Purification and Molecular and Catalytic Properties of Phosphoribulokinase from the Cyanobacterium *Chlorogloeopsis fritschii*

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Phosphoribulokinase (ATP:D-ribulose-5-phosphate 1-phosphotransferase, EC 2.7.1.19) was purified from the cyanobacterium *Chlorogloeopsis fritschii*. The enzyme had a molecular weight of 230000, as determined by gel filtration, and a pH optimum of 8.6. Divalent cations were essential for activity, maximal activity being supported by Mg\(^{2+}\), while Mn\(^{2+}\), Ca\(^{2+}\) and Co\(^{2+}\) were less effective. AMP, ADP, phosphoenolpyruvate, aspartate and malate inhibited enzyme activity completely at 1 mM. No effects on phosphoribulokinase activity were observed with NAD, NADH, NADP or NADPH at up to 10 mM or with glyoxylate at up to 20 mM. The enzyme was activated in semi-purified extracts by the addition of dithiothreitol and reduced glutathione. SDS-PAGE of SDS-dissociated enzyme revealed only one polypeptide band of molecular weight 40000. This suggests that *C. fritschii* phosphoribulokinase is a hexamer consisting of six subunits of identical size.

**INTRODUCTION**

Phosphoribulokinase (ATP:D-ribulose-5-phosphate 1-phosphotransferase, EC 2.7.1.19) and D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; 3-phospho-D-glycerate carboxylase, dimerizing, EC 4.1.1.39) are unique to, and essential for, the Calvin, or reductive pentose phosphate, cycle. Phosphoribulokinase catalyses the regeneration of the \(\text{CO}_2\) acceptor molecule, ribulose 1,5-bisphosphate (RuBP), from ribulose 5-phosphate (Ru5P) and ATP, and it follows that \(\text{CO}_2\) assimilation by autotrophic (Calvin cycle) organisms depends upon phosphoribulokinase activity (McFadden, 1978; Lorimer & Andrews, 1981).

Extensive research has been concentrated on the structure, functions and regulation of RuBisCO from plants and autotrophic micro-organisms (Lorimer, 1981; McFadden, 1978). However, relatively few studies have been made on phosphoribulokinase, whether from chloroplasts or autotrophic prokaryotes. Phosphoribulokinase is considered to be a principal control point of the Calvin cycle in purple photosynthetic and chemolithoautotrophic bacteria (Wolosiuk & Buchanan, 1978; Ohmann, 1979) and the enzyme has been purified and partially characterized from a few members of these groups of organisms (MacElroy et al., 1969, 1972; Rindt & Ohmann, 1969; Hart & Gibson, 1971; Joint et al., 1972; Abdelal & Schlegel, 1974; Siebert et al., 1981; Tabita, 1980, 1981). A general pattern of inhibition by AMP and phosphoenolpyruvate (PEP) and of activation by NADH has emerged from these studies. The activation of phosphoribulokinase by NADH in particular is seen to be consistent with the operation of the Calvin cycle, which requires reducing power in this form, which is the product of energy conversion in photosynthetic and chemoautotrophic bacteria. Little is known about cyanobacterial phosphoribulokinase, although, in contrast to the photosynthetic and chemoautotrophic bacterial enzymes, no activation by NADH was found by Tabita (1980, 1981) in dialysed crude extracts of *Anabaena CA* or *Agmenellum quadruplicatum*.

**Abbreviations:** BSA, bovine serum albumin; PEP, phosphoenolpyruvate; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; Ru5P, ribulose 5-phosphate.

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In this paper we report the first purification of phosphoribulokinase from a cyanobacterium, *Chlorogloeopsis fritschii*. Some molecular properties are described, together with studies on the effects of nucleotides, some intermediary metabolites and thiol compounds.

**METHODS**

**Organism and growth.** *Chlorogloeopsis fritschii* 1411/16, from the Culture Centre of Algae and Protozoa, Cambridge, UK, was grown phototrophically in pure batch culture in BG-11 medium including nitrate (Stanier *et al.*, 1971), as detailed earlier (Codd & Stewart, 1973).

