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Structure and Function of Chloroplast Proteins

V. Homotropic Effect of Bicarbonate in RuDP Carboxylase Reaction and the Mechanism of Activation by Magnesium Ions[1,2]

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Kinetic analyses of the CO_2-fixation reaction catalyzed by RuDP-carboxylase prepared from spinach leaves showed that the reaction rate ($^{14}CO_2$-fixation) *vs.* $NaHCO_3$ concentrations curve was deviated from the Michaelian type. From the analysis of data by the empirical Hill equation, the interaction coefficient, n^*, was calculated to be approximately 2, indicating a homotropic interaction of the $NaHCO_3$ molecule. It was thus inferred that $NaHCO_3$ molecules interact mutually between the multiple substrate-binding sites on the enzyme molecule. Activation of the carboxylation reaction by Mg^{++} was studied, and both *Km* ($NaHCO_3$) and *V*max values were found to be affected by increasing Mg^{++} concentrations. The *Km* value for $NaHCO_3$ was 2×10^{-2} M in the absence of Mg^{++} and 5.6×10^{-3} M at 2×10^{-2} M Mg^{++}. However, it was found that n^* numbers do not change by increasing Mg^{++} concentrations. This feature is in sharp contrast to the case of some other regulatory enzymes. Cooperativity of the enzyme molecule with $NaHCO_3$ was determined at different pH values, and the higher pH value the greater cooperativity (n^* numbers 2) and smaller the *Km* value to Mg^{++}. The nature of Mg^{++} activation was further studied as regards (1) the shift of optimum pH values of the enzyme; pH 7.5 at 10^{-3} M $MgCl_2$ and pH 6.5 at 10^{-2} M $MgCl_2$, and (2) the protective effect of Mg^{++} association to the enzyme molecule against the subsequent attack by the proteolytic enzyme (Nagarse). Some physiological implications have been made concerning the regulatory nature of RuDP carboxylase.

The elucidation of the control mechanism operative in the chloroplastic CO_2-fixation is an interesting object for better understanding the photosynthetic act. Thus, it will be recalled that classical observations of Warburg in 1919 (2) and others (3) demonstrating the maximum rate of the photosynthetic activity of plant cells at relatively low CO_2-concentrations under saturating light intensities have been a long-standing enigma of this field. Recent studies have established that the photosynthetic carbon-reduction cycle constitutes the major route of the chloroplastic CO_2-fixation and other related carbon assimilation reactions in photosynthetic organisms, and that ribulose-1,5-diphosphate (RuDP) carboxylase occupies a pivotal role in the cycle (4–6). There are several indications that the photosynthetic CO_2-fixation is regulated at the level of RuDP carboxylase based on both *in vivo* and *in vitro* experiments. Smillie and Fuller (7) presented experimental evidence showing that the RuDP carboxylase reaction may be the rate-limiting step in the CO_2-fixation by both isolated chloroplasts and intact leaves.

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[2] A part of this work has appeared in a preliminary form (1).

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Experimental results dealing with the transient change in metabolites of the photosynthesizing plant cells under light/dark conditions also suggest the regulatory function of RuDP carboxylase (4, 8). The following unique features of RuDP carboxylase may lead us to postulate its regulatory nature; (1) large molecular size consisting of subunits (9–14), (2) large negative ΔH value (-5000 cal/mole) of the carboxylation reaction (15), and (3) rather high *Km* value of the enzyme for $NaHCO_3$ and its relatively low molecular activity (15–17). Regardless of the validity of the recent prevailing hypothetical models concerning the subunit organization of the regulatory enzyme (18–22), the pursuit of reaction mechanism of RuDP carboxylase at the molecular level is certainly an intriguing problem. There have indeed been obtained some experimental evidences in this laboratory showing the important role of SH-groups in the catalysis of the RuDP carboxylase reaction (1, 23–25). As regards the thermodynamic irreversibility of the RuDP carboxylase reaction, it will be recalled that most enzymes known to be subject of metabolic control catalyze physiologically irreversible reactions (21). Finally, from the fact that RuDP carboxylase has an apparently low affinity for CO_2, one might speculate that some effector substance(s) in chloroplasts modulates the catalytic activity of the enzyme. Previous studies by Weissbach *et al.* (15) as well as by Racker (16) have shown unequivocally the requirement of Mg^{++} for the RuDP carboxylase activity. Pon *et al.* (26) have reported that the preliminary incubation of the carboxylase with bicarbonate in the presence of Mg^{++} induced a greater carboxylation activity than preincubation with RuDP. They thus indicated that an enzyme-Mg^{++}-CO_2 complex is formed just prior to the carboxylation of RuDP.

The purpose of the present investigation is to find a more substantial basis for above speculations concerning the facilitation of the CO_2 association to the enzyme molecule with Mg^{++} and to provide further experimental evidence to show that RuDP carboxylase is a type of regulatory enzyme. Based on kinetic analyses of the enzyme reaction, we will show that $NaHCO_3$ functions as a homotropic effector substance, and that Mg^{++} is a positive effector.

