A Kinetic Study of Ribulose Bisphosphate Carboxylase from the Photosynthetic Bacterium *Rhodospirillum rubrum*

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The activation kinetics of purified *Rhodospirillum rubrum* ribulose biphosphate carboxylase were analysed. The equilibrium constant for activation by CO_2 was $600\ \mu\text{M}$ and that for activation by Mg^{2+} was $90\ \mu\text{M}$, and the second-order activation constant for the reaction of CO_2 with inactive enzyme (k_{+1}) was $0.25 \times 10^{-3}\ \text{min}^{-1} \cdot \mu\text{M}^{-1}$. The latter value was considerably lower than the k_{+1} for higher-plant enzyme (7×10^{-3} – $10 \times 10^{-3}\ \text{min}^{-1} \cdot \mu\text{M}^{-1}$). 6-Phosphogluconate had little effect on the active enzyme, and increased the extent of activation of inactive enzyme. Ribulose biphosphate also increased the extent of activation and did not inhibit the rate of activation. This effect might have been mediated through a reaction product, 2-phosphoglycollic acid, which also stimulated the extent of activation of the enzyme. The active enzyme had a K_m (CO_2) of $300\ \mu\text{M}$ - CO_2 , a K_m (ribulose biphosphate) of 11 – $18\ \mu\text{M}$ -ribulose biphosphate and a $V_{\max}$ of up to $3\ \mu\text{mol/min}$ per mg of protein. These data are discussed in relation to the proposed model for activation and catalysis of ribulose biphosphate carboxylase.

Ribulose biphosphate carboxylase (EC 4.1.1.39) from the photosynthetic bacterium *Rhodospirillum rubrum* differs from ribulose biphosphate carboxylase from higher plants (McFadden, 1973). The *Rhodospirillum rubrum* enzyme has a mol.wt. of 120000 and consists of a dimer of identical subunits. In contrast, the higher-plant enzyme has a mol.wt. of 560000 and consists of eight large and eight small subunits (56000 and 13000 mol.wt.) (Kawashima & Wildman, 1970).

Stimulation of the activity of higher-plant ribulose biphosphate carboxylase by 6-phosphogluconate was noted by Chu & Bassham (1973, 1974, 1975), whereas 6-phosphogluconate has been reported to have no effect on the *Rhodospirillum rubrum* enzyme (Tabita & McFadden, 1972).

The kinetics of activation of the higher-plant ribulose biphosphate carboxylase by CO_2 and Mg^{2+} are well established (Laing *et al.*, 1975; Laing & Christeller, 1976; Lorimer *et al.*, 1976). In the present paper we studied the activation of the inactive bacterial enzyme and the catalysis by the pre-activated bacterial enzyme to allow a comparison with similar experiments performed with the higher-plant enzyme.

Experimental

Rhodospirillum rubrum (N.C.I.B. 8255), obtained from the Torrey Research Station, Aberdeen, Scotland, U.K., was grown in batch culture (Tabita & McFadden, 1974a), harvested in late exponential

phase by continuous-flow centrifugation and stored at -20°C until several batches had been accumulated. The cells were thawed, resuspended in a minimal volume of $50\ \text{mM}$ -Tris/HCl (pH 7.8)/ $25\ \text{mM}$ - NaHCO_3 / $10\ \text{mM}$ - MgCl_2 / $5\ \text{mM}$ -mercaptoethanol/ $1\ \text{mM}$ -EDTA (extraction buffer) and sonicated with an MSE 100 W sonicator, for ten 1 min periods at maximum power, jacketed by an ice bath at 0°C . The broken cells were centrifuged at $30000g$ for 10 min and the supernatant was treated at 50°C (Tabita & McFadden, 1974a). Heat-coagulated protein was removed by centrifugation ($30000g$, 10 min) and solid $(\text{NH}_4)_2\text{SO}_4$ added to bring the solution to 60% saturation. Precipitated protein was collected by centrifugation ($30000g$, 10 min), dissolved in the extraction buffer, dialysed overnight against the same buffer, and applied to a $4\ \text{cm} \times 30\ \text{cm}$ DEAE-cellulose column equilibrated with extraction buffer. The enzyme was eluted in 1.5 litres of a 0 – $0.3\ \text{M}$ -NaCl linear gradient, and the eluate treated with $(\text{NH}_4)_2\text{SO}_4$ to 60% saturation and centrifuged ($30000g$, 10 min). The pellet was dissolved in a minimal volume of extraction buffer and applied to a $4\ \text{cm} \times 50\ \text{cm}$ Sephadex G-150 column also equilibrated with extraction buffer. Ribulose biphosphate carboxylase was eluted after the void column indicating the purified enzyme has a mol.wt. of less than 150000, in agreement with the results of Tabita & McFadden (1974b). The purified enzyme was stored as a precipitate in 60%-saturated $(\text{NH}_4)_2\text{SO}_4$ at 4°C .