**Buffers.** Buffer A contained 10 mM-Tris/HCl, 10 mM-EDTA (disodium salt), 50 mM-NaHCO₃ and 80 mM-2-mercaptoethanol, pH 8.0 at 4°C. Buffer B contained 20 mM-Tris/HCl, 1 mM-EDTA (disodium salt), 1 mM-2-mercaptoethanol and 100 mM-KCl, pH 7.5 at 4°C. Buffer C was as for buffer B, plus 2 mM-ATP. Buffer D was as for buffer B, but minus KCl. Buffer E contained 150 mM-Tris/HCl, 1 mM-EDTA (disodium salt) and 1 mM-2-mercaptoethanol, pH 8.0 at 4°C.

**Enzyme extraction and purification.** Mid to late exponential phase cells were used throughout and all steps were done at 4°C unless stated otherwise. A yield of 15 g wet wt cells was obtained by centrifuging 18 l culture at 2000 g for 10 min. The cells were resuspended in 80 ml buffer A and broken by shaking in a Braun cell homogenizer (Braun MSK, Melsungen, FRG) using 0.2-0.4 mm diameter glass beads for 4 x 0.5 min, with 0.5 min intermittent cooling periods. Remaining whole cells and large membrane fragments were removed by centrifugation at 40000 g for 1 h. (NH₄)$_2$SO₄ was added to the supernatant to 35% saturation and the precipitated material removed by centrifugation at 40000 g for 20 min in an 8 x 50 ml angle rotor in a MSE Prep-spin 21. (NH₄)$_2$SO₄ was added to the resulting supernatant to 80% saturation and the precipitated material recovered by centrifugation at 40000 g for 20 min. The pellet was resuspended in 2 ml buffer B and dialysed against 1 l of buffer B for 1 h. The sample was applied to a Blue Sepharose CL-4B column (2.5 x 12 cm) equilibrated with buffer B. The column was eluted with 100 ml buffer B (flow rate 0.2 ml min⁻¹) followed by 50 ml buffer C. Peak phosphoribulokinase fractions were bulked and subjected to a 0-80% (NH₄)$_2$SO₄ fractionation. The precipitated material, recovered by centrifugation at 40000 g for 20 min, was resuspended in 0.5 ml buffer D and dialysed against 1 l buffer D for 1 h. The sample was applied to a Bio-Gel A-1.5 m gel filtration column (112 x 1.6 cm) equilibrated with buffer D and eluted with buffer D (flow rate 0.15 ml min⁻¹). Peak phosphoribulokinase fractions were collected and added to a DEAE-cellulose DE52 microgranular ion-exchange column (8 x 0.5 cm) equilibrated with buffer E. Phosphoribulokinase was eluted on a linear gradient (50 ml) of increasing ionic strength from buffer E to buffer F. Peak phosphoribulokinase fractions were collected and concentrated sixfold using Amicon Minicon concentrators (CS15) and stored at -20°C.

**Enzyme assays.** Phosphoribulokinase was routinely measured by coupling the formation of RuBP to the subsequent incorporation of NaH¹⁴CO₃ into acid-stable material in the presence of excess exogenous RuBisCO. Assays were done at 30°C in 5 ml plastic scintillation vials, in a final volume of 0.35 ml. Extract (0.1 ml) was incubated for 10 min in 100 mM-Tris/HCl (pH 8.6), 5 mM-ATP, 10 mM-MgCl₂, 1 mM-GSH, 50 mM-NaH¹⁴CO₃ (74 kBq pmol⁻¹) and 0.075 units spinach RuBisCO. The reaction was then initiated by addition of 50 µl Ru5P to a final concentration of 3 mM, allowed to run for 2 min and stopped by adding 50 µl 50% (w/v) TCA.

