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THE PURIFICATION AND SOME PROPERTIES OF RIBULOSEBISPHOSPHATE CARBOXYLASE AND OF ITS SUBUNITS FROM THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

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SUMMARY

A description is presented of a rapid and efficient method for large-scale preparation of ribulosebisphosphate carboxylase (EC 4.1.1.39) from the green alga, *Chlamydomonas reinhardtii*, and of the separation into two purified subunits. The purification of the enzyme was accomplished essentially by $(NH_4)_2SO_4$ fractionation and sucrose-density gradient centrifugation, while the separation into subunits has been done by chromatography on sodium dodecylsulfate–hydroxyapatite or by gel filtration on Sephadex G-150 in the presence of guanidine–HCl. The molecular weights of the subunits, as determined by sodium dodecylsulfate–polyacrylamide gel electrophoresis, were $55.0 \cdot 10^3$ and $16.5 \cdot 10^3$. The p*I* of the enzyme was found to be 6.25, while that of the subunits was in the range 6.0–7.0. No amino-terminal amino acid could be detected in either of the subunits. Amino acid composition of the whole enzyme and of the separated subunits were determined and compared to those from other plant species.

INTRODUCTION

Ribulose-1,5-bisphosphate (Ru-1,5-P_2) carboxylase (EC 4.1.1.39), an enzyme catalyzing the carboxylation reaction in the Calvin cycle, is widely distributed among various photosynthetic organisms [1]. In higher plants and most algae, this enzyme is located within the chloroplast and it accounts for up to 60% of the chloroplast stromal proteins [2]. In addition to its abundance, Ru-1,5-P_2 carboxylase has several distinctive properties: it has a large molecular weight of about $5 \cdot 10^5$ [1], a complex quaternary structure as revealed by electron microscopy [3, 4], and it can be dissociated by a variety of denaturing agents into two non-identical subunits [5, 6].

Results obtained by several laboratories indicate a remarkable similarity of the Ru-1,5-P_2 carboxylase large subunits derived from different plants, in terms of their amino acid compositions (cf. ref. 1), tryptic peptide maps [7], and immunological properties [8, 9]. The small subunits on the contrary, are markedly different (cf. refs 1, 7, 10). Recent evidence suggests that the carboxylase activity is a property

Abbreviation: Ru-1,5-P_2, ribulose-1,5-bisphosphate.

of the large subunits whereas the small subunits play a regulatory role [11, 12]. In the higher plants as well as algae, synthesis of Ru-1,5-P_2 carboxylase seems to depend upon the activities of both cytoplasmic (80-S) and chloroplast (70-S) ribosomes [13, 14]. Therefore the possibility exists that the large catalytic subunit, presumably synthesized by chloroplast ribosomes [13, 15], may be preserved during plant evolution. Thus, detailed comparative studies on the subunit properties of Ru-1,5-P_2 carboxylase from lower and higher autotrophic species may lead to an understanding of the phylogenetic development of CO_2 fixation in molecular terms. The research presented here is concerned with a rapid procedure for the purification of Ru-1,5P_2 carboxylase from the green alga, *Chlamydomonas reinhardtii*, and with the large-scale isolation of its subunits.

MATERIALS AND METHODS

Handling of the cells

Cells of *Chlamydomonas reinhardtii* (strain 137c) were used for the experiments. The cells were grown in Tris–acetate–phosphate medium under continuous light at 25 °C according to Gorman and Levine [16]. Cultures were harvested at final cell density of $3 \cdot 10^6$ to $6 \cdot 10^6$ cells/ml.

Ru-1,5-P_2 carboxylase purification procedure

The purification procedure is based on that of Wishnick and Lane [17], Goldthwaite and Bogorad [18], and Boynton et al. [19]. All steps of the purification were carried out at 0–4 °C; all buffers were titrated at 25 °C.

(1) 6–8 g (wet wt) of cells were collected by centrifugation and washed in Tris–Mg–dithiothreitol buffer (50 mM Tris–HCl buffer, pH 8.0, containing 5 mM $MgCl_2$ and 5 mM dithiothreitol). The cell pellet was resuspended in 40 ml of the same buffer, and broken by two passages through a French pressure cell at 3800 lb/inch^2. This homogenate was used as a starting material for Ru-1,5-P_2 carboxylase purification.

