Jap. J. Phycol. 26: 49-52. 1978

Partial purification and some properties of RuDP carboxylase from a green alga, *Bryposis maxima*

Takamichi YAMADA, Tomoyoshi IKAWA and Kazutosi NISIZAWA

YAMADA, T., T. IKAWA and K. NISIZAWA 1978. Partial purification and some properties of RuDP carboxylase from a green alga, *Bryopsis maxima*. Jap. J. Phycol. 26: 49-52.

RuDP carboxylase was extracted from the fresh fronds of *Bryopsis maxima* and purified partially to an extent being practically available for the investigations of some enzymatic properties. The enzyme was activated more remarkably by Mg^{2+} than Mn^{2+}, and showed a maximum activity at 10 mM Mg^{2+}, but its optimum pH was shifted depending on its concentration. The apparent Km values for RuDP and HCO_3^- were estimated to be 6.5×10^{-4} M and 6.0×10^{-3} M, respectively.

Takamichi Yamada, Tomoyoshi Ikawa, Department of Biological Sciences, The University of Tsukuba, Ibaraki-ken, 300-31 Japan; Kazutosi Nisizawa, Department of Fisheries, College of Agriculture and Veterinary Medicine, Nihon University, Shimouma, Setagaya-ku, Tokyo, 154 Japan.

In contrast to higher plants, RuDP carboxylases of algae have not been studied in detail except a few in green[1-5] and blue-green[1,5] algae. In view of this fact, we investigated precisely the RuDP carboxylase from a marine brown alga, *Spatogrossum pacificum*, and found that some of its properties are characteristic of the brown alga[6]. This work was carried out as a part of the studies on enzymes involved in CO_2 photoassimilation of brown algae[7,8].

In this connection, therefore, we extended similar study to the RuDP carboxylase of a marine green alga, *Bryopsis maxima* to compare the properties with those already reported. Thus, we obtained some interesting results, although the enzyme used was a partially purified preparation, and they are reported in this paper.

Materials and Methods

Algal material. The fronds of *Bryopsis maxima* were harvested in spring at Inubosaki, Choshi, Chiba-ken.

Chemicals. RuDP is the commercial product of Sigma Chemical Company, and all other chemicals were purchased according to need.

Enzyme assay. Following the methods of MENDIOLLA and AKAZAWA[9] and KIERAS and HASELKORN[1], reaction mixture was prepared. It contained 50 μmoles of Tris-HCl buffer (pH 8.3), 2.5 μmoles of $MnSO_4$ (or 5 μmoles of $MgSO_4$), 0.25 μmole of RuDP, 5 μmoles of $KH^{14}CO_3$ (1.25 μCi), and 0.1 ml of enzyme solution in a total volume of 0.5 ml. The mixture was incubated first without RuDP at 20°C for 20 min, and the reaction was then started at 30°C after addition of RuDP. After incubation for 20 min, 0.05 ml of 6 N acetic acid was added to the mixture and excess radioactive carbonate was removed by suction for 15 min. A 0.2 ml aliquot of the mixture was then placed

1) Contributions from the Shimoda Marine Research Center, the University of Tsukuba, No. 325.

2) This work was supported by Research Grant B-148014 from the Ministry of Education of Japan.

Abbreviations: EDTA; ethylenediaminetetraacetat (disodium), RuDP; ribulose-1,5-diphosphate, RuDP carboxylase (or RuDP Case); 3-phospho-D-glycerate carboxylyase (EC 4, 1, 1, 39).

on a planchet and the radioactivity was measured with a Aloka model FC-1E gassflow counter. All enzyme assays were duplicated and an average value was obtained.

Results and Discussion

Extraction and purification of RuDP carboxylase. Three handred grams of fresh algal fronds were minced with scissors, and squeezed out by hands through two layers of nylon cheesecloth into 600 ml of 0.1 M Tris-HCl buffer, pH 7.8, containing 0.4 M sucrose and 1 mM $MgCl_2$. The mixture was stirred throughly and centrifuged at 16,000 ×g at 0°C for 7 min. The precipitate was suspended in the same buffer and again centrifuged in the same way. The precipitate thus obtained was stored at −20°C.

