JOURNAL OF BACTERIOLOGY, Feb. 1976, p. 531-539

Vol. 125, No. 2
Printed in U.S.A.

Carbon Dioxide Assimilation in Blue-Green Algae: Initial Studies on the Structure of Ribulose 1,5-Bisphosphate Carboxylase

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Received for publication 20 October 1975

D-Ribulose 1,5-bisphosphate carboxylase was purified from the blue-green alga *Anabaena cylindrica* (Lemm) by procedures involving acid precipitation, ammonium sulfate fractionation, and Sephadex G-200 gel filtration. The enzyme was homogeneous by the criterion of polyacrylamide disc gel electrophoresis and was a multimer of a single-size polypeptide chain of 54,000 daltons. The carboxylases from four species of blue-green algae (*Anabaena*, *Nostoc* strain MAC, *Agmenellum quadruplicatum* strain PR-6, and *Anacystis nidulans* strain TX20) were closely similar in molecular size, since enzyme activity was eluted at the same volume after sucrose gradient centrifugation. Further analysis by gel filtration indicated that the four blue-green algal carboxylases were nearly identical in molecular weight, ranging from 449 to 453,000. The amino acid composition of the *Anabaena* carboxylase was determined and was found to resemble closely the composition of the large subunit from eukaryotic photosynthetic organisms.

Photolithotrophic organisms, such as the blue-green algae, obtain the necessary amounts of cell carbon needed for cell growth by reducing atmospheric carbon dioxide into organic matter. The pathway for this reductive biosynthesis is almost exclusively the Calvin cycle in blue-green algae (8, 16). The primary step in this biosynthetic pathway is the initial carboxylation, a step in which ribulose 1,5-bisphosphate (RuBP) is carboxylated to yield a 6-carbon carboxylated intermediate (2-carboxy-3-keto-D-ribitol 1,5-bisphosphate) (18, 19), which is subsequently cleaved between carbon atoms 2 and 3 to yield 2 molecules of 3-phosphoglyceric acid; the phosphoglyceric acid is then funneled into the mainstream of intermediary metabolism. Thus, for every molecule of RuBP carboxylated, 2 molecules of phosphoglyceric acid are produced as a result of CO_2 fixation.

The blue-green algae are prokaryotic organisms, yet the photosynthetic capacity of these organisms closely approximates the photosynthetic process catalyzed by eukaryotic algae and higher plants. For the past several years we have been interested in the structure, function, and regulation of RuBP carboxylase (3-phospho-D-glycerate carboxylase [dimerizing], EC 4.1.1.39) from photosynthetic microorganisms, the enzyme that catalyzes the primary carboxylation reaction. Thus, in order to gain some insight into the CO_2 fixation process of the ecologically important blue-green algae, we attempted in this investigation to elucidate some of the structural properties of RuBP carboxylase from four species of blue-green algae.

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MATERIALS AND METHODS

Growth of organisms. *Agmenellum quadruplicatum* strain PR-6 and *Nostoc* sp. strain MAC were obtained from C. Van Baalen. *Anacystis nidulans* strain TX20 was provided by Jack Myers, and *Anabaena cylindrica* (Lemm) was provided by G. A. Codd. *Agmenellum* was grown on the medium and under the conditions reported by Tabita et al. (26). *Nostoc* and *Anabaena* were grown on medium B of Stevens et al. (20) at 35 and 27 C, respectively, with 1% CO_2 in air (vol/vol) bubbled through the cultures. For *Nostoc*, illumination was provided by four F24T12 CW/HO fluorescent lamps, two on each side of the bath, 8 cm from the growth tubes. For *Anabaena*, illumination was provided by eight 60-W tungsten lamps, four on each side of the bath. *Anacystis* was grown on medium C_s of Stevens et al. (20) at 39 C, 1% CO_2, and illumination provided as above for *Nostoc*.

Preparation of cell extracts. Cells were harvested, washed, and frozen at −20 C as previously described (26). At the desired time, the frozen cells were thawed and suspended in 2 volumes of a buffer containing 20 mM tris(hydroxymethyl)aminomethane-sulfate, pH 8.0 (25 C), 1 mM ethylenediaminetetraacetate, 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM 2-mercaptoethanol, and 50 mM $NaHCO_3$ (TEMMB buffer). The cell suspension

was then disrupted by sonic oscillation with a Bronwill Biosonic III sonic oscillator using 1-min bursts of sonic energy; each sonic treatment period was followed by immersing the oscillator probe in ice water before starting the next period of sonic oscillation. The suspension was then centrifuged for 15 min at 15,000 × *g*, and the pellet was discarded. The resulting supernatant fluid was then further centrifuged at 100,000 × *g* for 60 min to remove all particulate material. The resulting high-speed supernatant fluid from this last centrifugation was the source of crude, soluble RuBP carboxylase.

Assay of protein. Protein was determined by the method of Lowry et al. (12), using bovine serum albumin to standardize the assay.

