Presence of Two Subunit Types in Ribulose-1,5-bisphosphate Carboxylase from Blue-Green Algae*

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Summary: Ribulose-1,5-bisphosphate carboxylase (E.C. 4.1.1.39) from 2 blue-green algae, Plectonema boryanum and Anabaena variabilis, was isolated by sucrose density gradient centrifugation. Both enzymes had a sedimentation value of about 18s, similar to that of Chromatium enzyme. The presence of two subunits (A, B) in the algal enzyme was demonstrated by Na-dodecyl sulfate polyacrylamide gel electrophoresis. The molecular weight of the two subunits was determined: for Plectonema A, 5.4×10^4 and B, 1.3×10^4 and Anabaena A, 5.2×10^4 and B, 1.3×10^4, respectively. The carboxylase reaction catalysed by the algal enzyme was similar to the higher plant enzyme in exhibiting the Mg^{2+}-effect, the optimal pH shifting from alkaline to neutral by elevating the concentration of Mg^{2+} in the assay mixture. The rabbit antisera developed against the spinach ribulose-1,5-bisphosphate carboxylase and its catalytic oligomer exhibited significant inhibitory effects on the carboxylation reaction catalysed by the algal enzyme.

It has been well established that RuDP carboxylase from higher plants as well as green algae has a large molecular weight (approx. 5×10^5) and contains two different types of subunits (A and B) (1-3). The enzyme from some bacterial sources such as Chromatium (4), Hydrogenomonas eutropha (5) and Ecthiorhodospira halophila (5) was found to be of the plant type, although the enzyme from Rhodospirillum rubrum (6) and Chlorobium thiosulfatophilum (7) was reported to contain only the large subunit. Experimental results from this laboratory clearly demonstrated that the catalytic site of both RuDP carboxylase and oxygenase reactions resides in

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Abbreviations: DTT, dithiothreitol; RuDP, ribulose-1,5-bisphosphate.

the large subunit A of the enzyme molecule (8-11), while the role of the small subunit appears to be closely associated with the regulatory mechanism of the enzyme reaction related to the Mg^{2+}-effect (8-10). Establishing the quarternary structure of the carboxylase molecule is of importance in order to analyse the intracellular subunit interactions in the process of photosynthetic CO_2-fixation. Comparative studies on the enzyme structure from procaryotic and eucaryotic organisms have value in characterizing the phylogenetic development of the enzyme as well as the mechanism of evolution of autotrophy in molecular terms (12-14). In this context, the structural characterization of RuDP carboxylase from procaryotic blue-green algae is particularly intriguing, because circumstantial evidence is accumulating which indicates the endosymbiotic origin of chloroplasts, presumably evolving from blue-green algae, in eucaryotic cells (15). Previously Kieras and Haselkorn (16) reported that RuDP carboxylases from the blue-green algae Anacystis nidulans and Plectonema boryanum, have a large molecular size like that of the plant enzyme. However, a recent report by Tabita et al. (17) showed that the enzyme isolated from a marine blue-green alga Agmenellum quardruplicatum has a molecular weight of 4.56×10^5, consisting only of the large subunit. In the work reported in this communication, we attempted to isolate the RuDP carboxylase from 2 filamentous blue-green algae, i.e., Plectonema boryanum and Anabaena variabilis, by sucrose density gradient centrifugation, and to characterize their subunit structures.

Materials and Methods

Algal cells of Plectonema boryanum and Anabaena variabilis used in the present investigation were kindly donated by Dr. K. Asada, Food Research Institute, Kyoto University. Algae cultured under the autotrophic growth conditions as described (18) were freshly harvested at the late-logarithmic to early stationary phase. The culture method for Chromatium was essentially the same as reported previously (4). After washing once with ice-chilled 50 mM Tris-HCl buffer (pH 7.0), 1 g of cells was suspended in 2 ml of 25 mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA, 1 mM DTT and 50 mM $NaHCO_3$ and disrupted for 10 min in a Kubota Insonator at the maximal input power at 2°C. The supernatant solution obtained by centrifugation of the whole homogenate at 97,000 ×g for 60 min at 4°C was made to 25% $(NH_4)_2SO_4$ saturation, then centrifuged again to separate the precipitate. The supernatant was made to 80% $(NH_4)_2SO_4$ saturation and the precipitate was dissolved in a small amount of 25 mM Tris-HCl buffer (pH7.0) containing 1 mM EDTA, 1 mM DTT and 1 mM $MgCl_2$,