Ribulose biphosphate was synthesized as previously described (Christeller *et al.*, 1976). $\text{Na}_2^{14}\text{CO}_3$

was from The Radiochemical Centre, Amersham, Bucks., U.K., and other biological compounds were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Ribulose biphosphate carboxylase was prepared for assay as described by Lorimer *et al.* (1976). Stored precipitated enzyme was centrifuged (30000g, 10min), resuspended in 50mM-Tris/HCl, pH8.2 (CO₂-free), and deactivated by Sephadex G-25 chromatography on a CO₂-free column to remove CO₂, Mg²⁺ and (NH₄)₂SO₄. The enzyme was stored under N₂ at 0°C and sampled under N₂. Protein was measured by absorbance at 280nm (Tabita & McFadden, 1974b).

Assay

Activation of inactive enzyme was measured by preincubating inactive enzyme in 50mM-Tris/HCl, pH8.2, with variable MgCl₂, NaHCO₃ and effector concentrations. At intervals samples (5–25μl) were injected into scintillation vials containing an assay mixture of 50mM-Tris/HCl, pH8.2, 0.7mM-NaH¹⁴CO₃ (5Ci/mol), 20mM-MgCl₂ and 0.5–0.92mM-ribulose biphosphate (see the Results section) and the reaction was stopped after 30s by adding 0.1ml of 2M-HCl. Another method used to measure activation was to add inactive enzyme to a complete assay mixture [50mM-Tris/HCl (pH8.2)/20mM-MgCl₂ containing variable ribulose biphosphate, NaH¹⁴CO₃ and effector concentrations] and inject 100μl samples at intervals into a scintillation vial containing 0.5ml of 1M-HCl in ethanol. A third method used was to inject enzyme and complete assay mixture simultaneously into a ratchet-controlled 5ml syringe (Laing & Christeller, 1976) and subsequently eject samples into 1M-HCl in ethanol at regular intervals. The vials were oven-dried, counted for radioactivity and corrected for quenching as described by Laing & Christeller (1976).

The specific activity of ribulose biphosphate carboxylase activated by preincubation in 20mM-NaHCO₃ and 10mM-MgCl₂ in Tris/HCl, pH8.2, and assayed at the same CO₂ and MgCl₂ concentration with 0.5mM-ribulose biphosphate was 0.4–0.6μmol/min per mg of protein. Most of the assays reported in this paper used a subsaturating CO₂ concentration during activation and catalysis to allow analysis of the activation process, so rates reported in the Tables and Figures are often below maximum specific activity. This is in accordance with the procedure described by Laing & Christeller (1976) and Lorimer *et al.* (1976).

In all experiments with effectors, the pH of the effector solution was in the range 6.5–7. Insignificant amounts of dissolved CO₂ would have been added along with the effector.

Electrophoresis

Electrophoresis in the presence of sodium dodecyl

sulphate was carried out with 12% gels as described by Weber *et al.* (1972).