Enzyme assay. RuBP carboxylase was assayed as previously described (22). In all cases, activities are reported in terms of RuBP-dependent CO_2 fixation; 1 unit of enzyme is the amount of enzyme needed to carboxylate 1 μmol of RuBP in 1 min.

Electrophoresis. Polyacrylamide disc gel electrophoresis was used to assess the purity of all preparations. Samples were loaded onto 7.5% acrylamide gels and subjected to electrophoresis at pH 9.3, using the Buchler anionic gel system (22). A current of 1 mA/gel was used until the bromophenol blue tracking dye (0.001%) entered the gels; at this time, the current was increased to 3 mA/gel and maintained at this current until the tracking dye was approximately 1 cm from the end of the gels. The gels were stained in 1% Amido Schwartz in 7.5% acetic acid for 30 min and then destained electrophoretically in 7.5% acetic acid.

To determine the subunit composition of the purified carboxylases, sodium dodecyl sulfate (SDS)-gel electrophoresis was used according to the procedures of Weber et al. (27), using 10% gels. A current of 8 mA/gel was used until the tracking dye was 1 to 2 cm from the end of the gel. The SDS-gels were then measured and stained in the Coomassie blue staining reagent (25) and then destained by the procedure of Fairbanks et al. (3).

Purification procedures. Two procedures were used to purify the RuBP carboxylase from blue-green algae. The first procedure, following conventional gel filtration techniques, was used to purify the RuBP carboxylase from *Anabaena cylindrica*. All purification steps and centrifugations were done at 0 to 2 C.

The crude, 100,000 × *g* supernatant fraction obtained from extracts of *Anabaena* was taken to pH 5.4 by the dropwise addition of 1 N acetic acid. After standing for 60 min in an ice bath, the highly pigmented cloudy suspension was centrifuged at 30,000 × *g* for 15 min. The deep-blue supernatant fraction, containing about 20% of the enzyme units and nearly all the C-phycocyanin, was discarded. The pellet fraction, containing 80% of the total units, was taken up in TEMMB buffer and resuspended in this buffer. To the resuspended pellet was added solid ammonium sulfate until 25% saturation was reached. This suspension, after standing in an ice bath for 60 min, was centrifuged at 30,000 × *g* for 15 min. The green pellet, which was devoid of RuBP carboxylase activity, was discarded. The supernatant fluid was further fractionated with solid ammonium sulfate to 45% saturation. After standing for 1 h as before, the cloudy suspension was centrifuged at 30,000 × *g* to obtain a greenish-blue pellet that contained all the remaining RuBP carboxylase activity. This pellet was resuspended in a small volume of TEMMB buffer (usually about 4 ml), recentrifuged to remove any denatured protein, and saved for further purification. At this point, the *Anabaena* RuBP carboxylase had a specific activity of 0.578, representing about a sevenfold purification over the crude 100,000 × *g* supernatant fraction, which had an initial specific activity of 0.085.

After ammonium sulfate fractionation, the greenish-blue enzyme solution (3 ml) was applied to a Sephadex G-200 column (2.5 by 90.5 cm). The column was equilibrated and eluted with TEMMB buffer in an upward direction at a flow rate of 21.4 ml/h, and 3-ml fractions were automatically collected. In some purifications, 0.5 ml was applied to a Sephadex G-200 column (1 by 100 cm) equilibrated with TEMMB buffer, and 1.6-ml fractions were collected from this column. This column was also used for molecular weight determinations. Active fractions eluted from the column were pooled and treated with solid ammonium sulfate to 0.60 saturation in ammonium sulfate. The precipitate was taken up in 1 to 2 ml of TEMMB buffer and dialyzed against this buffer overnight. This purified *Anabaena* RuBP carboxylase was stored at 2 C and remained stable for 2 weeks.

In some cases, RuBP carboxylase was purified from extracts of blue-green algae by a sucrose density gradient technique (24, 26). The 100,000 × *g* supernatant fluid obtained from cell extracts of *Anabaena*, *Agmenellum*, *Anacystis*, and *Nostoc* were layered onto discontinuous 0.2 to 0.8 M sucrose gradients as previously described (24, 26), and fractions were collected from the bottom of the centrifuge tubes after 20 to 24 h of centrifugation at 131,000 × *g*.

Molecular weight studies. Molecular weights were determined by gel filtration using a calibrated Sephadex G-200 column (1 by 100 cm) or by the electrophoretic technique of Hedrick and Smith (6). Standards of known molecular weight (2) were used to calibrate the column and to standardize the gels. When using the electrophoretic technique, the mobility of each standard protein was plotted against the percentage of gel concentration. Straight lines for each standard protein were obtained; a straight line was also obtained for the *Agmenellum* carboxylase. The slopes of these lines for each protein were then determined and plotted against the molecular weight for the protein in question (6). Thus, a standard curve was constructed that could be used to determine the molecular weight of the *Agmenellum* RuBP carboxylase.

