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THE EFFECT OF MAGNESIUM ION CONCENTRATION ON THE pH OPTIMUM AND MICHAELIS CONSTANTS OF THE SPINACH CHLOROPLAST RIBULOSE DIPHOSPHATE CARBOXYLASE (CARBOXYDISMUTASE)

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The effect of magnesium ion concentration on the pH optimum and Michaelis constants of the spinach chloroplast ribulose diphosphate carboxylase (carboxydismutase)

Kinetic tracer studies of levels of labeled metabolites in Chlorella pyrenoidosa¹ and in spinach chloroplasts² during light and dark gave evidence for the activation during photosynthesis of two enzymes of the photosynthetic carbon reduction cycle³. These enzymes were hexose diphosphatase (E.C.3.1.1.11) and ribulose diphosphate carboxylase (carboxydismutase) (E.C.3.1.3.11). Both enzymes are activated by Mg^{++} ions^{4,5}, and in view of the reported light-induced flow of H^+ and Mg^{++} ions in chloroplasts^{6,7}, it appears important to know in some detail the interaction of Mg^{++} and H^+ in affecting the activity of these enzymes.

Preiss, et al.⁴ have already shown that pH optimum of the diphosphatase is shifted from 8.5 to 7.5 by raising the Mg^{++} ion concentration from 5 mM to 40 mM. In the present study, raising the level of Mg^{++} ion from 1.8 mM to 45 mM shifted the pH optimum of the carboxylase from 8.5 to 7.7, and, at the same time, lowered the K_m for bicarbonate ion several fold.

Ribulose-1,5-diphosphate (Ru-1,5-P₂) was purchased as the dibarium salt at 72% purity. The free sugar phosphate was generated by treatment with the H^+ form of Dowex 50 resin.

Spinach chloroplasts were isolated from market spinach as described earlier⁸. The chloroplast pellets were sonicated for 1 min in H₂O. The resulting suspension was centrifuged at 36,000 x g for 30 min, and the supernatant solution was adjusted to 0.01 M tris at pH 7.6. To

this supernatant solution, $(\text{NH}_4)_2\text{SO}_4$ was added to 32% saturation and the precipitate discarded. Then $(\text{NH}_4)_2\text{SO}_4$ was added to 40%, and the resulting precipitate was stored at 5°C under a few ml of 50% ammonium sulfate. Before use, the pellet was dissolved in 10 ml of 0.001 M tris HCl at pH 8.0 containing 0.05 mM EDTA and dialyzed 48 h against the same buffer (with three changes) to remove the ammonium sulfate.

For assay, the enzyme was incubated at 23°C for 10 min (without preincubation) with Ru-1,5-P₂, MgCl_2 , and $\text{H}^{14}\text{CO}_3^-$ (32 $\mu\text{C}/\mu\text{mole}$), concentrations and pH as indicated in the figures, and tris buffer, 60 to 70 mM. In each incubation 85 μg of the enzyme was used, protein was determined by the method of Lowry *et al.*⁹ The incubation was stopped by addition of acetic acid and assayed for ^{14}C fixation into acid-stable products as described earlier.

In Fig. 1 is shown a series of curves of activity versus pH, all at 0.33 mM HCO_3^- and each curve at a different level of magnesium ion as indicated in the figure. The shift in the $\text{pH}_{\text{optimum}}$ from 8.5 at 1.8 mM Mg^{++} ion to 7.7 at 45 mM Mg^{++} ion is demonstrated. A similar result was obtained for a series of curves at 2.3 mM HCO_3^- , though there was some small variation in the shapes of the curves.

Fig. 2 shows the Lineweaver-Burk plot¹⁰ for the enzyme and Ru-1,5-P₂ with pH 7.7 and 45.4 mM Mg^{++} . The extrapolated $-1/K_m$ value of 4 (mM)⁻¹ gives a K_m of 2.5×10^{-4} in agreement with the value first reported by Weissbach *et al.*⁵, who assayed the enzyme at pH 7.7 and about 8.3 mM Mg^{++} . The present results, however, were obtained at 0.33 mM HCO_3^- .

Fig. 3 shows a similar plot for the enzyme and HCO_3^- at pH 7.7

and with 45.4 mM Mg^{++} . The value of $-1/K_m$ in this case was 0.4 (mM)^{-1} , giving $K_m = 2.5 \times 10^{-3}$, while K_m equal to the HCO_3^- concentration for half-maximal velocity was $1.8 \times 10^{-3} \text{ M}$.

