D-RIBULOSE 1, 5-DIPHOSPHATE CARBOXYLASE FROM BLUE-GREEN ALGAE[1]

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SUMMARY: D-Ribulose 1, 5-diphosphate carboxylase has been purified to a state of homogeneity from the marine blue-green alga Agmenellum quadruplicatum strain PR-6. The enzyme has been found to be easily separated from the bulk soluble protein by means of centrifugation into a sucrose gradient. RuDP carboxylase from Agmenellum, upon chromatography using a calibrated Sephadex G-200 column, exhibits a molecular weight of 456,000 daltons, considerably smaller than the protein from eucaryotic algae. Only one polypeptide of approximately 56,000 daltons was obtained upon dissociation in sodium dodecylsulfate.

D-Ribulose 1, 5-diphosphate (RuDP) carboxylase (3-phospho-D-glycerate carboxylase (dimerizing), EC 4.1.1.39) catalyzes primary carbon dioxide fixation in photosynthetic and chemosynthetic organisms (1). The enzyme has recently been purified and characterized from higher plants, green algae, and several photosynthetic and chemosynthetic bacteria (2-10). RuDP carboxylase ranges in size from: the small 114,000 dalton protein from Rhodospirillum rubrum (12); to the intermediate size protein from Chlorobium thiosulfatophilum (MW: 360,000) (13) and Thiobacillus dentrificans (MW: 350,000) (14); and the large molecular weight protein (MW$\geq$ 500,000 daltons) characteristic of higher plants, green algae and various chemosynthetic bacteria and photosynthetic bacteria belonging to the Thiorhodaceae (2-10). In addition, the large molecular weight carboxylases are composed of eight large polypeptide chains of approximately 56,000 daltons each and eight small subunits of about 15,000

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daltons each (3-7). The intermediate and small sized proteins thus far examined are devoid of the small subunit (12, 13). It will be the purpose of this paper to describe the facile isolation of homogeneous RuDP carboxylase from a marine blue-green alga, Agmenellum quadruplicatum strain PR-6. The blue-green algae are procaryotic organisms, yet they perform a plant-type photosynthesis, and thus presumably represent an evolutional link between the eucaryotic algae and the photosynthetic bacteria.

MATERIALS AND METHODS

Agmenellum quadruplicatum, strain PR-6 is a coccoid marine isolate provided by Van Baalen (15). The growth medium in g/l consisted of: Na_2EDTA, 0.03; NaCl, 18.0, $MgSO_4 \cdot 7H_2O$, 5.0; KCl, 0.6; $CaCl_2 \cdot 2H_2O$, 0.37; $NaNO_3$, 7.0; KH_2PO_4 1.1, tris (hydroxymethyl) amino methane, 1.0, adjusted to pH 8.2 with HCl. $FeCl_3 \cdot 6H_2O$ was used at a concentration of 8 mg/l. 10 ml of the trace metal mix for medium A (16) was used per liter of medium. After autoclaving, the pH of the medium was 6.5, but upon introduction of 5% CO_2 in air (v/v), the pH rose to 7.6± 0.1. PR-6 was grown in culture chambers scaled up (5:1) from the original design of Myers and Clark (17). The total culture volume was 2.5 l. The average cell yield was 13± 2 g dry weight of cells/day. Illumination was provided by four F48T12-WW-VHO fluorescent lamps. Cells were stored frozen at $-20°C$ until needed. At such time, cells were thawed in a buffer consisting of 20 mM Tris-SO_4, pH 8.0 (25°C), 1 mM EDTA, 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM 2-mercaptoethanol and 50 mM $NaHCO_3$ (TEMMB). The mass ratio of wet-packed cells to buffer was two to one. This cell suspension was then sonically disrupted for a period of 1 min/ml of cell suspension. The suspension was then centrifuged at 40,000 x g for 15 min. The resulting supernatant was then further centrifuged at 100,000 x g for 60 min. The high speed supernatant from this last centrifugation was the source of crude soluble RuDP carboxylase. Saturated ammonium sulfate (pH 7.7 with NH_4OH) was then added dropwise to 50% saturation. After standing for 60 min at 0°, the enzyme was collected by centrifugation as a 50% ammonium sulfate precipitate. The precipitate was then