(2) The cell debris was removed by centrifugation for 20 min at 27 000 $\times$ g. Approximately 8 ml of the supernatant containing about 6 mg protein per ml were then layered over 1 ml of 1 M sucrose and centrifuged for 3 h at 40 000 rev./min (105 000 $\times$ g_{av}) in a Spinco No. 40 rotor. The amber colored supernatant was carefully removed from the 1-M sucrose layer containing thylakoid membranes and ribosomes.

(3) A saturated solution of $(NH_4)_2SO_4$ (pH 7.5) was added to the supernatant from step 2, with constant stirring, to obtain 35% saturation. The precipitate which formed after 1 h was removed by centrifugation for 20 min at 37 000 $\times$ g, and saturated $(NH_4)_2SO_4$ solution was added to the resulting supernatant until 55% saturation was reached. After 1 h, the precipitate was recovered by centrifugation as above and was dissolved in 3–4 ml of Tris–Mg–dithiothreitol.

(4) A 0.5-ml sample of the dissolved pellet was loaded on top of a 10–30% continuous sucrose gradient containing the Tris–Mg–dithiothreitol buffer. The gradient was centrifuged in an International SB 283 rotor at 40 000 rev./min (180 000 $\times$ g_{av}) for 14–16 h. After the centrifugation the gradient was fractionated from the top by pumping 50% sucrose into the bottom of the tube. The absorbance of the fractions was monitored at 254 nm with an ISCO density gradient fractionator.

(5) The sucrose gradient fractions containing the enzyme were brought to 55% $(NH_4)_2SO_4$ saturation, and the precipitated suspension was kept at 4 °C for further use (for convenience, this suspension is named "purified enzyme"). For assay the Ru-1,5-P_2 carboxylase-containing fractions from the gradient were pooled and dialyzed for 6 h at 4 °C against 10 mM Tris–HCl, pH 8.0, 1 mM dithiothreitol.

Separation of subunits of Ru-1,5-P_2 carboxylase by chromatography on hydroxyapatite–sodium dodecylsulfate column

The subunits of Ru-1,5-P_2 carboxylase were separated by hydroxyapatite–sodium dodecylsulfate chromatography according to Moss and Rosenblum [20]. Purified enzyme samples containing 3–5 mg protein, treated as above, were mixed with an equal volume of a solution containing 2% sodium dodecylsulfate, 20% mercaptoethanol, 0.4 M bicarbonate buffer, pH 8.0, and placed immediately in a boiling water bath for 2 min. The sample was diluted with 20 mM sodium phosphate buffer, pH 6.4, to a final phosphate concentration of 10 mM and applied to a hydroxyapatite column (0.9 cm × 23 cm) previously equilibrated with 10 mM sodium phosphate buffer, pH 6.4, containing 0.1% sodium dodecylsulfate and 1 mM dithiothreitol. Proteins were eluted with a linear gradient established between 0.2 and 0.5 M sodium phosphate buffer, pH 6.4. Fractions corresponding to the absorbance peaks were pooled, dialyzed against distilled water and lyophilized.

Separation of subunits of Ru-1,5-P_2 carboxylase by gel filtration on Sephadex G-150 in the presence of 4.9 M guanidine–HCl

Purified enzyme samples, containing 20 mg protein, were centrifuged as above, and the resultant pellet was dissolved in 1 ml of 0.05 M acetate buffer, pH 4.8, containing 1 mM dithiothreitol. A solution of 6 M guanidine–HCl in the same buffer was then added to obtain a final concentration of 4.9 M guanidine–HCl. After dissociation (2 h at room temperature) the sample was applied to a Sephadex G-150 column (2.5 cm × 95 cm), previously equilibrated with a solution containing 0.05 M sodium acetate buffer, pH 4.8, 4.9 M guanidine–HCl and 1 mM dithiothreitol. Proteins were eluted with the same guanidine–HCl–acetate–dithiothreitol buffer and the absorbance of the eluate was measured at 280 nm.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis

A 1 mm thick, 11% polyacrylamide gel or a gradient (between 10 and 15% polyacrylamide) gel was run in a discontinuous buffer system as described by Laemmli [21]. Purified enzyme preparations were centrifuged to remove $(NH_4)_2SO_4$ and the protein pellet was dissolved in a small volume of Tris–Mg–dithiothreitol. An equal volume of a solution of 2% sodium dodecylsulfate, 20% β-mercaptoethanol, and 0.4 M bicarbonate buffer, pH 8.0, was added and the mixture was incubated for at least 15 min at room temperature or for 2 min in a boiling water bath. Gels were run at 10 mA for 15 h, and were stained with Coomassie Brilliant Blue and destained by the method of Weber and Osborn [22].