A 5 g aliquot of the above enzyme preparation was then sonicated in 25 ml of 0.05 M Tris-HCl buffer, pH 8.3, containing 5 mM $MgCl_2$, 50 mM KCl, 1 mM EDTA, and 30 mM 2-mercaptoethanol, at 0°C for 10 min. The mixture was centrifuged at 16,000×g at 0°C for 40 min. To the supernatant, was added solid ammonium sulfate to a 25% saturation, and the resulting precipitate was removed by centrifugation. By further addition of solid ammonium sulfate to the suppernatant, a 60% saturation was obtained. The resulting precipitate was collected by centrifugation at 16,000×g at 0°C for 30 min, and dissolved in 5 ml of 0.01 M Tris-HCl buffer, pH 7.5, containing 10 mM 2-mercaptoethanol and 0.1 mM EDTA.

The solution was applied to a Sephadex G-200 column (2.5×50 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 8.3, containing 5 mM $MgCl_2$, 1 mM EDTA, and 10 mM 2-mercaptoethanol (Buffer C), and eluted with the Buffer C at a flow rate of 0.8 ml/min. The eluates were collected every 10 ml in a tube. RuDP carboxylase was eluted into fractions (No's 24-27) following the void volume. These fractions were combined, concentrated in a collodion bag at 0°C for 12 hr, and then applied to a DEAE cellulose column (2.0×30 cm) equilibrated with Buffer C. A gradient elution was made with Buffer C containing linearly increasing NaCl concentrations from 0 to 1.0 M, as shown Fig. 1.

RuDP carboxylase was eluted at 0.5 M NaCl. The fractions (No's 23-27) were combined, concentrated in a collodion bag at 0°C for 12 hr, and the concentrated solution was used as RuDP carboxylase solution in this work.

Properties of partialy purified enzyme. First, the effect of Mg^{2+} at various concentrations on the enzyme activity was investigated at different pH values, as shown in Fig. 2.

The addition of Mg^{2+} caused remarkable shifts of the optimum pH of RuDP car-

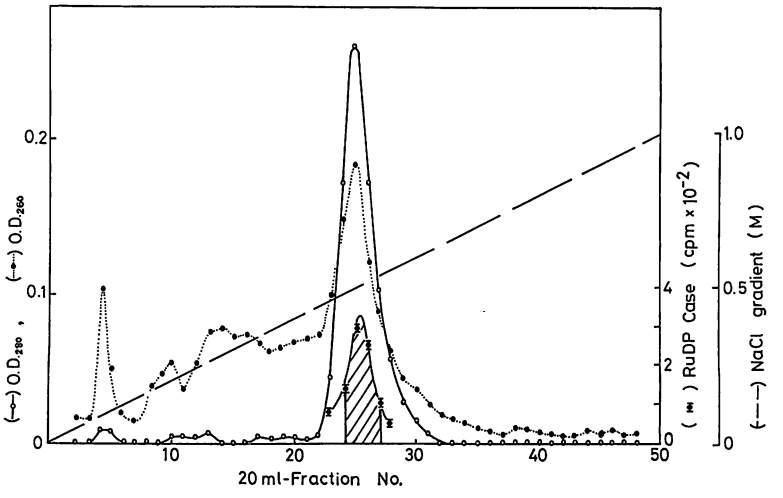

Fig. DEAE-cellulose column chromatography of RuDP carboxylase.

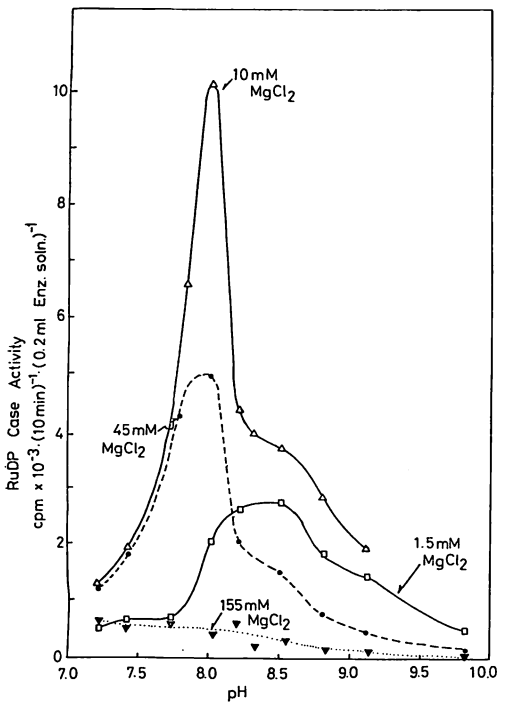

Fig. 2. Effect of concentration of Mg^{2+} on RuDP carboxylase activity.

boxylase toward neutral region. The optimum pH at 8.5 at 1.5 mM Mg^{2+} was shifted to pH 8.0 at 10 mM Mg^{2+}, and a maxmal activity was observed at this concentration of Mg^{2+} among several tests.

