commonly project externally into the surrounding chert matrix; if euhedral crystal encrustation had preceded microcrystalline permineralization and infilling of biological structures, one would instead expect these larger crystals intermixed within microfossil interiors. Formation of wellstructured, larger crystals may also not have been favorable until initial, microcrystalline apatite growth within microfossils and ooids provided suitable nucleation sites. It is also unlikely that crystal growth occurred after silicification, because a loosely consolidated, waterlogged sediment would have been necessary for the formation of large, euhedral apatite.

Phosphatization of microfossils and encrusting crystal growth most likely occurred at or immediately beneath the sediment-water interface where phosphate would have been



Figure 8. Apatite oxygen paleobarometer (AOP) values from an encrusting, Chulaktau crystal (left panel, also described in Figure 7) and averaged AOP values for each geologic setting (orange) and phosphatized specimen type (black) (right panel). Points A-E refer to similarly labelled points in Figure 7. Error bars in the left panel correspond to \pm standard error derived from the peak fitting algorithm, and those in the right panel correspond to \pm standard deviation describing dispersion of averaged values.

concentrated in surrounding pore waters (Krajewski et al., 1994). This may have also been at or proximal to the oxic-anoxic transition within the sediment based on analogy with modern phosphate deposition (Föllmi, 1996), though the fluorescence spectra and AOP values discussed here can further clarify this relation. Ooid formation likely occurred just above the sedimentwater interface, where agitation and transport by wave action in shallows would have promoted concentric phosphate accretion.

Unlike the diagenetically-formed phosphorite microfossil and ooid specimens discussed above, scale microfossils of the Fifteenmile Group preserve instead apatite biomineralization by protists inhabiting the water column. These specimens are also differentiated in terms of timing by the growth of apatite having occurred during the life of the host organism, rather than post mortem. Thus, if the interpretation of active, biologically controlled mineralization supported by various mineralogical and paleontological lines of evidence (Cohen et al., 2011; 2017) is presumed to be true, preserved apatite scale microfossils represent an entirely different depositional setting than that represented by phosphorite specimens.

Phosphorite Sm³⁺ emplacement

As experiments of artificial Sm³⁺-substitution of apatite have demonstrated (Chen et al., 2002a; 2002b; Gaft et al., 1997; Chapter 2 of this dissertation), oxygen availability during Sm³⁺ emplacement determines resulting fluorescence signatures. Therefore, the timing of Sm³⁺ emplacement relative to phosphorite and biomineral apatite formation must be considered for the interpretation of local environmental redox conditions. Sedimentary apatite can incorporate REEs available in seawater during crystal growth, presume to be true for the analyses of REE

patterns in ancient phosphorites (Ilyin, 1998; Mazumdar et al., 1999; Yang et al., 1999; Shields and Stille, 2001; Chen et al., 2003; Chunhua and Ruizhong, 2005; Jiang et al., 2007; Zhu et al., 2014).

The interpretation that apatite REE abundances record ancient seawater is complicated by the possibility of post-depositional diagenetic alteration and REE diffusion (e.g., Shields and Stille, 2001). However, the exceptional degree of microfossil preservation in all studied specimens is at odds with the possibility of post-depositional alteration of REE abundances, considering that experimentally determined apatite closure temperatures for Sm diffusion are on the order of ~500 to 900 °C (Cherniak, 2000). Additionally, measured specimens do not border fractures and infilled veins, nor do thin section samples exhibit high porosity. Together, these observations decrease the likelihood that measured Sm³⁺-attributed fluorescence signatures have been obtained from post-depositional, recrystallized apatite that might obscure those exhibited by early diagenetic apatite.

REE patterns in Doushantuo phosphorites have also previously been interpreted as being free from post-depositional alteration on the basis of comparable signatures between phosphates and their encompassing mineral cement, as well as the excellent fossil preservation discussed above (Chen et al., 2003). Though extensive studies of potential post-depositional REE alteration have not yet been performed on Chulaktau and Fifteenmile apatite, similar reasoning regarding lack of obvious recrystallization features and fine microfossil preservation likely preclude significant alteration effects. However, we do caution that it is currently unknown how postdepositional geologic processes affect, aside from relative abundances of various REEs, the Casite distribution of REEs including Sm^{3+} within sedimentary apatite, of significance to the interpretation of local marine O_2 concentrations.

Oxygenation across the Neoproterozoic and Cambrian transition

AOP values of Sm³⁺-substituted apatite have been directly correlated with oxygen availability during experimental heat treatments (see Chapter 2, Figure 6). If these correlations are directly and quantitatively applied to the AOP values calculated for phosphatized microfossils and ooids, all reflect generally anoxic conditions. These values fall within the experimental-derived AOP range for $\leq 2\%$ O₂, or conservatively of < 10% O₂. Substantially higher AOP values are only measured from the interiors of euhedral encrusting apatite crystals, which fall within experimentally derived ranges corresponding to $\geq 10\%$ O₂. It is, however, noteworthy that at this time, a full quantitative application of the experimental results described in Chapter 2 to ancient phosphorite and biomineral apatite specimens may not be entirely relevant due to the vast differences in mode, timescale, and environment of experimental Sm³⁺ emplacement to that for naturally precipitated apatite. However, because the mechanism for Sm^{3+} substitution at the Ca2 site, and therefore for the accompanying O^{2-} substitution of the neighboring A⁻, site is primarily determined by the apatite structure (Equation 2), the relative interpretations of local oxygen availability during phosphorite and biomineral apatite formation are assuredly relevant. For the expansion of the quantitative capabilities of this apatite oxygen paleobarometer approach, we expect these uncertainties to be resolved by further experiments exploring Sm³⁺ substitution in more directly analogous experimental conditions to those of Neoproterozoic-Cambrian phosphogenesis (e.g., experimental Sm³⁺-doped aqueous mineral growth).