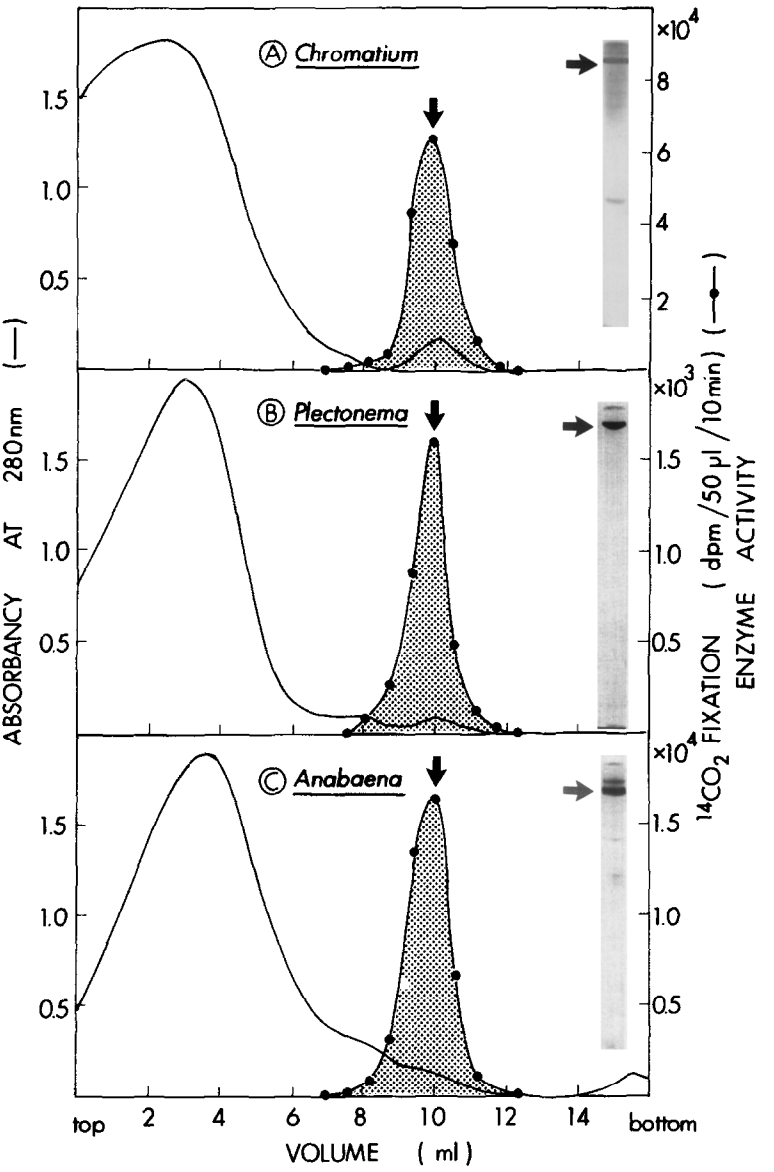

Fig. 1. Sucrose density gradient centrifugation patterns of RuDP carboxylases from (A) Chromatium, (B) Plectonema boryanum and (C) Anabaena variabilis. Details of experimental procedures are described in the text. Inset: Polyacrylamide gel electrophoretograms (pH 8.9) of enzymically active peak fractions (arrows) of each source. Samples applied: Chromatium and Anabaena, 10 µg and Plectonema, 25 µg.

and subsequently dialysed overnight at 4°C against the same solution. An aliquot (1 ml) was layered on top of 15 ml of sucrose density solution (0.2 - 0.8 M) dissolved in the above buffer solution and centrifuged in a Beckman Spinco SW 25.3 rotor at 78,700 ×g for 28 hr at 4°C. After centrifugation, the tube contents were separated with an ISCO fractionator equipped with UV absorptiometer (280 nm), and aliquots of the separated fractions (0.6 ml/tube)