In addition to the commercially available standards, *Rhodospirillum rubrum* RuBP carboxylase (molecular weight, 114,000) (23), *Thiobacillus* A2 protocatechuic acid oxygenase (molecular weight, 600,000) (M. Wells, personal communication), and *Agmenellum* C-phycocyanin (molecular weight, 210,000) (9; S. E. Stevens, Jr., and L. Fox, unpublished observations) were used as standards.

Amino acid analysis. Homogeneous *Anabaena*

RuBP carboxylase was dialyzed exhaustively against 0.05 M NH_4HCO_3. Samples of 88 μg were transferred to hydrolysis vials and lyophilized, and 0.5 ml of 6 N HCl was added. Vials were then degassed, sealed in vacuo, and hydrolyzed at 110 C for the listed times. Hydrolysates were analyzed with an automatic amino acid analyzer designed by J. L. Fox (4).

Reagents. All special chemicals were from the Sigma Chemical Co.; $Na_2{}^{14}CO_3$ (20 mCi/mmol) was obtained from Amersham-Searle, Inc.

RESULTS

Purification of RuBP carboxylase from blue-green algae. An alternative to the previously used sucrose density gradient purification was sought (26) for the purpose of obtaining quantities of homogeneous RuBP carboxylase suitable for physical and chemical analysis. It was found that acid treatment of *Anabaena cylindrica* extracts followed by ammonium sulfate fractionation and Sephadex G-200 gel filtration nicely separated the RuBP carboxylase from the bulk soluble proteins and the blue-pigmented C-phycocyanin (Fig. 1). The enzyme was eluted from the column as a sharply defined band of activity between a large-molecular-weight green-blue protein component that absorbed maximally at 680 nm and a darker-blue C-phycocyanin protein component that absorbed maximally at 625.5 nm. The fractions containing RuBP carboxylase were colorless and were all homogeneous by the criterion of polyacrylamide disc gel electrophoresis (Fig. 2). Similar results were obtained at several concentrations of acrylamide. RuBP carboxylase purified in this manner had a specific activity of 2.1, representing about a 25-fold purification over crude extracts. Usually a 10 to 15% recovery of enzyme units is obtained by this purification procedure. Similarly, the 45% ammonium sulfate precipitate fractions of *Agmenellum* RuBP carboxylase (without prior acid treatment) responded similarly upon gel filtration on columns of Sephadex G-200 (Fig. 3). However, results were variable concerning the homogeneity of the *Agmenellum* carboxylase fractions. Active fractions of impure *Agmenellum* RuBP carboxylase eluted from the G-200 column could, however, be pooled and rechromatographed on Sephadex or placed on sucrose gradients to obtain homogeneous RuBP carboxylase.

As previously reported, small amounts of homogeneous RuBP carboxylase could be obtained by centrifuging crude extracts of *Ag-*

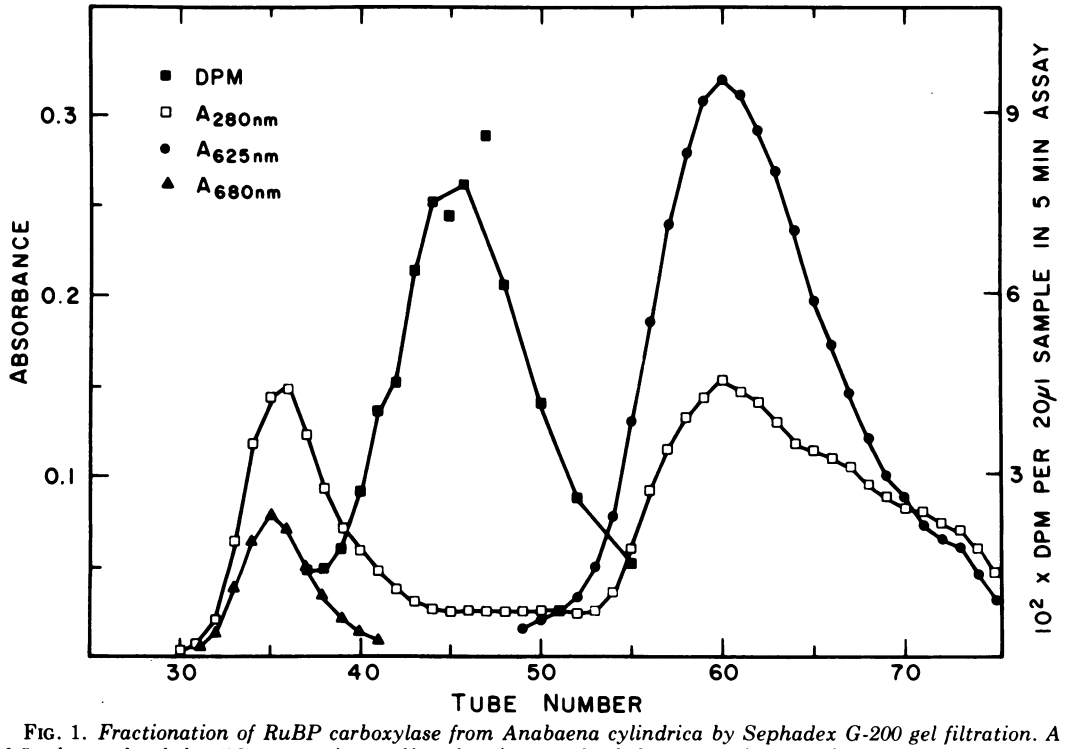

FIG. 1. *Fractionation of RuBP carboxylase from Anabaena cylindrica by Sephadex G-200 gel filtration. A 0.5-ml sample of the 45% ammonium sulfate fraction was loaded onto a column (1 by 100 cm), and 1.6-ml fractions were collected.*