Relatively anoxic values are not unexpected for phosphatization at or immediately below the sediment-water interface, as might be expected for mineralized phosphorite microfossils of the Doushantuo and Chulaktau Formations, and are consistent with models of modern sedimentary apatite formation at or near the sediment redox boundary (Föllmi, 1996). Comparable anoxic conditions interpreted for apatite scale fossil AOP values, which would be reflective of the water column environment rather than that of the sediment, is also consistent with geochemical evidence suggesting the Fifteenmile basin was largely anoxic during the mid-Neoproterozoic (Cohen et al., 2011).

The highest observed AOP values are sampled from Chulaktau Formation ooids and encrusting apatite crystal interiors. That ooids record the highest AOP values of the nonencrusting Chulaktau apatite specimens may result from their formation directly above the sediment-water interface; measurements thus perhaps sample a moderately more oxygenated, lower water column setting rather than at shallow depth within the sediment. If euhedral apatite encrustation occurred secondarily to microfossil and ooid phosphatization, as well as winnowing and redeposition as evidenced by fragmented euhedral crystals freed from their original nucleating substrate, the early stages of crystal growth may record a secondary, more oxygenated and agitated environment. The concentrically zoned, gradual decrease in AOP values from crystal interior to exterior is then consistent with increasing anoxia that would have resulted from increasing sediment burial and lithification.

Though both datasets may be characterized as generally representative of local anoxic conditions, there exists a significant distinction between mean Doushantuo and Chulaktau AOP

values. Sampled from phosphatized microfossils and ooids, the greater Chulaktau AOP values may indicate moderately greater oxygenation of the shallow sediment environment relative to that indicated by Doushantuo microfossil AOP values. Mean Chulaktau ooid AOP values are similarly greater than those of the Fifteenmile scale microfossils, though the latter likely formed higher in the water column. In the context of the postulated increased global oxygenation between the Neoproterozoic and Cambrian, the moderately greater local marine O₂ concentrations evidenced by Chulaktau AOP values is intriguingly consistent. However, these results must be considered initial, "proof of concept" measurements that must await further confirmation by more extensive experimental calibration and measurements at larger sampling scales, standard for established redox proxies, that would allow further extrapolation to basinand regional-scale marine, and perhaps atmospheric interpretations of O₂ concentration.

The characterization of Neoproterozoic- and Cambrian-age, microfossil-associated apatite specimens described here details the presence of fluorescence signatures attributed to Sm^{3+} -substitution of apatite, the observed spectral variability of which has been correlated with varying local marine O₂ concentrations on the basis of their comparison with experimental calibrations of said signatures (Chapter 2). That the distribution of AOP values from the studied geologic settings can be quantitatively distinguished despite correlating qualitatively with anoxic conditions demonstrates the potential for expanded quantitative refinement of the apatite oxygen paleobarometer. The strategy used here also establishes the advantage of these techniques in providing a finer investigative tool to target specific mineralogical phases of apatite, whose diagenetic and depositional context can be determined by association with well-preserved microfossils and other informative sedimentological features. Finally, though this study has

focused on Sm³⁺ fluorescence signatures, comparable oxygen-associated fluorescence spectral characteristics have been documented for other apatite-substituting REEs (Gaft et al., 1997). An expanded apatite oxygen paleobarometer based on a suite of REE fluorescence signatures might be envisioned, each providing complimentary evidence of local O₂ concentrations during apatite precipitation. The application of these analytical methods to numerous shallow-water phosphorites deposited during a ~200 Ma span of the Neoproterozoic-Cambrian transition would provide a large and detailed dataset representative of regional and global conditions of marine oxygenation. These results reported here are an initial step toward a novel approach for an improved quantitative and age-constrained understanding of environmental oxygenation associated with the Cambrian Explosion of life at the close of the Precambrian.

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CHAPTER 4:

Reconstructed ancestral enzymes suggest long-term cooling of Earth's photic zone since the Archean

4.1 – ABSTRACT

Paleotemperatures inferred from the isotopic compositions (δ^{18} O and δ^{30} Si) of marine cherts suggest that Earth's oceans cooled from 70 + 15 °C in the Archean to the present ~15 °C. This interpretation, however, has been subject to question due to uncertainties regarding oceanic isotopic compositions, diagenetic or metamorphic resetting of the isotopic record, and depositional environments. Analyses of the thermostability of reconstructed ancestral enzymes provide an independent method by which to assess the temperature history inferred from the isotopic evidence. Though previous studies have demonstrated extreme thermostability in reconstructed archaeal and bacterial proteins compatible with a hot early Earth, taxa investigated may have inhabited local thermal environments that differed significantly from average surface conditions. We here present thermostability measurements of reconstructed ancestral enzymatically active nucleoside diphosphate kinases (NDKs) derived from light-requiring prokaryotic and eukaryotic phototrophs having widely separated fossil-based divergence ages. The ancestral environmental temperatures thereby determined for these photic zone organisms – shown in modern taxa to correlate strongly with NDK thermostability – are inferred to reflect ancient surface-environment paleotemperatures. Our results suggest that Earth's surface temperature decreased over geological time from ~65-80 °C in the Archean, a finding consistent both with previous isotope-based and protein reconstruction-based interpretations. Interdisciplinary studies such as those reported here integrating genomic, geologic and

paleontologic data hold promise for providing new insight into the co-evolution of life and environment over Earth history.

