Activation of Spinach Leaf Ribulose-1,5-Diphosphate Carboxylase Activities by Magnesium Ions*

by

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The initial study of Weissbach et al. (1) on spinach ribulose-1,5-diphosphate (RuDP) carboxylase showed that Mg^{++} was required for maximum enzyme activity. This finding was confirmed by Racker (2), also using RuDP carboxylase isolated from spinach. The present communication describes the kinetics of the activation of spinach leaf RuDP carboxylase activities by Mg^{++}. Our experimental results suggest that Mg^{++} behaves as an allosteric activator for the enzyme reaction.

RuDP carboxylase was prepared from spinach leaves as reported previously (3,4). The enzyme preparation used in the present studies was homogeneous as judged from analytical ultracentrifugation and polyacrylamide gel electrophoresis. The standard reaction mixture described previously was used, except for the varying concentrations of $MgCl_2$ and $NaHCO_3$ as indicated in each experiment. The reaction was carried out for 10 minutes at 25°C, and radioactivity measurement of the fixed $C^{14}O_2$ was carried out using a liquid scintillation counter (3,4).

The effect of Mg^{++} concentrations on the carboxylase activities was determined at pH values of 7.0, 7.8, and 9.0 respectively. Results are shown in Fig. 1. At each pH value, the enzyme activity was stimulated by addition of Mg^{++}. Using the double reciprocal plot to express the data (inset), it may be seen that as the pH values increased, the Km values to $MgCl_2$ decreased; 1.1×10^{-3} M at pH 7.0, 5.0×10^{-4} M at pH 7.8, and 1.5×10^{-4} M at pH 9.0.

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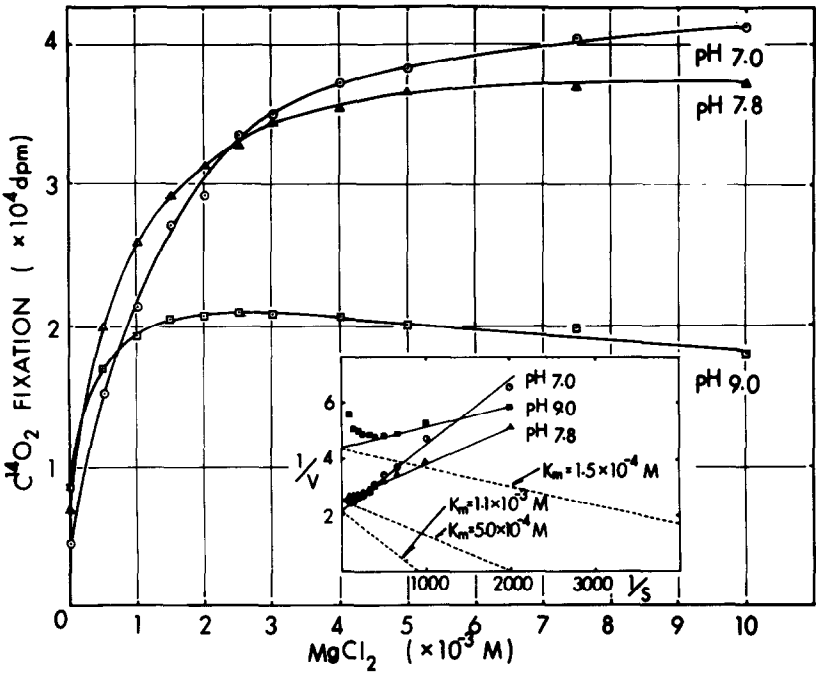

Fig. 1. Effect of Mg^{++} concentrations on the reaction velocity of RuDP carboxylase

Reaction mixture contained 20 µmoles of Tris buffer (pH 7.0, 7.8 and 9.0), 0.35 µmole of RuDP, 25 µmoles (2.0 µc) of $NaHC^{14}O_3$, various amounts of $MgCl_2$ (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.75 and 5.0 µmoles), and 0.1 ml (0.05 mg) of enzyme protein in a total volume of 0.5 ml.

Table I. Effect of $MgCl_2$ on the Apparent Km ($NaHCO_3$) and Vmax Values of Spinach Leaf RuDP Carboxylase

$MgCl_2$ (M)	Apparent Km ($\times 10^{-2}$ M)	Vmax ($C^{14}O_2$ fixation d.p.m. $\times 10^3$/10 min.)
0	2.00	4.2
1.25×10^{-3}	1.44	14.7
5×10^{-3}	1.20	17.0
1×10^{-2}	0.99	14.2
2×10^{-2}	0.56	10.3