resuspended in ice-cold TEMMB and dialyzed overnight. The dialyzed ammonium sulfate fraction was then applied to a 0.2-0.8 M sucrose density gradient exactly as described by Tabita and McFadden (18); 1 ml fractions were collected after 20-24 hrs of centrifugation at 131,000 x g.

RuDP carboxylase was assayed as previously described (10); one unit of enzyme is the amount of enzyme needed to carboxylate one μmole of RuDP in one min. Protein was determined by the method of Lowry et al (19).

Disc gel and sodium dedecylsulfate polyacrylamide gel electrophoresis were performed as previously described (11).

RESULTS

Sucrose density gradient centrifugation nicely separated the RuDP carboxylase contained in the crude ammonium sulfate fraction from the bulk protein (Fig. 1).

Prior to density gradient centrifugation, the ammonium sulfate fraction

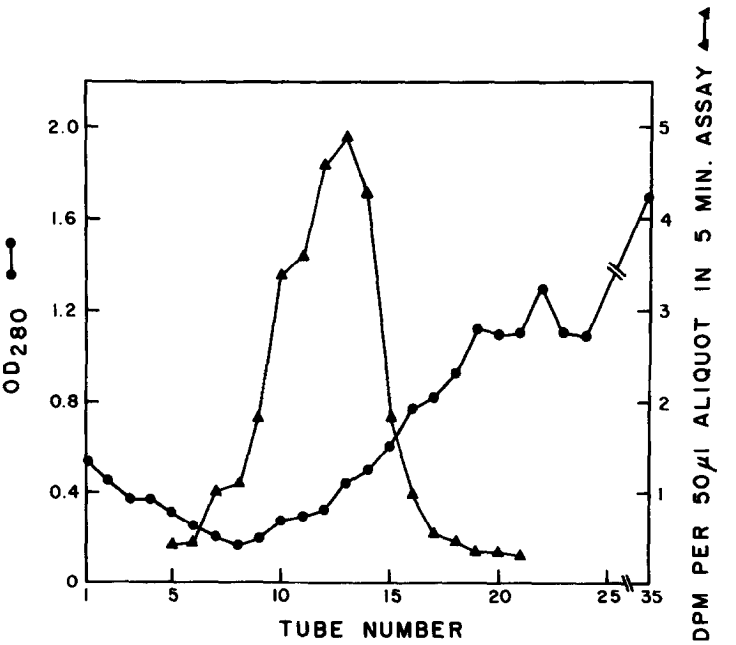


Fig. 1. Sucrose density gradient fractionation of Agmenellum RuDP carboxylase. 1 ml. fractions were collected from the bottom of the centrifuge tube (tubes 1-35).

had a specific activity of 0.031 units/mg protein. Upon elution from the sucrose gradient, peak fractions had a specific activity of 0.95 representing a 47-fold purification over the crude high speed supernatant which had a specific activity of 0.02. Moreover, over 60% of the enzyme units initially applied to the gradient were recovered in the peak fractions.