At pH 7.7 and 2.0 mM Mg^{++} ion, HCO_3^- concentration for half-maximal velocity was found to be $5.4 \times 10^{-3} \text{ M}$, which may be compared with $11 \times 10^{-3} \text{ M}$, reported by Weissbach et al.

The high value for the K_m of this enzyme for HCO_3^- has for some time seemed a problem, when one considers the low level of CO_2 required for high rates of photosynthesis in vivo, and even in isolated chloroplasts⁸. From the results shown here, it appears that part of the activation and K_m lowering of the enzyme in vivo could be caused by a high local concentration of Mg^{++} ion.

The shifting of the pH optimum of the carboxylation enzyme to physiological pH by the use of high levels of magnesium ion, which parallels very closely the behavior of the diphosphatase enzyme, when considered in the context of the regulatory roles of these two enzymes in the photosynthetic carbon reduction cycle, seems significant. Whether or not such high levels of magnesium ion as 40 mM can be generated locally in the stroma region of the chloroplasts as a result of ion pumping through the thylakoid membrane during the light reactions

of photosynthesis remains to be seen. Dilley and Vernon⁷ indicated a light-induced influx of protons into the thylakoids and an efflux of K^+ and Mg^{++} ions from the thylakoids. It seems likely that from the method of preparation of chloroplasts used by Dilley and Vernon the outer membrane was not intact. Thus their observations with the broken system may be a reflection of a somewhat different process that occurs with the intact chloroplasts.

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FIGURE CAPTIONS

Fig. 1. Effect of several levels of Mg^{++} ion concentration on curves of activity vs. pH for ribulosediphosphate carboxylase.

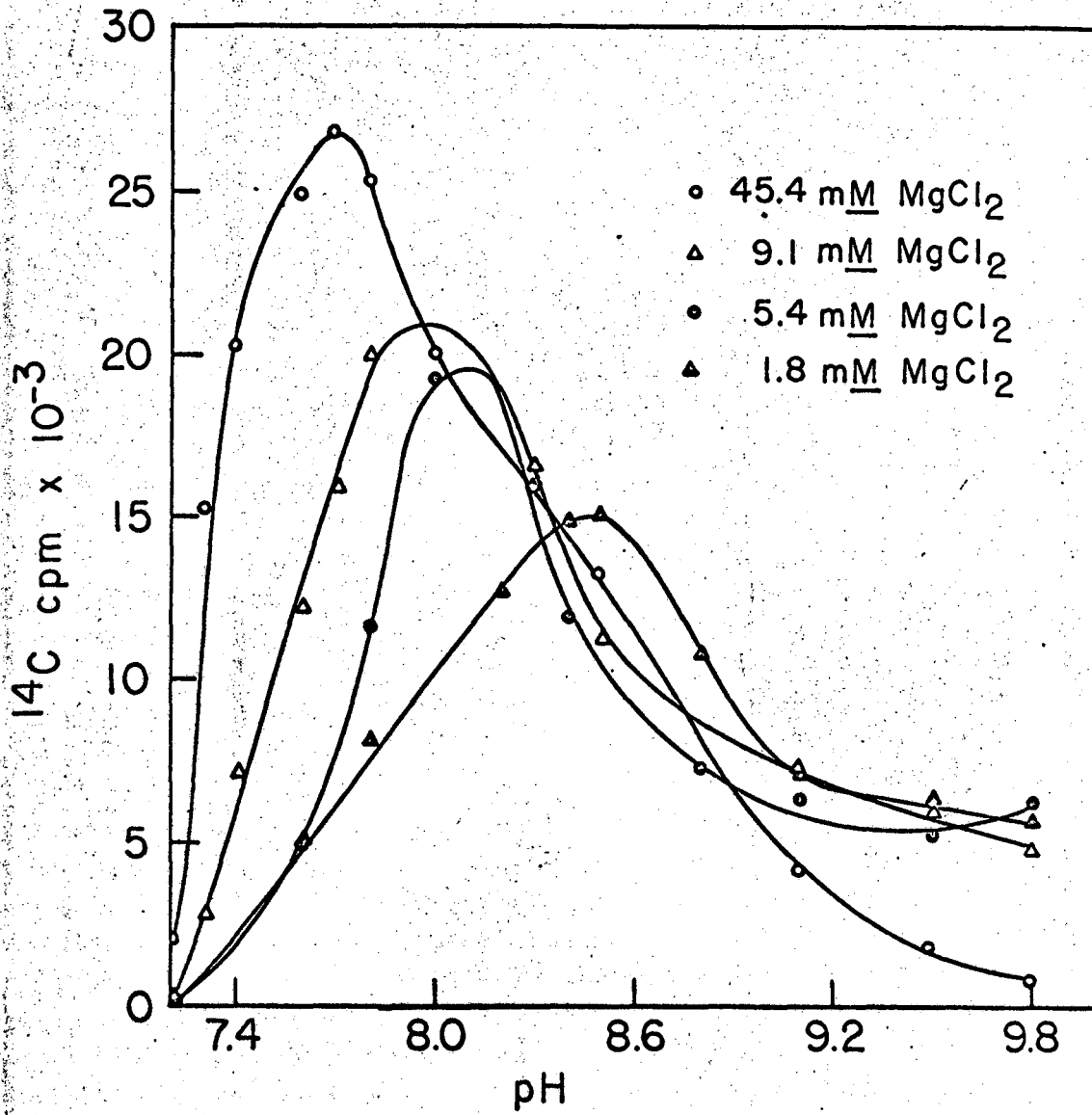
Protein, 85 μ g in 0.275 ml; 10 min incubation with $H^{14}CO_3^-$, 0.33 mM, 32.4 μ C/ μ mole, counter sensitivity, 0.15 cpm/dpm. No preincubation.