MATERIALS AND METHODS

Enzyme purification. Details of the enzyme purification from spinach leaves have already appeared in previous papers of this series (24, 27). Homogeneous fraction-I protein as judged by analytical ultracentrifugation (Fig. 1) as well as polyacrylamide gel electrophoresis (cf. Figs. 5 and 7 of Ref. 28) was used throughout the experiments.

Assay of RuDP carboxylase activity. The standard reaction mixture contained the following components (in μmoles): Tris buffer, 100; $MgCl_2$, 5; RuDP, 0.35; $NaH^{14}CO_3$ (2.0 μC), 25; and enzyme protein (about 0.05 mg), 0.1 ml in a total volume of 0.5 ml. For the kinetic studies, the level of $NaH^{14}CO_3$, RuDP, and $MgCl_2$ were varied as indicated in each case. In some experiments, Mg^{++} was replaced by other metal ions of varying concentrations (cf. Fig. 4). Incubation was carried out at 25°C for 10 min, and the fixation of $^{14}CO_2$ was determined according to the method reported previously, using a Packard Tri-Carb Liquid Scintillation Spectrometer. All the assays were in duplicate and average values are presented.

The concentrations of CO_2 and HCO_3^- in the reaction system vary as a function of environmental pH ($pK_a = 6.5$). However, it has not been established yet to distinguish the precise molecular species engaged in the carboxylase reaction between CO_2 and HCO_3^-. Consequently all the data are expressed using the concentrations of $NaHCO_3$ added to the reaction mixture.

Nagarse digestion of RuDP carboxylase. The proteolytic digestion of the carboxylase molecule has been described in previous reports (24, 28). At different times during incubation with Nagarse, aliquots of the mixture were withdrawn and assayed for residual RuDP carboxylase activity, using the standard assay system.

Analytical ultracentrifugation. This followed the method reported previously (13), using a Beckman Spinco Model E analytical ultracentrifuge at the Institute of Molecular Biology of this university.

RESULTS

Effect of bicarbonate concentration on the reaction kinetics. We examined the effect of different $NaHCO_3$ concentrations on the reaction velocities at 3 different levels of RuDP. Figure 2 A shows a plot of reaction velocity *vs.* substrate concentrations. The anomalous kinetic order with respect to substrate clearly deviates from the Michaelis-Menten type reaction. This fact is further

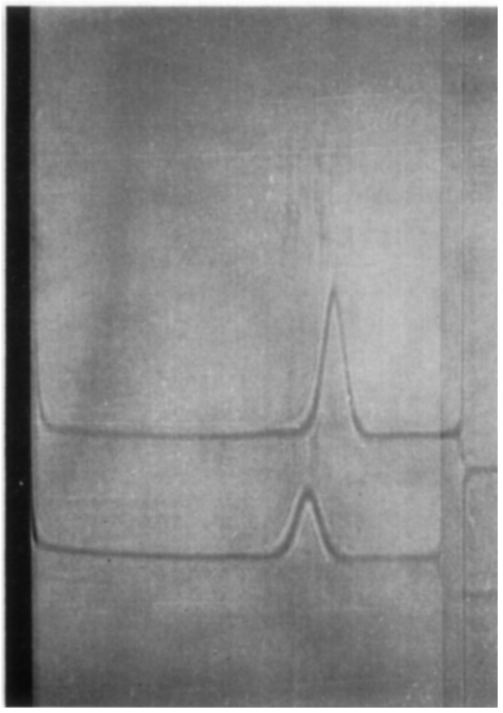

FIG. 1. Ultracentrifugation of RuDP carboxylase. Spinach leaf RuDP carboxylase dissolved in 0.05 M Tris buffer (pH 7.5) was dialized over night against the same buffer solution. Picture was taken at 24 min after reaching the maximum rotor speed (42,040 rpm) with a bar-angle setting of 70° and temperature at 19°C. Protein concentration was 10 mg/ml (upper) and 5 mg/ml (lower).

