

# Purification and Some Properties of *Chlorella fusca* Ribulose 1,5-Diphosphate Carboxylase

Received for publication August 1974 and in revised form October 18, 1974

J. MICHAEL LORD AND RICHARD H. BROWN

Postgraduate School of Biological Sciences, University of Bradford, Bradford, Yorkshire BD7 1DP, England

## ABSTRACT

Ribulose 1,5-diphosphate carboxylase has been purified from extracts of autotrophically grown *Chlorella fusca* by ammonium sulfate precipitation and centrifugation on a linear sucrose density gradient. The enzyme was homogeneous by the criterion of polyacrylamide gel electrophoresis. The molecular weight of the enzyme was 530,000, and it was composed of two types of subunit of molecular weight 53,000 and 14,000. Ribulose 1,5-diphosphate, CO<sub>2</sub>, and Mg<sup>2+</sup> had Michaelis constant values of 15 μM, 0.3 mM, and 0.37 mM, respectively. At high bicarbonate concentration (17 mM and 50 mM), 6-phosphogluconate inhibited the enzyme, the inhibition being noncompetitive with respect to ribulose 1,5-diphosphate (K<sub>i</sub> 0.065 mM), whereas at low bicarbonate concentration (1 mM), 6-phosphogluconate activated the enzyme. Oxygen was a competitive inhibitor with respect to CO<sub>2</sub>, suggesting the enzyme also functions as an oxygenase. This was confirmed by direct assay, a 1:1 stoichiometry between ribulose 1,5-diphosphate consumed and O<sub>2</sub> uptake being observed.

The introduction of CO<sub>2</sub> into the reductive pentose phosphate (Calvin) cycle of autotrophs is accomplished by the carboxylation of RuDP<sup>1</sup> catalyzed by the enzyme RuDPCase (3-phospho-D-glycerate carboxylase (dimerizing), EC 4.1.1.39). The occurrence of this pathway and its key enzyme is now well documented in a diversity of organisms ranging from prokaryotes to higher plants (for review, see McFadden [9]). Comparison of the molecular structure and regulatory properties of RuDPCase from such organisms is important to current concepts regarding the evolution of autotrophs (19).

Studies on the molecular size of diverse RuDPCase have shown that three size categories exist: small (mol wt = 110,000 [3, 28]), intermediate (mol wt 240,000-360,000 [1, 4, 20]) and large (mol wt > 500,000 [17]).

The enzymes isolated from the unicellular green algae *Chlamydomonas reinhardtii* (13) and *Euglena gracilis*<sup>2</sup> fall into the large mol wt category typically found for the enzyme from higher plants, and are composed of two types of subunit (approximate mol wt 55,000 and 15,000). Slightly lower mol wt have been reported for RuDPCase from two strains of

*Chlorella*; 470,000 from *Chlorella ellipsoidea* (25) and 460,000 from *Chlorella pyrenoidosa* (21), although the former enzyme might be significantly larger (26).

In the present study, we have examined RuDPCase purified from *Chlorella fusca* (formerly *C. pyrenoidosa*). The mol wt and quaternary structure are typical of the large carboxylase purified, for comparison, from tobacco leaves and some properties of the *Chlorella* enzyme are reported.

## MATERIALS AND METHODS

**Growth of Organism.** *Chlorella fusca* (formerly *C. pyrenoidosa*), strain 211/8p, obtained from the Cambridge Culture Collection, Cambridge, England, was grown photoautotrophically in the medium described by Gouling and Merrett (15). For RuDPCase isolation, 8-liter cultures were grown in 10-liter aspirators at a constant temperature of 25 C. A mixture of 5% CO<sub>2</sub> in air was continuously bubbled through the cultures, which were illuminated at a light intensity of 5000 lux. The cells were harvested during the exponential phase of growth by centrifugation for 10 min at 500g.

**Preparation of Extracts.** *Chlorella* cells were washed once in 20 mM tris-HCl, pH 8, containing 1 mM MgCl<sub>2</sub> and 1 mM dithiothreitol, and were then resuspended in the same buffer to give a dense cell suspension in a final volume of approximately 30 ml. Cells were broken by passing this suspension through a chilled French pressure cell at 20,000 p.s.i. Cell debris was removed by centrifuging at 20,000g and 4 C for 10 min, and the homogenate was further centrifuged at 100,000g and 4 C for 1 hr.

