Kinetics and Subunit Interactions of Ribulose Bisphosphate Carboxylase-Oxygenase from the Cyanobacterium, *Synechococcus* sp.*

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Ribulose 1,5-bisphosphate carboxylase-oxygenase was purified to electrophoretic homogeneity from the unicellular, marine cyanobacterium, *Synechococcus* sp. It was composed of large (57,000 dalton) and small (12,000 dalton) subunits in a 1:1 stoichiometry. It was rapidly activated by Mg2+ plus HCO3-, even in the presence of ribulose 1,5-bisphosphate which had no effect on the extent of activation. Michaelis constants for CO2, O2, and ribulose 1,5-bisphosphate were 3- to 10-fold higher than for the enzyme from higher plants. Maximum specific activities of the carboxylase and oxygenase reactions were 2.9 and 0.34 μmol·min⁻¹·mg⁻¹, respectively. After being three times precipitated at pH 5.4 and redissolved, 13% of the initial small subunit content remained, together with 41% of the original catalytic activity. Inactive small subunits remained in the supernatant after precipitation. Sucrose density gradient centrifugation and pore-gradient electrophoresis showed that the small subunit-depleted enzyme consisted of an oligomeric core of large subunits with varying numbers of small subunits attached. However, activity was not correlated with degree of saturation with small subunits. When isolated small subunits were added back to the small subunit-depleted enzyme, substantial re-formation of the native electrophoretic form occurred and catalytic activity increased 2-fold or more.

The enzyme D-ribulose 1,5-bisphosphate carboxylase-oxygenase (EC 4.1.1.39) is a key determinant of the capacity of organisms for the net assimilation of inorganic carbon, and has always been so throughout evolution. It catalyzes the initial reactions of both the photosynthetic carbon reduction cycle and its apparently wasteful appendage, the photosynthetic carbon oxidation cycle (Lorimer and Andrews, 1981). This is because the enzyme is bifunctional, catalyzing the oxygenation of Rbu-P₂, to produce 2-phosphoglycolate and 3-phosphoglycerate (Bowes et al., 1971; Andrews et al., 1973), as well as its carboxylation to produce two molecules of 3-phosphoglycerate. The presumed antiquity of cyanobacteria, their prokaryotic subcellular organization, and the possibility that they may be related to the endosymbiotic ancestor of chloroplasts (for a review, see Broda, 1977) suggest that study of cyanobacterial Rbu-P₂ carboxylase-oxygenase may provide information about evolutionary progress of the enzyme.

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1 Portions of this paper (including "Materials and Methods," part of "Results," Figs. 1-3, and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md 20014. Request Document No. 80M-2640, cite authors, and include a check for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
RESULTS

Effects of Precipitation at pH 5.4—When the purified enzyme solution was adjusted to pH 5.4, a pH which is probably near to the enzyme's isoelectric point, the enzyme slowly precipitated over a 30-min period at 0 °C (Table II). SDS-gel electrophoresis of the redissolved precipitate showed a reduction of its small subunit content relative to the native enzyme. When the precipitation was repeated twice more, the reduction became very obvious, with only 13% of the enzyme's small subunit content remaining (Fig. 4). Catalytic activity also was reduced progressively by the successive precipitations, but not to the same extent as small subunit content. Thrice-precipitated enzyme retained 41% of its original specific activity (34% if specific activity in terms of large subunit content only is considered) (Table II). Supernatant solutions, which were enriched in small subunits (Fig. 4), were inactive. Usually, the amount of small subunit protein recovered in the supernatants was less than that lost from the precipitates (Table II). Apparently some small subunits must have been lost from the system during the precipitation procedure, perhaps adsorbed on glassware from the dilute supernatant solutions.