Kinetic measurements on whole organisms

Cells of *R. rubrum* were harvested by centrifugation (5000g, 10min) and resuspended in CO₂-free growth media (Tabita & McFadden, 1974a). Light-dependent CO₂ fixation was measured under saturating light by adding variable quantities of NaH¹⁴CO₃ to shaking flasks containing 3ml of the bacterium at 25°C. Samples (0.5ml) were transferred at regular intervals to 100μl of 2M-HCl and their radioactivity was determined. Assays were linear with time for about 10min.

Equilibrium constants for the H₂O+CO₂ ⇌ HCO₃⁻+H⁺ equilibrium, obtained from Hodgman *et al.* (1958) (see Laing, 1974), were (temperatures in parentheses): K (9°C) = 3.40×10^{-7} ; K (20°C) = 4.45×10^{-7} ; K (30°C) = 4.71×10^{-7} .

Non-linear parameter estimation (Bard, 1974) was used to fit the pseudo-first-order exponential equation (Newton-Raphson method). Asymptotic standard errors of the estimated parameters were calculated as described by Bard (1974). Unweighted-linear-regression analyses using the Eadie-Hofstee transformation were used to determine equilibrium and Michaelis-Menten affinity constants.

Results

Gel electrophoresis in the presence of sodium dodecyl sulphate confirmed the previous report (Tabita & McFadden, 1974a) that purified *R. rubrum* ribulose biphosphate carboxylase consisted of only large subunits.

Lorimer *et al.* (1976) (Scheme 1) postulated a model of activation for spinach ribulose biphosphate carboxylase whereby the enzyme is activated in two second-order reactions. If CO₂ is represented by C, Mg²⁺ by M and enzyme by E, they suggested that EC and ECM equilibrated rapidly and that the activation of ribulose biphosphate carboxylase could be described as a pseudo-first-order reaction when $[C] \gg [E]$. They, and Laing & Christeller (1976), showed that $k_{obs.}$, the pseudo-first-order rate constant, was a function of $[C]$ and $[M]$:

$$k_{obs.} = k_{+1} \left([C] + \frac{K_C K_{Mg}}{[M]} \right) \quad (1)$$

Some activation curves for *R. rubrum* ribulose biphosphate carboxylase are shown in Fig. 1. Although the curves followed first-order kinetics,

Scheme 1.

Fig. 1. Influence of HCO_3^- concentration on the activation of *R. rubrum* ribulose biphosphate carboxylase

Inactive enzyme (2.59 mg) was added to 50 mM-Tris/HCl (pH 8.2)/20 mM- MgCl_2 containing variable NaHCO_3 at 18°C , the samples (30 μl) were injected, at the times indicated, into 1.0 ml of 50 mM-Tris/HCl (pH 8.2) / 20 mM- MgCl_2 / 1 mM- $\text{NaH}^{14}\text{CO}_3$ / 0.92 mM-ribulose biphosphate at 18°C and the reaction was stopped after 30 s. $\bullet$, 1 mM- NaHCO_3 ; $\blacklozenge$, 2 mM- NaHCO_3 ; $\triangle$, 4 mM- NaHCO_3 .

Fig. 2. Plot of $k_{\text{obs.}}$ as a function of CO_2 concentration and the reciprocal of Mg^{2+} concentration

Inactive enzyme (2.3 mg) was added to 50 mM-Tris/HCl, pH 8.2, containing variable MgCl_2 and NaHCO_3 at 9°C . At regular intervals samples (20 μl) were injected into 1 ml of 50 mM-Tris/HCl (pH 8.2)/21 mM- MgCl_2 / 0.74 mM-ribulose biphosphate / 0.73 mM- $\text{NaH}^{14}\text{CO}_3$ at 9°C and the reaction was terminated after 30 s. Exponential curves were fitted to the time courses and calculated $k_{\text{obs.}}$ ($\pm$ asymptotic S.E. as bars) was plotted (Bard, 1974). $\circ$, $k_{\text{obs.}}$ as a function of CO_2 ; $\bullet$, $k_{\text{obs.}}$ as a function of the reciprocal of the Mg^{2+} concentration.

because full activation did not occur during preincubation before assay, it was necessary to use a non-linear-parameter-estimation method (Bard, 1974) to evaluate $k_{\text{obs.}}$ and n_e , the asymptotic or equilibrium value of the enzyme's activity. This method of parameter estimation is likely to give less biased estimates of $k_{\text{obs.}}$, even if n_e is known, than the logarithmic transform linearization method.