Isoelectric focusing of Ru-1,5-P_2 carboxylase

Purified samples (in $(NH_4)_2SO_4$ or from the sucrose gradient directly (cf. above)) were focused on a 110-ml column (standard LKB model) in the Ampholine 3–10

pH range. A linear density gradient was obtained by mixing equal volumes of 0.05 and 1 M sucrose by means of a gradient mixer. A 1-ml sample containing 0.4–0.8 mg of protein in 0.5 M sucrose was layered in the middle of the column. The electrofocusing procedure was carried out at 10 °C at 2 W for 72 h. Gel electrofocusing was performed in pH range 3–10 (1% Ampholine) according to Wrigley [23], except that 5% rather than 8% gel was used. Riboflavin served as a catalyst for gel polymerization. For electrofocusing of subunits, the sample was dissolved in freshly prepared solution containing 8 M urea, 0.05 M Tris–HCl buffer, pH 8.0, 0.01 M dithiothreitol, and incubated for 1 h at room temperature to allow for complete dissociation of subunits prior to the application of the sample. The urea-containing gel was polymerized with ammonium persulfate. Gels were stained according to the method of Spencer and King [24].

Amino acid analysis of Ru-1,5-P_2 carboxylase and its subunits

For amino acid and amino-terminal analysis, the purified enzyme preparation was dialyzed as indicated above for enzyme assay and further dialyzed against double distilled water for at least 12 h at 4 °C and finally lyophilized. Subunit preparations obtained by either method were similarly treated. Aliquots of the lyophilized material were divided into two parts. One portion was first oxidized with performic acid; both portions were then hydrolyzed in 6 M HCl in the presence of phenol and mercaptoacetic acid according to the method of Moore and Stein [25].

Determination of amino terminal

Grey's method for dansylation was used [26]. Approximately 0.2–0.5 mg protein were dissolved in 0.1 ml of 0.2 M $NaHCO_3$ in 2% sodium dodecylsulfate, 50 μl of dansyl chloride solution (20 mg/ml) was added and the reaction was allowed to proceed for 1 h at 37 °C. Dansylated proteins were hydrolyzed in 6 M HCl for 3 or 16 h at 110 °C. Two-dimensional chromatography on 15 cm × 15 cm polyamide (Chen-Ching) sheets was carried out according to Woods and Wang [27]. To analyze small protein samples Stark's cyanate procedure [28] was modified by decreasing the column diameter as well as the effluent volume by 10-fold, and then subsequently calibrated with hydantoins of a variety of amino acids, and with ribonuclease as a standard protein.

Ru-1,5-P_2 carboxylase assay

The Ru-1,5-P_2 carboxylase activity was measured by the incorporation of $NaH^{14}CO_3$ into acid stable counts in the presence of Ru-1,5-P_2 [19]. The incubation mixture contained the following components: 50 μmoles Tris–HCl, pH 8.0, 2.5 μmoles $MgCl_2$, 1 μmole dithiothreitol, 40 μmoles $NaH^{14}CO_3$ (specific activity 0.2 Ci/μmole), 1 μmole Ru-1,5-P_2 and 5–100 μl of the enzyme in a final volume of 0.5 ml. Each sample was assayed at several concentrations to insure that the reaction rate is not limited by the substrate concentration. The reaction was carried out at room temperature for 15–20 min and was terminated by pouring aliquots of the incubation mixture into a scintillation vial containing 0.5 ml of glacial acetic acid. The vials were incubated in an oven at 85 °C to drive off unfixed CO_2 and to dry the remaining material. After cooling, 0.1 ml of water was added to each vial to redissolve the residue, followed by 10 ml of Bray's solution [29]. The samples were counted in a Nuclear-Chicago liquid scin-

tillation spectrometer. The counts were corrected for background, for zero-time controls, and for quenching by the channel ratio method.