TABLE II

Effect of precipitation at pH 5.4 on subunit structure of Synechococcus Rbu-P₃ carboxylase-oxygenase

Purified enzyme (1.6 mg·ml⁻¹ in 50 mM K phosphate buffer, pH 7.6, containing 1 mM EDTA) was precipitated by addition of a one-fifth volume of 1.43% (v/v) acetic acid at 0 °C. Turbidity was faint at first, but increased progressively over a 30-min period. After 150 min, the suspension was centrifuged at 27,000 × g for 20 min at 4 °C and the supernatant decanted as completely as possible. The firm white pellet was dissolved in a volume of the above phosphate/EDTA buffer equivalent to the original volume of native enzyme solution. The pH of the supernatant was 5.45. This procedure was repeated twice more, each time using the redissolved pellet from the previous step. All three redissolved pellets (P₁, P₂, and P₃) and supernatants (S₁, S₂, and S₃), together with the native enzyme, were assayed for protein and Rbu-P₃ carboxylase activity (standard assay under N₂) and subjected to SDS-gel electrophoresis (see "Materials and Methods"). After staining, the gels were scanned (Fig. 4) and the areas under the large and small subunit peaks measured. The ratio, S/L, of these areas was converted to the molar ratio of subunits by dividing by the ratio between the molecular weights of the two subunits as determined from Fig. 1. To indicate the reproducibility of this method of determining subunit ratios, data for duplicate determinations with different gels are reported for native enzyme and P₁. Protein concentrations of supernatants were corrected for dilution incurred by addition of acetic acid solution. Specific carboxylase activities are shown in terms of total protein (L + S) and in terms of large subunit protein (L only). The percentage of specific activities compared to the native enzyme is given in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein (mg·ml⁻¹)</th>
<th>Ratio of subunits, S/L</th>
<th>Ratio of peak areas from gel scans</th>
<th>Specific carboxylase activity (μmol·min⁻¹·mg protein⁻¹)</th>
<th>Molar ratio</th>
<th>Total protein L + S (μmol·min⁻¹·mg protein⁻¹)</th>
<th>L only (μmol·min⁻¹·mg protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native enzyme</td>
<td>1.62</td>
<td>1.46</td>
<td>0.202</td>
<td>0.216</td>
<td>0.116</td>
<td>0.103</td>
<td>0.069</td>
</tr>
<tr>
<td>S₁</td>
<td>1.15</td>
<td>0.069</td>
<td>0.33</td>
<td>0.05 (48)</td>
<td>1.02 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₂</td>
<td>1.00</td>
<td>0.028</td>
<td>0.13</td>
<td>0.80 (41)</td>
<td>0.82 (34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₃</td>
<td>0.038</td>
<td>ND*</td>
<td>ND</td>
<td>&lt;0.02 (&lt;1)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₄</td>
<td>0.066</td>
<td>4.17</td>
<td>19.8</td>
<td>&lt;0.02 (&lt;1)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₅</td>
<td>0.030</td>
<td>1.27</td>
<td>6.04</td>
<td>&lt;0.02 (&lt;1)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ND, not determined.

Lack of an obligate relationship between catalytic activity and small subunit content was further shown by linear sucrose density gradient centrifugation of the native and small subunit-depleted enzymes according to the procedure of Martin et al. (1981).

FIG. 4. Absorbance scans of stained gels after SDS electrophoresis of native Synechococcus Rbu-P₃ carboxylase-oxygenase and two of the fractions produced by precipitation of the enzyme at pH 5.4. Top, native enzyme (8 μg); middle, thrice-precipitated and redissolved enzyme (P₃, 5 μg); bottom, supernatant from second precipitation (S₃, 3 μg). Further details are given in the legend to Table II. The sharp spikes at 0 cm and approximately 6.7 cm are caused by the top of the gel and the dye front marker, respectively.
and Ames (1961) (Fig. 5). The small subunit-depleted enzyme sedimented more slowly than the native enzyme. Furthermore, the 289-nm absorbance peak for the small subunit-depleted enzyme was much broader, suggesting that it did not represent a homogeneous population of molecules. This heterogeneity was presumably the result of partial saturation of an oligomeric large subunit core with varying numbers of small subunits. However, the carboxylase activity profile of the small subunit-depleted enzyme on the sucrose gradient coincided exactly with the 280-nm absorbance profile (Fig. 5). This constancy of specific activity across the heterogeneous peaks suggests that all species were approximately equally active regardless of their degree of saturation with small subunits. When referred to catalase as standard (data not shown) the specific activity values (Martin and Ames, 1961) for the native and small subunit-depleted enzymes were 17.6 and 15.8, respectively. This corresponds roughly to an average molecular weight difference from peak to peak of 70,000 (Martin and Ames, 1961).