The sucrose gradient fractions were homogeneous by the criterion of polyacrylamide disc gel electrophoresis (Fig. 2). The experiment shown in

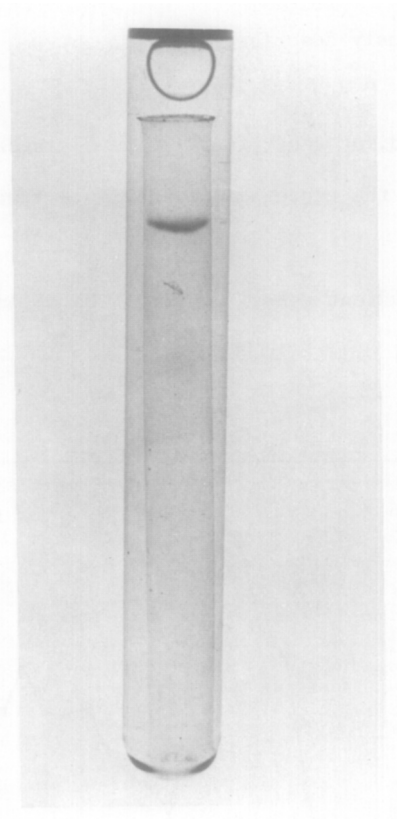


Fig. 2. Polyacrylamide disc gel electrophoretogram of Agmenellum RuDP carboxylase. 10 µg of protein were applied to 7.5% gels as previously described (11).

Fig. 2 was performed using a gel polymerized with 7.5% acrylamide. Subsequent experiments using gels polymerized from several concentrations of acrylamide, also showed one stained band after electrophoresis, eliminating the possibility of a contaminant of similar charge but different molecular weight (19).

From the enzyme elution profile in the sucrose gradient experiment and the protein migration in polyacrylamide gels, the Agmenellum RuDP carboxylase appears to be a fairly large protein (18). Molecular weight studies, using a calibrated Sephadex G-200 column, indicate that the Agmenellum RuDP carboxylase has a molecular weight of 456,000 (Fig. 3). These results have been corroborated by

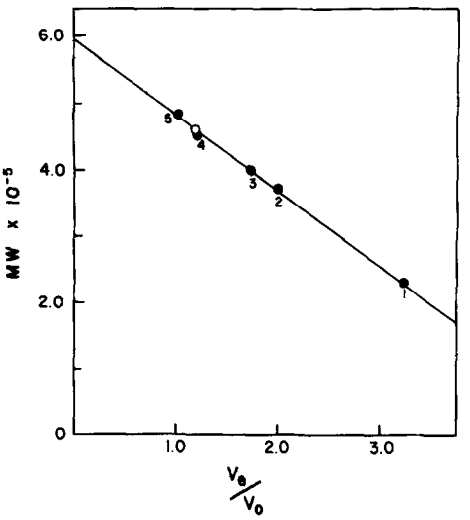
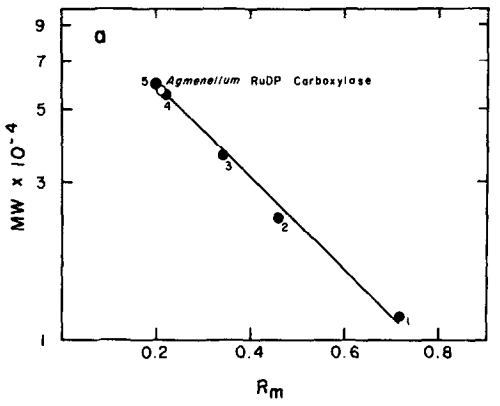


Fig. 3. Molecular weight determination of Agmenellum RuDP carboxylase by Sephadex G-200 gel filtration. A column (2.5 x 90.5 cm) of Sephadex G-200 was run against gravity at a flow rate of 21.4 ml/hr.; 3.2 ml. fractions were collected. The following standards were employed: catalase (1), L-glutamate decarboxylase (2), Agmenellum C-phycocyanin (3), ferritin (4), and urease (5). The elution of a 3 ml. aliquot of an ammonium sulfate fraction from Agmenellum was compared to the elution of the standard proteins. The experimentally generated line was drawn by the method of least squares. The equation describing this straight line is $y = (-110,841)x + 590,432$.

determinations using the electrophoretic method of Hedrick and Smith (20) (data not shown).