Protein and chlorophyll determination

Protein determination was carried out by the method of Lowry et al. [30] using bovine serum albumin as a standard. Chlorophyll was measured according to Arnon [31].

Chemicals

The chemicals used in this work were obtained as follows: $NaH^{14}CO_3$, spec. act. 57 μCi/mmole, sucrose, $(NH_4)_2SO_4$ and guanidine–HCl, all of ultra pure grade from Schwarz-Mann Biochemicals; polyamide layer sheets (Chen-Ching) from Callard-Schlesinger Corporation; ampholine from LKB Company; Sephadex gel from Pharmacia, hydroxyapatite (Bio-Gel HTP) from Bio-Rad Laboratories; Spinach Ru-1,5-P_2 carboxylase from Sigma was further purified by fractionation on sucrose gradient as described above.

RESULTS

Table I presents the purification data of Ru-1,5-P_2 carboxylase. A 20-fold purification was obtained from the cell homogenate (containing both soluble and insoluble proteins) and a 10-fold purification from the soluble protein fraction (105 000 $\times$ *g* supernatant). The most efficient step in the purification procedure is the final

TABLE I

PURIFICATION OF Ru-1,5-P_2 CARBOXYLASE FROM *C. REINHARDTII*
Methods are given in Materials and Methods.

Step of purification	Total protein (mg)	Chlorophyll (mg/ml)	Specific activity*	Yield (%)
1 Homogenate	501.2	35.36	31.2	100
2 27 000 $\times$ *g* supernatant	202.2	0.52	49.1	63
3 105 000 $\times$ *g* supernatant	135.8	0.0	71.4	61
4 35% $(NH_4)_2SO_4$ supernatant	102.3	0.0	88.6	57
5 55% $(NH_4)_2SO_4$ pellet	22.3	0.0	257.2	36
6 Sucrose gradient fraction	6.2	0.0	635.9	25

* μmoles of CO_2 fixed per min per g of protein at 25 °C.

fractionation on a continuous sucrose-density gradient (Step 6). A well separated absorption peak corresponding to the enzyme activity is observed at about two-thirds of the way down the gradient (Fig. 1). Similar separation was obtainedby Goldthwaite and Bogorad [18] for spinach Ru-1,5-P_2 carboxylase and by Boynton et al. [19] for *Chlamydomonas* Ru-1,5-P_2 carboxylase on a comparable sucrose gradient.

Fig. 2 shows the dissociation by sodium dodecylsulfate of purified Ru-1,5-P_2

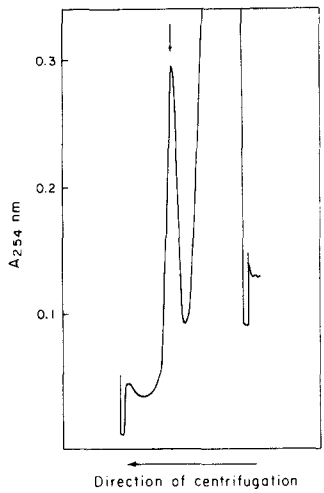

Fig. 1. Fractionation of Ru-1,5-P_2 carboxylase from *C. reinhardtii* by centrifugation in linear sucrose gradient, as described in Materials and Methods. Arrow indicates position of Ru-1,5-P_2 carboxylase.

carboxylase (Step 6, Table I) into two distinct subunits. The pattern obtained after electrophoretic separation of the protein on sodium dodecylsulfate–polyacrylamide gels indicated that the enzyme obtained after Step 6 (Table I) is quite homogeneous. However, at higher protein concentrations additional faint bands were detected on the