4.2 – INTRODUCTION

Understanding of the interrelated evolution of life and environment requires knowledge both of biology and the associated physical setting, the latter traditionally provided by paleontological and geological evidence. Though in recent decades the known fossil record has been extended well into the Archean (Schopf, 1993; Wacey et al., 2006), significant aspects of the development of Earth's environment – most notably, changes in day length and surface temperature (Schopf, 2013) – remain to be fully explored. We here focus on the history of Earth's surface temperature, poorly resolved for the formative Precambrian seven-eighths of Earth history due to a lack of paleoclimate proxies like those available for the more recent ~550 Ma of the Phanerozoic (e.g., Veizer and Prokoph, 2015).

To date, the most complete temperature record for the Precambrian is that inferred from oxygen and silicon isotope compositions of marine cherts, cryptocrystalline siliceous rocks typically chemically precipitated from associated pore-waters. δ^{18} O and δ^{30} Si measurements of such sediments from the 3500–3200 Ma Barberton greenstone belt register a notably hot 55-85 °C marine environment for the Archean that, coupled with measurements in younger cherts, suggest a geologically long-term decrease to the present ~15 °C (Knauth and Lowe, 1978; Knauth and Lowe, 2003; Robert and Chaussidon, 2006). This interpretation, however, has engendered skepticism due to uncertainties associated with possible changes in oceanic isotopic compositions (e.g., Perry, 1967; Kasting et al., 2006; Hren et al., 2009), diagenetic and/or

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metamorphic resetting of the reported isotopic signatures by exchange with groundwater and/or recrystallization, respectively (e.g., Degens and Epstein, 1962; Weis and Wasserburg, 1987; Chakrabarti et al., 2012; de Wit and Furnes, 2016), and ambiguities regarding the depositional environment of some of the cherts analyzed that, if hydrothermal, would not be indicative of global surface conditions (e.g., de Wit and Furnes, 2016). To resolve such questions, an independent line of evidence is necessary.

In recent years, molecular biology has advanced understanding of the history of life and its environment, supplementing traditional geology- and paleontology-based approaches. Initially conceived as "chemical paleogenetics" by Pauling and Zuckerlandl (1963), ancestral sequence reconstruction (ASR) estimates the monomeric sequences of ancient biomolecules by use of standard evolutionary statistics (e.g., maximum likelihood and Bayesian) applied to phylogenies constructed from the genomics of extant descendants. Based on the assumption that molecular sequence determines biological function and that the ancient molecules would have necessarily been adapted to and functional in their surroundings, the reconstructed molecules should evidence past environmental conditions.

The basal presence in molecular phylogenies of hyperthermophilic bacterial and archaeal lineages suggests an early-evolved tolerance to high environmental temperatures (Woese, 1987). Previous ASR studies have evaluated this possibility by inferring the thermostabilities of ancestral reconstructed rRNA and protein amino acid sequences (Galtier et al., 1999; Boussau et al., 2008), or by measuring the temperature-dependent functionality of experimentally "resurrected" ancient proteins (Gaucher et al., 2003; Gaucher et al., 2008; Hobbs et al., 2011;

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Akanuma et al., 2013; Akanuma et al., 2015). Most notably, Gaucher et al. (2008) used the thermostabilities of experimentally reconstructed bacterial elongation factors to infer variations in paleotemperature over Precambrian time, data producing a temperature trend similar to that suggested by the isotopic compositions of marine cherts (Knauth and Lowe, 1978; Knauth and Lowe, 2003; Robert and Chaussidon, 2006) and interpreted to evidence a gradual cooling from 60–70 °C in the Archean.

The thermostabilities exhibited by reconstructed biomolecules can reflect the range of temperatures only of their immediate ancient surroundings (Hobbs et al., 2011). Thus, accurate assessment of variations over time of Earth's surface temperature requires evaluation of the reconstructed molecular constituents of widespread inhabitants of near-surface environments, thereby excluding the inclusion of data from thermophilic microorganisms prevalent in localized submarine or terrestrial hydrothermal settings. In this study, we accomplish this goal by including reconstructed ancestral proteins of phototrophs only, light-requiring organisms that, like their extant descendants, would have been restricted to photic-zone near-surface environments. Possible inhabitants of terrestrial hot springs are excluded by restricting our study to land plants and marine cyanobacteria and green algae.

The biomolecule selected to be reconstructed for this study is nucleoside diphosphate kinase (NDK), an enzyme that catalyzes the transfer of a phosphate group from nucleoside triphosphate to nucleoside diphosphate, is virtually ubiquitous among extant organisms, and the thermostability of which has been shown previously to correlate strongly with organismal growth temperature (Akanuma et al., 2013; Akanuma et al., 2015). Thus, measurement of the thermostabilities of reconstructed ancestral NDK from cyanobacteria, green algae and land plants, groups and subgroups of which have temporally widely spaced fossil record-indicated divergence ages, should provide a firm basis for analysis of the history of Earth's surface temperature over geological time. Such data would afford an independent set of data by which to assess the paleotemperature trend previously inferred from mineralogical isotope-based evidence (Knauth and Lowe, 1978; Knauth and Lowe, 2003; Robert and Chaussidon, 2006).

