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Direct Detection of Oxygen Ligation to the Mn_4Ca Cluster of Photosystem II by X-ray Emission Spectroscopy

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Ligands play critical roles during the catalytic reactions in metalloproteins through bond formation/breaking, protonation/deprotonation, and electron/spin delocalization. While there are well-defined element-specific spectroscopic handles, such as X-ray spectroscopy and EPR, to follow the chemistry of metal catalytic sites in a large protein matrix, directly probing particular ligand atoms like C, N, and O is challenging due to their abundance in the protein. FTIR/Raman and ligand-sensitive EPR techniques such as ENDOR and ESEEM have been applied to study metal-ligand interactions. X-ray absorption spectroscopy (XAS) can also indirectly probe the ligand environment; its element-specificity allows us to focus only on the catalytic metal site, and EXAFS and XANES provide metal-ligand distances, coordination numbers, and symmetry of ligand environments. However, the information is limited, since one cannot distinguish among ligand elements with similar atomic number (i.e. C, N. and O). As an alternative and a more direct method to probe the specific metal-ligand chemistry in the protein matrix, we investigated the application of X-ray emission spectroscopy (XES). Using this technique we have identified the oxo-bridging ligands of the Mn_4Ca complex of photosystem II (PS II), a multisubunit membrane protein, that catalyzes the water oxidizing reaction.[1] The catalytic mechanism has been studied intensively by Mn XAS.[2] The fundamental question of this reaction, however, is how the water molecules are ligated to the Mn_4Ca cluster and how the O-O bond formation occurs before the evolution of O_2.[3-5] This implies that it is necessary to follow the chemistry of the oxygen ligands in order to understand the mechanism.

XES which is a complementary method to XAS, has the potential to directly probe ligation modes.[6] Among the several emission lines, $K\beta_{1,3}$ and $K\beta'$ lines originate from the metal 3p to 1s transition, and they have been used as an indicator of the charge and spin states on Mn in the OEC (Figure 1).[7,8] The higher energy region corresponds to valence to core transitions just below the Fermi level, and can be divided into the $K\beta''$ and the $K\beta_{2,5}$ emission (Fig.1 left scheme). $K\beta_{2,5}$ emission is predominantly from ligand 2p (metal 4p) to metal 1s, and the $K\beta''$ emission is assigned to a ligand 2s to metal 1s, and are referred to as crossover transitions.[9-11] Therefore, only direct ligands to the metal of interest are probed with $K\beta_{2,5}/K\beta''$ emission; i.e. other C, N, and O atoms in the protein media do not contribute to the spectra. In this report, we focus on the $K\beta''$ spectral region to characterize metal-ligand interactions, in particular contributions from ligated oxygens. The energy of the $K\beta''$ transition is dependent on the difference between the metal 1s and ligand 2s binding energies, which is dependent on the environment of the ligand due to orbital hybridization. Therefore the $K\beta''$ energy is affected by the charge density on the metal, the ligand protonation state, and changes in the coordination environment. The $K\beta''$ intensity is influenced by the spatial overlap between the wavefunction that describes the Mn 1s orbital and the molecular orbitals on the ligands. The $K\beta''$ intensity is affected by the metal to ligand distance, and the number of ligands per metal ion. Shorter distances (e.g. from higher bond order or deprotonation) result in increased $K\beta''$ intensity with an approximate exponential dependence.[9] On the other hand, a spread of the molecular wavefunction over next-nearest neighbor atoms will decrease the $K\beta''$ spectral intensity. Therefore contribution from single atom ligands such as oxo-bridges, or terminal oxo ligands bonded to Mn is predominant (see below). These combination of factors makes the $K\beta''$ spectrum a powerful tool for detection and characterization of oxo-bridges in the Mn_4Ca cluster of PS II.