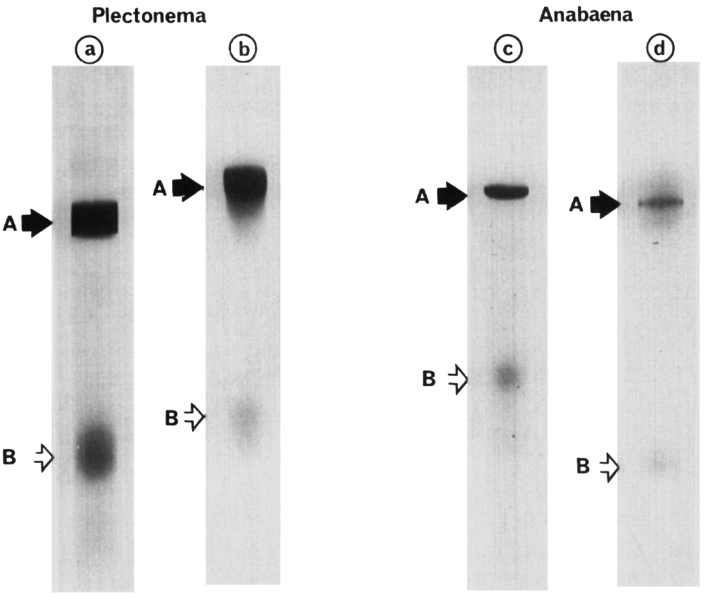

Fig. 2. Na-dodecyl sulfate polyacrylamide gel electrophoresis of RuDP carboxylases from Plectonema boryanum and Anabaena variabilis.
(a) and (c). Enzymically active peak fractions shown in Fig. 1. Enzymically active fractions from Plectonema were repeatedly applied to the density gradient centrifugation under the same conditions, giving almost pure enzyme. The final enzyme preparation was then subjected to electrophoresis. Protein content applied was 60 μg (Plectonema) and 25 μg (Anabaena), respectively.
(b) and (d). Eluates from gel segments shown in Fig. 1 were dialyzed against 10 mM phosphate buffer (pH 7.0) containing 0.1% Na-dodecyl sulfate and 1 mM DTT and aliquots applied to the electrophoresis. Protein content applied was 50 μg (Plectonema) and 20 μg (Anabaena), respectively.

were assayed for RuDP carboxylase activity according to the procedure reported previously (4). Incubation was done at 25°C for 10 min.

Standard polyacrylamide gel electrophoresis (7.5% cross linkage) at pH 8.9 (19) and Na-dodecyl sulfate polyacrylamide gel electrophoresis after Weber and Osborn (20) were employed to characterize the enzyme proteins.

The details of immunochemical experiments to examine the effect of antisera against the spinach RuDP carboxylase (anti-[N]) and the catalytic large subunit (anti-[A]) were reported previously (4, 8). The γ-globulin fraction of each antiserum prepared by the $(NH_4)_2SO_4$ precipitation method was used throughout the investigation. The protein content was determined by the method of Lowry et al. (21).

Results and Discussion

By means of the separation method described above, RuDP carboxylase was obtained from Chromatium and blue-green algal extracts. Centrifugation patterns as shown in Fig. 1 were obtained. In every case the enzyme activities are clearly located in the fractions of about 18s, in agreement with the pre-

vious report of Kieras and Haselkorn (16) and ours (4). Specific activity of the algal extracts was lower than that of the Chromatium extract, and Anabaena contained approximately 5 times more enzyme activity (1.47 unit/mg protein) than Plectonema (0.32 unit/mg protein). The gel electrophoretograms in Fig. 1 show that the relative mobilities of the major protein constituent correspond closely to that of the plant type RuDP carboxylase. As shown in Fig. 2 (a, c), Na-dodecyl sulfate polyacrylamide gel electrophoresis shows the presence of two types of subunits designated as A and B in both algal enzymes. To confirm this finding, the dye-stained gel segments as marked by arrow in Fig. 1 were eluted with 50 mM phosphate buffer containing 1% Na-dodecyl sulfate and 50 mM DTT, and the eluates were applied to Na-dodecyl sulfate polyacrylamide gel electrophoresis. Separation of two subunits is clearly seen [Fig. 2 (b, d)], although in such experiments the mobilities of the proteins are somewhat altered, presumably because of dye binding. By comparing with several standard proteins, molecular weights of subunits A and B were estimated to be 5.4×10^4 and 1.3×10^4 for Plectonema, and 5.2×10^4 and 1.3×10^4 for Anabaena, respectively.

We previously demonstrated that the plant type RuDP carboxylase from various sources exhibits a Mg^{2+}-effect (8-10), in the presence of Mg^{2+} the optimal pH of the enzyme reaction shifts towards neutral from alkaline in the absence of Mg^{2+}, while the property is not present in the enzyme reaction catalysed by the catalytic oligomer (A_8). As presented in Fig. 3, the carboxylase reactions catalysed by the preparations from Plectonema and Anabaena have similar properties, indicating that the enzyme from these algae contains the regulatory small subunit related to the Mg^{2+}-effect.