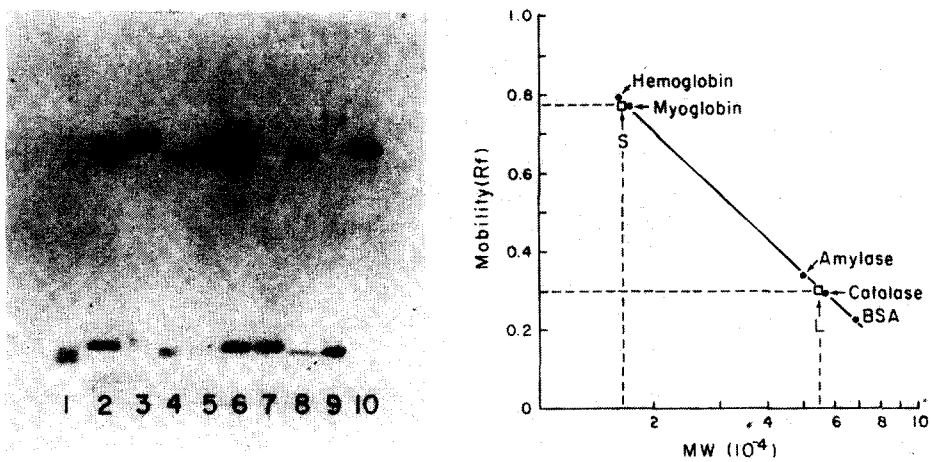

Fig. 2. Electrophoretic mobility of Ru-1,5-P_2 carboxylase subunits and standard proteins on dodecylsulfate-polyacrylamide slab gel. Methods as in Materials and Methods. On the left (2a) is a photograph of the 1-mm-thick gradient slab gel after electrophoresis of Ru-1,5-P_2 carboxylase, of its subunits, and of several standard proteins. Numbers refer to: (1) hemoglobin (5 μg); (2) Ru-1,5-P_2 carboxylase (10 μg); (3) catalase (2 μg) and myoglobin (1 μg); (4) amylase (2 μg) and cytochrome *c* (2 μg); (5) Ru-1,5-P_2 carboxylase-isolated subunits (2 μg each); (6) Ru-1,5-P_2 carboxylase (10 μg); (7) Ru-1,5-P_2 carboxylase, small subunit (5 μg); (8) spinach Ru-1,5-P_2 carboxylase (5 μg); (9) lysozyme (2 μg) and bovine serum albumin (1 μg); (10) Ru-1,5-P_2 carboxylase, large subunit (4 μg). On the right (2b) is shown the graphic determination of molecular weights of the subunits of Ru-1,5-P_2 carboxylase (L = large and S = small), after dodecylsulfate–gel electrophoresis of these subunits and some standard proteins.

sodium dodecylsulfate gels (estimated as less than 5% of the total protein on the basis of Coomassie Brilliant Blue staining).

The elution pattern of the enzyme from hydroxyapatite–sodium dodecylsulfate column showed also two distinct peaks of protein (Fig. 3). The first peak, which eluted between 0.34 and 0.37 M sodium phosphate buffer was subsequently found by sodium dodecylsulfate–polyacrylamide electrophoresis to be the small subunit of Ru-1,5-P_2 carboxylase, while the second peak, which was found to be the large subunit

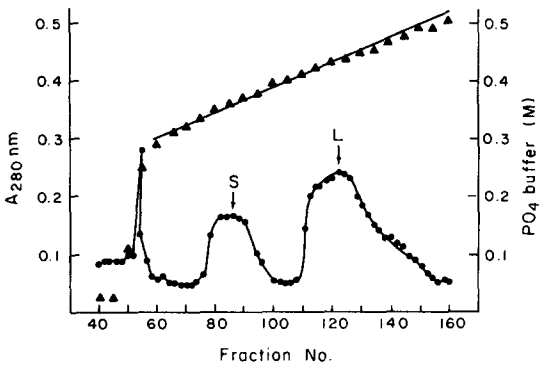

Fig. 3. Separation of subunits of Ru-1,5-P_2 carboxylase by chromatography on hydroxyapatite–dodecylsulfate column, as described in Materials and Methods. S and L refer to small and large subunits; triangles refer to buffer concentration. The flow rate is 27 ml/h and sample volume is 1.5 ml.

by the same method, was eluted between 0.41–0.46 M sodium phosphate. The separation of subunits of Ru-1,5-P_2 carboxylase by gel filtration on Sephadex G-150 after treatment with 4.9 M guanidine–HCl is shown in Fig. 4. The large subunit was eluted slightly after the void volume, well separated from the small subunit. The peak fractions obtained by either method were analyzed by polyacrylamide–sodium dodecyl-

