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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)

J. A. Bassham, Pamela Sharp, and Ian Morris

January 1968

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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 pyrenoidosa<sup>1</sup> and in spinach chloroplasts<sup>2</sup> during light and dark gave evidence for the activation during photosynthesis of two enzymes of the photosynthetic carbon reduction cycle<sup>3</sup>. 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<sup>4,5</sup>, and in view of the reported light-induced flow of  $H^+$  and  $Mg^{++}$  ions in chloroplasts<sup>6,7</sup>, it appears important to know in some detail the interaction of  $Mg^{++}$  and  $H^+$  in affecting the activity of these enzymes.

Preiss, et al.<sup>4</sup> 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  $K_{mH}$  for bicarbonate ion several fold.

Ribulose-1,5-diphosphate (Ru-1,5-P<sub>2</sub>) 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<sup>8</sup>. The chloroplast pellets were sonicated for 1 min in H<sub>2</sub>O. 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  $5^\circ\text{C}$  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  $23^\circ\text{C}$  for 10 min (without preincubation) with Ru-1,5-P<sub>2</sub>,  $\text{MgCl}_2$ , 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  $\mu\text{g}$  of the enzyme was used, protein was determined by the method of Lowry *et al.*<sup>9</sup> The incubation was stopped by addition of acetic acid and assayed for  $^{14}\text{C}$  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  $\text{HCO}_3^-$  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  $\text{Mg}^{++}$  ion to 7.7 at 45 mM  $\text{Mg}^{++}$  ion is demonstrated. A similar result was obtained for a series of curves at 2.3 mM  $\text{HCO}_3^-$ , though there was some small variation in the shapes of the curves.

Fig. 2 shows the Lineweaver-Burk plot<sup>10</sup> for the enzyme and Ru-1,5-P<sub>2</sub> with pH 7.7 and 45.4 mM  $\text{Mg}^{++}$ . The extrapolated  $-1/K_m$  value of 4 (mM)<sup>-1</sup> gives a  $K_m$  of  $2.5 \times 10^{-4}$  in agreement with the value first reported by Weissbach *et al.*<sup>5</sup>, who assayed the enzyme at pH 7.7 and about 8.3 mM  $\text{Mg}^{++}$ . The present results, however, were obtained at 0.33 mM  $\text{HCO}_3^-$ .

Fig. 3 shows a similar plot for the enzyme and  $\text{HCO}_3^-$  at pH 7.7

and with 45.4 mM  $Mg^{++}$ . The value of  $-1/K_m$  in this case was  $0.4 \text{ (mM)}^{-1}$ , 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 chloroplasts<sup>8</sup>. 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 Vernon<sup>7</sup> 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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Chemical Biodynamics Laboratory  
Lawrence Radiation Laboratory  
University of California  
Berkeley, Calif. (U.S.A.)

J. A. BASSHAM  
PAMELA SHARP  
IAN MORRIS\*

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\*Present address: Botany Department, University College, London.