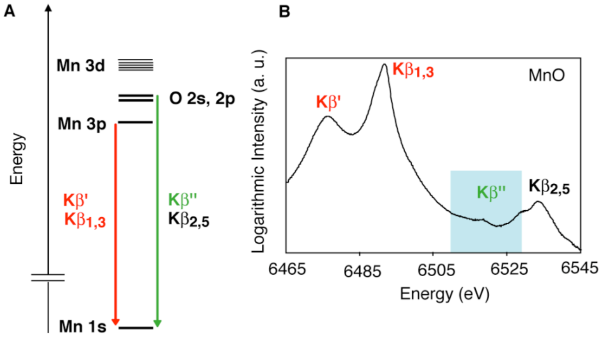

Figure 1. (A) Energy diagram of Mn Kβ transitions in MnO. The $K\beta''$ and $K\beta_{2,5}$ transitions are from valence molecular orbitals, $K\beta''$ is O 2s to Mn 1s 'cross-over' transition. (B) Logarithmic plot of MnO Kβ spectrum. The O-Mn cross-over $K\beta''$ transition is highlighted.

However, because of the weak intensity of the $K\beta''$ spectrum obtaining such spectra from biological samples as dilute as PS II (800μM Mn) has been difficult. For O ligation in a typical model compound, the signal is ~10^3 times weaker than that of Kα and there is an additional large background from both the $K\beta_{1,3}$ and the $K\beta_{2,5}$ spectral features (Fig. 1). Furthermore the work is challenging because of the high sensitivity of the Mn_4Ca cluster to radiation damage.[12] This study of PS II became possible by using a new high resolution spectrometer equipped with 8-14 analyzer crystals collecting a large solid angle (Suppl. Info.).

Fig. 2 shows the $K\beta''$ spectrum of a sample of PS II in the S_1 state compared with a series of Mn oxide spectra. Each spectrum is normalized by the $K\beta_{1,3}$ peak intensity which is proportional to the number of Mn atoms in the system. The 1st moment energy of

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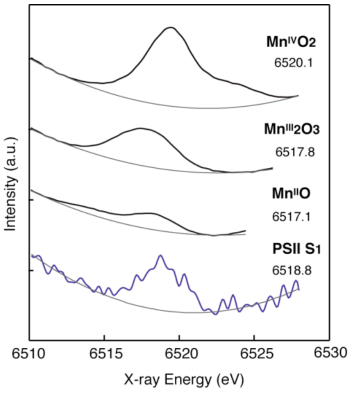

Figure 2. Mn Kβ″ emission spectra from Mn oxides and PS II S_1 state and 1st moments. These spectra referred to as crossover peaks are assigned to emission from ligand 2s2p levels to the Mn 1s core level.

the PS II S_1 state, 6518.8 eV, falls in between the Mn^{III} and Mn^{IV} oxides. The energy of the Kβ″ feature may be influenced by charge screening effects that depend on the charge density on the Mn ion, i.e. the Mn oxidation state. The actual charge density, however, can only be approximated based on the formal oxidation state and the Kβ″ peak position thus exhibits the correct trend.[13]

Fig. 3 shows a comparison of the PS II S_1 state spectrum with those of a series of Mn coordination compounds, Mn^{V}-oxo (**a**), di-μ-oxo bridged Mn$_2^{III,IV}$ and Mn$_2^{IV}$ (**b, c**), cubane-type Mn$_2^{III}$Mn$_2^{IV}$ and Mn^{III}Mn$_3^{IV}$ (**d, e**), and a μ-alkoxide bridged Mn$_2^{II}$ (**f**). These compounds have oxo-bridged Mn (except for Mn^{V}-oxo) with O or N/O terminal ligands. In Fig. 3, there are no detectable Kβ″ peaks from the μ-alkoxide/carboxylate bridged Mn$_2^{II}$ complex (**f**). This is likely due to delocalization of the oxygen 2s electron which is part of a molecular orbital that is spread over the whole methyl/carboxylate group, i.e. atoms that are next-nearest to Mn, and to a lesser degree due to the longer Mn-ligand bridging interactions (~2.0-2.1 Å).[14] Similarly, contributions from O and N terminal ligands from carboxylates or histidine/amines are weak, because the molecular orbitals that contain O and N 2s are strongly delocalized. Moreover, terminal ligands are generally at longer distances to Mn than the bridging ligands and their contributions are smaller.[9] Theoretical studies regarding X-ray emission spectra from transition metal complexes are emerging,[14] yet at present the interpretation of the Kβ″ spectra has remained largely empirical. There is a strong peak in the Mn^{V}-oxo compound, indicating that the Kβ″ peak intensity is predominantly sensitive to single atom ligands and short metal ligand atom distances, namely, bridging O ligands and double/triple bonds which all have localized 2s orbitals. The sensitivity of the spectra to even one electron changes is illustrated in Fig. 3. The spectra are different for the two binuclear and the two cubane molecules in which one out of two or four Mn is oxidized from (III) to (IV). The Kβ″ peak of the PS II S_1 state is relatively intense compared to other Mn$_2^{III,IV}$ di-μ-oxo bridged compounds. This indicates that there are several μ-oxo bridged Mn-O bonds in the S_1 state.[2]