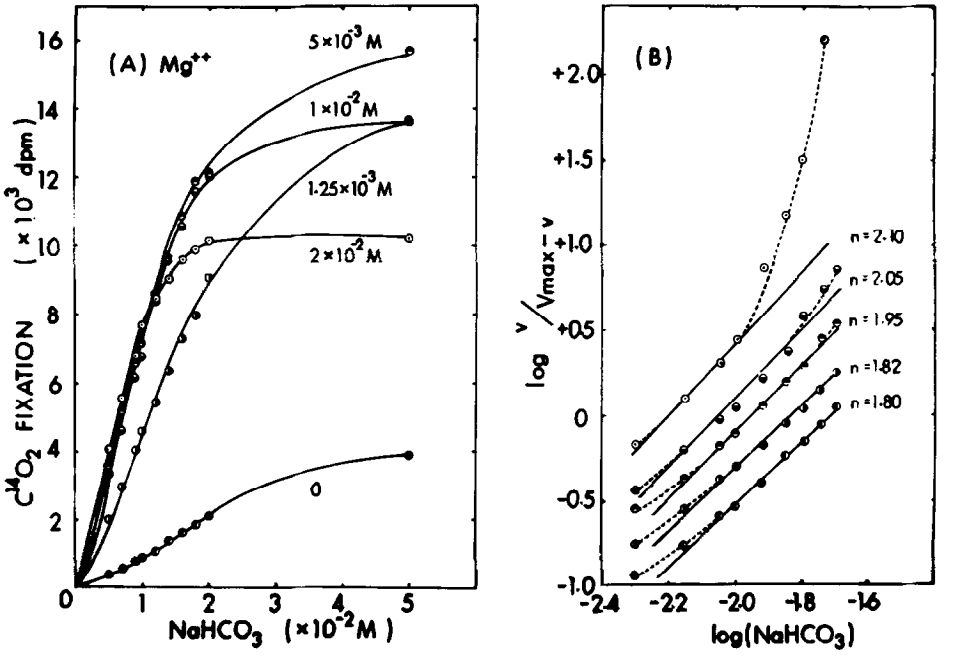

Fig. 2. Effect of Mg^{++} on the kinetic behavior of RuDP carboxylase

A. Direct plot; reaction rate ($C^{14}O_2$ fixation) vs. $NaHCO_3$ concentrations.

B. Hill plot; $\log {}^{v}/_{Vmax - v}$ vs. $\log (NaHCO_3)$.

Reaction mixture contained 100 μmoles of Tris buffer (pH 8.5), 0.35 μmole of RuDP, various amounts of $NaHC^{14}O_3$ (2.0 μc) (2.5, 3.5, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 25.0 μmoles) and $MgCl_2$ (0, 0.625, 2.5, 5.0, and 10.0 μmoles), and 0.1 ml (0.05 mg) of enzyme protein in a total volume of 0.5 ml.

The effect of different Mg^{++} concentrations on the rate of CO_2-fixation was determined at pH 8.5. The sigmoid curve of the reaction rate, as a function of $NaHCO_3$ concentrations, strongly suggests a homotropic interaction of $NaHCO_3$ in the CO_2-fixation reaction, indicating more than one substrate binding sites interact cooperatively in the enzyme molecule (Fig. 2A). This view was further supported by applying the Hill plot analysis of the data, giving interaction coefficient (n*) approximately 2 (Fig. 2B). It will be noted that the addition of effector, Mg^{++}, does not affect the substrate interaction. Regardless of the concentrations of Mg^{++}, n* numbers were calculated to be 2. The reaction rate vs. $NaHCO_3$ concentrations curve seems normalized by addition of Mg^{++}. However, as has been discussed by Wyman (5), deviation of the reaction

kinetics from the Michaelis-Menten type is suggested from the non-linearity of the Hill slope in certain concentration ranges of $NaHCO_3$ shown in the figure. Unique behavior of RuDP carboxylase showing the negative response of the interaction coefficient to Mg^{++}, is similar to that of yeast phosphofructokinase and yeast DPN isocitrate dehydrogenase studied by Atkinson and his associates (6-8). On the other hand, the present data are in sharp contrast to Mg^{++}-activation of fructose-1,6-diphosphatase (9) and Li^+- and K^+- activation of adenylate deaminase (10,11), in which the interaction coefficient is changed due to metal addition.