Since Mn^{2+} activated the activity of RuDP carboxylase from *S. pacificum*[6] more effectively than Mg^{2+}, the effect on Mn^{2+} on the activity of this algal enzyme was investigated, as shown in Fig. 3. The optimum pH at 8.5 at 2 mM Mn^{2+} was shifted to pH 7.6 at 6 mM and 11 mM Mn^{2+} concentrations, respectively, but the activation by Mn^{2+} was far lower than Mg^{2+} in contrast to the enzyme of the brown alga[6]. Effect of substrate concentration on RuDP carboxylase activity was examined to obtain Km values. As shown in Figs. 4 and 5, the Lineweaver-Burk plots for RuDP and HCO_3^- each showed sigmoid curve, as with the RuDP carboxylase of *S. pacificum*[6], and the Km values could not exactly obtained. However, apparent Km values calculated at lower concentrations of substrates were 6.5×10^{-4} M and 6.0×10^{-3} M for RuDP and

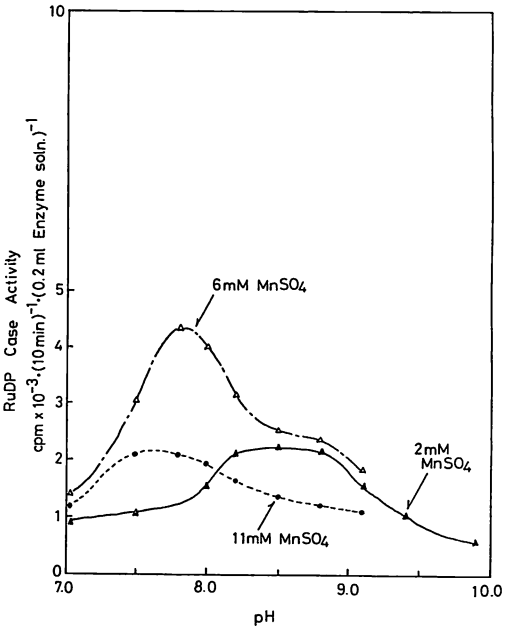

Fig. 3. Effect of concentration of Mn^{2+} on RuDP carboxylase activity.

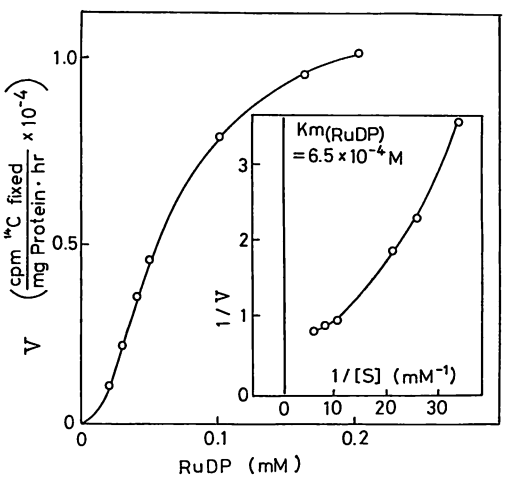

Fig. 4. Effect of concentration of RuDP on RuDP carboxylase activity.

HCO_3^-, respectively. These values were of almost the same order as those for brown algal enzyme[6]. Thus, the RuDP carboxylase of *B. maxima* was elucidated to be activated remarkably by Mg^{2+} and its optimum pH was shifted depending on its concentration as with the enzyme of spinach[10,11] while Km values for RuDP and HCO_3^- are rather simi-

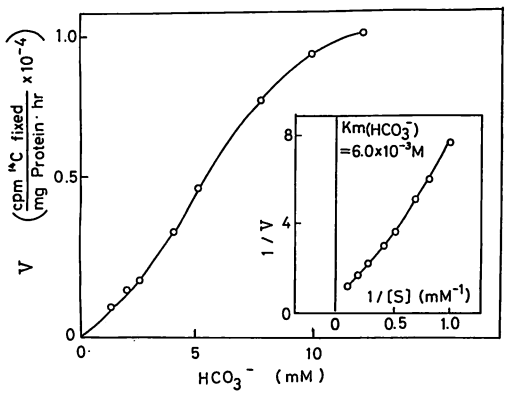

Fig. 5. Effect of concentration of HCO_3^- on RuDP carboxylase activity.

lar to those for the enzyme of *S. pacificum*[6], the values for spinach enzyme being 1.0-2.5×10^{-4} M and 5.6×10^{-3} M, respectively[12]. Therefore, the properties of subunits A and B of RuDP carboxylase of *B. maxima* would be more similar to those of the higher plants than to those of the brown algal enzyme. This is particularly interesting upon taking into account the phylogenetic position of green algae.