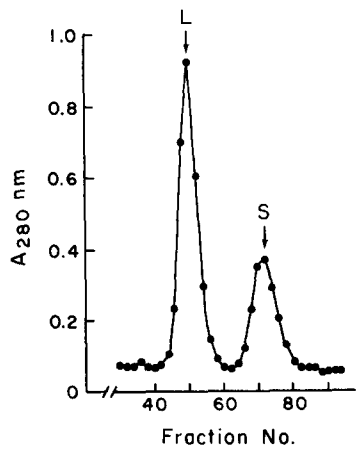

Fig. 4. Separation of subunits of Ru-1,5-P_2 carboxylase by gel filtration on Sephadex G-150 in the presence of 4.9 M guanidine–HCl, as described in Materials and Methods. S and L refer to small and large subunits. The flow rate is 12 ml/h and the sample volume is 3 ml.

sulfate gel electrophoresis as above and found to contain clean preparations of either the small or large subunits.

In order to determine the molecular weight of each subunit, the relative migration of the subunits on the sodium dodecylsulfate–polyacrylamide slab gel electrophoresis (Fig. 2a) were compared with those of marker proteins of known molecular weight (Fig. 2b). The molecular weights of large and small subunits were found to be 55 000 and 16 500, respectively (Fig. 2b). The large subunit showed the same mobility as that from the spinach Ru-1,5-P_2 carboxylase while the small subunit moved slower than that from the spinach enzyme.

Amino acid composition, expressed in molar ratio relative to phenylalanine, of the whole algal enzyme and of its subunits, is presented in Table II. The analysis of the total enzyme is similar to that reported for other plants (cf. ref. 1). Notable differences between the two subunits of the *C. reinhardtii* enzyme are in their glycine:histidine:

TABLE II

AMINO ACID COMPOSITION OF *C. REINHARDTII* Ru-1,5-P_2 CARBOXYLASE AND OF ITS TWO SUBUNITS

Methods are given in Materials and Methods. Data are in moles, relative to phenylalanine

	Enzyme	Large subunit	Small subunit
Phenylalanine	(1)	(1)	(1)
Tyrosine	1.00	0.96	0.91
Glycine	2.56	2.62	1.09
Lysine	1.85	1.25	1.05
Histidine	0.64	0.63	0.18
Arginine	1.74	1.62	1.02
Aspartic acid	2.64	2.42	2.14
Threonine	1.46	1.39	0.96
Serine	1.24	0.87	1.08
Glutamic acid	2.53	2.40	2.08
Proline	1.47	1.27	1.16
Alanine	2.67	2.61	1.96
Valine	1.91	1.80	1.83
Methionine	0.95	0.66	0.65
Isoleucine	1.16	1.07	0.74
Leucine	1.90	2.02	1.19
Cysteine*	0.67	0.93	0.72

* Determined as cysteic acid.

arginine content. It is 2.62:0.63:1.62 in the large subunit, while the molar ratio of these residues in the small subunit is 1.09:0.18:1.02. Further comparison of data in Table II indicates other compositional differences between the two subunits. These differences in amino acid content of the subunits from the algal Ru-1,5-P_2 carboxylase (Table II) showed that the bands on polyacrylamide gel were in fact different polypeptides and not aggregates of the same basic unit.

Amino-terminal determination resulted in no stoichiometric yield of amino acid residue for either subunit. This may be due to the presence of a blocking group or of pyrollidone glutamic acid as an N-terminal amino acid. The inability to detect

N-terminal amino acids was also reported by Moon and Thompson [32] for spinach beet Ru-1,5-P_2 carboxylase.

Isoelectric focusing of Ru-1,5-P_2 carboxylase from *C. reinhardtii* in a sucrose density gradient proved to be unsuccessful because of protein precipitation in the column after 16–18 h of focusing, even at low enzyme concentration. However, isoelectric focusing in 5% polyacrylamide gels (Fig. 5a) did resolve 2 bands in pH range