Heterogeneity of the small subunit-depleted enzyme was confirmed by pore-gradient gel electrophoresis under nonde-naturing conditions (Fig. 6, lanes 3 and 7). At least three clearly separated bands were obvious, spanning a region corresponding to a molecular weight difference of approximately 50,000. Traces of aggregated protein which barely entered the gel were also noticeable.

Reconstitution—When the small subunit-depleted enzyme was mixed at 0 °C with a supernatant fraction containing isolated small subunits in proportions designed to recreate the subunit ratio of the native enzyme, a substantial portion of the activity lost during the original separation was regained (Table III). Activity after reconstitution was double or more than that before. Reactivation could have occurred either during the incubation period at 0 °C or during the 15-minute preincubation at 25 °C which was part of the standard assay procedure. The picture is complicated by the observation that, unlike the native enzyme, small subunit-depleted enzyme lost activity slowly during storage at 4 °C (compare Tables II and

![Fig. 5. Linear sucrose density gradient centrifugation (see "Materials and Methods") of native Synechococcus Rbu-P2 carboxylase-oxygenase and after three times repeated acid precipitation and re-solution as described in the legend to Table II. The UV absorbance monitor traces were digitized and replotted (as dashed and solid lines) for the native and acid-precipitated preparations, respectively) so that the areas under the peaks are equal. This was achieved by dividing the native enzyme trace by 1.8, which was approximately the ratio between the amounts of protein applied to the two gradients. Sample volume was 100 µl and fraction volume was 110 µl. Carboxylase activities of the fractions of the gradient for the acid-precipitated enzyme are shown also (O).

![Fig. 6. Pore-gradient gel electrophoresis of Synechococcus Rbu-P2 carboxylase-oxygenase. See "Materials and Methods" for details of the method and legends to Tables II and III for details of the samples. Lanes 2, 4, 6, and 8, standard proteins (from top: thyroglobulin, ferritin, catalase, lactate dehydrogenase, bovine serum albumin; molecular weights as indicated); lanes 1 and 9, native enzyme (8 and 16 µg); lanes 3 and 7, thrice-precipitated and redisolved enzyme (P5, 5 and 10 µg); lane 5, 3 µg P5 plus 0.5 µg neutralized S7 mixed together and stored at 0 °C for 30 min before application. P5 and S7 fractions had been prepared 21 days previously.)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time since preparation</th>
<th>Time between mixing and assay</th>
<th>Carboxylase activity</th>
<th>Specific activity in terms of large subunit content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days min</td>
<td></td>
<td>µmol·min⁻¹·mg⁻¹</td>
<td>µmol·min⁻¹·mg⁻¹</td>
</tr>
<tr>
<td>Native enzyme</td>
<td>22 2.28</td>
<td></td>
<td>1.97</td>
<td>0.47</td>
</tr>
<tr>
<td>P5 (5 µg)</td>
<td>22 &lt;0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S5 (1.1 µg)</td>
<td>14 5.92</td>
<td></td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>P5 (5 µg) + S5 (1.1 µg)</td>
<td>14 5.95</td>
<td></td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>P5 (5 µg) + S5 (1.1 µg)</td>
<td>14 6.14</td>
<td></td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>P5 (5 µg) + S5 (1.1 µg)</td>
<td>22 4.55</td>
<td></td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>P5 (5 µg) + S5 (1.1 µg)</td>
<td>22 4.47</td>
<td></td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

III: note that assays for Table II were performed under N2 and those for Table III under air. Whereas, for the native enzyme, the difference was only that expected between O2-free assays and those in air (see later), the small subunit-depleted enzyme lost about 30% of its activity during 22 days storage. This may explain the observed reduction in degree of reactivation achieved by reconstitution experiments as the small subunit-depleted enzyme aged (Table III).
Reconstitution of enzymatic activity was accompanied by substantial reformation of the native electrophoretic form of the enzyme, a finding only a trace of the predominant middle band and none of the smallest molecular weight band of the small subunit-depleted enzyme (Fig. 5, lane 5).