The involvement of bridging oxo groups[4] or the high valent Mn^{IV}=O• or Mn^{V}≡O species[3,5] have all been implicated in the mechanism for the formation of the critical O-O bond in the water-oxidation reaction of PS II. As we have demonstrated here, it is now feasible to obtain such spectra for the Mn_4Ca cluster in PS II and the outlook for Kβ″ spectroscopy as a tool for studying the nature of the O ligand binding modes and therefore the mechanism of the water-splitting reaction seems promising.

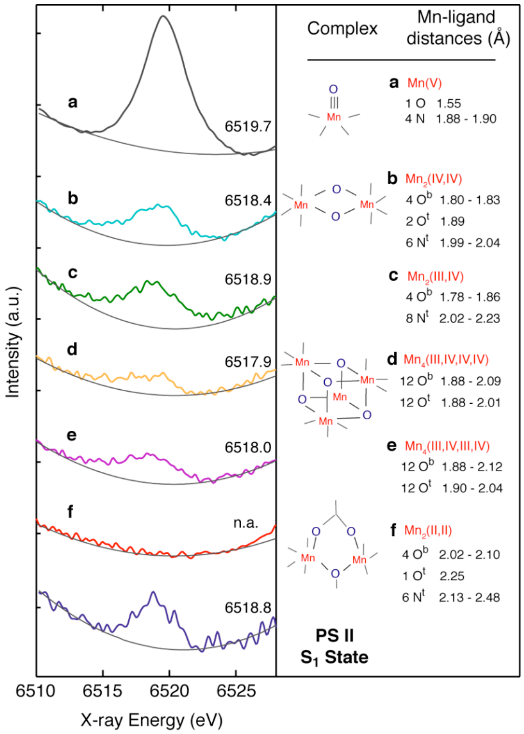

Figure 3. The Kβ″ emission spectra from a series of multinuclear Mn complexes (Suppl. Info. for details) with oxo-bridging groups, a Mn^{V}-oxo complex and PS II in the S_1 state. The crossover peak from the O ligand is prominent when short bridging Mn-O distances are present.

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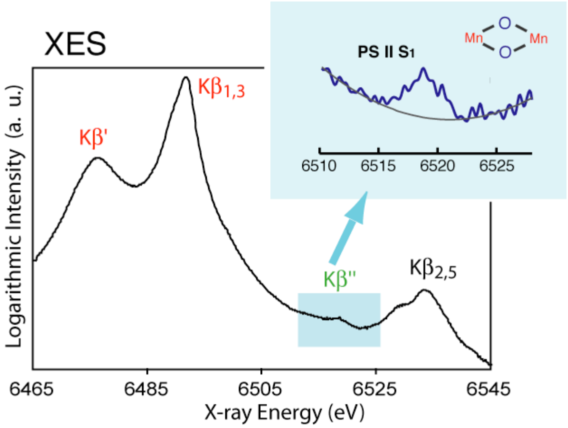
Supporting Information Available: Materials and methods.