Experimental results from this laboratory have demonstrated interspecies immunological cross-reactivities of RuDP carboxylases, reflecting probable sequence homology in the catalytic moiety in the enzyme molecules from various origins (14). As shown in Fig. 4 (a, b) the carboxylase reaction was strongly inhibited by treating with the anti-[N]. Furthermore, the in-

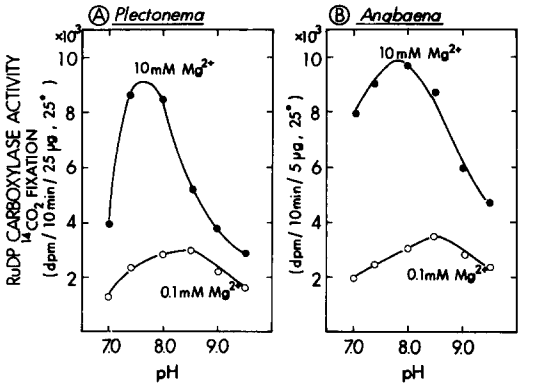

Fig. 3. Shift in optimum pH of RuDP carboxylase reaction of (A) Plectonema boryanum and (B) Anabaena variabilis. Enzyme activities were measured at different pH employing the standard assay mixture in the presence of 0.1 mM or 10 mM $MgCl_2$.

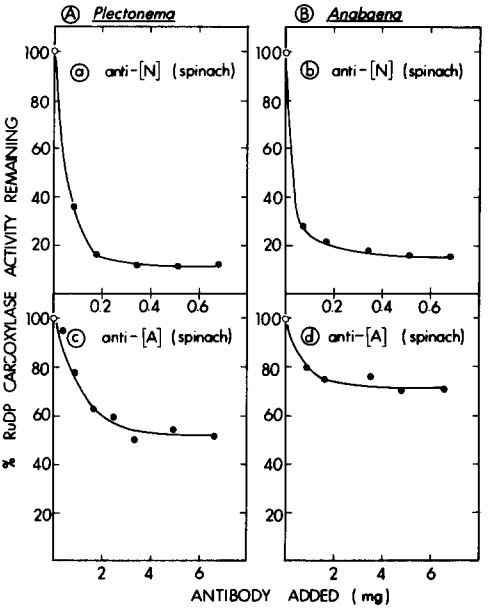

Fig. 4. Inhibitory effect of rabbit anti-spinach RuDP carboxylase serum (anti-[N]) and anti-catalytic subunit of spinach RuDP carboxylase (anti-[A]) on RuDP carboxylase reactions from (A) Plectonema boryanum and (B) Anabaena variabilis. About 35 µg each of Plectonema or Anabaena RuDP carboxylase was first treated with various quantities of antisera as indicated at 25°C for 30 min. Afterwards, aliquots withdrawn were used for the enzyme activity measurements employing the standard assay conditions. In the figure, % remaining enzyme activity was calculated by taking the value of the control system, in which an equal quantity of non-immunized rabbit serum was added, as 100%. The control serum showed almost no inhibitory effect.

hibitory effect exhibited by the anti-[A] treatment was quite significant, about 50% with Plectonema enzyme [Fig. 4 (c)] and 30% with Anabaena enzyme [Fig. 4 (d)]. Since it has been found that the treatment by the anti-[B] (spinach) is not effective in preventing the spinach RuDP carboxylase reaction (8), we did not test its effect on the algal enzyme system.

The phylogenetic relationship among RuDP carboxylases of different origins may assist us in unveiling the mechanism of molecular evolution of autotrophy. Results of our present research show that the structural make-up of the enzyme from two blue-green algae is of the plant-type, and thus that the finding of Tabita et al. (17) is not typical for this class of organisms. Current investigations (22, 23) dealing with the extensive homology between oligonucleotide sequences of chloroplastic 16s ribosomal RNA and those obtained from procaryotes including blue-green algae appear to support the hypothesis of the endosymbiotic origin of the chloroplasts (15), and our observation is consistent with this conceptual mechanism. Future studies on primary sequence determination of the RuDP carboxylases from procaryotic cells in comparison with the enzyme from eucaryotes may provide additional information on this hypothesis.

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