From $k_{\text{obs.}}$ values typical of the lower values reported in these results, 99% activation of the initially inactive enzyme would have taken over 100 min. This made it difficult to do complete time courses. However, statistical analysis showed that the exponential fit to the time courses was highly significant. Plots of the calculated values of $k_{\text{obs.}}$ as a function of the CO_2 concentration and the reciprocal of the Mg^{2+} concentration (Fig. 2) were both linear. The observed kinetics of *R. rubrum* enzyme were therefore consistent with the activation model proposed by Lorimer *et al.* (1976).

Calculated values of k_{+1} , K_C and K_{M_g} from eqn. (1) and the data of Fig. 2 are compared in Table 1 with values obtained with the enzyme from soya bean and from spinach. The discrepancies in calculated k_{+1} and $K_C K_{M_g}$ by using different methods were discussed by Laing & Christeller (1976). The values calculated at constant and high MgCl_2 concentrations are considered the most significant.

The rate of activation of inactivated ribulose biphosphate carboxylase from spinach and from soya bean is inhibited by ribulose biphosphate (Chu & Bassham, 1975; Laing & Christeller, 1976), the activation rate being inversely related to the ribulose biphosphate concentration. In contrast, inactive ribulose biphosphate carboxylase from *R. rubrum* showed a different response (Fig. 3). When inactive ribulose biphosphate carboxylase was added to a complete reaction mixture the reaction rate during the following incubation was directly related in a hyperbolic manner to ribulose biphosphate (results not shown). There was little inhibition when 6-phosphogluconate was present during the assay, in agreement with Tabita & McFadden (1972).

When inactive *R. rubrum* ribulose biphosphate carboxylase was preincubated with 6-phosphogluconate in the presence of CO_2 and Mg^{2+} , but before adding ribulose biphosphate, 6-phosphogluconate increased the extent of activation (Fig. 4). The degree of stimulation decreased as the CO_2 concentration was increased. This result is similar to that observed with higher-plant enzyme by Chu & Bassham (1975) except that high concentrations of 6-phosphogluconate did not decrease the extent of activation. The response of extent of activation to CO_2 is a measure of the product $K_C K_{M_g}$ (Laing & Christeller, 1976), showing that 6-phosphogluconate

Table 1. Some kinetic and equilibrium constants for the activation process of ribulose biphosphate carboxylase from several sources

Data were calculated by the methods indicated below from experiments described in the text and in the papers referred to.

Constant	<i>R. rubrum</i> (see Figs. 1 and 2)	Temperature (°C)	Soya bean (Laing & Christeller, 1976; mean values)	Temperature (°C)	Spinach (Lorimer <i>et al.</i> , 1976; mean values)	Temperature (°C)
k_{+1} ($\text{min}^{-1} \cdot \mu\text{M}^{-1}$)	0.25×10^{-3} * $0.93 \times 10^{-3}\dagger$	9 9	7.11×10^{-3}	18	10.3×10^{-3}	10
$K_C K_{Mg}$ (μM^2)	$4.6 \times 10^5 \ddagger$ $1.3 \times 10^5 \S$ $0.54 \times 10^5 $	9 9 19	1.43×10^5	18	1.635×10^5	10
K_C (μM)	607¶	19	91	18	309	10
K_{Mg} (μM)	89**	19	1130	18	529	10

* Calculated from slope of line k_{obs} , as a function of CO_2 concentration (see Fig. 2).

† Calculated from intercept of line k_{obs} , as a function of the reciprocal of Mg^{2+} concentration (see Fig. 2).

‡ Calculated from intercept of line k_{obs} , as a function of CO_2 concentration (see Fig. 2).