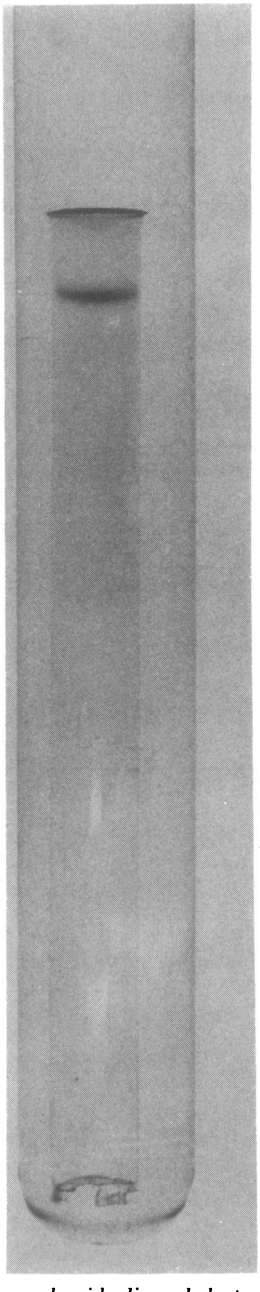

FIG. 2. *Polyacrylamide disc gel electrophoretogram of Anabaena cylindrica RuBP carboxylase. A 20-μg sample was applied to a 7.5% acrylamide gel as previously described (22).*

menellum into a sucrose gradient (26). We have extended these results and have found that the RuBP carboxylase in crude extracts of *Anabaena*, *Anacystis*, and *Nostoc* is well separated from the bulk soluble and pigmented proteins upon sucrose density gradient centrifugation (Fig. 4). Moreover, peak fractions of *Anabaena* RuBP carboxylase after density gradient centrifugation were found to be homogeneous, with specific activities ranging from 1.3 to 1.7. Crude extracts from *Nostoc* and *Anacystis* had very low initial specific activities for RuBP carboxylase, ranging from 0.005 to 0.011. Upon elution from the sucrose gradients, neither the *Nostoc* nor the *Anacystis* carboxylase-containing fractions were homogeneous. Moreover, the specific activities obtained were low, ranging from 0.4 to 0.6. Thus, the *Nostoc* and *Anacystis* carboxylases were not further studied in this investigation. The sucrose gradient experiment does illustrate, however, that the RuBP carboxylase from four different species of blue-green algae is constant in molecular size; maximal enzyme activity was eluted at 15 to 16 ml in each gradient (Fig. 4).

Molecular weight studies. The elution profile obtained from the sucrose gradients indicated that the RuBP carboxylase from the blue-green algae is a fairly large protein. Thus, molecular weight studies were initiated to further delineate the molecular size of this protein. Using a calibrated Sephadex G-200 column, the molecular weights for the carboxylase from the four species of blue-green algae were determined to range from 449,000 to 453,000 (Fig. 5). These experiments were performed several times, using partially purified extracts (through the ammonium sulfate step). It should be noted that upon elution from the calibrated column, the carboxylases were highly purified and, in the case of *Anabaena* and *Agmenellum*, homogeneous. Table 1 summarizes the gel filtration molecular weight data obtained for each carboxylase.

The gel filtration data were further corroborated by the electrophoretic technique of Hedrick and Smith (6) for molecular weight determinations. With homogeneous *Agmenellum* RuBP carboxylase and several standards of known molecular weight at six different concentrations of acrylamide, a molecular weight of approximately 460,000 was obtained (Fig. 6). This value is quite close to the molecular weight determined by gel filtration (Fig. 5).

Quaternary structure. RuBP carboxylase from eukaryotic algae (5, 7, 10, 13, 21) is a large molecule (molecular weight ≅ 550,000) and is composed of two different-size subunits, a large catalytic subunit of about 55,000 daltons and a small polypeptide of about 15,000 daltons. By contrast, however, the carboxylases from the photosynthetic bacteria *R. rubrum* (23) and *Chlorobium thiosulfatophilum* (25) lack the small subunit. Since the blue-green algae are