Vials were heated to 100°C in a water bath for 1 h and the acid-stable radioactivity incorporated was measured by liquid scintillation counting in a Packard Tri-carb 2660 liquid scintillation system. Specific radioactivity of the NaH¹⁴CO₃ was determined; controls lacked Ru5P.

Activity was also determined in a two stage assay. The primary reaction mixture contained 0.1 ml extract in 0.3 ml 100 mM-Tris/HCl, (pH 8.6), 5 mM-ATP, 10 mM-MgCl₂, and 1 mM-GSH. The reaction was initiated with 0.05 ml Ru5P to a final concentration of 3 mM and terminated after 2 min by heating in a boiling water bath for 5 min. After cooling, the mixture was incubated for 10 min with 0.1 ml 100 mM-Tris/HCl (pH 8.6), 5 mM-ATP, 1 mM-GSH and 50 mM-NaH¹⁴CO₃ (74 kBq µmol⁻¹). The reaction was initiated by addition of 0.05 ml activated spinach RuBisCO (0.075 units) and 20 mM-MgCl₂. Acid-stable radioactivity was determined as before. Preliminary investigations revealed the assays to be linear up to 5 min, with semi-purified extracts, following pre-incubation for at least 10 min.

**Catalytic and regulatory properties of phosphoribulokinase.** General properties (pH optimum, divalent cation specificity, nucleoside, glyoxylate and thiol effects) were determined using semi-purified enzyme obtained after dialysis of 35-80% (NH₄)$_2$SO₄-precipitated material. Substrate affinities and effects of ADP, AMP, L-aspartate, L-malate and PEP were determined on dialysed 35-80% (NH₄)$_2$SO₄ extracts before and after Sephadex G-75 gel filtration (150 x 1.5 cm, equilibrated with buffer D lacking 2-mercaptoethanol). For divalent
cation studies the enzyme was dialysed for 24 h at 4 °C against buffer B lacking MgCl₂ and pre-incubated with divalent cations (at 5 mM) for 30 min at 30 °C. Phosphoribulokinase activity was assayed using the two stage assay where the exogenous RuBisCO was pre-activated in 20 mM-MgCl₂. For ATP concentration studies, the MgCl₂ concentration was kept in constant excess of 5 mM over the ATP concentration.

**Electrophoresis.** Standard PAGE was carried out by the method of Davis (1964), omitting the spacer gel, in 6-5% (w/v) acrylamide cylinders (6 × 0.4 cm). Bromophenol blue was used as a tracking dye and electrophoresis was done at a constant current of 3 mA per tube for 1.5 h.

SDS-PAGE was done according to Weber & Osborne (1969) using 10% (w/v) acrylamide cylinders (6 × 0.4 cm) at 8 mA per tube constant current for about 5 h; SDS dissociation of the protein was carried out at 100 °C for 2 min. Gels were stained for protein using Coomassie Brilliant Blue R250 stain and enhancer (Fairbanks et al., 1971). Gels were soaked for 12 h in stain containing 0.025% (w/v) Coomassie Brilliant Blue R250, 25% (w/v) propan-2-ol and 10% (v/v) acetic acid, and then incubated for 4 h in enhancer containing 0.0025% (w/v) Coomassie Brilliant Blue R250, 10% (v/v) propan-2-ol and 10% (v/v) acetic acid. Gels were destained in 16-5% (v/v) methanol plus 5% (v/v) acetic acid and either photographed or scanned to provide a permanent accurate record.

**Protein determination.** Protein concentrations were measured by the method of Bradford (1976) with 10 μl samples and 1 ml reagent. Concentrations were calculated using crystalline BSA as a standard.

**Molecular characteristics of phosphoribulokinase.** The molecular weight of the enzyme was determined by gel filtration using a Bio-Gel A1.5 m column calibrated with the following standards (molecular weights in parentheses): spinach RuBisCO (560000); apo-ferritin (470000); bovine liver catalase (232000); BSA (68000); cytochrome c (13000); and vitamin B₁₂ (1165). The molecular weight of the subunits was measured by comparing their relative mobilities after SDS-PAGE relative to SDS-dissociated standards.