§ Calculated from slope of line k_{obs} , as a function of the reciprocal of Mg^{2+} concentration (see Fig. 2).

|| Enzyme (18 μg) was added to preincubation mixture (50 mM-Tris/HCl, pH 8.3, variable MgCl_2 and NaHCO_3 concentrations) at 19°C, and after 115 min 10 μl was added to the assay mixture (50 mM-Tris/HCl, pH 8.3, 20 mM- MgCl_2 , 0.6 mM-ribulose biphosphate, 0.74 mM- $\text{NaH}^{14}\text{CO}_3$) at 19°C and assays were terminated after 30 s. $K_C K_{Mg}$ was calculated as described in Laing & Christeller (1976).

¶ K_C was calculated as described for $K_C K_{Mg}$.

** K_{Mg} was calculated from $K_{Mg} = K_C K_{Mg}/K_C$.

Fig. 3. Time course of CO_2 fixed as a function of ribulose biphosphate concentration and 6-phosphogluconate concentration

Inactive enzyme (303 μg) was added to 1 ml of 50 mM-Tris/HCl (pH 8.2)/20 mM- MgCl_2 /0.96 mM- $\text{NaH}^{14}\text{CO}_3$ containing variable ribulose biphosphate and 6-phosphogluconate at 20°C. At minute intervals samples (100 μl) were injected into acid to stop the reaction. Filled symbols represent assays with 0.2 mM-6-phosphogluconate added, and the open symbols assays containing only the indicated ribulose biphosphate concentration: $\circ$, $\bullet$, 0.01 mM; Δ , $\blacktriangle$, 0.05 mM; $\diamond$, $\blacklozenge$, 0.25 mM; $\square$, $\blacksquare$, 0.5 mM.

decreased this product. Ribulose biphosphate also increased the extent of activation of *R. rubrum* ribulose biphosphate carboxylase (Table 2).

Fig. 4. Effect of 6-phosphogluconate and CO_2 concentration on the extent of activation of *R. rubrum* ribulose biphosphate carboxylase

Inactive enzyme (285 μg) was incubated in 50 mM-Tris/HCl (pH 8.27)/20 mM- MgCl_2 containing variable CO_2 and 6-phosphogluconate for 96 min at 30°C. Five samples (25 μl) were then taken over the next 35 min and injected into 1 ml of 50 mM-Tris/HCl (pH 8.27)/20 mM- MgCl_2 /0.53 mM- $\text{NaH}^{14}\text{CO}_3$ /0.654 mM-ribulose biphosphate at 13°C and the reaction was terminated after 30 s. Bars represent ± 1 S.E.M. ($n = 5$). The numbers beside the curve are 6-phosphogluconate concentrations.

In these assays with effectors precautions were taken. After equilibrium had been reached the activity was constant for at least 20min, indicating the enzyme was not deactivating or denaturing. No effect of 6-phosphogluconate during the 30s assay was detected.

During preincubation with CO₂ and Mg²⁺ containing ribulose biphosphate, the latter would be consumed by catalytic reaction. The increase in extent of activation during preincubation may have been caused by the products of the ribulose biphosphate carboxylase/oxygenase reaction, phosphoglyceric acid and phosphoglycollic acid (Bowes

et al., 1971), or by ribulose biphosphate itself if the binding coefficient of ribulose biphosphate by ribulose biphosphate carboxylase at the effector site is very small. Both explanations are compatible with the data. Phosphoglycollic acid increased the extent of activation much more than phosphoglyceric acid (Table 3).

Table 2. Effect of ribulose biphosphate on extent of activation of ribulose biphosphate carboxylase

Inactive enzyme was preincubated in 50mm-Tris/HCl (pH8.2)/20.9mm-MgCl₂/1.36mm-NaHCO₃ containing variable ribulose biphosphate at 25°C. After 41min, 100μl samples were injected into 900μl of 50mm-Tris/HCl (pH8.2)/20mm-MgCl₂/1.044mm-NaH¹⁴CO₃/0.667mm-ribulose biphosphate at 14°C. Assays were terminated after 30s. Further samples were taken at 45, 72, 77 and 81min. Values are means±s.e.