4.3 – RESULTS AND DISCUSSION

rRNA and NDK Phylogenies

Ancestral NDK reconstructions were targeted to represent the molecular phylogenydetermined last common ancestors of cyanobacteria (oxygenic photosynthetic prokaryotes; ~3100–2700 Ma), nostocaleans (later-evolved cyanobacteria characterized by morphologically distinctive cells; ~2300–2100 Ma), Viridiplantae (green algae and land plants; ~850–700 Ma), and Embryophyta (land plants only; ~460–440 Ma). The estimated ~2900 Ma age for the origin of oxygen-producing cyanobacteria precedes the earliest widespread occurrence of geochemical evidence of oxygenic photosynthesis (e.g., banded iron formations and subaerial red beds) and the subsequent ~2400 Ma Great Oxidation Event, the "GOE" (Farquhar et al., 2000; Anbar et al., 2007), suggesting that older Archean stromatolitic mat-forming microbes may have been anoxygenic phototrophs (cf. Schopf, 2012). The origin of nostocalean cyanobacteria at ~2100 Ma is based on microfossils interpreted to be akinetes (Golubic et al., 1995; Tomitani et al., 2006), resting cells characteristic of the Nostocales as are heterocysts, differentiated trichomic thick-walled cells that shielded the oxygen-labile N₂-fixing nitrogenase enzyme system from the increase of ambient oxygen at the GOE (Holland and Beukes, 1990). The earliest strong fossil evidence of green algae places the origin of the Viridiplantae in the late Proterozoic (Butterfield, 2009), and that of isolated trilete spores dates the divergence of land plants as perhaps as early as the mid-Ordovician (Wellman and Gray, 2000).

Following Akanuma et al. (2013) and due to concerns that NDK sequences alone might not accurately depict evolutionary relationships, we used 16S rRNA or 18S rRNA to construct two separate phylogenetic trees, 16S for prokaryotic cyanobacteria and nostocaleans and 18S for eukaryotic Viridiplantae and Embryophyta (Fig. 1A, 1B). To further evaluate the eukaryotic ancestral NDKs, we constructed a third tree (Fig. 1C) based on NDK from the same Viridiplantae taxa used to construct the 18S rRNA tree.

In some respects the eukaryotic 18S rRNA (Fig. 1B) and NDK (Fig. 1C) trees differ. Most such variation occurs in the clustering of angiosperm taxa, differences that are relatively insignificant due to the lack of resolution for short angiosperm branch-lengths in both trees. More significant variation occurs in the topological differences among major Viridiplantae lineages. Phylogenetic relations depicted in the 18S tree that are supported by previously published plant phylogenies (e.g., Ruhfel et al., 2014) include the monophyletic clustering of chlorophyte green algae and monocotylendonous angiosperms. These relations are not shown by the NDK tree in which the Chlorophyta is paraphyletic and monocots are split into two distinct monophyletic groups. The most significant departure of the 18S tree (Fig. 1B) from accepted

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Figure 1. Phylogenetic trees used to evaluate ancestral NDK sequences. Nodes from which ancestral NDK sequences were calculated are indicated by red arrows; scale bars indicate the number of substitutions per site. (A) A maximum likelihood (ML) 16S rRNA tree of cyanobacteria based on the NDK sequences of outgroups (archaea and bacteria, black-filled), non-nostocalean cyanobacteria (white-filled), and nostocaleans (gray-filled). (B) An ML 18S rRNA tree of Viridiplantae, based on the NDK sequences showing non-Viridiplantae outgroups (black-filled), non-embryophyte Viridiplantae (white-filled), and Embryophyta (gray-filled). (C) An ML NDK tree constructed using NDK sequences of the Viridiplantae taxa used to construct (B), the various parts colored like those in (B).

plant phylogenies is the occurrence of a gymnosperm sequence within the angiosperms rather than being a sister branch as in the NDK tree (Fig. 1C). The unsupported placement of this deepbranching sequence within the Embryophyta may be a source of significant sequence inaccuracy for our reconstruction of the Embryo18S NDK.

Ancestral NDK Sequences

To calculate the ancestral amino acid sequences of the NDKs to be reconstructed, we used a maximum likelihood (ML) algorithm shown to yield reliable reconstructions and thermostability inferences comparable or even preferable to more computationally expensive Bayesian methods (Hanson-Smith et al., 2010; Hobbs et al., 2011; Akanuma et al., 2015; Eick et al., 2016), specifically CODEML of the PAML program package for phylogenic analysis that incorporates a homogenous amino acid substitution model (Yang, 1997). Ancestral NDK sequences were estimated using prokaryotic and eukaryotic NDK and rRNA reference trees. The amino acid sequences of six ancestral NDKs were determined, two from ancestral cyanobacterial and nostocalean nodes within the 16S rRNA prokaryote tree (NDKs referred to here, respectively, as "Cyano16S" and "Nosto16S"); two from ancestral Viridiplantae and Embryophyta nodes of the 18S rRNA eukaryote tree ("Viridi18S" and "Embryo18S"); and two from the eukaryotic NDK tree ("ViridiNDK" and "EmbryoNDK").

Experimental NDK Thermostability Measurements

Genes coding for the six ancestral NDKs were synthesized and cloned into expression vectors, expressed in *Escherichia coli*, and purified. NDKs from extant cyanobacteria (*Nostoc punctiforme*, *Synechocystis sp. PCC6803*, and *Thermosynechococcus elongatus*), a green alga

(*Chlamydomonas reinhardtii*), and three land plants (*Physcomitrella patens, Oryza sativa*, and *Picea sitchensis*) were also constructed to provide taxon-specific calibration of NDK thermostability and organismal environmental temperature (cf. Akanuma et al., 2013).