**Chemicals.** NaH¹⁴CO₃ was obtained from Amersham and Bio-Gel A1.5 m from Bio-Rad. Blue Sepharose CL-6B and Sephadex G-75 were purchased from Pharmacia and AMP, ADP, NAD, NADH, NADP, NADPH, PEP, Ru5P, RuBP and RuBisCO (from spinach) from Sigma. ATP was from Boehringer-Mannheim and Coomassie Brilliant Blue R250 was from Serva, Heidelberg, FRG. All other chemicals were from BDH and were used at the highest purity commercially available.

**RESULTS**

**Purification and structural characterization of phosphoribulokinase**

Phosphoribulokinase activity was readily detectable in cell-free extracts of photoautotrophically grown *Chlorogloeopsis fritschii*. Most (about 90%) of the total extractable activity from this organism is soluble (Lanaras & Codd, 1981) and a supernatant obtained after centrifuging a cell-free extract at 40000 g for 1 h was used as the starting point for enzyme purification. A typical purification protocol is given in Table 1. The enzyme was purified approximately 216-fold with an activity yield of 3.1%, which compares with that obtained by Siebert *et al.* (1981) using *Alcaligenes eutrophus* extracts. Enzyme activity was maintained after storage at −20 °C for at least four weeks. Attempts to increase yield resulted in co-purification of several proteins since increasing protein loading on to the columns resulted in a decrease in resolution. Pulsed elution

**Table 1. Purification summary of phosphoribulokinase from Chlorogloeopsis fritschii**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total vol (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity*</th>
<th>Specific activity*</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude supernatant (40000 g supernatant)</td>
<td>80</td>
<td>89-6</td>
<td>5-90</td>
<td>0-07</td>
<td>100</td>
<td>1-0</td>
</tr>
<tr>
<td>35-80% (NH₄)₂SO₄ precipitation</td>
<td>1</td>
<td>55-3</td>
<td>3-63</td>
<td>0-07</td>
<td>61-61</td>
<td>1-0</td>
</tr>
<tr>
<td>Blue-Sepharose CL-6B affinity chromatography</td>
<td>18</td>
<td>0-32</td>
<td>1-83</td>
<td>5-70</td>
<td>30-99</td>
<td>86-66</td>
</tr>
<tr>
<td>Bio-Gel A1-5 gel filtration chromatography</td>
<td>24</td>
<td>0-12</td>
<td>1-23</td>
<td>10-33</td>
<td>20-76</td>
<td>156-86</td>
</tr>
<tr>
<td>DEAE-cellulose DE52 ion-exchange chromatography</td>
<td>5</td>
<td>0-01</td>
<td>0-19</td>
<td>14-24</td>
<td>3-13</td>
<td>216-24</td>
</tr>
</tbody>
</table>

* Activity is expressed as μmol substrate transformed (mg protein)⁻¹ min⁻¹. Enzyme was determined by a single stage radiometric assay, as detailed in Methods.
Fig. 1. DEAE-cellulose DE52 elution profile of phosphoribulokinase from *C. fritschii*. The column was eluted with a linear Tris gradient of increasing ionic strength. ●, Phosphoribulokinase activity. The fractions were 8.8 ml.

Fig. 2. SDS-polyacrylamide gel electrophoresis of SDS-dissociated *C. fritschii* phosphoribulokinase. (a) Coomassie Blue R250-stained gel after electrophoresis of 10 μg dissociated protein. The front of the tracking dye was close to the bottom of the gel. (b) Relative mobility versus $\log_{10}$ molecular weight of the SDS-dissociated phosphoribulokinase subunits (■) and dissociated standards (●). Molecular weights in parentheses: 1, BSA (66000); 2, catalase (58000); 3, $\gamma$-globulin heavy chain (49000); 4, aldolase (31500); 5, chymotrypsinogen (25700); cytochrome $c$ (13000).