DISCUSSION

Although Rbu-P₂ carboxylase-oxygenase represents a much smaller proportion of total cellular protein in Synechococcus and other cyanobacteria than it does in leaves of higher plants, it nevertheless appears to be the predominant large protein in extracts. The efficacy of the zonal ultracentrifuge purification step stems from this. The DEAE-Sephadex step was also efficient because the enzyme was unusually tightly bound. Either step by itself was capable of achieving substantial purification, and in combination they provided a very reliable procedure which yielded 30-50% of the crude extract's carboxylase activity (data not shown). The product was homogeneous, had a high specific activity, and was stable in dilute solution at 4°C.

Synechococcus Rbu-P₂ carboxylase-oxygenase is composed of both large (M₀ = 57,000) and small (M₀ = 12,000) subunits (Fig. 1) in a 1:1 molar ratio (Table II). The number of L-S pairs comprising the native enzyme molecule is less certain. Comparison of its mobility with those of standard proteins on pore-gradient electrophoresis gels showed that it was apparently slightly smaller than ferritin, i.e. M₀ = 490,000. This seems more in keeping with a hexameric (Lawlis et al., 1979; Taylor and Dow, 1980) than an octameric structure but, since shape plays such an important part in the pore exclusion process, further studies with other techniques for molecular weight estimation are required. These will be the subject of a separate communication.

Two aspects of the Mg²⁺-plus HCO₃⁻-induced activation of Synechococcus Rbu-P₂ carboxylase-oxygenase are noteworthy (Fig. 2). First, the rate of activation was rapid, being at least as fast as that of the higher plant enzyme under optimum conditions. Furthermore, this rapid activation occurred in the presence of Rbu-P₂, whereas the higher plant enzyme activated only very slowly when Rbu-P₂ was present (Andrews et al., 1975; Lorimer et al., 1976; Laing and Christeller, 1976; Gibson and Tabita, 1979). The Synechococcus enzyme's activation rate was much faster than that reported by Christeller and Laing (1978) for the Rhodospirillum rubrum enzyme. However, faster rates of activation of the R. rubrum enzyme have been observed more recently (Gibson and Tabita, 1979; Whitman et al., 1979). Second, the extent of activation under given conditions was unaffected by the presence of Rbu-P₂. This again contrasts with the deleterious effect of Rbu-P₂ on activation of the higher plant enzyme and is also unlike the R. rubrum enzyme, where Rbu-P₂ increased the extent of activation (Christeller and Laing, 1978). The data indicate that the inactive form of the Synechococcus enzyme may be unable to bind Rbu-P₂.

Kinetic data obtained for both carboxylase and oxygenase activities of the Synechococcus enzyme (Table I) confirm and extend those of Badger (1980) for the A. variabilis enzyme. The Kₘ(CO₂) value, 240 μM, was an order of magnitude greater than that of the fully activated, higher plant enzyme (Jensen and Bahr, 1977) and resembled those of the Rbu-P₂ carboxylases from a variety of other prokaryotes.3 The affinity

3 It is difficult to compare Kₘ(CO₂) values between laboratories because of the lack of standardization in the method of computing CO₂ concentrations from HCO₃⁻ concentrations. The main uncertainty surrounds the choice of the appropriate value for the pH of the CO₂ ↔ HCO₃⁻ interconversion. Most workers appear to have used the value for distilled water solution (6.35 at 25°C, Edsall and Wyman, 1958), although this is often not stated. We have followed this convention. However, Edsall and Wyman's value for 0.1 M NaCl solution (6.12 at 25°C) is probably more appropriate to the composition of assay solutions commonly used. If this value is adopted, the CO₂ concentrations, and therefore the Kₘ(CO₂) values, are reduced by approximately 40%.

From studies with the two variants of Rhodopseudomonas sphaeroides Rbu-P₂ carboxylase-oxygenase, which either possess or lack small subunits, Gibson and Tabita (1979) suggested that presence of small subunits (i) causes an accelerated rate of Mg²⁺- plus HCO₃⁻-induced activation; (ii) inhibits this activation when Rbu-P₂ is present; and (iii) causes a high affinity for CO₂. Present data concerning activation and kinetics of the Synechococcus enzyme are in accord with (i) but opposed to (ii) and (iii).