**Purification of RuDPCase.** The highspeed, Chl-free *Chlorella* supernatant was fractionated with solid ammonium sulfate, the bulk of the RuDPCase activity being precipitated between 30 and 48% salt saturation. This fraction, recovered by centrifugation, was redissolved in 5 ml of the homogenization buffer. Two ml of this solution were layered onto a 34-ml gradient increasing linearly in concentration from 0.2 M to 0.8 M sucrose. Sucrose solutions were prepared in 20 mM tris-HCl, pH 8, containing 1 mM MgCl<sub>2</sub> and 1 mM dithiothreitol. Gradients were contained in 38.5-ml polyallomer tubes and were centrifuged for 19 hr at 65,000g (average) and 4 C in an SW27 rotor on a Beckman L2 65B centrifuge. After centrifugation, gradients were collected from the bottom of the tubes in 2-ml fractions using a Beckman density gradient fractionator.

In order to compare the mol wt of the *Chlorella* RuDPCase with that of the enzyme from tobacco, 10 to 15 leaves from a young tobacco (*Nicotiana tabacum*) plant were chopped with scissors and homogenized in 50 ml of buffer (as above) in an M.S.E. top-drive homogenizer operating at top speed for 1 min. The homogenate was filtered through four layers of cheesecloth, and centrifuged at 100,000g and 4 C for 1 hr. RuDPCase was purified from this Chl-free supernatant by

<sup>1</sup> Abbreviations: RuDP: ribulose-1,5-diphosphate; RuDPCase: ribulose 1,5-diphosphate carboxylase; 6-P-gluconate: 6-phosphogluconate.

<sup>2</sup> McFadden, B. A., J. M. Lord, A. Rowe, and S. Dilks, submitted for publication.

ammonium sulfate fractionation and sucrose density gradient centrifugation exactly as described above for the *Chlorella* enzyme.

**RuDPCase Assay.** The RuDP-dependent incorporation of  $\text{NaH}^{14}\text{CO}_3$  into an acid-stable product was determined in a reaction mixture, of 0.3 ml final volume, which contained 33 mM tris-HCl, pH 8, 6.6 mM  $\text{MgCl}_2$ , 2 mM reduced glutathione, 0.33 mM EDTA, 17 mM  $\text{NaH}^{14}\text{CO}_3$  (specific radioactivity, 0.4  $\mu\text{Ci}/\mu\text{mole}$ ), enzyme (up to 0.1 ml) and 1 mM RuDP. The enzyme was incubated with all components except RuDP for 5 min at 30 C, and then the reaction was started by the addition of this compound and allowed to proceed for 5 min at 30 C. The reaction was stopped by adding 0.1 ml of 50% (w/v) trichloroacetic acid. After allowing sufficient time for the removal of unused  $\text{NaH}^{14}\text{CO}_3$  (overnight at room temperature or 2–3 hr at 60 C), an aliquot of the reaction mixture was added to 10 ml of scintillation fluid (9) in a counting vial, and its radioactivity was determined in a Packard Tricarb liquid scintillation counter at an efficiency of 50%.

In order to assay the enzyme in an  $\text{O}_2$  atmosphere, the glass tubes in which the assays were routinely performed were closed with rubber Suba seals, evacuated, and refilled with 100%  $\text{O}_2$  via a syringe needle. A unit of enzyme activity was defined as that amount catalyzing the fixation of 1  $\mu\text{mole}$  of  $^{14}\text{CO}_2/\text{min}$ .

**RuDP Oxygenase Assay.** RuDP-dependent  $\text{O}_2$  uptake by purified *Chlorella* RuDPCase was measured using a Rank  $\text{O}_2$  electrode. The reaction mixture contained, in a final volume of 1 ml, 80 mM glycylglycine (pH 9.3), 10 mM  $\text{MgCl}_2$ , 6 mM reduced glutathione, 1 mM EDTA, enzyme (about 1 mg), and 75  $\mu\text{M}$  RuDP. The reaction was started by adding RuDP. Controls, run simultaneously, contained all components except RuDP. Results were recorded on a Rikadenki model B241 two-pen recorder.