Ribulose biphosphate concentration (mm)	CO ₂ fixed (nmol/min per mg of protein)	Percentage
0	1.95±0.12	100
0.173	2.50±0.10	128
0.348	3.12±0.21	160
0.522	3.59±0.12	184
0.696	3.58±0.18	184
0.870	3.63±0.19	186

Table 3. Effect of preincubation in 2-phosphoglycollic acid and 3-phosphoglyceric acid on the extent of activation of *R. rubrum* ribulose biphosphate carboxylase

Inactive enzyme (142μg) was added to 50mm-Tris/HCl (pH8.2)/2mm-NaHCO₃/20mm-MgCl₂ containing variable amounts of 3-phosphoglyceric acid or 2-phosphoglycollic acid and incubated for 96min. Samples (20μl) were injected into 1ml of 50mm-Tris/HCl (pH8.2)/21mm-MgCl₂/0.74mm-ribulose biphosphate/0.73mm-NaH¹⁴CO₃ at 9°C and the assays terminated after 30s. Values are means±s.e. of five determinations.

Effector	Effector concentration (mm)	Activity (nmol/min per mg of protein)	Percentage
—	—	0.819±0.04	100
3-Phosphoglyceric acid	0.067	0.712±0.04	87
	0.133	0.770±0.02	94
	0.267	0.836±0.05	102
	0.667	0.885±0.04	108
	1.33	0.917±0.11	112
2-Phosphoglycollic acid	0.067	1.24±0.06	151
	0.133	1.10±0.07	134
	0.267	1.41±0.11	172
	0.667	2.33±0.10	284
	1.33	2.91±0.10	353

Table 4. Effect of 6-phosphogluconate and ribulose biphosphate on the pseudo-first-order rate constant of activation (k_{obs.}), the equilibrium activation value (n_e), and the initial rate of activation (k_{obs.} n_e)

Inactive enzyme (530μg) was added to 50mm-Tris/HCl (pH8.2)/20mm-MgCl₂/1mm-NaHCO₃ containing variable ribulose biphosphate or 6-phosphogluconate at 30°C. Samples (50μl) were taken each minute for 8min and then several samples were taken at times between 40min and 60min. They were injected into 950μl of 50mm-Tris/HCl (pH8.2)/20mm-MgCl₂/0.703mm-NaH¹⁴CO₃/0.667mm-ribulose biphosphate and the assays terminated after 30s. The time courses were fitted to curves of the form $n = n_e [1 - \exp(-k_{obs.} t)]$, where n is nmol of CO₂ fixed and t is time. Asymptotic standard errors were calculated (Bard, 1974). Values are means±s.e. k_{obs.} n_e is the initial rate of activation.

Effector	Concentration (mm)	k _{obs.} (min ⁻¹)	n _e (nmol of CO ₂ fixed/min per mg of protein)	k _{obs.} n _e (nmol of CO ₂ fixed/min per mg of protein)
Ribulose biphosphate	0	0.292±0.0021	1.68±0.189	0.491
	0.1	0.172±0.0017	3.33±0.34	0.573
	0.2	0.160±0.0013	4.20±0.332	0.672
	0.3	0.119±0.0013	5.00±0.377	0.595
	0.4	0.121±0.0013	5.17±0.368	0.626
	1.0	0.129±0.0015	4.17±0.350	0.538
6-Phosphogluconate	0	0.292±0.0021	1.68±0.189	0.491
	0.25	0.112±0.0025	4.16±0.521	0.466
	0.5	0.107±0.0015	4.96±0.377	0.531
	1.0	0.066±0.038	11.61±4.22	0.766
	5.0	0.120±0.010	12.50±1.25	1.50

Table 5. Affinity constants *in vitro* (a) for the substrates CO₂ and ribulose biphosphate for ribulose biphosphate carboxylase and affinity constants *in vivo* (b) for CO₂ in light-dependent CO₂ fixation by intact *R. rubrum*

Inactive enzyme was added to 50mM-Tris/HCl, pH 8.2, NaHCO₃ and MgCl₂ as indicated and preincubated for at least 60min. Samples were added to 50mM-Tris/HCl, pH 8.2, and variable MgCl₂, NaH¹⁴CO₃ and ribulose biphosphate. Assays were terminated after 30s. K_m and V_{max} were determined by linear regression by using the Eadie-Hofstee transformation. Kinetic experiments *in vivo* on intact *R. rubrum* were performed at 30°C as described in the Experimental section.