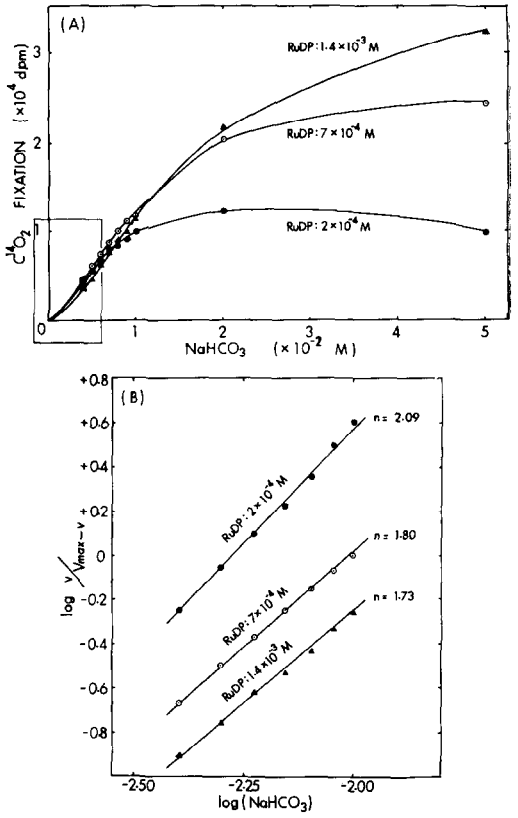

FIG. 2. Reaction kinetics of RuDP-carboxylase as a function of $NaHCO_3$ concentrations. Reaction mixture contained (in μmoles): Tris buffer (pH 7.8), 100; RuDP, 0.10, 0.35, 0.70; $MgCl_2$, 5; $NaH^{14}CO_3$ (2.5 μC), 2.5, 6, 7, 8, 9, 10, 20, 50, and 0.1 ml of enzyme (0.04 mg) in a total volume of 0.5 ml. A, Direct plot: reaction rate ($^{14}CO_2$ fixation) *vs.* $NaHCO_3$ concentrations. B, Hill plot: log v/Vmax-v *vs.* log ($NaHCO_3$).

supported by applying the Hill equation to the analytical data (18–22) (Fig. 2B). At three different levels of RuDP, the interaction coefficient (n^*) was calculated to be approximately 2, suggesting the cooperative interaction between homologous sites for $NaHCO_3$ in the enzyme molecule. Previous workers have used higher than physiological levels of $NaHCO_3$; Horecker's group (15) reported half maximal velocity of spinach RuDP carboxylase at 1.1×10^{-2} M $NaHCO_3$ (pH 7.7, 25°), and Paulsen and Lane (17) obtained a Km value ($NaHCO_3$) of 2.2×10^{-2} M (pH 7.9). We then examined the reaction kinetics at the concentration ranges of $NaHCO_3$ as encircled in Fig. 2A. It can be seen from Fig. 3 that even at extremely low bicarbonate concentrations, the carboxylase reaction displays the second-order kinetics regardless of the presence of Mg^{++}. The effect of Mg^{++} will be discussed in subsequent sections.

Effects of pH and Mg^{++} on the reaction kinetics of RuDP carboxylase. In order to examine the reaction kinetics of RuDP carboxylase on a more quantitative basis, the effect of varying Mg^{++} concentrations and different pH values was studied. As shown in Fig. 4, the carboxylation reaction is activated only by Mg^{++}, while Mn^{++}, Ni^{++}, and Co^{++} are entirely inactive (cf. 26). We examined the rate of CO_2-fixation at 3 different pH values, 7.0, 7.8, and 9.0, also varying the concentrations of Mg^{++}. The enzyme activities were invariably stimulated by

Mg^{++} at each pH value; raising the Mg^{++} concentration from 0 to 10^{-2} M increased the specific activity of the enzyme nearly 10-fold at pH 7.0. Using the double reciprocal plot to express the data (cf. Fig. 1 inset of Ref. 1), it is seen that the *Km* values for Mg^{++} decrease with increasing pH; 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.

We then determined the effects of varying the pH on the cooperativity between the carboxylase and $NaHCO_3$, at a fixed concentration of Mg^{++} (10^{-2} M). The results presented in Fig. 5A show that at each pH value, the reaction curve is non-Michaelian. However, by applying the Hill equation and

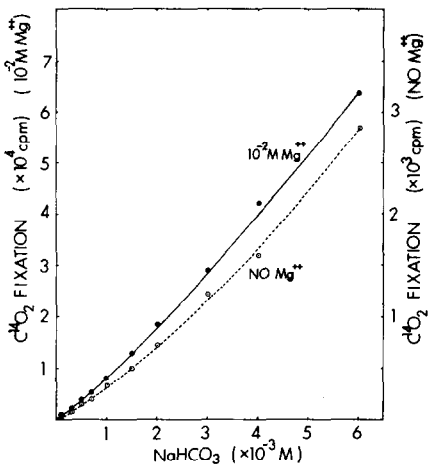

FIG. 3. RuDP carboxylase activity at the low concentration levels of $NaHCO_3$. Reaction mixture contained (in μmoles): Tris buffer (pH 7.0), 100; RuDP, 0.7; $NaHCO_3$ (2.5 μC), 0.05, 0.15, 0.25, 0.35, 0.5, 0.75, 1.0, 1.5, 2.0; $MgCl_2$, 0, 5.0; and 0.1 ml of enzyme (0.05 mg) in a total volume of 0.5 ml.