Table 1 lists the measured thermostability of the reconstructed NDK enzymes. The temperature-induced denaturation both of reconstructed and extant NDKs was monitored by circular dichroism (CD) spectroscopy at pH 6.0 and 7.6, their thermostability being defined as the midpoint unfolding temperature (T_m) evidenced by their two-state denaturation curves. Although all NDKs produced clear two-state curves at pH 6.0, several yielded atypical denaturation curves at pH 7.6 (Table 1) that nevertheless exhibited CD signal shifts coinciding within experimental error at the T_m s measured for the same NDK at pH 6.0.

The plausibility of the reconstructed enzymes accurately representing their original ancestral configuration can be assessed by comparison of the thermostability of NDKs reconstructed from the same ancestral node in topologically differing phylogenetic trees. For example, the Viridiplantae ancestral NDKs calculated from the 18S (Fig. 1B) and NDK (Fig. 1C) trees, Viridi18S and ViridiNDK, coincide at all but eight of 152 amino acid residues and have essentially identical T_m values (81.7 ± 0.4 and 81.2 ± 0.5 °C, respectively; Table 1), notable similarities that support the accuracy of their reconstruction despite the differences in tree topologies used to determine their sequences. Such similarities, however, are not exhibited by the Embryophyta NDKs calculated from the two eukaryotic trees, Embryo18S and EmbryoNDK, reconstructed enzymes that differ at 17 of 153 residues and have T_m values that diverge by ~15

Reconstructed NDK Enzyme	Median Divergence	$T_m \pm 1$ S	Environmental Temperature	
	Age (Ma)	pH 6.0	pH 7.6	Range (°C)
Cyano16S	~2900	100.1 ± 0.4	n.d.*	64–82
Nosto16S	~2200	77.8 ± 0.1	n.d.*	36–56
Viridi18S	~775	81.7 ± 0.4	82.4 ± 1.2	24–61
ViridiNDK	~775	81.2 ± 0.5	83.4 ± 0.5	24–62
Embryo18S	~450	80.0 ± 0.3	n.d.*	24–58
EmbryoNDK	~450	64.3 ± 0.6	n.d.*	20–41

Table 1. Reconstructed ancestral NDK divergence ages, thermostabilities (T_m) , and environmental temperature ranges inferred from Fig. 3.

n.d.* T_m not determined at pH 7.6 due to atypical denaturation curves.

°C. These differences seem likely to be a result of the misplacement discussed above of a deepbranching gymnosperm sequence within the Embryophyta 18S rRNA tree (Fig. 1B). Because this may be a significant source of sequence inaccuracy for Embryo18S, we have greater confidence that the T_m of ~64 °C measured for EmbryoNDK more accurately represents the thermostability of ancestral Embryophyta NDK. Such disparities highlight the need for the testing of multiple phylogenetic models in ASR studies.

NDK Enzymatic Activity

All of the enzymes were assayed for the production of ATP by the NDK-catalyzed transfer of phosphate from GTP to ADP, specific activities measured in triplicate at 10 °C intervals, typically from 40 to 90 °C (Fig. 2). Consistent with the plausible accuracy of the reconstructions, all NDKs, ancestral and extant, were enzymatically active with the experimentally induced loss of their specific activity generally coinciding with their denaturation as inferred from measured T_m values. The order of magnitude difference between the specific activities of the reconstructed prokaryotic and eukaryotic NDK (Fig. 2) is characteristic both of the ancestral and extant NDKs studied here, perhaps evidencing an inheritably lower catalytic activity of the eukaryotic enzyme.

Ancestral Environmental Temperature

The T_m values listed in Table 1 show a marked overall decrease in the thermostability of the reconstructed NDKs with increasingly younger fossil-based divergence ages. Previous studies have demonstrated a strong correlation in extant archaea and bacteria between such thermostability and organismal environmental growth temperature, data that have provided the



Figure 2. Temperature dependence of ancestral NDK specific activity measured by the ATP produced, each value being the average of three or more measurements and the dotted lines spanning activity values measured at temperature intervals greater than the T_ms analyzed by circular dichroism spectroscopy. Data are not available for the specific activity of Cyano16S at temperatures greater than its T_m (~100 °C).

basis for construction of a calibration curve from which to infer paleoenvironmental temperatures (Akanuma et al., 2013; Akanuma et al., 2015). The present work extends such studies to include prokaryotic cyanobacteria and eukaryotic green algae and land plants of the Viridiplantae, light-requiring phototrophs from which surface or near-surface photic zone temperatures can be inferred.

In Figure 3, the ranges of thermostabilities determined for the NDKs of extant archaea and bacteria (Akanuma et al., 2013), cyanobacteria, and Viridiplantae are plotted relative to their mean organismal environmental temperatures, using values for archaea and bacteria as an upper limit and those either for cyanobacteria (for Cyano16S and Nosto16S) or Viridiplantae (for ViridiNDK, Viridi18S, EmbryoNDK, and Embryo18S) as a lower limit. The calculated combined correlation for all taxa between NDK T_m and organismal environmental temperature is strong (r = 0.92), the two parameters consistently offset by ~20 °C, and is particularly strong for the cyanobacterial NDK (r = 1.00). For the Viridiplantae, however, the correlation is less robust (r = 0.21), possibly because thermophiles, including hot spring chlorophytes (Brock, 1967), were purposefully not included in this study. In addition, the geologically relatively recent (~775 Ma; Table 1) divergence of the Viridiplantae resulted in the absence of modern mesophilic taxa that, evidently like extant archaeans, bacteria and cyanobacteria, were derived from ancestors adapted to an earlier, appreciably hotter Earth (Fig. 4).