Fig. 2. Effect of Ru-1,5- P_2 concentration on the activity of ribulosediphosphate carboxylase.

Protein, 85 μ g in 0.275 ml; pH 7.7; Mg^{++} ion, 45.4 mM; $H^{14}CO_3^-$, 0.33 mM.

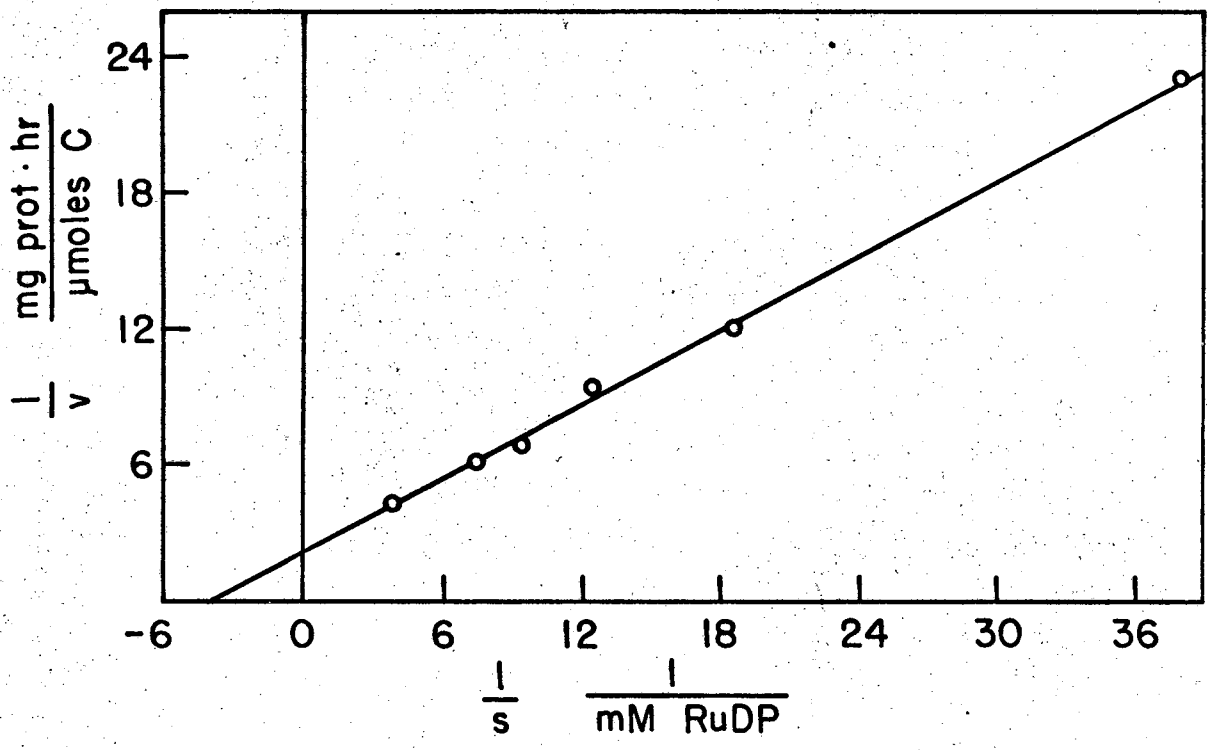
Fig. 3. Effect of HCO_3^- ion concentration on the activity of ribulosediphosphate carboxylase.