(a)

Substrate	Temperature (°C)	K_m (μM)	V_{max} (μmol/min per mg of protein)
Ribulose bisphosphate	25*	18	—
	30†	11	0.967
CO ₂	30‡	310	2.53
	30§	291	2.99

(b)

pH	K_m (mM)		V_{max} (μmol/min per mg of protein)
	HCO ₃ ⁻	CO ₂	
6.24	3.4	1.87	72
6.9	2.9	0.61	—
7.11	1.5	0.21	287
8.24	5.0	0.060	301

* Preincubated in 9.5mM-MgCl₂/19mM-NaHCO₃, assayed in 9.3mM-MgCl₂/18mM-NaH¹⁴CO₃.

† Preincubated in 20mM-MgCl₂/24mM-NaHCO₃, assayed in 25mM-MgCl₂/25mM-NaH¹⁴CO₃.

‡ Preincubated in 16mM-MgCl₂/20mM-NaHCO₃, assayed in 20mM-MgCl₂/0.5mM-ribulose biphosphate.

§ Preincubated in 20mM-MgCl₂/25mM-NaHCO₃, assayed in 20mM-MgCl₂/0.5mM-ribulose biphosphate.

The activation of ribulose biphosphate carboxylase in the presence of 6-phosphogluconate or ribulose biphosphate still appeared to follow pseudo-first-order kinetics. Consequently, k_{obs} and n_e can be calculated as a function of ribulose biphosphate and 6-phosphogluconate concentrations (Table 4). The product $k_{obs} \cdot n_e$, the initial rate of activation, increased with 6-phosphogluconate concentration, but remained constant with ribulose biphosphate concentration.

The values of the K_m (CO₂) and K_m (ribulose biphosphate) were determined for active enzyme (Table 5). The enzyme was preincubated in CO₂ and Mg²⁺ until activated and assayed as a function of CO₂ and ribulose biphosphate concentration for 30s. Time courses over about 40s with the ratchet-controlled syringe showed the assay was linear over the entire range of CO₂ concentrations used (results not shown). Deactivation did not occur at the low CO₂

concentrations. The CO₂ affinity of light-dependent CO₂ fixation by intact *R. rubrum*, measured at several pH values, is shown in Table 5.

Discussion

Ribulose biphosphate carboxylase from *R. rubrum* appears to follow the same activation scheme as the enzyme from higher plants (Table 1; Figs. 1 and 2) (Laing & Christeller, 1976; Lorimer *et al.*, 1976).

The bacterial enzyme is activated more slowly than is the enzyme from higher plants (Table 1). The equilibrium constants for activation (K_C and K_{Mg}) appear to be different from the values for higher plants. K_C is somewhat higher, and K_{Mg} is about 10% of the higher-plant value (Table 1). The K_{Mg} value is similar to the apparent K_m (Mg²⁺) of 0.21 mM measured by Tabita & McFadden (1974b). The low value of k_{obs} for *R. rubrum* compared with higher-plant enzyme is explained by the low values of k_{+1} and the product $K_C K_{Mg}$ we observed for the bacterial enzyme. The low activation rate may be a considerable disadvantage *in vivo*, especially since effectors that increase n_e do not increase k_{obs} (Table 4), and therefore do not affect the time necessary for the increased activation.

Another major difference between *R. rubrum* and higher-plant enzymes is that ribulose biphosphate has little effect on the rate of activation of *R. rubrum* enzyme (Table 4; Fig. 3) though strongly increasing the extent of activation (Tables 2 and 4). On the other hand, ribulose biphosphate strongly inhibits the initial rate of activation of higher-plant enzyme (Chu & Bassham, 1975; Laing & Christeller, 1976).