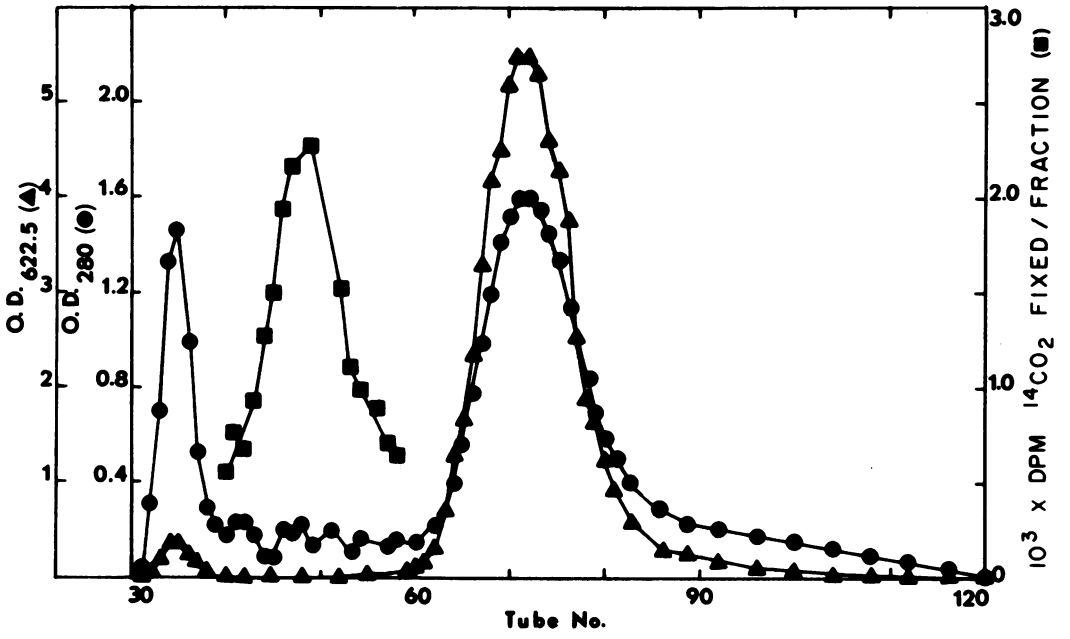

FIG. 3. *Fractionation of RuBP carboxylase from Agmenellum quadruplicatum by Sephadex G-200 gel filtration. A 3-ml portion of the 45% ammonium sulfate fraction was applied to a column (2.5 by 90.5 cm), and 3-ml fractions were collected.*

prokaryotic organisms but resemble the eukaryotic algae and higher plants in their ability to generate oxygen during photosynthesis, it was of interest to examine the subunit composition of the blue-green algal enzyme. In these experiments, *Anabaena* RuBP carboxylase was used, since it could be obtained in appreciable amounts. After denaturation of the protein in SDS in the presence of 2-mercaptoethanol, the *Anabaena* carboxylase was subjected to electrophoresis in the presence of SDS according to the procedures of Weber et al. (27). Only one electrophoretic species (Fig. 7) was obtained, as would be expected if there is a single type of polypeptide in the native protein. Comparison of the mobility of this polypeptide to the mobility of several standards of known subunit molecular weight (Fig. 8) showed the *Anabaena* RuBP carboxylase subunit to have a molecular weight of 54,000.

Amino acid composition. Table 2 shows the results of timed hydrolyses of RuBP carboxylase from *Anabaena*. Values for amino acids exhibiting changes with hydrolysis time were obtained by the appropriate extrapolation. When the amino acid composition of the *Anabaena* enzyme was normalized with respect to the phenylalanine content and compared with the compositions of the large subunits of spinach and *Chlorella*, the data in Table 3 were obtained. As a further comparison, the composition of the *R. rubrum* enzyme is included in this table. These data show that the amino acid composition of the *Anabaena* enzyme resembles the composition of the large subunit from the eukaryotic protein and the enzyme from *R. rubrum*, a protein that lacks the small subunit (23).

DISCUSSION

Considerable interest has recently been shown concerning the physiology and biochemistry of the blue-green algae, particularly since these organisms have been implicated in the formation of natural blooms in freshwater, estuarine, and marine environments. In order for these organisms to be biologically successful, they must incorporate atmospheric carbon dioxide efficiently into cellular material, the initial step of which is catalyzed by RuBP carboxylase. Previously, it was found that small amounts of *Agmenellum* RuBP carboxylase could be isolated in homogeneous form simply by centrifuging crude extracts into a sucrose gradient (26). In the present investigation, we were able to obtain sufficient quantities of homogeneous RuBP carboxylase from *Anabaena* for the desired structural studies. The key step in this purification involved a preliminary acid treatment to separate the enzyme from the bulk of the ubiquitous blue C-phycocyanin. After treat-