Protein, 85 μ g in 0.275 ml; Ru-1,5- P_2 , 0.136 mM; pH 7.7.



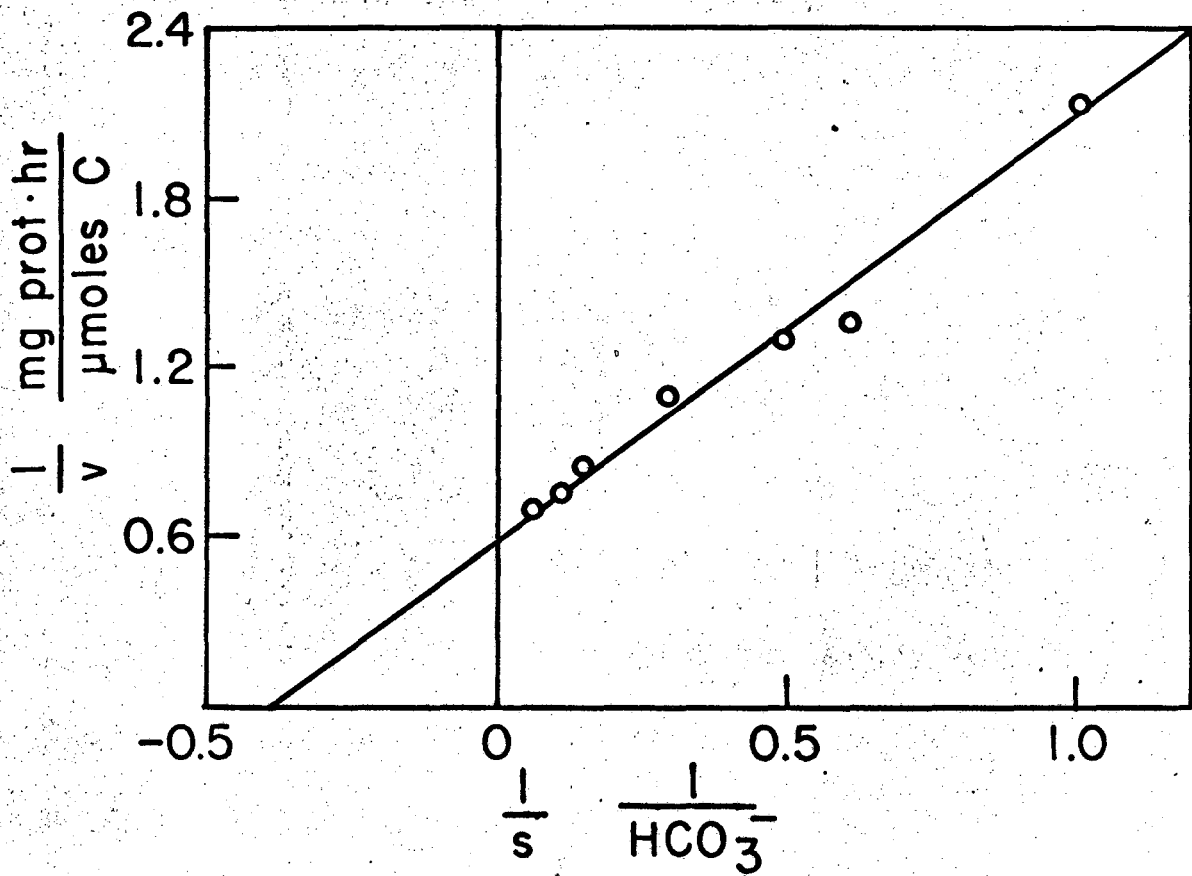
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Fig. 1



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Fig. 2



XBL 681-4003

Fig. 3

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