**Polyacrylamide Gel Electrophoresis.** Purified RuDPCase was examined electrophoretically according to the method of Davis (12) on gels polymerized from various acrylamide concentrations using a bis to acrylamide ratio of 1:37. Bromophenol blue was used as tracking dye, and electrophoresis was carried out at a constant current of 2 mamp/tube.

For quaternary structure studies, the enzyme was incubated for 3 hr at 37 C in 10 mM sodium phosphate, pH 7, containing 1% (w/v) sodium dodecyl sulfate, and 1% (v/v)  $\beta$ -mercaptoethanol. Electrophoresis (on gels polymerized from 10% acrylamide) was performed as described by Weber and Osborne (29) at a constant current of 8 mamp/tube.

All gels were stained overnight in Coomassie blue.

**Gel Filtration.** The 30 to 48% ammonium sulfate precipitates containing RuDPCase were redissolved in 3 ml of 50 mM tris-HCl, pH 7, containing 50 mM KCl, and applied to a column (2 cm  $\times$  100 cm) of Sepharose 6B (Pharmacia). The column was eluted with 50 mM tris HCl, pH 7, containing 50 mM KCl. Three-ml fractions were collected and assayed for RuDPCase activity.

**Materials.**  $\text{NaH}^{14}\text{CO}_3$  was obtained from the Radiochemical Centre, Amersham (U.K.). RuDP, 6-P-gluconate, and proteins of known molecular weight used to calibrate gels and the column were obtained from Sigma, London. Sodium dodecyl sulphate and reagents for polyacrylamide gel electrophoresis were supplied by British Drug Houses, Speke, Liverpool. All other compounds were commercial preparations of the highest quality available.

## RESULTS

**Purification of RuDPCase.** RuDPCase present in a 100,000g *Chlorella* supernatant was enriched by precipitation between

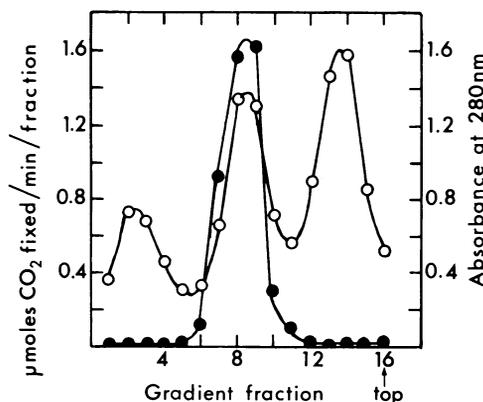


FIG. 1. Sucrose density gradient centrifugation of the redissolved 30–48% ammonium sulfate fraction of *Chlorella fusca*. ●: RuDPCase activity; ○:  $A_{280}$ .

30 and 48% ammonium sulfate saturation and separated from the residual soluble proteins by sucrose density gradient centrifugation (Fig. 1). The enzyme present in the fractions showing maximum activity was homogenous by the criterion of electrophoresis in gels polymerized from a range of acrylamide concentrations. Over-all, the purification procedure yielded a 40% recovery of enzyme activity, and the specific activity in the peak fractions (refs. 8 and 9; Fig. 1) was 1 unit/mg protein. However, the enzyme was relatively unstable and only 60% of the activity added to the gradient was recovered after centrifugation. Assuming 100% recovery had been achieved after the step, the calculated specific activity of the purified *Chlorella* enzyme was 1.66 units/mg, which is in good agreement with that of the purified enzyme from other sources (17). On a protein basis, the enzyme was purified about 10-fold, indicating that it represents approximately 10% of the total soluble protein in extracts of autotrophically grown *Chlorella*.