of the Blue Sepharose CL-6B affinity column was chosen since analytical separations showed that the enzyme was weakly bound. In the absence of ATP, 20% of the total enzyme activity was eluted from the column, which is perhaps indicative of a non-specific association with membrane fragments. The active phosphoribulokinase from *C. fritschii* bound to the affinity gel, although it is recognized that Tris/HCl buffer at pH 7.5 has little buffering capacity. The enzyme retarded by the affinity column was eluted free of the dominant water-soluble cyanobacterial pigment, phycocyanin. Ion-exchange chromatography on DEAE-cellulose DE52 produced a sharp phosphoribulokinase activity peak between 0.3 and 0.33 M-Tris/HCl at pH 8.0 (Fig. 1).

Standard PAGE of up to 15 μg of the purified enzyme yielded a single protein band and only one peptide was obtained after dissociation with SDS and SDS-PAGE (Fig. 2a). The molecular weight of the native enzyme was determined to be 230000 ± 6000, using a Bio-Gel A1.5 m column. The single polypeptide revealed by SDS-PAGE had a molecular weight of 40000 as determined using dissociated standards (Fig. 2b). This suggests that intact *C. fritschii* phosphoribulokinase is a hexamer, probably consisting of six subunits of identical size.
Cyanobacterial phosphoribulokinase

Table 2. Effects of divalent cations and various compounds on phosphoribulokinase activity

<table>
<thead>
<tr>
<th>Assay conditions</th>
<th>Specific activity*</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete (including Mg^{2+} at 5 mM)</td>
<td>24.50</td>
<td>100</td>
</tr>
<tr>
<td>Complete, minus Mg^{2+}</td>
<td>1.30</td>
<td>5</td>
</tr>
<tr>
<td>Complete, minus Mg^{2+}, plus Mn^{2+} (5 mM)</td>
<td>19.35</td>
<td>79</td>
</tr>
<tr>
<td>Complete, minus Mg^{2+}, plus Ca^{2+} (5 mM)</td>
<td>18.62</td>
<td>76</td>
</tr>
<tr>
<td>Complete, minus Mg^{2+}, plus Co^{2+} (5 mM)</td>
<td>1.23</td>
<td>5</td>
</tr>
<tr>
<td>Complete plus ADP (1 mM)</td>
<td>0.40</td>
<td>2</td>
</tr>
<tr>
<td>Complete plus AMP (1 mM)</td>
<td>1.10</td>
<td>4</td>
</tr>
<tr>
<td>Complete plus PEP (1 mM)</td>
<td>1.40</td>
<td>6</td>
</tr>
<tr>
<td>Complete plus L-aspartate (1 mM)</td>
<td>1.40</td>
<td>6</td>
</tr>
<tr>
<td>Complete plus L-malate (1 mM)</td>
<td>0.80</td>
<td>3</td>
</tr>
</tbody>
</table>

* Expressed as nmol substrate transformed (mg protein)^{-1} min^{-1}. All assays were performed with resuspended (NH_{4})_{2}SO_{4} extracts after exhaustive dialysis (see Methods).

Catalytic and regulatory properties

Phosphoribulokinase activity showed a sharp maximum at pH 8.6 in Tris/Cl buffer. The enzyme showed a divalent cation requirement, maximum activity being obtained with Mg^{2+} (Table 2). Mg^{2+} could be substituted by 5 mM-Mn^{2+} or Ca^{2+} which largely restored activity, but Co^{2+} was ineffective. Mn^{2+}, Ca^{2+} and Co^{2+} at 20 mM were inhibitory.