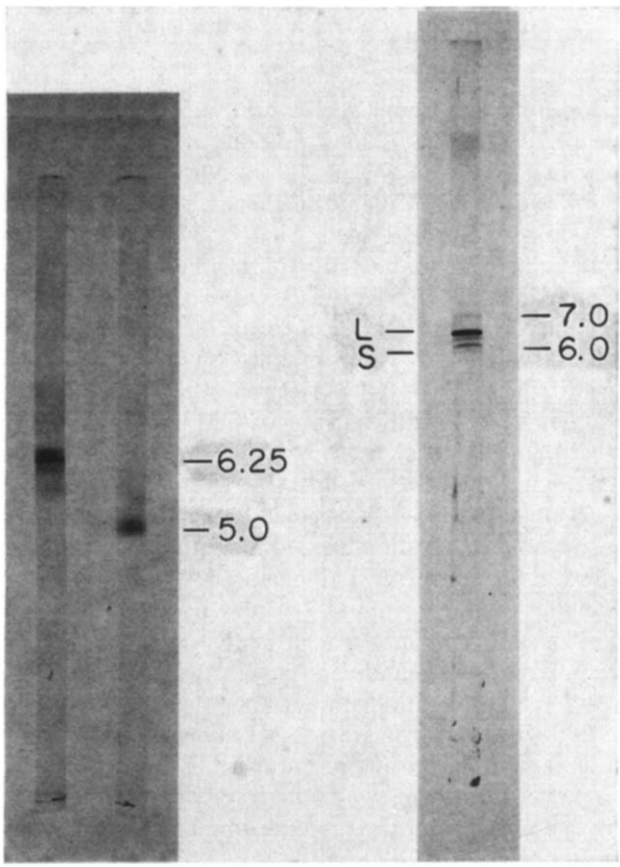

Fig. 5. (a) Isoelectric focusing in pH range 3–10, on 5% polyacrylamide gel, of Ru-1,5-P_2 carboxylase isolated from *C. reinhardtii* and from spinach. Left gel is Ru-1,5-P_2 carboxylase from *C. reinhardtii* (5 μg); right gel, Ru-1,5-P_2 carboxylase from spinach (4 μg). Numbers refer to pH. (b) Isoelectric focusing in the pH range 3–10, on 5% polyacrylamide gel, of Ru-1,5-P_2 carboxylase from *C. reinhardtii* in the presence of 8.0 M urea. Numbers refer to pH. L = large subunit; S = small subunit.

6.25–6.30 for Ru-1,5-P_2 carboxylase from *C. reinhardtii*, while at the same time Ru-1,5-P_2 carboxylase from spinach (purified from Sigma Co. product) was electrofocused to pH value 5.0, similar to that reported by Matsumoto et al. [33]. With aging of the preparation, more protein bands appear in the same region and this result can be explained by aggregates formed during storage [34, 35]. Isoelectric focusing of subunits of Ru-1,5-P_2 carboxylase in the presence of 8 M urea resulted in a separation of 2 major and 2 minor proteins (Fig. 5b). The two major components can be tentatively

identified as the large and the small subunit on the basis of staining intensity with Coomassie Brilliant Blue. The two minor proteins are possibly altered forms of the subunits, either due to some limited proteolytic digestion of the enzyme in early steps of purification or to the presence of a subpopulation of the subunits with a non-blocked amino terminus. All resolved proteins were localized in close proximity to each other in the 6–7 pH region; thus both subunits have p*I* values close to that of the native enzyme.

DISCUSSION

The Ru-1,5-P_2 carboxylase from *C. reinhardtii* can be rapidly purified to almost homogeneity by means of $(NH_4)_2SO_4$ precipitation and subsequent linear sucrose density gradient centrifugation, resulting in 20-fold purification of the enzyme from the cell homogenate. The analysis of the purified enzyme by sodium dodecylsulfate–polyacrylamide gel electrophoresis and isoelectric focusing suggests that this preparation is contaminated by not more than 5–10%. The same level of contamination was reported by Givan and Criddle [14] for Ru-1,5-P_2 carboxylase from *C. reinhardtii* after a more lengthy procedure. The high concentration of the acidic amino acids, aspartic and glutamic acid, in the whole enzyme, as revealed by amino acid composition studies, is in agreement with the acidic p*I* (6.25) of the enzyme. The p*I* values of Ru-1,5-P_2 carboxylases from *Chlorella* [33] and from spinach [33] were also shown to be in the acidic range but lower than that of *C. reinhardtii*.