References

1. Wydrzynski, T.; Satoh, S., *Photosystem II: The Light-Driven Water:Plastoquinone Oxidoreductase*. Springer: Dordrecht, 2005.
2. Yano, J.; Yachandra, V. K., *Inorg. Chem.* **2008,** *47*, 1711-1726.
3. Messinger, J., *Phys. Chem. Chem. Phys.* **2004,** *6*, 4764-4771.
4. Yachandra, V. K.; Sauer, K.; Klein, M. P., *Chem. Rev.* **1996,** *96*, 2927-2950.
5. McEvoy, J. P.; Brudvig, G. W., *Chem. Rev.* **2006,** *106*, 4455-4483.
6. Glatzel, P.; Bergmann, U., *Coord. Chem. Rev.* **2005,** *249*, 65-95.
7. Bergmann, U.; Grush, M. M.; Horne, C. R.; DeMarois, P.; Penner-Hahn, J. E.; Yocum, C. F.; Wright, D. W.; Dube, C. E.; Armstrong, W. H.; Christou, G.; Eppley, H. J.; Cramer, S. P., *J. Phys. Chem. B* **1998,** *102*, 8350-8352.
8. Messinger, J.; Robblee, J. H.; Bergmann, U.; Fernandez, C.; Glatzel, P.; Visser, H.; Cinco, R. M.; McFarlane, K. L.; Bellacchio, E.; Pizarro, S. A.; Cramer, S. P.; Sauer, K.; Klein, M. P.; Yachandra, V. K., *J. Am. Chem. Soc.* **2001,** *123*, 7804-7820.
9. Bergmann, U.; Horne, C. R.; Collins, T. J.; Workman, J. M.; Cramer, S. P., *Chem. Phys. Lett.* **1999,** *302*, 119-124.
10. Bergmann, U.; Glatzel, P.; Robblee, J. H.; Messinger, J.; Fernandez, C.; Cinco, R.; Visser, H.; McFarlane, K.; Bellacchio, E.; Pizarro, S.; Sauer, K.; Yachandra, V. K.; Klein, M. P.; Cox, B. L.; Nealson, K. H.; Cramer, S. P., *J. Syn. Rad.* **2001,** *8*, 199-203.
11. Bergmann, U.; Bendix, J.; Glatzel, P.; Gray, H. B.; Cramer, S. P., *J. Chem. Phys.* **2002,** *116*, 2011-2015.
12. Yano, J.; Kern, J.; Irrgang, K.-D.; Latimer, M. J.; Bergmann, U.; Glatzel, P.; Pushkar, Y.; Biesiadka, J.; Loll, B.; Sauer, K.; Messinger, J.; Zouni, A.; Yachandra, V. K., *Proc. Natl. Acad. Sci. USA* **2005,** *102*, 12047-12052.
13. Glatzel, P.; Bergmann, U.; Yano, J.; Visser, H.; Robblee, J. H.; Gu, W. W.; de Groot, F. M. F.; Christou, G.; Pecoraro, V. L.; Cramer, S. P.; Yachandra, V. K., *J. Am. Chem. Soc.* **2004,** *126*, 9946-9959.
14. de Groot, F., *Chem. Rev.* **2001,** *101*, 1779-1808.



Mn $K\beta$ X-ray emission spectroscopy is the study of transitions from the 3p level to the core 1s level of Mn. $K\beta_{1,3}$ and $K\beta'$ have been used to probe the oxidation state and spin state of the Mn atom. Mn $K\beta_{2,5}$ and $K\beta''$ spectra arise from emissions from the valence shells of the metal complexes to the core 1s levels. The valence shells comprise of ligand 2s2p levels and hence this spectroscopy provides a method to study the ligands of Mn. In biological systems, it is particularly difficult to specifically probe the O and N ligands which are ubiquitous in the protein. Mn $K\beta_{2,5}$ and $K\beta''$ emission provides a methodology for specifically obtaining information about the ligand atoms. In this study we have used a new spectroscopic technique to study the O ligands of Mn and have been able to detect for the first time the bridging oxo groups of Mn. The bridging oxo groups have been implicated in the mechanism and this direct spectroscopic technique provides a method for studying these O ligand in the mechanism of the O-O bond formation and water oxidation mechanism.