The C. fritschii enzyme was not activated by pre-incubation with NADH up to 10 mM. NAD, NADP and NADPH also had no effect. The enzyme was, however, completely inhibited when preincubated with 1 mM-AMP, ADP, L-aspartate, L-malate or PEP (Table 2). The effects of up to 20 mM-glyoxylate were determined by preincubation with dialysed semi-purified extracts for up to 30 min. Glyoxylate effects on C. fritschii and spinach RuBisCO activities were also investigated, since glyoxylate has been reported to inhibit the higher plant RuBisCO in vitro (Cook & Tolbert, 1982). Glyoxylate inhibited the carboxylase activity of the commercial purified spinach RuBisCO (from 72.0 to 65.0 nmol RuBP consumed min^{-1} (mg protein)^{-1} at 20 mM-glyoxylate) and the exogenous RuBisCO activity present in the dialysed (NH_{4})_{2}SO_{4} preparation from C. fritschii (from 89.0 to 78.0 nmol consumed min^{-1} (mg protein)^{-1} at 20 mM-glyoxylate). Since only 10% inhibition by glyoxylate occurred and RuBisCO was added in 10–30-fold excess over that required to consume all of the RuBP produced by phosphoribulokinase in the assays, any glyoxylate effect can be attributed to action on phosphoribulokinase. There was, however, no evidence for glyoxylate inhibition of C. fritschii phosphoribulokinase [14.3 nmol Ru5P consumed min^{-1} (mg protein)^{-1} at 0–20 mM-glyoxylate].

Preincubation with the thiol reagent DTT had a stimulatory effect (2–2-fold) upon the activity of C. fritschii phosphoribulokinase in the dialysed (NH_{4})_{2}SO_{4} preparation (Fig. 3). Similar
effects were observed using reduced glutathione at the same concentrations. The same degree of stimulation of phosphoribulokinase activity by DTT (2.3-fold) was found after gel filtration of the enzyme preparation through Sephadex G-75, which effectively separates cyanobacterial thioredoxin from glucose-6-phosphate dehydrogenase, an enzyme of similar molecular weight to phosphoribulokinase (P. Rowell, personal communication). Phosphoribulokinase activity after passage through Sephadex G-75 was not affected by the addition of the low molecular weight eluate fraction.

DISCUSSION

A number of kinases and dehydrogenases have been successfully purified by affinity chromatography on blue dye-substituted gel matrices (Easterday & Easterday, 1974; Siebert et al., 1981). The present study demonstrates the usefulness of Blue Sepharose CL-6B in purifying phosphoribulokinase from Chlorogloeopsis fritschii. Addition of EDTA Na2 and 2-mercaptopethanol to the elution buffers involved in the affinity step increased activity yields. Activity losses during purification can be attributed to enzyme not being retarded by the affinity gel and possibly partial inactivation of the enzyme.

The pH optimum of 8.6 for the C. fritschii phosphoribulokinase is identical to that reported for Alcaligenes eutrophus (Siebert et al., 1981), but is considerably higher than that reported for the enzyme from Chromatium D (Hart & Gibson, 1971), Thiobacillus neapolitanus (MacElroy et al., 1972), spinach (Hurwitz et al., 1956) and pea (Anderson, 1973). The C. fritschii enzyme resembles the phosphoribulokinases from the photosynthetic and chemoautotrophic bacteria examined in its requirement for divalent cations, with Mg2+ as the preferred species.

NADH regulation of the phosphoribulokinase of chemoautotrophic bacteria is well established (MacElroy et al., 1969; Abdelal & Schlegel, 1974; Kiesow et al., 1977; Siebert et al., 1981) and requirement for NADH is also a general feature of the phosphoribulokinases from purple non-sulphur bacteria (Joint et al., 1972; Rindt & Ohmann, 1969; Tabita, 1980, 1981). In contrast, C. fritschii phosphoribulokinase was not influenced by NADH, or NAD, NADP or NADPH. Similar findings have been reported for the enzymes from the cyanobacteria Agmenellum quadruplicatum and Anabaena CA (Tabita, 1980). In this respect the cyanobacterial enzymes resemble the phosphoribulokinase from Chlorella (Tabita, 1980) and spinach chloroplasts (Kiesow et al., 1977).