**Molecular Weight.** Comparative studies indicated that the mol wt of *Chlorella* RuDPCase is very close to that of the enzyme from *N. tabacum*, which is known to be 525,000. When the tobacco enzyme was centrifuged into a sucrose density gradient under the conditions employed to purify the *Chlorella* enzyme, its position after centrifugation was indistinguishable from that obtained for the latter enzyme (data not shown). The close similarity in mol wt of the enzyme from these two sources was confirmed by (a) comparing their electrophoretic mobilities in gels polymerized from various concentrations of acrylamide; Hendrick-Smith plots (16) gave identical slopes for these enzymes and (b) the elution profiles of the two carboxylases during gel filtration on Sepharose 6B were almost identical. Calibration of the Sepharose column with proteins of known mol wt established that the mol wt of *Chlorella* RuDPCase was 530,000.

**Quaternary Structure.** Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of purified *Chlorella* RuDPCase showed that the enzyme was comprised of two types of subunit. Calibration of the gels with proteins of known monomer mol wt established that the mobilities of the *Chlorella* RuDPCase subunits corresponded to mol wt of 53,000 and 14,000 (Fig. 2). Assuming the native enzyme is composed of 8 large and 8 small subunits (22), the mol wt calculated on the basis of the experimentally determined subunit mol wt is 536,000, in close agreement with the value obtained by gel filtration.

Comparative quaternary structure studies with the *N. tabacum* carboxylase established that the *Chlorella* and tobacco enzymes contain large subunits of identical molecular weight, whereas the small subunit of the tobacco enzyme was repeat-

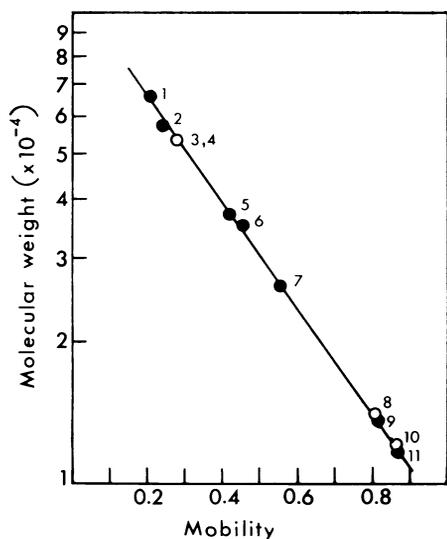


FIG. 2. Relationship between mobility and log molecular weight obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified *Chlorella* and *N. tabacum* RuDPCase and various marker proteins. 1: BSA; 2: pyruvate kinase; 3: large subunit of tobacco RuDPCase; 4: large subunit of *Chlorella* RuDPCase; 5: alcohol dehydrogenase; 6: malate dehydrogenase; 7: chymotrypsinogen; 8: small subunit of *Chlorella* RuDPCase; 9: ribonuclease; 10: small subunit of tobacco RuDPCase; 11: Cyt *c*.

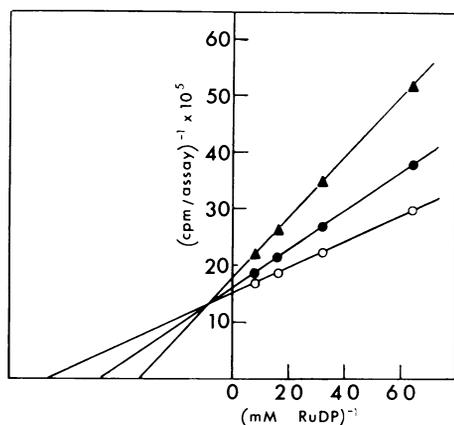


FIG. 3. Double reciprocal plot showing the inhibition of carboxylation at various RuDP concentrations by 6-P-gluconate. ○: control (no inhibitor); ●: 0.1 mM; ▲: 0.2 mM 6-P-gluconate.

edly shown to be slightly smaller (mol wt 12,000) than the *Chlorella* small subunit (Fig. 2).

**Properties of *Chlorella* RuDPCase.** Under the standard assay conditions, preincubation of the enzyme with  $Mg^{2+}$  and  $NaH^{14}CO_3$  for a few minutes was necessary for maximum activity. In 5-min assays with no preincubation (reaction started by adding enzyme) the measured activity was about 50% of that with the preincubated enzyme. Observed rates were proportional to the amount of enzyme assayed in all cases. With the preincubated enzyme, the rate became linear with time for assay times greater than 2 min; higher rates were obtained during the first 2 min. Purifying the enzyme in buffers lacking  $Mg^{2+}$  established an absolute requirement for this divalent cation. Control assays in which RuDP was omitted showed less than 5% of the activity of the complete system.