NDK Paleotemperature Trend

In Figure 4, paleoenvironmental temperature ranges inferred from the modern taxa calibration (Fig. 3) are plotted relative to the estimated geological age of lineage-origination and



Figure 3. Calibration curves showing the correlation between NDK T_ms and the organismal environmental growth temperatures of extant taxa of archaea and bacteria (22), cyanobacteria, and Viridiplantae. The best-fit linear regression lines for each group were constructed from the indicated data, the black-dashed line being the best fit through all available data and "r" specifying the calculated correlation coefficients. Environmental temperature ranges inferred from ancestral NDK T_ms are indicated by red bars for Cyano16S and Nosto16S and by pink bars for eukaryotic Viridi18S, ViridiNDK, Embryo18S, and EmbryoNDK.

the paleotemperature trend suggested by δ^{18} O and δ^{30} Si measurements of marine cherts (Knauth and Lowe, 1978; Robert and Chaussidon, 2006). The data from both our reconstructed NDK studies and the isotopic geochemical record suggest a general cooling of Earth's environment over geological time, the datasets exhibiting notable agreement for an estimated Archean temperature of ~65 to 80 °C.

The ASR methods used in our study incorporate two principal sources of uncertainty, the accuracy of the reconstructed ancestral enzyme sequences and the paleotemperatures inferred from the environmental temperature ranges of extant taxa (Fig. 3). As noted above, the plausibility of the reconstructed sequences can be evaluated by the functionality of the reconstructed enzymes and the degree of similarity of the measured thermostability of the same ancestral enzyme constructed based on differing phylogenetic trees. All NDKs reconstructed in our study are demonstrably enzymatically active, satisfying the first of these criteria. The second criterion, previously demonstrated to be met by archaea and bacteria (Akanuma et al., 2013) but not investigated for cyanobacteria, is shown to be satisfied here by the reconstructed NDKs of Viridiplantae for which the enzymes calculated both from 18S rRNA- and NDK-based phylogenies exhibit essentially identical thermostabilities (Table 1). And although the difference of ~15 °C between the T_m values of the reconstructed Embryo18S and EmbryoNDK enzymes (Table 1) signifies a need for further detailed study, it does not alter significantly the geologically long-term cooling trend indicated by the available data. Similarly, the ~20–40 °C range of environmental temperature derived from the extant organism-based calibration curve (Fig. 3) does not overprint this first-order trend of gradually decreasing paleotemperature.



Figure 4. Environmental temperature ranges inferred from reconstructed ancestral NDK T_ms plotted against fossil record-indicated first appearance of the various groups. Paleotemperatures inferred from $\delta^{18}O$ (5) and $\delta^{30}Si$ (7) in marine cherts are included for comparison. Blue boxes show the inferred NDK-based temperature ranges (Fig. 3) and fossil-based age uncertainties, the red diamonds denoting temperature and age midpoint values for which ViridiNDK and Viridi18S have been combined due to the similarity of their T_ms .

The hot, ~65–80 °C Archean temperatures inferred from stable isotopes in marine cherts (Knauth and Lowe, 1978; Robert and Chaussidon, 2006), reconstructed archaeal and bacterial enzymes (Gaucher et al., 2008; Akanuma et al., 2013; Akanuma et al., 2015), and here from the reconstructed ancestral NDK of cyanobacteria are consistent with the upper environmental temperature limits of comparable modern taxa. For example, diverse extant hypertheromophilic prokaryotes, including methanogens, thrive in such settings (Woese, 1987) and some modern cyanobacteria inhabit thermal springs as hot as ~75 °C (Brock, 1967). Given that methanogens were evidently a significant component of the pre-2400 Ma biosphere (Hayes, 1983) and that early cyanobacterial fossils are known solely from shallow marine sediments (Hofmann, 1976; Klein et al., 1987; Golubic and Lee, 1999; Schopf, 2012), it is plausible and perhaps likely that thermophily was ecologically widespread during early Earth history. Interestingly, although eukaryotic algae originated much later in geological time than their prokaryotic precursors, the ~40–60 °C upper temperature limit for extant taxa (Brock, 1967) is similarly consistent with the NDK-based temperature estimates derived here for ancestral Viridiplantae (Table 1).

The ASR methods used here to determine the thermostabilities of reconstructed ancestral NDKs of photic zone-inhabiting cyanobacteria, algae, and land plants provide a promising approach to analyses of the long-term temperature history of Earth's surface environment. Interdisciplinary studies such as this, combining the newly available data and techniques of genomic molecular biology with the more traditional findings of geology and paleontology can be expected to play an increasingly significant role in understanding the interrelated evolution of life and its environment.

4.4 – MATERIALS AND METHODS

rRNA and NDK Phylogenetic Tree Building

Following the methods of Akanuma et al. (2013), NDK amino acid sequences were acquired from the NCBI Protein database for 170 taxa of extant cyanobacteria (including 31 nostocaleans) and 69 Viridiplantae (including 58 embryophytes). Cyanobacterial genomes include a single NDK gene. Of the multiple NDK genes occurring in the genomes of plants, the cytosolic Viridiplantae NDKI isoform (referred to here simply as "NDK") was selected for this study because of its similarity in sequence length and composition to cyanobacterial NDK. To serve as outgroups for the analyzed cyanobacteria and Viridiplantae, NDK sequences were obtained from the database for 193 extant taxa of prokaryotes and 24 eukaryotes, respectively. Cyanobacteria and Viridiplantae NDK sequences were aligned separately by MAFFT (Katoh et al., 2002) version 7.222 and adjusted to correct for gap positions.