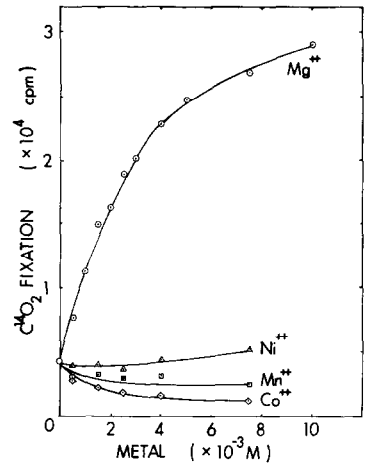

FIG. 4. Effect of divalent cations on RuDP carboxylase activity. Reaction mixture contained (in μmoles): Tris buffer (pH 7.0), 100; RuDP, 0.7; $NaHCO_3$ (2.0 μC), 25; either one of $MgCl_2$, $MnCl_2$, $NiCl_2$ and $CoCl_2$ at different final concentrations shown in the figure; and 0.1 ml of enzyme (0.05 mg) in a total volume of 0.5 ml.

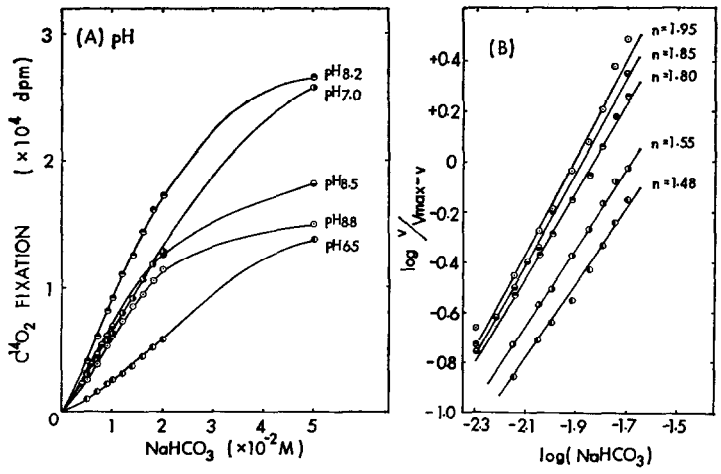

FIG. 5. Reaction kinetics of RuDP carboxylase as a function of pH values. Reaction mixture contained (in μmoles): Tris buffer (pH 6.5, 7.0, 8.2, 8.5, 8.8), 100; RuDP, 0.35; $NaHCO_3$ (2.0 μC), 2.5, 3.5, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 25.0; $MgCl_2$, 0.625; and 0.1 ml of enzyme (0.05 mg) in a total volume of 0.5 ml. A, Direct plot: reaction rate ($^{14}CO_2$ fixation) *vs.* $NaHCO_3$ concentrations. B, Hill plot: log c/Vmax-v *vs.* log ($NaHCO_3$).

replotting the data, we find that smaller n^* values are obtained at lower pH's (Fig. 5B). It thus appears evident that the cooperative interaction between the bicarbonate molecules and the enzyme varies as a function of pH. The apparent Km ($NaHCO_3$) and Vmax values at different pH's were calculated by inserting the n^* numbers obtained into the equation, $1/v = 1/Km \times 1/S^n + 1/V\text{max}$. The results summarized in Table I show that the Km values increase with decreasing pH.

The effect of different Mg^{++} concentrations on the rate of CO_2-fixation was determined at pH 8.5. The anomalous reaction curve of the CO_2-fixation *vs.* $NaHCO_3$ concentrations (Fig. 6A), as well as the result of the Hill plot of the data (Fig. 6B) again indicate the homotropic interaction of $NaHCO_3$ molecules in the CO_2-fixation reaction. It will be noted from Fig. 6B, however, that the addition of the activator, Mg^{++}, does not appreciably affect the cooperativity between substrate and the enzyme. At the different levels of Mg^{++} tested, the n^* values obtained are approximately the same ($n^* = 2$). The reaction rate *vs.* $NaHCO_3$ concentration curve appears to be normalized by the addition of higher levels of Mg^{++}. However, as has been discussed by Wyman (29), a deviation of the reaction kinetics from the Michaelian type can be inferred from the non linearity of the Hill slope at certain concentration ranges of $NaHCO_3$, as shown in Fig. 6B. The results summarized in Table II show that the apparent Km values are decreased about 4-fold by raising the Mg^{++} concentration from 0 to 2×10^{-2} M; Vmax is also affected (cf. Table I).

TABLE I

EFFECT OF pH ON THE APPARENT Km ($NaHCO_3$) AND $Vmax$ VALUES OF SPINACH LEAF RuDP CARBOXYLASE

pH	n^*	Km ($\times 10^{-2}$ M)	Vmax ($^{14}CO_2$ fixation, dpm $\times 10^{-4}$/10 min)
6.5	1.48	2.90	1.65
7.0	1.55	2.17	2.86
8.2	1.80	1.47	2.70
8.5	1.85	1.43	2.02
8.8	1.95	1.34	1.67

The shift in the optimum pH values for RuDP carboxylase as a function of Mg^{++} concentration can be seen in the results shown in Table III. Experimental data of previous workers concerning the optimum pH values of RuDP carboxylase are presented also for comparison. In the absence of Mg^{++} the enzyme activity is low and the optimum pH is near 8.5. At 10^{-3} M and 10^{-2}