**Effect of 6-Phosphogluconate.** 6-P-gluconate was a mixed inhibitor of *Chlorella* RuDPCase with respect to RuDP (Fig.

3), in confirmation of the potent inhibition by this compound reported for other high mol wt carboxylases (10, 27). The  $K_i$  was  $65 \mu M$ . However, Chu and Bassham (11) have shown that this compound has a dual effect on spinach RuDPCase, inhibiting at high bicarbonate concentrations but activating at low bicarbonate concentrations. We have confirmed this interesting observation using the *Chlorella* enzyme (Fig. 4). At 1 mM  $NaH^{14}CO_3$  concentration, preincubation of the enzyme with 6-P-gluconate,  $MgCl_2$ , and  $NaH^{14}CO_3$  resulted in a striking activation which was maximal at 0.1 mM 6-P-gluconate and was still pronounced at 0.5 mM. However, repeating these assays with 50 mM  $NaH^{14}CO_3$  resulted in inhibition by 6-P-gluconate which increased with concentration (Fig. 4).

**Oxygen Inhibition of *Chlorella* RuDPCase.** When *Chlorella* RuDPCase was assayed in an atmosphere of 100%  $O_2$ , activity was markedly inhibited in comparison with assays performed simultaneously in air (21%  $O_2$ ). Activities recorded in 100%  $O_2$  were about 60% of those in air over a range of bicarbonate concentrations. A double reciprocal plot (Fig. 5) emphasized the competitive nature of the  $O_2$  inhibition (8, 23), and indicated that *Chlorella* RuDPCase was also functioning as an oxygenase (5, 18). RuDP oxygenase activity was confirmed by direct assay using an  $O_2$  electrode. Figure 6 shows the RuDP-dependent  $O_2$  uptake catalyzed by *Chlorella* RuDPCase. The reaction was allowed to proceed to completion when a 1:1 stoichiometry between RuDP consumption and  $O_2$  uptake was observed.

## DISCUSSION

RuDPCase has been purified in good yield from *Chlorella fusca* by the sucrose density gradient method of Goldthwaite and Bogorad (14). This procedure offers a simple one step purification for high molecular weight RuDPCase and has been successfully used to obtain pure enzyme from higher plant (14), *Euglena*<sup>a</sup> and microbial extracts (24, 27, 28).

In the present work, the yield of purified RuDPCase from the gradients was increased by enriching prior to centrifugation by ammonium sulfate fractionation.

The mol wt of *Chlorella* RuDPCase was determined to be

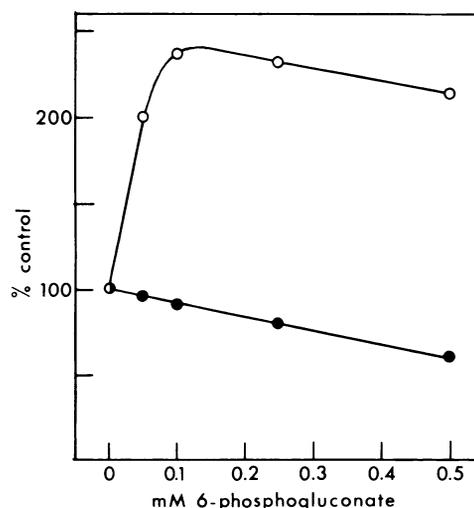


FIG. 4. Inhibition and activation of *Chlorella* RuDPCase preincubated with 6-P-gluconate in the presence of high (50 mM; specific radioactivity 0.266 Ci/mole)  $NaH^{14}CO_3$  (●), or low (1 mM; 13.33 Ci/mole)  $NaH^{14}CO_3$  (○). The activity recorded in the controls (no 6-P-gluconate) in these assays were 15,570 cpm at 50 mM  $NaH^{14}CO_3$  and 54,303 cpm at 1 mM  $NaH^{14}CO_3$ .