The present study of Ru-1,5-P_2 carboxylase from *C. reinhardtii* also showed that the non-identical subunits of the enzyme can be readily separated and obtained as highly pure preparations by sodium dodecylsulfate–hydroxyapatite chromatography and by gel filtration in the presence of guanidine–HCl. The dissociation of the subunits by alkaline buffer treatment (pH 11.2) as described by Takabe and Akazawa [11] proved to be unsuccessful for *Chlamydomonas* Ru-1,5-P_2 carboxylase. The subunits apparently are not held by disulfide bridges because the presence of sulfhydryl reagents is not necessary for separation; moreover, longer incubation without such reagents results in the formation of aggregates of subunits, as revealed by sodium dodecylsulfate–polyacrylamide electrophoresis (unpublished observation). The subunit preparations obtained either by chromatography on sodium dodecylsulfate–hydroxyapatite or by gel filtration in the presence of guanidine–HCl are identical as judged by the electrophoretic mobilities and the amino acid compositions of the subunits. The molecular weight of the small and large subunits was estimated to be $16.5 \cdot 10^3$ and $55 \cdot 10^3$ respectively, and these values are in agreement with data obtained by Givan and Criddle [14] after sodium dodecylsulfate–electrophoresis. The small subunit from *C. reinhardtii* was found to be of similar size as that from *Chlorella elipsoidea* mol. wt $15.5 \cdot 10^3$ [36], but larger than that from spinach, $13 \cdot 10^3$ [6].

A comparison of the amino acid compositions of large and small subunits of Ru-1,5-P_2 carboxylases from *C. reinhardtii*, *Chlorella elipsoidea*, and spinach is shown in Fig. 6. The data reveal a similarity in overall composition of the large subunits, particularly with respect to the relative abundance of the following amino acids: histidine, aspartic acid, glycine and valine. However, the composition of the small subunits differed markedly from one another, particularly in their glycine, serine and glutamic acid contents. The similarity of the large subunits of Ru-1,5-P_2 carboxylase

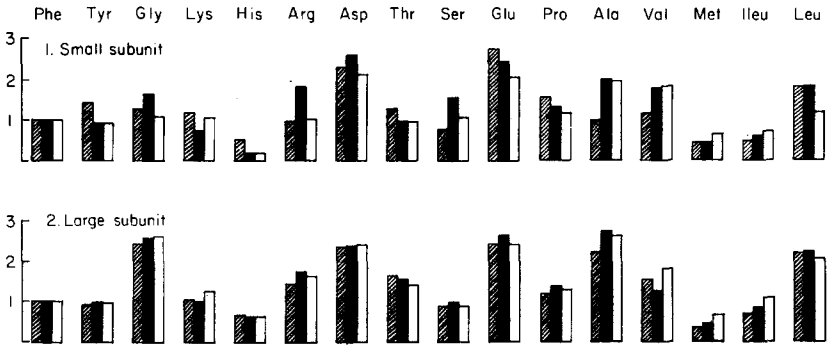

Fig. 6. Amino acid composition of subunits of Ru-1,5-P_2 carboxylase from spinach, *Chlorella* and *C. reinhardtii*. Amino acid composition of *C. reinhardtii* enzyme subunits were obtained as described in Materials and Methods; the amino acid composition of spinach (cf. ref. 1) and *Chlorella* (cf. ref. 1) were from the literature. Phenylalanine was set as unity (one mole per subunit). Open bar = *Chlamydomonas*; black bar = *Chlorella*; hatched bar = spinach.

among different plant species was noted by many investigators (cf. ref. 1) and is believed to reflect the preservation of primary structure during the evolution of photosynthetic organisms, while the small subunit shows more variability in amino acid composition (cf. ref. 1). A similarity between the algal small subunits can be noted in that both have a lower histidine content than the spinach small subunit and they both have a higher molecular weight than that of the spinach subunit. Moreover, a possibly common feature of all Ru-1,5-P_2 carboxylases from different algal and plant species is the presence of blocked amino terminal.

The separation and characterization of the subunits of Ru-1,5-P_2 carboxylase from *C. reinhardtii* will be of value in further studies examining the regulation of the biosynthesis of the separate subunits by chloroplastic and extra-chloroplastic ribosomes.

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