Complimentary 16S rRNA or 18S rRNA nucleotide sequences from the same taxa used for the NDK alignments were obtained from the SILVA database, which for eight of the Viridiplantae were genus- rather than species-matched due to missing entries in SILVA (Quast et al., 2012). ML rRNA phylogenetic trees (Fig. 1A-1B) were constructed using RAxML (Stamatakis, 2014) and the GTR+G model for cyanobacteria and LG + Gamma model for Viridiplantae; an NDK ML tree (LG + Gamma model) was also constructed using RAxML for the Viridiplantae (Fig. 1C). Best-fit evolutionary models were selected using jModelTest or ProtTest (Guindon and Gascuel, 2003; Darriba et al., 2012).

Ancestral NDK Sequence Calculation

Ancestral NDK sequences were inferred by use of CODEML in the PAML program package (Yang, 1997). A 16S rRNA tree topology was used as a reference tree to infer the NDK sequences of the last common ancestors of cyanobacteria (Cyano16S) and nostocaleans (Nosto16S). The same procedure was performed using an 18S rRNA tree topology to infer the NDK sequences of the last common ancestors of Viridiplantae (Viridi18S) and Embryophyta (Embryo18S). Viridiplantae and Embrophyta ancestral NDK sequences were also inferred using an NDK tree topology (for ViridiNDK and EmbryoNDK).

Ancestral NDK Gene Construction

Ancestral NDK gene sequences were determined by reverse translation of the inferred amino acid sequences with optimal codon usage for gene expression in *E. coli*. Cyano16S and Nosto16S gene constructs and the NDKs of three extant cyanobacterial taxa were prepared by Eurofins Scientific and ligated into pTAKN-2 (BioDynamics Laboratory Inc.). Viridi18S, ViridiNDK, Embryo18S, and EmbryoNDK gene constructs and the NDKs of four extant Viridiplantae taxa were prepared by GenScript and ligated into pET23a(+) (Novagen).

NDK Expression and Purification

pTAKN-2 constructs were digested with *Nde*I and *Bam*HI (New England Biolabs), purified by agarose gel electrophoresis, and ligated to pET23a(+), a ligation step not necessary for GenScript constructs. *E. coli* Rosetta2 (DE3) (Novagen) cultures transformed with the expression plasmids were cultivated in Luria-Bertani medium supplemented with ampicillin (150 µg/mL). Gene expression was induced using Overnight Express Autoinduction system reagents (Novagen) at 37 °C. Cells were then disrupted by sonication and heated at 70 °C (for cyanobacterial proteins) or 60 °C (for Viridiplantae proteins) for 15 minutes to denature *E. coli* proteins. The heated suspensions were then centrifuged at 60,000 x g, and to purify the NDK, the resulting supernatants were chromatographed through HiTrapQ, ResourceQ, and Superdex 200 columns (GE Healthcare Biosciences). Protein homogeneity was confirmed by SDS-PAGE followed by Coomassie Brilliant Blue staining.

Thermostability and Enzyme Activity Analyses

Purified NDK protein concentrations were analyzed by spectrophotometric absorbance at 280 nm. For thermostability analyses, each NDK protein was dissolved in 20 mM KPi (pH 6.0 or 7.6), 50 mM KCl, and 1 mM EDTA to a final concentration of 20 μ M. Thermal denaturation experiments were performed in a 0.1 cm path-length pressurized cell using a J-720 spetropolarimeter (Jasco) equipped with a programmable temperature controller. For each measurement, temperature was typically increased at a rate of 1.0 °C/min from 40 - 110 °C and protein denaturation was monitored by CD spectral changes of the proteinaceous solution at 222 nm. The midpoint unfolding temperature, T_m, was calculated from two-state denaturation curves normalized to their native- and denatured-state baselines, measurements made for each protein at pH 6.0 and pH 7.6.

Enzyme activity assays were performed in a solution of 50 mM Hepes (pH 8.0), 25 mM KCl, 10 mM (NH₄)₂SO₄, 2.0 mM Mg(CH₃COO)₂, 1.0 mM DTT, 5.0 mM ADP, and 5.0 mM GTP. Increases in ATP, produced by the NDK-catalyzed transfer of phosphate from GTP to ADP, were monitored with the Kinase-Glo Luminescence Kinase Assay Kit (Promega) and a

Perkin Elmer Wallac 1420 Victor2 microplate reader (Winpact Scientific) was used to measure the luminescence. Measurements were typically taken in triplicate at 10 °C intervals from 40 to 90 °C.

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CHAPTER 5:

Conclusion

The development of novel strategies for the investigations of Neoproterozoic-Cambrian environmental oxygenation and of Precambrian surface temperature evolution described in this work together contribute to both present and continued improved understanding of major geobiological transitions during the evolution of the early Earth.

The experimental characterization of heat-promoted Sm³⁺-substitution in apatite and calibration to ambient oxygen availability during experimental Sm³⁺-emplacement provide a basis for the comparison of similar signatures reported from Neoproterozoic- and Cambrian- age phosphorite and biomineral apatite specimens associated with a globally extensive phosphogenic event. The results of these experiments demonstrate that Sm³⁺-attributed fluorescence spectral variability arises from the presence of oxygen during Sm³⁺ emplacement, and that a gradual spectral transition can be recorded for progressively increasing oxygen concentrations during experimental heat treatment. An apatite oxygen paleobarometer ("AOP") metric, devised to quantitatively represent relative fluorescence peak intensities associated with anoxic or oxic conditions, robustly correlates with the percentage of available O₂.