530,000, somewhat higher than a previously reported value of 460,000 from this strain of *Chlorella* (21). The *Chlorella* enzyme is thus very similar in size to the enzyme from higher plants (17) and the unicellular green algae *Chlamydomonas reinhardtii* (13) and *Euglena gracilis*.<sup>2</sup> This was confirmed by comparative studies with tobacco RuDPCase.

The quaternary structure of *Chlorella* RuDPCase is also the same as that of the enzyme from higher plants (17), green algae thus far examined (13, 26), and the purple sulphur bacterium, *Chromatium D* (2). Two types of subunit are present, and the molecular size of the *Chlorella* subunits reported here (53,000 and 14,000) fall within the size range typically reported for the large, multimeric carboxylase (mol wt > 500,000). These subunit molecular weights are consistent with a stoichiometry of subunit combination of (L<sub>6</sub>S<sub>6</sub>) for the native enzyme, where L represents the large and S the small mol wt subunits respectively.

The kinetic properties of *Chlorella fusca* RuDPCase, summarized in Table I, are, in general, similar to those of the enzyme from other sources. The exception is the *K<sub>m</sub>* for

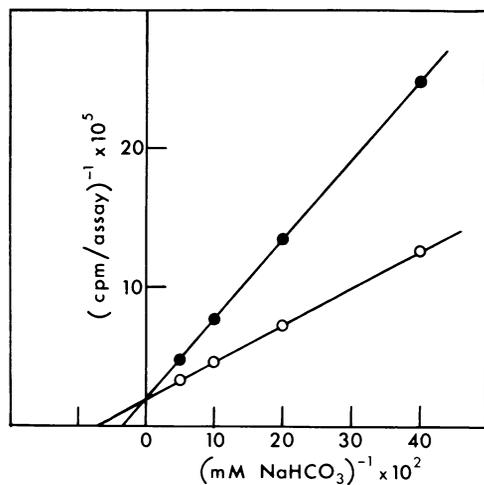


FIG. 5. Double reciprocal plot showing the inhibition of carboxylation at various CO<sub>2</sub> (NaHCO<sub>3</sub>) concentrations by an atmosphere at 100% O<sub>2</sub> during the assays (●) as compared to 21% O<sub>2</sub> (air) (○).

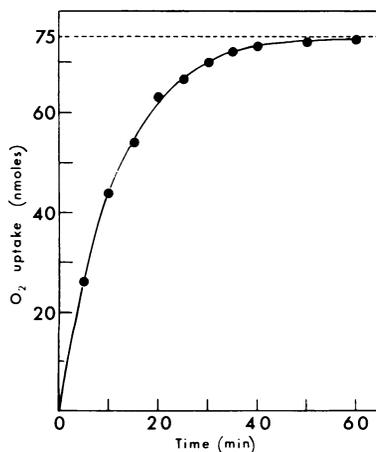


FIG. 6. Oxygen electrode determination of RuDP oxygenase activity of *Chlorella* RuDPCase showing the stoichiometric consumption of O<sub>2</sub> in a reaction initiated by adding 75 nmoles of RuDP. The plot has been corrected for the slight uptake of O<sub>2</sub> recorded in the control (minus RuDP).

Table I. Kinetic Constants Determined for Purified *Chlorella* RuDPCase

The gas phase was air.

Reactant	<i>K<sub>m</sub></i>
	$\mu\text{M}$
RuDP	15
RuCP + 0.1mM 6-P-gluconate	21
RuDP + 0.2mM 6-P-gluconate	30
MgCl <sub>2</sub>	370
CO <sub>2</sub>	300
CO <sub>2</sub> (in 100% O <sub>2</sub> )	650

RuDP of 15  $\mu\text{M}$ , which is approximately an order of magnitude lower than that of the higher plant enzyme (17), but is identical to that reported recently for purified *Euglena* RuDPCase.<sup>2</sup> A low *K<sub>m</sub>* for RuDP may thus be a property of the enzyme from aquatic green algae.