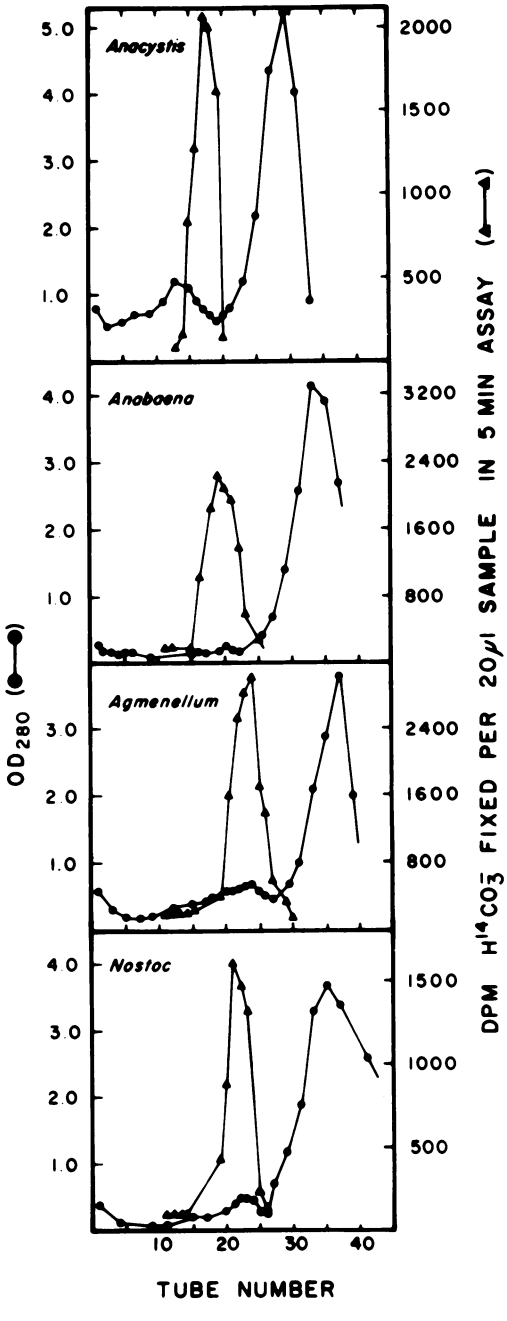

FIG. 4. *Sucrose density gradient fractionation of Anacystis nidulans, Anabaena cylindrica, Agmenellum quadruplicatum, and Nostoc sp. RuBP carboxylase. Approximate 1-ml fractions were collected from the bottom of the centrifuge tube. Tube fractions from the gradients do not correspond exactly since the volumes of the fractions vary slightly from gradient to gradient. Maximal RuBP carboxylase activity was eluted at a volume between 15 to 16 ml for each gradient.*

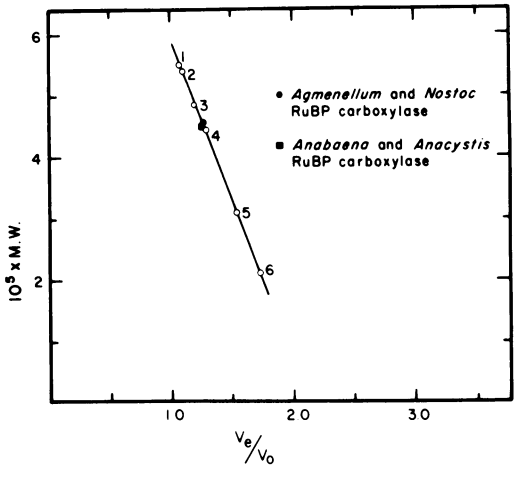

FIG. 5. *Molecular weight determination of Anacystis nidulans, Anabaena cylindrica, Agmenellum quadruplicatum, and Nostoc sp. RuBP carboxylase by Sephadex G-200 gel filtration. A column (1 by 100 cm) of Sephadex G-200 was run against gravity at a flow rate of 11.7 ml/h, and 1.5 ml fractions were collected. The following standards were used: (1) Spinach RuBP carboxylase (molecular weight, 560,000); (2) Escherichia coli β-galactosidase (molecular weight, 540,000); (3) jack bean urease (molecular weight, 483,000); (4) horse spleen apoferritin (molecular weight, 445,000); (5) glutamate decarboxylase (molecular weight, 310,000); (6) Agmenellum C-phycocyanin (molecular weight, 210,000). Symbols: ●, Agmenellum and Nostoc RuBP carboxylase; ■, Anabaena and Anacystis RuBP carboxylase.*

TABLE 1. *Summary of Sephadex G-200 gel filtration molecular weight determinations*

Enzyme source	Mol wt
Agmenellum quadruplicatum (PR-6)	453,000 (7)[a]
Anabaena cylindrica	449,000 (4)
Anacystic nidulans (TX20)	449,000 (2)
Nostoc sp. (MAC)	453,000 (2)

[a] Numbers in parentheses indicate the number of determinations for each protein.