The potent stimulation by ribulose biphosphate (Tables 2 and 4) may be significant *in vivo*. The ribulose biphosphate concentration in *Chlorella pyrenoidosa* is about 2mM (Bassham & Krause, 1969), sufficient to increase significantly the extent of activation. The ribulose biphosphate concentration *in vivo* in *R. rubrum* is unknown.

Laing & Christeller (1976) postulated that both the inactive and active forms of the higher-plant enzyme bound ribulose biphosphate, the inactive enzyme-ribulose biphosphate complex being unable to be activated. They also suggested various effectors such as 6-phosphogluconate and NADPH bound with high affinity to an effector site on the higher-plant enzyme promoting activation. However, it was suggested that 6-phosphogluconate competed with ribulose biphosphate for the ribulose biphosphate catalytic site on both inactive and active plant enzyme and decreased the rate of activation of inactive enzyme and catalysis by active enzyme. As 6-phosphogluconate and ribulose biphosphate, in contrast, increase the final activation state (n_e) of the enzyme from *R. rubrum* without inhibiting the rate of activation or catalysis (Fig. 3), there is

probably no catalytic binding site for ribulose biphosphate on the inactive enzyme. The lack of inhibition of active enzyme by 6-phosphogluconate indicates the ribulose biphosphate catalytic site on the active enzyme cannot bind 6-phosphogluconate and is therefore different from the equivalent higher-plant site. Furthermore, it appears the effector site promoting activation on the enzyme is able to bind both ribulose biphosphate and 6-phosphogluconate. In Fig. 3, 6-phosphogluconate did not promote the rate of activation over the controls. The 6-phosphogluconate concentration in Fig. 3 was only 0.2mM, a concentration that has little effect on the rate of activation though having considerable effect on the extent of activation (Table 4).

The decrease in $K_c K_m$ by 6-phosphogluconate would increase the extent of activation at physiological concentrations of CO_2 and perhaps increase photosynthesis *in vivo*.

The $K_m(\text{CO}_2)$ for *R. rubrum* (370 μM) is very high compared with the $K_m(\text{CO}_2)$ for higher-plant enzymes (20 μM) (Laing *et al.*, 1975). The *R. rubrum* enzyme was preincubated to about 95% activation and assayed under conditions analogous to those where a low K_m for higher-plant enzyme is observed, so, unless effectors lower the $K_m(\text{CO}_2)$, or the CO_2 concentration of the natural habitat of *R. rubrum* is considerably higher than CO_2 concentrations in the air, this enzyme is apparently operating in a very inefficient manner. The affinity for CO_2 by intact *R. rubrum* grown in high CO_2 concentrations was found by us to be dependent on pH, whereas the affinity for HCO_3^- was independent of pH. This suggests HCO_3^- is probably the species transported into this bacterium [cf. Berry *et al.* (1976), where CO_2 appears to be the transported species in *Chlamydomonas reinhardtii*]. However, the $K_m(\text{HCO}_3^-)$ at all pH values and the $K_m(\text{CO}_2)$ at pH 8.24 shown by the cells are much lower than for the enzyme *in vitro*. It is possible this organism concentrates HCO_3^- internally, thus enhancing ribulose biphosphate carboxylase activity.

The measured values of K_m (ribulose biphosphate) are similar to the value reported by Tabita & McFadden (1974b), which was expected as they partially activated the enzyme by preincubating it at the assay CO_2 concentration.

Since only the large subunit is present in the *R. rubrum* enzyme, these studies show that the

activation processes (like the catalytic processes) are a function of the large subunit. However, the interaction of eight large subunits in the plant enzyme does not seem important in gross regulation of activation, because the *R. rubrum* enzyme is a dimer. Although there are several major differences between the bacterial and higher-plant enzymes, these do not allow us to ascribe any definitive role to the small subunit of the latter.

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