Removal of small subunits from Synechococcus Rbu-P₂ carboxylase-oxygenase by repeated isoelectric precipitation at pH 5.4 is particularly interesting. However, unless cyanobacterial Rbu-P₂ carboxylase-oxygenases differ with respect to the ease with which small subunits are lost, it seems an unlikely explanation for the inability of some previous authors to detect small subunits in preparations which had been subjected to this procedure (Tabita et al., 1976; Codd and Stewart, 1977). These authors precipitated the enzyme only once, which according to present data removes only about half of the small subunits (Table II). Furthermore, Tabita et al. (1974) did not observe small subunits even when the precipitation step was omitted. Although a single precipitation resulted in only partial separation of large and small subunits, repetition produced a preparation greatly depleted in small subunits (13% remaining after 3 precipitations, Table II). This preparation apparently consisted of an oligomeric core of large subunits, similar to that of the native enzyme, with varying degrees of saturation with small subunits (see 'Results'). It seems that 11% of the native enzyme's catalytic activity, but this activity was not preferentially associated with any particular molecular species, apparently being borne equally by all large subunit cores regardless of their degree of saturation with small subunits (Fig. 5). Although the small subunit-depleted preparation lost activity slowly with storage at 4°C, re-addition of sufficient small subunits to restore the native L:S ratio of 1:1 resulted in a doubling, or more, in activity (Table III) accompanied by a substantial re-formation of the native configuration (Fig. 6).

Akazawa and co-workers dissociated the small subunits from the oligomeric large subunit core of Rbu-P₂ carboxylase-oxygenases from Chromatium (Akazawa et al., 1972; Takabe and Akazawa, 1973; 1975) and spinach (Nishimura et al., 1973; 1974; Kobayashi et al., 1979) by treatment at alkaline pH and, in the case of spinach, with p-chloromercuribenzoate. The
percentage of native activity retained by these preparations (10-15%) was lower than that of the small subunit-depleted Synechococcus enzyme (41%) and their specific activities were much lower. Furthermore, the alkaline procedures tended to disrupt the integrity of the reactor and to promote aggregation. No such disruption was apparent with the small subunit-depleted preparations, in marked contrast with the alkali-dissociated preparations, as evidenced by the reconstitution data. The difficulty may be regained only when sufficient small subunits are present in marked contrast with the alkali-dissociated preparations, resulting in at least a doubling of catalytic activity, could be demonstrated even after several weeks of separate storage.

At first sight, the observation that the specific activities of the various species present in the small subunit-depleted preparation were independent of their L:S ratio (Fig. 5) seems difficult to reconcile with the reconstitution data. The difficulty may be overcome by postulating that any degree of undersaturation with small subunits, even the loss of only one per molecule, forces the large subunit core to adopt a different, less active conformation. The native, high activity conformation may be regained only when sufficient small subunits are bound to reconstitute the native L:S ratio. We are thus led to hypothesize that the small subunit of Rbu-P₂ carboxylase-oxygenase, while not itself bearing the active site, nevertheless plays a role in catalysis. Its binding also changes substrate affinities, or the Mg²⁺-plus CO₂-induced activation process, (Kobayashi et al., 1979) are questions requiring further research. In view of the obvious disadvantages in requiring two polypeptides to express fully a single (albeit bi-functional) catalytic activity, might it not be possible to accommodate these disadvantages in the primary sequence of the large subunit (presumably by changes in the primary sequence of the large subunit) as the more advanced?

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Cyanobacterial Rbu-P2 Carboxylase-Oxygenase

SUPPLEMENTARY MATERIAL

KINETICS AND SUBUNIT INTERACTIONS OF RIBULOSE BISPHOSPHATE CARBOXYLASE-OXYGENASE FROM THE CYANOBACTERIUM, SYNECHOCOCUS sp.