ment with ammonium sulfate, the *Anabaena* RuBP carboxylase was nicely separated from any remaining protein contaminants (mainly C-phycocyanin) by Sephadex G-200 chromatography and was obtained in a homogeneous form of very high specific activity. Sucrose density gradient centrifugation established that the RuBP carboxylase from *Anabaena cylindrica*, *Agmenellum quadruplicatum*, *Anacystis nidulans*, and *Nostoc* sp. (MAC) were of similar large molecular weight. Indeed, values obtained from gel filtration molecular weight analysis of all four proteins were extremely close, ranging

from 449,000 to 453,000. In addition, an electrophoretic molecular weight determination gave a value of 460,000 for the *Agmenellum* protein. Thus, with four carboxylases isolated from four taxonomically diverse blue-green algae, molecular weights consistently lower than that reported for the enzyme from eukaryotic algae and higher plants were found, the carboxylase from eukaryota having a molecular weight of about 550,000 (14). This difference is particularly evident since spinach RuBP carboxylase was used as a standard in the molecular weight studies. Moreover, the enzyme from eukaryotic sources is composed of both large, catalytic (~55,000 daltons) and small (~15,000 daltons) subunits. Presumably eight of the large and eight of the small subunits comprise the native enzyme in eukaryota (1). Noteworthy is the quaternary structure of the *Anabaena* carboxylase. Only one subunit of approximately 54,000 daltons was found, similar to a previous report concerning the structure of the *Agmenellum* protein (26). Thus, it is evident from these results that, with at least two of the blue-green algal RuBP carboxylases, only one size of polypeptide comprises the native protein. It is possible, however, that during isolation of the pure enzyme the small subunit is lost. Presumably, then, the RuBP carboxylases isolated from *Anabaena* and *Agmenellum* in this investigation are octomeric proteins containing eight of the large type subunits to form a stable oligomeric form of RuBP carboxylase. Whether or not the subunits are chemically identical awaits

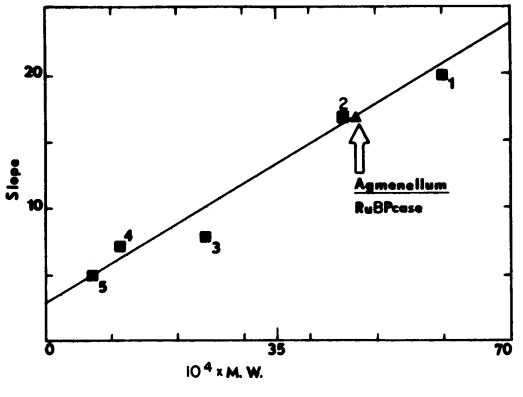

FIG. 6. *Electrophoretic determination of the molecular weight of Agmenellum RuBP carboxylase. A 10-μg portion of homogeneous RuBP carboxylase was subjected to electrophoresis at acrylamide concentrations ranging from 4 to 7.5% In addition, five standards of known molecular weight were subjected to electrophoresis at the same time. The standards used were: (1) protocatechuic acid oxygenase; (2) apoferritin; (3) catalase; (4) R. rubrum RuBP carboxylase; and (5) malate dehydrogenase.*

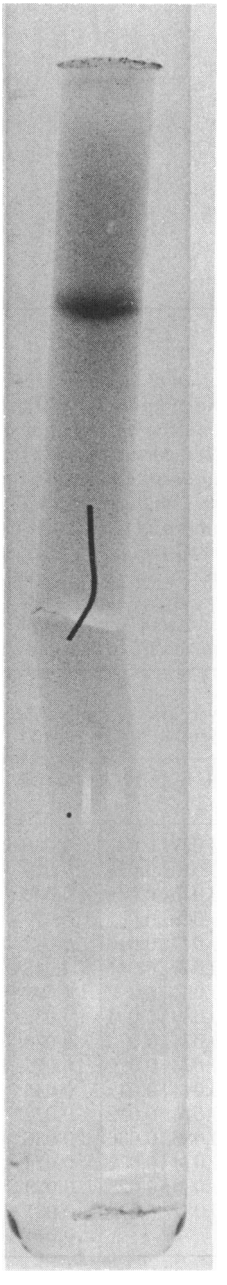

FIG. 7. *SDS electrophoretogram of RuBP carboxylase from Anabaena cylindrica. A 40-μg sample of enzyme was dissociated and subjected to electrophoresis as discussed in the text.*

further experimentation. Moreover, it appears that the blue-green algal enzyme is smaller than the enzyme from eukaryota by virtue of lacking the small-type subunit. It will be fascinating to determine what properties are conferred upon this protein by virtue of the presence of the

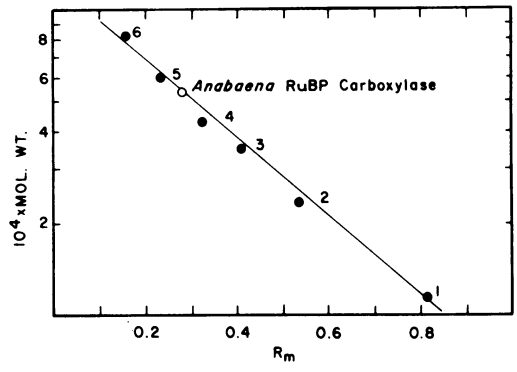

FIG. 8. *Estimation of molecular weight of SDS-dissociated Anabaena cylindrica RuBP carboxylase. The logarithm of the molecular weight of several standard proteins was plotted versus the electrophoretic mobility (R_m) of the standards and the Anabaena cylindrica RuBP carboxylase. Standards used were: (1) cytochrome c; (2) gamma globulin; (3) lactate dehydrogenase; (4) ovalbumin monomer; (5) catalase; and (6) ovalbumin dimer.*