References

1) KIERAS, F. J. and HASELKORN, R. 1968. Properties of ribulose-1, 5-diphosphate carboxylase (Carboxydismutase) from Chinese cabbage and phostosynthetic microorganisms. Plant Physiol. 43: 1264-1270.
2) SUGIYAMA, T., MATSUMOTO, C., AKAZAWA, T. and MIYACHI, S. 1969. Structure and function of chloroplast proteins. VII. Ribulose-1, 5-diphosphate carboxylase of *Chlorella ellipsoidea*. Arch. Biochem. Biophys. 129: 597-602.
3) AKAZAWA, T., SATO, K. and SUGIYAMA, T. 1969. Structure and function of chloroplast proteins. VIII. Some properties of ribulose-1, 5-diphosphate carboxylase of Athiorhodaceae in comparison with those of plant enzyme. Arch. Biochem. Biophys. 132: 255-261.
4) WILSON, J. H. and MCCALLA, D. R. 1968. A simple method for the isolation of fraction I protein of chloroplasts. Can. J. Biochem. 46: 441-445.
5) ANDERSON, L., PRICE, G. B. and FULLER, R. C. 1968. Molecular diversity of the ribulose-1, 5-diphosphate carboxylase from photosynthetic microorganisms. Science 161: 482-484.
6) YAMADA, T., IKAWA, T. and NISIZAWA, K. 1978. Purification and properties of RuDP carboxylase from a brown alga, *Spatoglossum pacificum*. Botanica Marina, in press.
7) YAMAGUCHI, T., IKAWA, T. and NISIZAWA, K. 1969. Pathway of mannitol formation during photosynthesis in brown algae. Plant & Cell Physion. 10: 425-440.
8) IKAWA, T., WATANABE, T. and NISIZAWA, K. 1972. Enzymes involved in the last step of the biosynthesis of mannitol in brown algae. Plant & Cell Physiol. 13: 1017-1029.
9) MENDIOLA, L. and AKAZAWA, T. 1964. Partial purification and the enzymatic nature of fraction I protein of rice leaves. Biochemistry 3: 174-179.
10) WEISSBACH, A., HORECKER, B. L. and HURWITZ, J. 1956. The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide. J. Biol. Chem. 218: 759-810.
11) SUGIYAMA, T., NAKAMURA, N. and AKAZAWA, T. 1968. Activation of spinach leaf ribulose-1, 5-diphosphate carboxylase activities by magnesium ions. Biochem. Biophys. Res. Comm. 30: 118-123.
12) KAWASHIMA, N. and WILDMAN, S. G. 1970. Fraction I protein. Ann. Rev. Plant Physiol. 21: 325-358.

山田剛通*・猪川倫好*・西澤一俊：オオハネモ (*Bryopsis maxima*) の RuDP カルボキシラーゼの部分精製とその二・三の性質**

褐藻における CO_2 光同化酵素系のうち，その最初の固定酵素 RuDP カルボキシラーゼの研究はつい最近筆者らにより行われたが，海産の緑藻や紅藻のものについてはまだ残っていたので，まず緑藻の酵素について研究した。オオハネモを材料に選び，抽出精製を試みたが，今回は試料の関係上褐藻や既報の高等植物やクロレラの酵素ほど純度の高いものは得られなかった。しかし，二・三の性質を調べるには十分の標品が得られた。その結果，褐藻のものとは違い Mn^{2+} より Mg^{2+} によってよく活性化されたり，Mg^{2+} の濃度により至適酸度の変動がみられた点はむしろ高等植物のものに似ていたが，RuDP や HCO_3^- に対する Km 値 (6.5×10^{-4}M と 6.0×10^{-3}M) はむしろ褐藻のものに近かった。(*300-31 茨城県新治郡桜村，筑波大学生物系，**145 東京都世田谷区下馬 3，日本大学農獣医学部水産学科)