FIGURE CAPTIONS

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

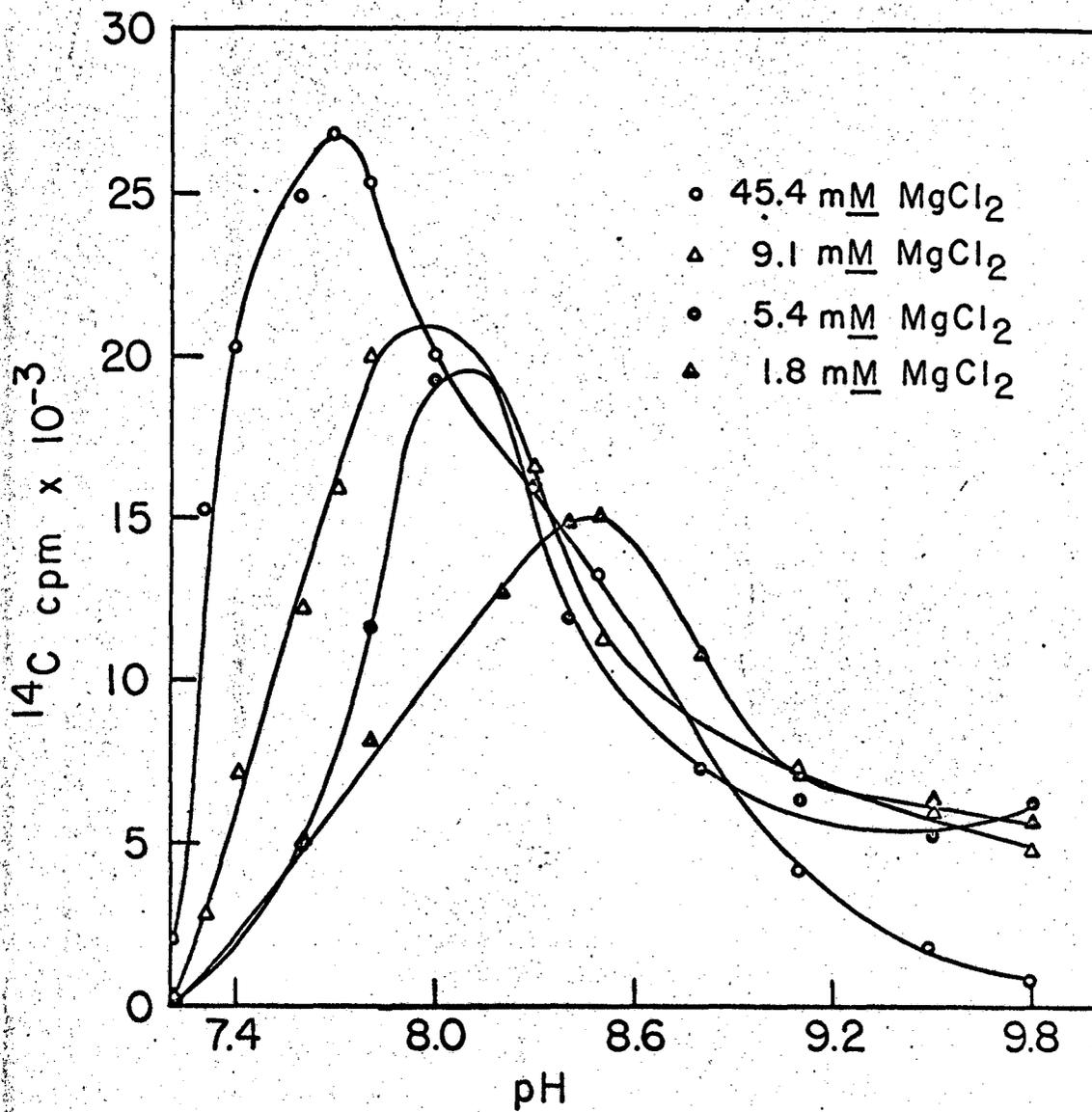
Protein, 85  $\mu$ g in 0.275 ml; 10 min incubation with  $H^{14}CO_3^-$ , 0.33 mM, 32.4  $\mu$ C/ $\mu$ mole, counter sensitivity, 0.15 cpm/dpm. No preincubation.

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

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

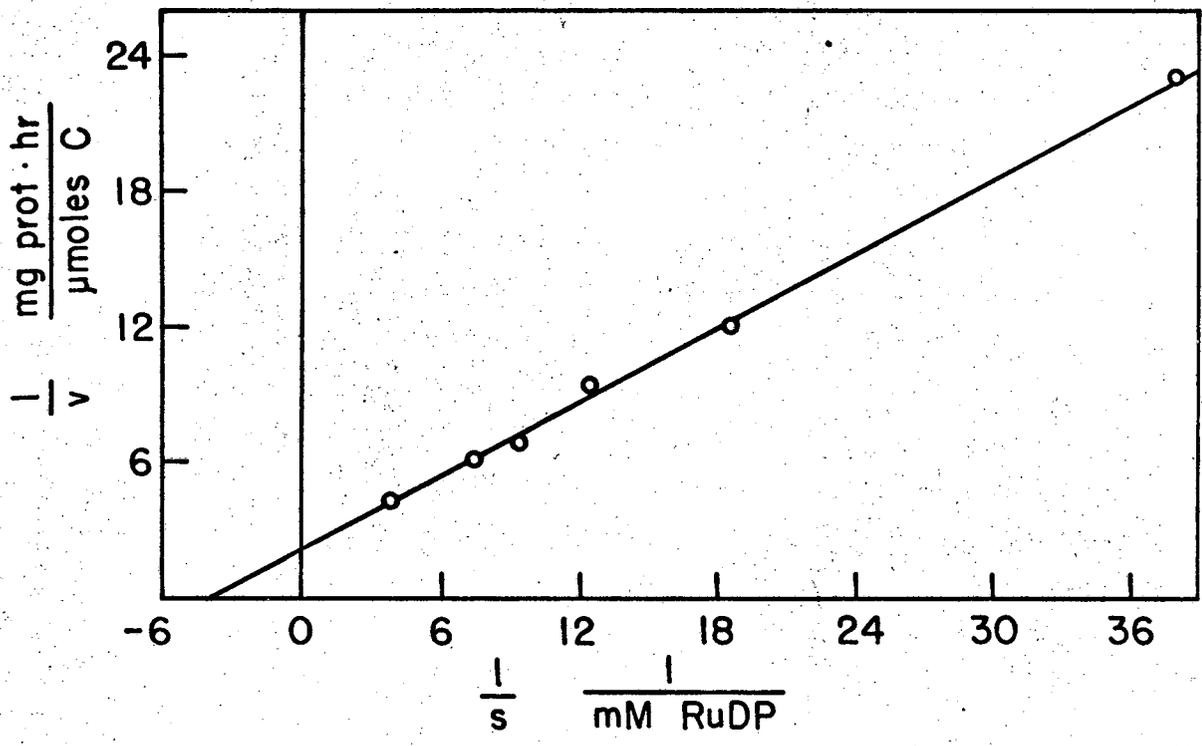
Fig. 3. Effect of  $HCO_3^-$  ion concentration on the activity of ribulosediphosphate carboxylase.