Under the standard assay conditions used here, RuDPCase activity was determined in the presence of 17 mM NaH<sup>14</sup>CO<sub>3</sub>. Maximum activity was observed only after the enzyme had been preincubated with this concentration of NaH<sup>14</sup>CO<sub>3</sub> plus Mg<sup>2+</sup> for a few minutes. The nonpreincubated system showed 50% of the activity of the preincubated system. Chu and Bassham (11) reported a similar difference when comparing the nonpreincubated and preincubated spinach carboxylase assayed in the presence of 50 mM NaHCO<sub>3</sub>, but found a 10-fold activation as a result of preincubation at 1 mM NaHCO<sub>3</sub>.

At relatively high bicarbonate concentration, 6-P-gluconate was a potent inhibitor of *Chlorella* RuDPCase confirming earlier reports with diverse, high molecular weight carboxylases (10, 27). At low bicarbonate concentration, preincubation of the enzyme with low concentrations of 6-P-gluconate resulted in pronounced activation of the enzyme, confirming the findings obtained using spinach RuDPCase by Chu and Bassham (11). During steady state photosynthesis in *Chlorella*, Bassham and Kirk (6) have shown that after the light to dark transition, 6-P-gluconate rapidly accumulates in the dark in the presence of vitamin K. Thus 6-P-gluconate may be an important *in vivo* regulator of high molecular weight RuDPCase. A possible mechanism for this regulation and its physiological significance have been discussed by Chu and Bassham (11).

*Chlorella* RuDPCase was inhibited by O<sub>2</sub> and was shown to possess oxygenase activity. This could account, in part, for the rapid formation of phosphoglycolate observed when *Chlorella pyrenoidosa* photosynthesizing in 1.5% CO<sub>2</sub> in air were gassed with 100% O<sub>2</sub> (7).

#### LITERATURE CITED

- AKAZAWA, T., T. SUGIYAMA, AND H. KATOAKA. 1970. Further studies on ribulose 1,5-diphosphate carboxylase from *Rhodospseudomonas spheroides* and *Rhodospirillum rubrum*. *Plant Cell Physiol.* 11: 541-550.
- AKAZAWA, T., H. KONDO, T. SHIMAZUE, M. NISHIMURA, AND T. SUGIYAMA. 1972. Further studies on ribulose 1,5-diphosphate carboxylase from *Chromatium* strain D. *Biochemistry* 11: 1298-1303.
- ANDERSON, L. E. AND R. C. FULLER. 1969. Photosynthesis in *Rhodospirillum rubrum*. IV. Isolation and characterization of ribulose 1,5-diphosphate carboxylase. *J. Biol. Chem.* 244: 3105-3109.
- ANDERSON, L. E., G. B. PRICE, AND R. C. FULLER. 1968. Molecular diversity of ribulose 1,5-diphosphate carboxylase from photosynthetic microorganisms. *Science* 161: 482-484.
- ANDREWS, T. J., G. H. LORIMER, AND N. E. TOLBERT. 1973. Ribulose 1,5-diphosphate oxygenase. 1. Synthesis of phosphoglycolate by fraction 1 protein of leaves. *Biochemistry* 12: 11-18.
- BASSHAM, J. A. AND M. KIRK. 1968. Dynamic metabolic regulation of the photosynthetic carbon reduction cycle. In: K. Shibita, A. Takamiya, A. T. Jagendorf and R. C. Fuller, eds., *Comparative Biochemistry and Biophysics of Photosynthesis*. University of Tokyo Press, Tokyo. pp. 365-378.