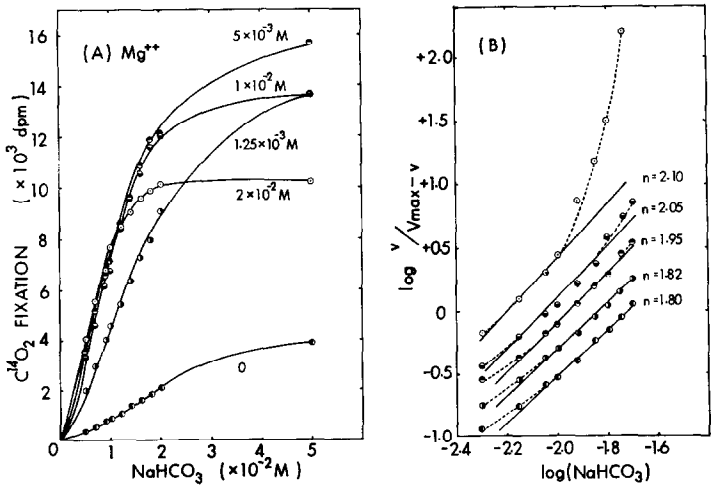

FIG. 6. Reaction kinetics of RuDP carboxylase as a function of Mg^{++} concentrations. Reaction mixture contained in (μmoles): Tris buffer (pH 8.5), 100; RuDP, 0.35; $NaH^{14}CO_3$ (2.0 μC), 2.5, 3.5, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 25.0; $MgCl_2$, 0, 0.625, 2.5, 5.0, 10.0; and 0.1 ml of enzyme (0.05 mg) in a total volume of 0.5 ml. A, Direct plot: reaction rate ($^{14}CO_2$ fixation) *vs.* $NaHCO_3$ concentrations. B, Hill plot: log v/Vmax-v *vs.* log ($NaHCO_3$).

TABLE II

EFFECT OF $MgCl_2$ ON THE APPARENT *Km* ($NaHCO_3$) AND *V*max VALUES OF SPINACH LEAF RuDP CARBOXYLASE

$MgCl_2$ (M)	n^*	Apparent *Km* ($\times 10^{-2}$ M)	*V*max ($^{14}CO_2$ fixation cpm $\times 10^3$/10 min)
0	1.80	2.00	4.2
1.25×10^{-3}	1.82	1.44	14.7
5×10^{-3}	1.95	1.20	17.0
1×10^{-2}	2.05	0.99	14.2
2×10^{-2}	2.10	0.56	10.3

TABLE III

OPTIMUM pH VALUES OF RuDP CARBOXYLASE

Mg^{++} (mM)	Optimum pH	Buffers used	Authors
9.6	About 8.0	Triethanolamine Succinate Bicarbonate Glycylglycine Glycine Tris	Weissbach *et al.* (15)
10.0	7.8[a]	Tris	Racker (16)
10.0	7.8	Tris	Pon (30), Pon *et al.* (26)
16.0	8.0[a]	Tris	Trown (10)
10.0	7.8[a]	Tris	Paulsen and Lane (17)
0	About 8.5	Succinate	Present
1.0	7.5	Histidine	
10.0	6.5	Tris Glycylglycine	

[a] In these experiments no specific emphasis was made in determining the optimum pH values of the enzyme reaction.

M Mg^{++}, the optimum pH is now shifted to 7.5, and 6.5, respectively.

Proteolytic digestion of RuDP carboxylase-effect of pretreatment with Mg^{++} and $NaHCO_3$. One of the possible indications about structural conformational change of the enzyme molecule induced by Mg^{++} binding comes from studies on Nagarse digestion of RuDP carboxylase. We have shown that pretreatment of the carboxylase enzyme with either RuDP or $NaHCO_3$ and Mg^{++} protects this protein against attack by Nagarse, urea, or SDS (28). We found in previous experiments that Mg^{++} alone exhibited an appreciable protective effect. In the present study