After dissociation and electrophoresis of the Agmenellum RuDP carboxylase in sodium dodecylsulfate-polyacrylamide gels, only one polypeptide species was observed (Fig. 4a). This protein, when compared to the electrophoretic mobilities of several standards, gave a molecular weight of about 56,000 (Fig. 4b). Moreover, the RuDP carboxylase from Agmenellum did not contain a



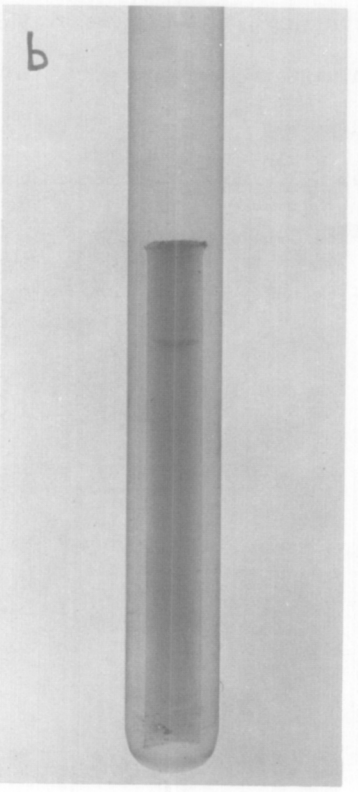


Fig. 4. (a) Log molecular weight vs. electrophoretic mobility (Rm) of standards and Agmenellum RuDP carboxylase in sodium dodecylsulfate polyacrylamide gels. Standards employed were: catalase (1), R. rubrum RuDP carboxylase (2), phosphoglyceraldehyde dehydrogenase (3), γ-globulin, L-chain (4), cytochrome C (5). (b) Sodium dodecylsulfate electrophoretogram of 20 μg of Agmenellum RuDP carboxylase.

small type subunit typical of the enzyme from eucaryotic algae and higher plants (1, 3-6).

DISCUSSION

This investigation reports the first isolation of homogeneous RuDP carboxylase from blue-green algae. Advantage has been taken of the large molecular size of the carboxylase in separating this enzyme from the bulk soluble protein. Moreover, we have succeeded in using the technique of sucrose density gradient centrifugation in purifying the RuDP carboxylase from several species of blue-green algae in addition to Agmenellum (21).

The molecular weight of the Agmenellum carboxylase (456,000 daltons) is considerably less than the values reported for the higher plant and algal proteins (2-7). Of interest is the quaternary structure of the Agmenellum RuDP carboxylase. Only one subunit of approximately 56,000 daltons was obtained when the Agmenellum protein was dissociated in sodium dodecylsulfate, whereas the plant and eucaryotic algal RuDP carboxylases contain a small protein of approximately 15,000 daltons in addition to the larger subunit (1). Thus, the plant and algal carboxylases possess eight large and eight small subunits, giving a native molecular weight of approximately 550,000 (1, 3-6). With the absence of a smaller type subunit, apparently eight of the larger-type subunits compose the native protein from Agmenellum, yielding a calculated molecular weight of 448,000, a value quite close to the experimentally generated value of 456,000.

Recently, it has been shown that the two different subunits of the plant and algal RuDP carboxylase are synthesized at separate locations in the cell (22, 23). Moreover, there is evidence in support of the fact that chloroplast DNA codes for the synthesis of the large subunit of RuDP carboxylase in eucaryotic organisms (23, 24). If the present day chloroplast is a descendant of a primitive blue-green alga which invaded a primitive eucaryotic host (25, 26), it would be expected that the blue-green algal RuDP carboxylase would structurally resemble the protein synthesized in the chloroplast. Our finding that the Agmenellum RuDP carboxylase is composed only of the large type subunit, similar to the protein coded for by chloroplasts of eucaryotic algae may be of deep evolutional significance. Future investigations will seek to compare the blue-green algal and chloroplast RuDP carboxylases in greater detail.

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