T. John Andrews and Kay M. Arol

MATERIALS AND METHODS

Materials. The unicellular marine cyanobacterium, Synechococcus sp. (strain R256) was kindly supplied by Dr. W. D. Bergersen. Tricine pH 8.3 buffer, and standard proteins for SDS gel electrophoresis were obtained from Sigma Chemical Co., St. Louis, Mo. Succinyl-CoA (50 μmol) was obtained from New England Nuclear. Standard proteins were supplied by the Sigma Chemical Co., St. Louis, Mo. Bovine serum albumin (70 mg/ml) was prepared as a 20% (w/v) solution in Tricine buffer (pH 8.3). Standard protein solutions were dialyzed against a large volume of Tricine buffer (pH 8.3) and used within 24 hr after preparation.

Results. 1. Temporal Changes in Carboxylase Activity in Whole Cells. Whole cells were harvested with a modification of the method of Mettenleiter and Hesse (1972) in 0.29-0.3 M sucrose/mannitol buffer, pH 7.4, and stored at -20°C.

2. Purification of Rbu-P2 carboxylase-oxygenase. All procedures were carried out at 4°C. Buffer solutions were prepared by mixing Tricine, 40 mM; Bicine, 80 mM; and Bicine-HCl, 20 mM, containing 50 mM imidazole, pH 7.4. All solutions were adjusted to pH 7.4 with Tricine buffer (pH 8.3). The bacterial concentration was determined by a spectrophotometric method at 280 nm. The enzyme was assayed in a reaction mixture containing 0.3 mg protein and 0.3 mM sodium phosphate buffer, pH 7.4.

3. Results and Discussion. The carboxylase-oxygenase activity of the cyanobacterium was determined using the method of Mettenleiter and Hesse (1972) with a slight modification. The enzyme was partially purified by the method of Mettenleiter and Hesse (1972). The purified enzyme was used for the kinetic studies. The enzyme was assayed using the method of Mettenleiter and Hesse (1972). The enzyme was found to catalyze the reaction between ribulose 1,5-bisphosphate and oxygen to form ribulose 1,5-bisphosphate and dihydroxyacetone phosphate. The reaction was followed spectrophotometrically at 340 nm.

4. Conclusion. The results presented in this study demonstrate that the cyanobacterial Rbu-P2 carboxylase-oxygenase is a potential source of a novel enzyme which can catalyze the conversion of ribulose 1,5-bisphosphate to ribulose 1,5-bisphosphate and dihydroxyacetone phosphate. This enzyme may have potential applications in the field of biochemistry and medicine.
Stability. When stored at 2-4°C in 50 mM phosphate buffer, pH 7.6, containing 1 mM EDTA, at a concentration of 1.5 mg protein/ml, the purified enzyme lost little or no activity over a 5-month period. Furthermore, no changes in its electrophoretic or sedimentation properties were detected. The enzyme was less stable when 20 mM phosphate and 10 mM EDTA was also included in the storage buffer or when stored at 20°C. However, when stored at 20°C, the enzyme was stable as long as the pH was maintained at 7.6. A proteinase inhibitor (1 mg/ml) was added to the control samples to prevent proteolysis. Under these conditions 75% of the carboxylase activity was lost over a 3-month period at 4°C and this was accompanied by massive aggregation to very high molecular weight material which hardly entered the gel-filtration electrophoresis wells (data not shown).

Activation. Like all RuBP carboxylases-oxygenases so far studied, this enzyme was activated by MgCl2 and CO2, and was insensitive to their absence. The rate of activation increased linearly with the concentration of MgCl2, reaching a maximum at about 0.2 M MgCl2. The time required for full activation ranged from about 40 min at 25°C to 3 hours at 12°C. The final extent of activation reached at a particular MgCl2 concentration was the same regardless of whether or not RuBP was present during activation.

Table 1. Summary of kinetic properties of "Rb" carboxylase-oxygenase.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Vmax</th>
<th>Km</th>
<th>Kcat</th>
<th>Vmax/Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>RuBP</td>
<td>23.9</td>
<td>1.4</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>CO2</td>
<td>25.0</td>
<td>1.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>O2</td>
<td>8.0</td>
<td>2.0</td>
<td>4.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Figure 1. Lineweaver-Burk plots of carboxylase activity versus CO2 concentration at varying concentrations of RuBP. Assays were performed in triplicate as described in "Materials and Methods." The enzyme solutions were equilibrated with the respective CO2 concentrations and the lines represent the best fit to the data points according to the statistical method of Wilkinson (1961).