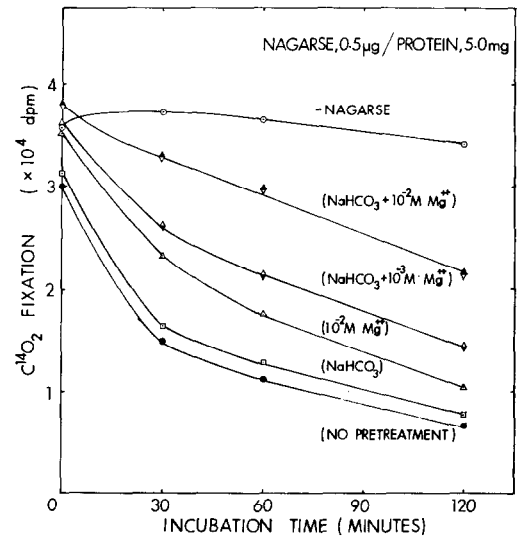

FIG. 7. Effect of RuDP carboxylase pretreatment on the Nagarse digestion. Enzyme preparation (about 0.5 mg) was incubated with each of (1) $NaHCO_3$ (4 μC), 50 μmoles, (2) $MgCl_2$ (1.0 μmole), (3) $NaH^{14}CO_3$ (50 μmoles) and $MgCl_2$ (1.0 μmole), (4) $NaH^{14}CO_3$ (50 μmoles) and $MgCl_2$ (10 μmoles) in a total volume of 1.0 ml. In the nonpretreatment system, Tris buffer (pH 7.0) was added. Then, 0.1-ml aliquots were taken out at each of 0, 30, 60, and 120 minutes, and added to the reaction mixture of the RuDP carboxylase assay containing the following compositions (in μmoles): Tris buffer (pH 7.0), 20; RuDP, 0.35; $NaH^{14}CO_3$, 25; and $MgCl_2$, 5.0 in a total volume of 0.5 ml.

the carboxylase was pretreated with Mg^{++} and/or $NaHCO_3$, and the effect on the proteolytic digestion by Nagarse was determined. The results presented in Fig. 7 demonstrate that pretreatment with both Mg^{++} (10^{-2} M) and $NaHCO_3$ prevents proteolytic digestion to a greater extent than either Mg^{++} or $NaHCO_3$ alone.

Effect of urea and PCMB treatment on the reaction kinetics. Pretreatment of the enzyme with urea (2.0 M) and PCMB (1.6×10^{-4} M) results in a partial normalization of the reaction kinetics (Fig. 8). From the Hill plot analysis of the data (insert), a decline in n^* numbers is observed: 1.30 and 1.40 for urea and PCMB-pretreated enzyme, respectively. This phenomenon is basically similar to the one observed for desensitized allosteric enzymes (18, 31).

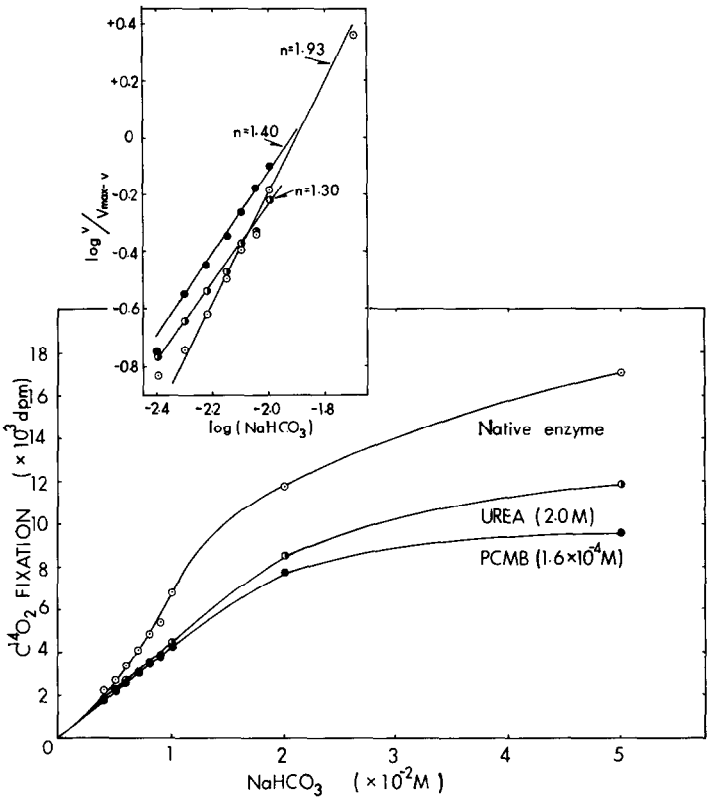

FIG. 8. Effect of PCMB- and urea-pretreatment on reaction kinetics of RuDP carboxylase. Stock enzyme solution (4.7 mg/ml) was treated as follows. (A) Native enzyme: 0.5 ml enzyme solution was diluted to 2.35 ml with 0.05 M Tris buffer (pH 7.8). (B) Urea-treatment: stock enzyme preparation was first diluted to 2.0 mg/ml, followed by the incubation with an equal volume of 4.0 M urea dissolved in 0.05 M Tris buffer (pH 7.8) at 25°C for 15 min. (C) PCMB-treatment: 0.45 ml enzyme solution was incubated with PCMB (1.6×10^{-4} M) at 25°C for 30 min, followed by the dilution with 0.05 M Tris buffer (pH 7.8) to the final enzyme concentration at 1.0 mg/ml. A 0.1-ml aliquot of the variously treated enzyme protein was added to the following reaction mixture of the RuDP carboxylase assey (in μmoles): Tris buffer (pH 8.5), 100; RuDP, 0.35; $MgCl_2$, 0.625; $NaH^{14}CO_3$ (2.0 μC), 2.25, 3, 3.5, 4, 4.5, 5, 10, 25, in a total volume of 0.5 ml.