The results from the experimental calibration of oxygen-associated fluorescence signatures of Sm³⁺-substituted apatite permit the interpretation of comparable detected signatures in microfossil-associated apatite specimens of the late Neoproterozoic Doushantuo and Early Cambrian Chulaktau Formation phosphorites, as well as mid-Neoproterozoic-age apatite scale microfossils of the Fifteenmile Group previously interpreted as the earliest examples of eukaryotic biomineralization. The AOP values calculated from the fluorescence spectral features acquired from analyses of such specimens demonstrate variable, but relatively low local marine O₂ concentrations during apatite mineralization for all geologic settings. However, distinct total AOP value distributions derived from Doushantuo and Chulaktau analyses are consistent with increased oxygenation of the apatite-precipitating, shallow sediment environment in the latter.

Lastly, experimental characterization of ancestral enzymes, reconstructed by the genomic information provided by extant, photosynthetic descendants, demonstrate increasing enzymatic thermostability with increasing fossil-evidenced divergence age. These reconstructed enzymes have been determined to be robust to the uncertainty inherent to ancestral sequence reconstruction methods on the basis of comparable catalytic activity to that of modern enzymes, and the comparison of distinct phylogenetic methods for sequence reconstruction. Correlation of experimentally-determined enzyme thermostability with environmental growth temperature of photosynthetic host taxa suggest that the ambient photic zone environment temperature broadly decreased through time since the Archean.

APPENDIX A:

Geologic setting	Thin section	Specimen type	Specimen #	Measurement #	AOP	SE	r ² of modeled spectrum (600-612 nm)
Fifteenmile	UT1-7a	Scale	4	2535	0.4434	0.04433	0.9999
				2536	0.5110	0.07218	1.000
				2537	0.3708	0.1154	0.9999
				2539	0.2233	0.05496	0.9999
				2540	0.2046	0.05354	0.9999
	UT1-7b		1	2153	0.3511	0.05634	0.9999
				2168	0.4284	0.04193	1.000
				2169	0.2333	0.05464	1.000
				2170	0.3700	0.02800	1.000
				2173	0.3770	0.06623	1.000
Doushantuo	WSB-60-120-2	Embryo	1	9451	0.1706	0.05764	0.9996
		•		9454	0.2219	0.09079	0.9999
				9457	0.1754	0.04782	0.9998
				9460	0.2222	0.06644	0.9998
			2	9477	0.2413	0.05590	0.9998
				9479	0.2323	0.05713	1.000
				9481	0.2376	0.05968	0.9997
			17	9471	0.1523	0.04650	0.9999
				9473	0.2150	0.05733	1.000
				9475	0.1907	0.04331	1.000
		Algae	14	9483	0.2630	0.03808	0.9996
		-		9485	0.2996	0.1174	0.9998
				9487	0.2989	0.05722	0.9997
			15	9463	0.3074	0.06400	0.9997
				9466	0.2966	0.05180	0.9999
				9469	0.2879	0.04545	0.9999

Peak fitting and AOP data for fossil-associated apatite specimens (Chapter 3)

			16	9489	0.3415	0.07626	0.9996
				9491	0.2577	0.04766	0.9999
				9493	0.2109	0.07788	0.9997
Chulaktau	CHUL-4681-273-D	Cyano sheath	86	9130	0.3316	0.08429	1.000
		-		9133	0.3374	0.06284	0.9997
				9136	0.2938	0.1100	0.9999
			89	9140	0.2578	0.06354	0.9998
				9143	0.3312	0.1313	0.9998
				9146	0.3883	0.03748	1.000
			90	9150	0.2620	0.08167	1.000
				9153	0.2609	0.06836	1.000
				9156	0.3299	0.1118	1.000
		Cyano helical filament	73	9083	0.3369	0.08652	1.000
		-		9089	0.2621	0.09598	1.000
				9092	0.3703	0.2296	0.9999
			74	9097	0.3877	0.06104	1.000
				9100	0.3170	0.02221	0.9999
				9103	0.3675	0.06212	1.000
			82	9107	0.3045	0.09141	0.9997
				9110	0.4396	0.04787	0.9999
				9113	0.3183	0.03146	1.000
		Spicule	56	9162	0.3051	0.05161	1.000
		-		9165	0.3812	0.09799	0.9999
				9168	0.4394	0.05668	0.9999
			72	9173	0.4017	0.07961	0.9998
				9176	0.4721	0.07449	0.9999
				9179	0.3552	0.03871	0.9999
			81	9185	0.4607	0.06119	0.9999
				9188	0.5901	0.05340	0.9997
				9191	0.4773	0.02328	1.000
	PPRG-P 1476 1-A	Cyano straight filament	7	9045	0.5760	0.03795	0.9999
				9048	0.5411	0.03087	1.000
				9051	0.5251	0.03624	0.9999

		19	9060	0.3789	0.07877	1.000
			9063	0.4463	0.06634	0.9999
			9066	0.3544	0.09750	0.9999
		38	9029	0.4261	0.06746	0.9998
			9069	0.3555	0.03254	1.000
			9075	0.3125	0.06808	0.9998
PPRG-P 1476 1-B	Ooid	15	9006	0.5628	0.08469	0.9999
			9008	0.5356	0.05554	0.9997
			9010	0.7733	0.03541	0.9999
			9012	0.5226	0.06612	1.000
			9014	0.6536	0.03539	0.9999
			9016	0.41304	0.04801	1.000
		16	9022	0.5579	0.04973	1.000
			9024	0.4848	0.09739	0.9998
			9026	0.4090	0.04809	1.000
		17	9029	0.4502	0.02939	1.000
			9031	0.7008	0.05929	0.9996
			9040	0.6275	0.02050	0.9999