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The effect of Mg^{2+} concentration on the pH optimum and Michaelis constants of the spinach chloroplast ribulosediphosphate carboxylase (carboxydismutase)

Kinetic tracer studies of levels of labeled metabolites in *Chlorella pyrenoidosa*[1] and in spinach chloroplasts[2] during light and dark gave evidence for the activation during photosynthesis of two enzymes of the photosynthetic carbon reduction cycle[3]. These enzymes were hexosediphosphatase (EC 3.1.3.11) and ribulosediphosphate carboxylase (carboxydismutase) (EC 4.1.1.39). Both enzymes are activated by Mg^{2+} (refs. 4, 5), and in view of the reported light-induced flow of H^+ and Mg^{2+} in chloroplasts[6,7], it appears important to know in some detail the interaction of Mg^{2+} and H^+ in affecting the activity of these enzymes.

PREISS, BIGGS AND GREENBERG[4] have already shown that the pH optimum of the diphosphatase is shifted from 8.5 to 7.5 by raising the Mg^{2+} concentration from 5 mM to 40 mM. In the present study, raising the level of Mg^{2+} 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 HCO_3^- several fold.

Ribulose 1,5-diphosphate (Ribul-1,5-P_2) 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 earlier[8]. The chloroplast pellets were sonicated for 1 min in H_2O. The resulting suspension was centrifuged at 36000 × *g* for 30 min, and the supernatant solution was adjusted to 0.01 M Tris at pH 7.6. To this supernatant solution, $(NH_4)_2SO_4$ was added to 32 % satn. and the precipitate discarded. Then $(NH_4)_2SO_4$ was added to 40 %, and the

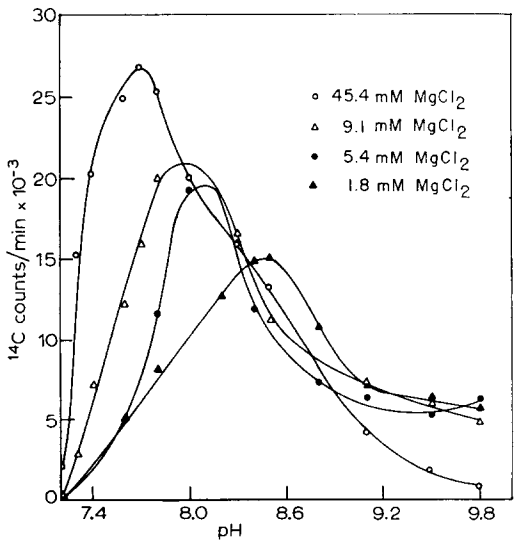

Fig. 1. Effect of several levels of Mg^{2+} concentration on curves of activity *vs.* pH for ribulosediphosphate carboxylase. Protein, 85 μg in 0.275 ml; 10 min incubation with 0.33 mM $H^{14}CO_3^-$, 32.4 μC/μmole; counter sensitivity, 0.15 counts/min per disint./min. No preincubation.

resulting precipitate was stored at 5° under a few ml of 50% $(NH_4)_2SO_4$. 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 $(NH_4)_2SO_4$.

For assay, the enzyme was incubated at 23° for 10 min (without preincubation) with Ribul-1,5-P_2, $MgCl_2$, and $H^{14}CO_3^-$ (32 μC/μmole), concentrations and pH as indicated in the figures, and 60–70 mM Tris buffer. 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 Mg^{2+} as indicated in the figure. The shift in the pH optimum from 8.5 at 1.8 mM Mg^{2+} to 7.7 at 45 mM Mg^{2+} 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 plot[10] for the enzyme and Ribul-1,5-P_2 with pH 7.7 and 45.4 mM Mg^{2+}. The extrapolated $-1/K_m$ value of 4 mM^{-1} gives a K_m

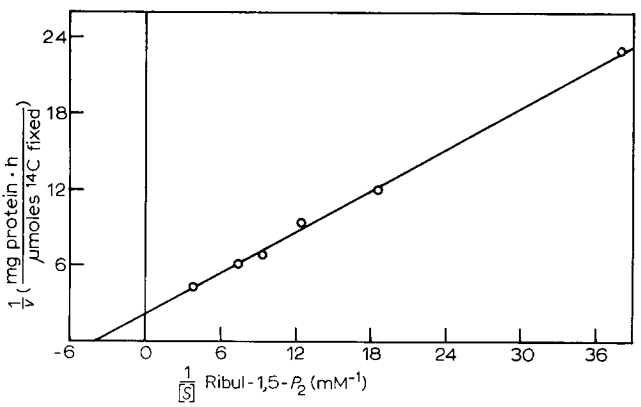

Fig. 2. Effect of Ribul-1,5-P_2 concentration on the activity of ribulosediphosphate carboxylase. Protein, 85 μg in 0.275 ml; pH 7.7; Mg^{2+}, 45.4 mM; $H^{14}CO_3^-$, 0.33 mM.

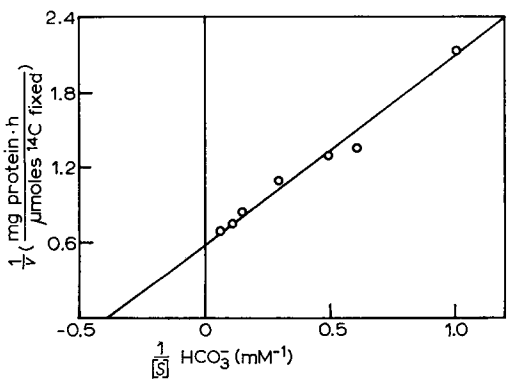

Fig. 3. Effect of HCO_3^- concentration on the activity of ribulosediphosphate carboxylase. Protein, 85 μg in 0.275 ml; Ribul-1,5-P_2, 0.136 mM; pH 7.7; Mg^{2+}, 45.4 mM.

of $2.5 \cdot 10^{-4}$ in agreement with the value first reported by WEISSBACH, HORECKER AND HURWITZ[5], who assayed the enzyme at pH 7.7 and about 8.3 mM Mg^{2+}. 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^{2+}. The value of $-1/K_m$ in this case was 0.4 mM^{-1}, giving $K_m = 2.5 \cdot 10^{-3}$, while K_m equal to the HCO_3^- concentration for half-maximal velocity was $1.8 \cdot 10^{-3}$ M.

At pH 7.7 and 2.0 mM Mg^{2+}, HCO_3^- concentration for half-maximal velocity was found to be $5.4 \cdot 10^{-3}$ M, which may be compared with $11 \cdot 10^{-3}$ M, reported by WEISSBACH, HORECKER AND HURWITZ[5].

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 chloroplasts[8]. 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^{2+}.

The shifting of the pH optimum of the carboxylation enzyme to physiological pH by the use of high levels of Mg^{2+}, which parallels very closely the behaviour of the diphosphate 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 Mg^{2+} 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 VERNON[7] indicated a light-induced influx of protons into the thylakoids and an efflux of K^+ and Mg^{2+} from the thylakoids. It seems likely that from the method of preparation of chloroplasts used by DILLEY AND VERNON[7] 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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