DISCUSSION

Recent studies have demonstrated the regulatory characteristics of certain enzymes in a variety of metabolic sequences. These enzymes were found to be allosteric proteins,[4] containing stereospecific binding sites for substrates as well as for effector or inhibitor metabolites which regulate the activity of the enzyme (18–22, 31, 32). Cooperative interaction between substrate molecules, as indicated by deviations from Michaelis-Menten kinetics, can be tested for by applying the empirical Hill equation to the data (18–22). From the Hill plot, the interaction coefficient, referred to as n^* number, may be obtained.

With the above considerations in mind, the results presented here suggest strongly that RuDP carboxylase is a regulatory enzyme, in which $NaHCO_3$ functions as a homotropic effector substance. The activation of the carboxylase reaction by CO_2 at its low concentrations is considered to be the most

[4] In the literature many workers have used the term "allosteric" without referring to the original contention of Monod and his associates (see 21). Thus we have used the word regulatory enzyme throughout the paper.

notable finding (cf. Fig. 3). The results of the kinetic analyses further provide evidence that Mg^{++} acts as a metabolic activator, supporting the original finding of Pon *et al.* (26). We have shown that raising the Mg^{++} concentration from 0 to 2×10^{-2} M resulted in a 4-fold decrease in the apparent *Km* for $NaHCO_3$. However, the n^* numbers remained essentially unchanged with increasing Mg^{++} concentrations, in sharp contrast to the phenomenon typically observed for the metal activation of regulatory enzymes. It has been reported that either positive or negative effector substances influence the kinetic order of the enzyme reaction, the addition of effector molecules resulting in a reduction of n^* numbers (21); *e.g.*, Mg^{++}-activation of chloroplastic FDPase (33), Li^+- and K^+-activation of muscle and calf brain adenylate deaminase (34, 35), and K^+-activation of pyruvic kinase (36). The relative constancy in the n^* numbers that we obtained appears kinetically analogous to the case in yeast phosphofructokinase and yeast NAD-specific isocitrate dehydrogenase as reported by Atkinson and his associates (21, 37–39).

Several hypothetical schemes, based on model structures, have been proposed for the mechanism of action of regulatory enzymes at the molecular level (18–22, 31, 40). Although our knowledge of the subunit conformation of RuDP carboxylase is too scanty (11–14, 24), it is tempting to speculate on the significance of such a conformation. It will be recalled that Anderson *et al.* (41) reported that RuDP carboxylase isolated from the photosynthetic bacteria, *Rhodospirillum rubrum*, has a much smaller molecular weight than the enzyme from higher plants. Interestingly, Mg^{++} was not required for the activity of the bacterial enzyme, whereas other kinetic constants were found to be quite similar for the plant and bacterial carboxylases.

There are obvious limitations in relating the information gained from *in vitro* experiments to the regulatory mechanisms that actually operate *in vivo*. However, some inferences might be made concerning the physiological regulation of photosynthetic CO_2-fixation in chloroplasts, based on the kinetic behaviors of RuDP carboxylase. It has often been implied that the relatively low activity of RuDP carboxylase at saturating concentrations of $NaHCO_3$ may be compensated for by the high concentrations of fraction I protein in the chloroplasts of green leaf tissues. However, the regulatory nature of the enzyme, its strong cooperativity with respect to $NaHCO_3$ as well as its interaction with respect to activator molecules Mg^{++}, may constitute a more effective and crucial mechanism of control of photosynthetic CO_2-fixation. It has been shown in this investigation that besides decreasing the apparent *Km* values for $NaHCO_3$, higher levels of Mg^{++} caused the optimum pH of enzymic reaction to shift to the neutral range, despite the fact that it is known that pH optimum can vary quite widely with the buffer employed (cf. Table III). The new pH optimum (6.5) established by 10^{-2} M Mg^{++} contradicts the work done by Pon (30), in which the optimum pH at 10^{-2} M Mg^{++} was found to be 7.8. Nevertheless, it is interesting to note that Preiss *et al.* (33) reported that another enzyme of the photosynthetic carbon cycle, alkaline FDPase of spinach chloroplasts was activated by Mg^{++}, and that the pH optimum of the enzyme reaction was found to shift from 8.5 to 7.5 by raising the Mg^{++} concentration. It is difficult to evaluate the discrepancy in the pH optimum shifts between the two enzymes. However, in view of the fact that these enzymes are possibly regulated *in vivo*, being metabolically active in the light and inactive in the dark, the future work is certainly needed to disclose the nature of the pH optimum shifts. In this connection it might be of value to speculate that the activation of the RuDP carboxylase reaction by Mg^{++} is probably linked to the light-induced pH decrease in the internal chloroplasts accompanied by the conformational change of chloroplast structure (42, 43).