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



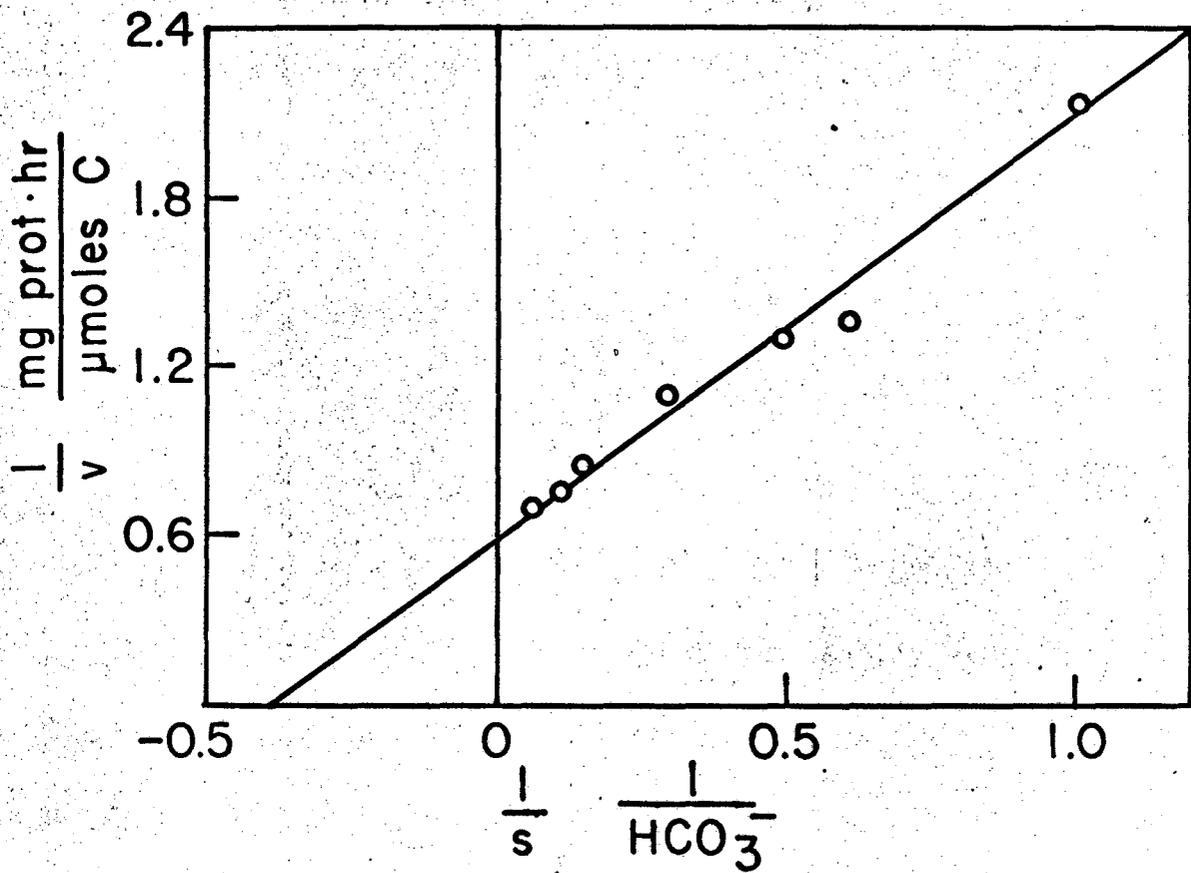
XBL 6711-6249

Fig. 1



XBL 681-4002

Fig. 2



XBL 681-4003

Fig. 3

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