TABLE 2. *Amino acid composition of Anabaena cylindrica RuBP carboxylase*[a]

Amino acid residue	Recovery after hydrolysis (μmol)			Extrapolated[*] or avg value (μmol)	Nearest whole no. of amino acid residues/ molecule of protein (mol wt, 449,000)
	24 h	48 h	72 h		
Lys	0.040	0.037	0.037	0.038	195
His	0.021	0.019	0.018	0.019	98
Arg	0.049	0.045	0.044	0.046	236
Asp	0.075	0.068	0.067	0.077*	396
Thr	0.047	0.047	0.046	0.047	241
Ser	0.046	0.041	0.041	0.046*	236
Glu	0.096	0.093	0.092	0.094	483
Pro	0.032	0.030	0.034	0.032	164
Gly	0.073	0.074	0.073	0.073	375
Ala	0.074	0.074	0.072	0.073	375
Val	0.046	0.044	0.045	0.045	231
Met	0.018	0.014	0.012	0.022*	113
Ile	0.030	0.036	0.036	0.036*	185
Leu	0.076	0.074	0.072	0.074	380
Tyr	0.030	0.030	0.028	0.029	149
Phe	0.031	0.032	0.030	0.031	159
Cys				0.014[b]	72

[a] Protein sample size, 88 μg.
[b] Measured as cysteic acid (in dimethyl sulfoxide).

second polypeptide. Comparisons of properties of the blue-green algal enzyme with the enzyme from eukaryotic algae should thus prove rewarding, particularly since antisera to *Euglena* carboxylase strongly cross-react with extracts of blue-green algae (11). Certainly it is not surprising that the amino acid compositions of the large subunits of spinach and *Chlorella* carboxylase closely resemble the composition of the *Anabaena* enzyme. Indeed, using the statistical analysis of Marchalonis and Weltman (15), an SΔQ value of 16 is obtained when the compositions of the *Anabaena* enzyme and *Chlorella* large subunit are compared. Similar comparisons yielded values of 20 for *R. rubrum* and *Chlorella*, 15 for *Chlorella* and spinach, 35 for *R. rubrum* and spinach, 20 for *Anabaena* and spinach, and 36 for *Anabaena* and *R. rubrum*. Values of SΔQ less than 50 are indicative of amino acid homology (15). Thus the structural resemblance, similar amino acid composition, and immunological relatedness between the protein found in the chloroplasts of eukaryotic algae (large subunit of RuBP carboxylase) and the prokaryotic *Anabaena* carboxylase might have deep significance in developing a coherent picture of the evolution of this important catalyst.

TABLE 3. *Comparison of amino acid compositions of RuBP carboxylase from eukaryotic and prokaryotic species*[a]

Amino acid	Source			
	Anabaena cylindrica	*Chlorella* subunit 1[b]	Spinach subunit 1[c]	*Rhodospirillum rubrum*[d]
Asp	2.48	2.37	2.18	2.54
Thr	1.52	1.57	1.75	1.40
Ser	1.48	0.98	0.81	1.39
Glu	3.03	2.63	2.20	2.44
Pro	1.03	1.36	1.13	1.53
Gly	2.36	2.57	2.10	2.77
Ala	2.36	2.72	2.16	3.47
Val	1.45	1.24		1.62
Ile	1.16	0.84	0.88	1.11
Leu	2.39	2.23	2.09	1.96
Tyr	0.93	0.98	0.92	0.61
Phe	(1.00)	(1.00)	(1.00)	(1.00)
Lys	1.23	1.01	1.18	1.05
His	0.61	0.63	0.67	0.54
Arg	1.48	1.74	1.44	1.54
Cys	0.45			0.25
Met	0.71	0.45	0.42	0.65

[a] Compositions normalized with respect to mole content of Phe.
[b] Data of Sugiyama et al. (21) for the large subunit of *Chlorella ellipsoidea* RuBP carboxylase.
[c] Data of Rutner and Lane (17) for the large subunit of spinach RuBP carboxylase.
[d] Data of Tabita and McFadden (23) for RuBP carboxylase.

ACKNOWLEDGMENTS

This investigation was supported by National Science Foundation grant GB-43268 and by a grant from the University Research Institute of the University of Texas at Austin to F.R.T. S.E.S. was supported by Public Health Service

postdoctoral fellowship GM-52117 from the National Institute for General Medical Sciences.

We would like to thank Jack Myers for the generous loan of his growth facilities. Larry Fox for the use of his amino acid analyzer, and Catherine Stevens for culturing the *Anacystis* cells. The senior author is particularly grateful to the staff of the University of Texas Marine Science Institute at Port Aransas for many courtesies extended during the time when this manuscript was completed.

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