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REFERENCES

1. SUGIYAMA, T., NAKAYAMA, N., AND AKAZAWA, T. *Biochem. Biophys. Res. Commun.* (1968). In press.
2. WARBURG, O., *Biochem. Z.* **100,** 230 (1919).
3. HILL, R., AND WHITTINGHAM, C. P., "Photosynthesis," Menthuen Monographs on Biochemical Subjects, pp. 41–73 (1953).
4. CALVIN, M., *Science* **135,** 879 (1962).
5. BASSHAM, J. A., Advan. Enzymol. **25,** 39 (1963).
6. BASSHAM, J. A., "Plant Biochemistry," p. 875 (1965).
7. SMILLIE, R. M., AND FULLER, R. C., *Plant Physiol.* **34,** 551 (1959).
8. SANTARIUS, K. A., AND HEBER, U., *Biochim. Biophys. Acta* **102,** 39 (1965).
9. MENDIOLA, L., AND AKAZAWA, T., *Biochemistry* **3,** 174 (1964).
10. TROWN, P. W., *Biochemistry* **4,** 908 (1965).
11. RIDLEY, S. M., THORNBER, J. P., AND BAILEY, J. L., *Biochim. Biophys. Acta* **140,** 62 (1967).
12. HASELKORN, R., FERNANDEZ-MORAN, H., KIERAS, F. J., VAN BRUGGEN, E. F. J., *Science* **150,** 1598 (1965).
13. SUGIYAMA, T., AND AKAZAWA, T., *J. Biochem. (Tokyo)* **62,** 474 (1967).
14. RUTNER, A. C., AND LANE, M. D., *Biochem. Biophys. Res. Commun.* **28,** 531 (1967).
15. WEISSBACH, A., HORECKER, B. L., AND HURWITZ, J., *J. Biol. Chem.* **28,** 795 (1956).
16. RACKER, E., *Arch. Biochem. Biophys.* **69,** 300 (1957).
17. PAULSEN, J. M., AND LANE, D. M., *Biochemistry* **5,** 2350 (1966).
18. CHANGEUX, J. P., *Brookhaven Symp. Biol.*, **17,** 232 (1964).
19. MONOD, J., WYMAN, J., AND CHANGEUX, J. P., *J. Mol. Biol.* **12,** 88 (1965).
20. MONOD, J., *Science* **154,** 475 (1966).
21. ATKINSON, D. E., *Ann. Rev. Biochem.* **35,** 85 (1966).
22. MAHLER, H. A., AND CORDES, E. H., "Biological Chemistry," pp. 316–321 (1967).
23. AKAZAWA, T., SUGIYAMA, T., AND NAKAYAMA, N., *Proc. Inter. Congr. Biochem. 7th Congress (Tokyo)*, **V1.** 2.
24. SUGIYAMA, T., NAKAYAMA, N., OGAWA, M., AKAZAWA, T., AND ODA, T., *Arch. Biochem. Biophys.* **125,** 98 (1968).
25. SUGIYAMA, T., AKAZAWA, T,. AND NAKAYAMA, N., *Arch. Biochem. Biophys.* **121,** 522 (1967).
26. PON, N. G., RABIN, B. R., AND CALVIN, M., *Biochem. Z.*, **338,** 7 (1963).
27. SUGIYAMA, T., AKAZAWA, T., NAKAYAMA, N., AND TANAKA, Y., *Arch. Biochem. Biophys.* **125,** 107 (1968).
28. SUGIYAMA, T., NAKAYAMA, N., TANAKA, Y., AND AKAZAWA, T., *Arch. Biochem. Biophys.* in press (1968).
29. WYMAN, J., *Cold Spring Harbor Symp. Quant. Biol.* **28,** 483 (1963).
30. PON, N. G., Ph.D., Dissertation, University of California (1960).
31. GERHART, J. C., *Brookhaven Symp. Biol.* **17,** 222 (1964).
32. STADTMAN, E. R., *Adv. Enzymol.* **28,** 41 (1966).
33. PREISS, J., BIGGS, M. L., AND GREENBERG, E., *J. Biol. Chem.* **242,** 2292 (1967).
34. SETLOW, B., AND LOWENSTEIN, J. M., *J. Biol. Chem.* **242,** 607 (1967).
35. SMILEY, K. D. JR., AND SUELTER, C. H., *J. Biol. Chem.*, **242,** 1980 (1967).
36. HUNSLEY, J. R., AND SUELTER, C. H., Cited by Preiss *et al.* (33).
37. ATKINSON, D. E., HATHAWAY, J. A., AND SMITH, E. C., *Biochem. Biophys. Res. Commun.* **18,** 1 (1965).
38. HATHAWAY, J. A., AND ATKINSON, D. E., *J. Biol. Chem.* **238,** 2875 (1963).
39. ATKINSON, D. E., HATHAWAY, J. A., AND SMITH, E. C., *J. Biol. Chem.* **240,** 2682 (1965).
40. KOSHLAND, D. E., Nemethy, G., AND FILMER, D., *Biochemistry* **5,** 365 (1965).
41. ANDERSON, L., personal communication (1968).
42. JAGENDORF, A. T., AND URIBE, E., *Brookhaven Symp. Biol.* **19,** 215 (1966).
43. PACKER, L., DEAMER, D. W., AND CROFTS, A. R., *Brookhaven Symp. Biol.* **19,** 281 (1966).