Effect of different Mg^{++} concentrations on the Km ($NaHCO_3$) and Vmax values was examined by inserting n* numbers obtained into the equation, $1/v = 1/Km \times 1/S^n + 1/Vmax$, and results are summarized in Table 1. It can be seen that the apparent Km values were decreased about 4 fold by raising Mg^{++} concentrations from 0 to 2×10^{-2} M. These results suggest that Mg^{++} may facilitate the binding of $NaHCO_3$ to the enzyme molecule. In fact the optimum pH values of RuDP carboxylase were found to shift as a function of Mg^{++} concentrations (Fig. 3). In the absence of Mg^{++} the enzyme activity was low and optimum pH was near 8.5. Increasing the Mg^{++} concentration to 10^{-3} M to 10^{-2} M, the optimum pH was shifted to 7.5 and 6.5 respectively. The pH shift is considered to indicate a possible conformation change of the enzyme molecule induced by the Mg^{++} binding. This view was strengthened by the fact that the enzyme protein was protected from Nagarse hydrolysis by the prior incubation with both $NaHCO_3$ and Mg^{++} (12). The protective effect was much less when the enzyme was incubated with $NaHCO_3$ and Mg^{++} separately. Provided $NaHCO_3$ was incubated in the preincubation mixture, the higher the Mg^{++} concentrations the greater the protective effect on the enzyme molecule. Preliminary treatment of the enzyme with Mg^{++} and $NaHCO_3$ also protected the enzyme from urea and SDS inactivation (13).

All the findings described above support the notion that RuDP carboxylase is a type of regulatory enzyme in which CO_2 behaves as a homotropic effector substance and Mg^{++} an allosteric activator. It is difficult to assess the physiological status of Mg^{++} in chloroplasts and its relation to the photosynthetic CO_2-fixation. Nevertheless physiological implication can be made concerning the activating effect of Mg^{++} on the RuDP carboxylase reaction. Most previous experiments of RuDP carboxylase have been conducted at the pH value above 7.0. Horecker's group (1) obtained the half maximal velocity of the spinach enzyme to $NaHCO_3$ 2×10^{-2} M at pH 7.7, and Paulsen and Lane (14) reported the Km ($NaHCO_3$) value 2.2×10^{-2} M at pH 7.9. The present experiments demonstrate that increase in

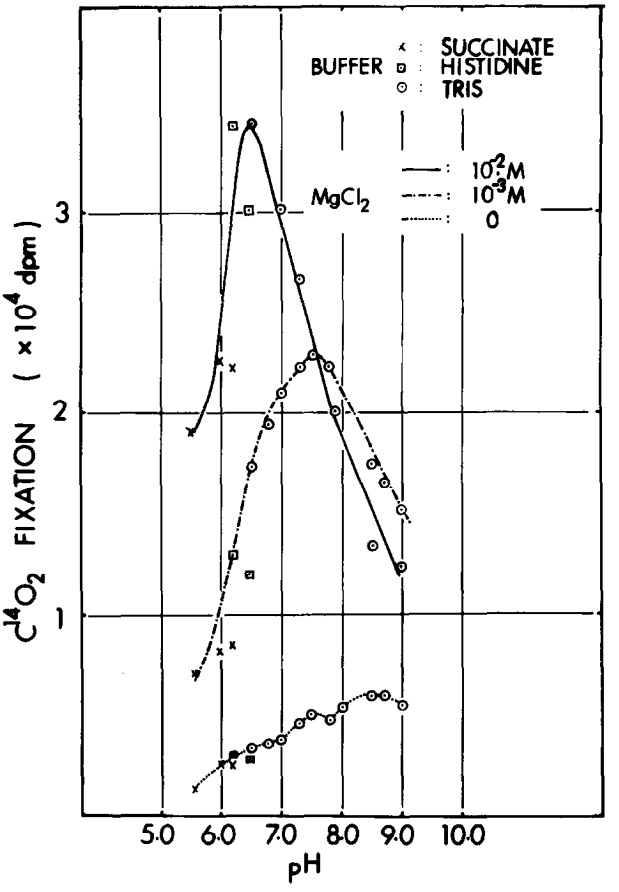

Fig. 3. Shift of optimum pH values of RuDP carboxylase reaction as affected by the addition of $MgCl_2$

Reaction mixture contained 20 µmoles each of Tris, histidine and succinate buffer, 0.35 µmole of RuDP, 25 µmoles (2.0 µc) of $NaHC^{14}O_3$, different amounts of $MgCl_2$ (0, 0.25 and 5.0 µmoles) and 0.1 ml (0.05 mg) of enzyme protein in a total volume of 0.5 ml. Ranges of pH changes were 6.5, 6.8, 7.0, 7.3, 7.5, 7.8, 8.0, 8.5, 8.7 and 9.0.

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the Mg^{++} concentrations brings about (a) an increase of the specific enzyme activities, (b) a decrease in the apparent Km ($NaHCO_3$) value, and (c) a shift of optimum pH value toward the neutral ranges. Experiments of Calvin's group (15) have shown the enhancement of dark $C^{14}O_2$-fixation of Chlorella cells grown in nutrient solution containing $MgSO_4$. More recently Keller and Huffaker (16) reported the supplement of $MgCl_2$ to the detached barley leaves in dark resulted in an enhancement of RuDP carboxylase activity, almost equal to that of the illuminated water control.

Correlation of our understanding of the molecular mechanism of Mg^{++}-activation of RuDP carboxylase with the physiological role of the metal in the photosynthetic act remains to be elucidated.

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