7. BASSHAM, J. A. AND M. KIRK. 1973. Sequence of formation of phosphoglycolate and glycolate in photosynthesizing *Chlorella pyrenoidosa*. *Plant Physiol.* 52: 407-411.
8. BOWES, G. AND W. L. OGREN. 1972. Oxygen inhibition and other properties of soybean ribulose 1,5-diphosphate carboxylase. *J. Biol. Chem.* 247: 2171-2176.
9. BRAY, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* 1: 279-285.
10. CHU, D. K. AND J. A. BASSHAM. 1972. Inhibition of ribulose 1,5-diphosphate carboxylase by 6-phosphogluconate. *Plant Physiol.* 50: 224-227.
11. CHU, D. K. AND J. A. BASSHAM. 1973. Activation and inhibition of ribulose 1,5-diphosphate carboxylase by 6-phosphogluconate. *Plant Physiol.* 52: 373-379.
12. DAVIS, B. J. 1964. Disc electrophoresis. II. Methods and application to human serum proteins. *Ann. N.Y. Acad. Sci.* 121: 404-427.
13. GIVAN, A. L. AND R. S. CRIDDLE. 1972. Ribulose 1,5-diphosphate carboxylase from *Chlamydomonas reinhardtii*: purification, properties and its mode of synthesis in the cell. *Arch. Biochem. Biophys.* 149: 153-163.
14. GOLDTHWAITE, J. J. AND L. BOGORAD. 1971. A one step method for the isolation and determination of leaf ribulose 1,5-diphosphate carboxylase. *Anal. Biochem.* 41: 57-66.
15. GOULDING, K. H. AND M. J. MERRETT. 1966. The photometabolism of acetate by *Chlorella pyrenoidosa*. *J. Exp. Bot.* 17: 678-689.
16. HENDRICK, J. L. AND A. J. SMITH. 1968. Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. *Arch. Biochem. Biophys.* 126: 155-164.
17. KAWASHIMA, N. AND S. G. WILDMAN. 1970. Fraction 1 protein. *Annu. Rev. Plant Physiol.* 21: 325-358.
18. LORIMER, G. H., T. J. ANDREWS AND N. E. TOLBERT. 1973. Ribulose 1,5-diphosphate oxygenase. II. Further proof of reaction products and mechanism of action. *Biochemistry* 12: 18-23.
19. MCFADDEN, B. A. 1973. Autotrophic CO<sub>2</sub> assimilation and the evolution of ribulose 1,5-diphosphate carboxylase. *Bact. Rev.* 37: 289-319.
20. MCFADDEN, B. A. AND A. R. DENEND. 1972. Ribulose 1,5-diphosphate carboxylase from autotrophic micro-organisms. *J. Bact.* 110: 633-642.
21. MORAN, M. AND P. C. L. JOHN. 1973. Ribulose 1,5-diphosphate carboxylase of *Chlorella pyrenoidosa*. *J. Gen. Microbiol.* 75: xx.
22. NISHIMURA, M., T. TAKABE, T. SUGIYAMA, AND T. AKAZAWA. 1973. Structure and function of chloroplast proteins. XIX. Dissociation of spinach leaf ribulose 1,5-diphosphate carboxylase by *p*-mercuribenzoate. *J. Biochem.* 74: 945-954.
23. OGREN, W. L. AND G. BOWES. 1971. Ribulose 1,5-diphosphate carboxylase regulates soybean photorespiration. *Nature* 230: 159-160.
24. ROMANOVA, A. K., I. Y. VEDENINA, V. M. KORNITSKAYA, AND N. G. DOMAN. 1971. Purification of ribulose 1,5-diphosphate carboxylase from hydrogen bacteria *Hydrogenomonas entrophia* Z-1. *Biokhimiya (Transl.)* 36: 339-345.
25. SUGIYAMA, T., C. MATSUMOTO, T. AKAZAWA, AND S. MIYACHI. 1969. Structure and function of chloroplast proteins. VII. Ribulose 1,5-diphosphate carboxylase of *Chlorella ellipsoidea*. *Arch. Biochem. Biophys.* 129: 597-602.
26. SUGIYAMA, T., T. ITO, AND T. AKAZAWA. 1971. Subunit structure of ribulose 1,5-diphosphate carboxylase from *Chlorella ellipsoidea*. *Biochemistry* 10: 3406-3411.
27. TABITA, F. R. AND B. A. MCFADDEN. 1972. Regulation of ribulose 1,5-diphosphate carboxylase by 6-phosphogluconate. *Biochem. Biophys. Res. Commun.* 48: 1153-1159.
28. TABITA, F. R. AND B. A. MCFADDEN. 1974. One-step isolation of microbial ribulose 1,5-diphosphate carboxylase. *Arch. Microbiol.* 99: 231-240.
29. WEBER, K. AND OSBORNE, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244: 4406-4412.