Allosteric inhibition of phosphoribulokinase by PEP and AMP is known from several photosynthetic bacteria (Rindt & Ohmann, 1969; Hart & Gibson, 1971; Ohmann, 1979) and chemoautotrophic bacteria (MacElroy et al., 1969; Abdelal & Schlegel, 1974; Siebert et al., 1981). The C. fritschii phosphoribulokinase was also inhibited by PEP, AMP and ADP, but in this respect the cyanobacterial enzyme differs from the phosphoribulokinase of chloroplasts, which is not apparently influenced by these compounds (MacElroy et al., 1972; Anderson, 1973).

Glyoxylate is an intermediate of the C-2 photorespiratory cycle of plants (Lorimer & Andrews, 1981) and of glycollate metabolism in cyanobacteria (Codd & Stewart, 1973). This compound has been reported to stimulate photosynthetic CO2 assimilation by tobacco leaf discs (Oliver & Zelitch, 1977), soybean mesophyll cells (Oliver, 1980) and spinach mesophyll cells (Ishibashi et al., 1978) and to stimulate both photosynthesis and N2 fixation in cyanobacteria (Bergman, 1980, 1981). More recently, however, inhibitory effects of glyoxylate have been found using isolated chloroplasts and spinach mesophyll cells (Mulligan et al., 1983; Lawyer et al., 1983). The mode(s) of action of glyoxylate on photosynthesis are not understood. The stimulatory effect of glyoxylate on net CO2 assimilation may be due to a selective inhibition of photorespiration (Oliver & Zelitch, 1977; Bergman, 1981). The inhibitory effect of glyoxylate on chloroplast photosynthesis may be due to stromal acidification, NADPH depletion, or the inhibition of carboxylation by RuBisCO (Cook & Tolbert, 1982). Whether glyoxylate exerts a regulation of the Calvin and/or C-2 photosynthetic cycles via phosphoribulokinase has not been reported. However, we have found no effects of glyoxylate on the C. fritschii enzyme and our data also indicate that neither C. fritschii nor spinach RuBP carboxylase inhibition by 20 mM-glyoxylate was substantial.
Cyanobacterial phosphoribulokinase

Table 3. Summary of molecular weight and presumed quaternary structures of phosphoribulokinases from various sources

<table>
<thead>
<tr>
<th>Organism</th>
<th>Native enzyme mol. wt</th>
<th>Subunit mol. wt</th>
<th>Suggested quaternary structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatium sp.</td>
<td>240000</td>
<td>Not reported</td>
<td>–</td>
<td>Hart &amp; Gibson (1971)</td>
</tr>
<tr>
<td>Rhodopseudomonas capsulata</td>
<td>220000</td>
<td>36000</td>
<td>6 subunits</td>
<td>Tabita (1981)</td>
</tr>
<tr>
<td>Alcaligenes eutrophus</td>
<td>256000</td>
<td>33000</td>
<td>8 subunits</td>
<td>Siebert et al. (1981)</td>
</tr>
<tr>
<td>Chlorogloeopsis fritschii</td>
<td>230000</td>
<td>40000</td>
<td>6 subunits</td>
<td>This paper</td>
</tr>
<tr>
<td>Tobacco</td>
<td>90000</td>
<td>46000</td>
<td>2 subunits</td>
<td>Kagawa (1982)</td>
</tr>
</tbody>
</table>

Chloroplast phosphoribulokinase is light-activated via a membrane-bound vicinal-dithiol-containing factor, or via reduced thioredoxin (Anderson, 1979; Flügge et al., 1982). Duggan & Anderson (1975) suggested that the cyanobacterial enzyme may be similarly modulated, since phosphoribulokinase in crude extracts of Anacystis nidulans was light-activated. The low degree of stimulation by light could be mimicked in the dark by DTT (Duggan & Anderson, 1975). Phosphoribulokinase from C. fritschii was clearly activated by DTT and by GSH in vitro. However, the lack of effect of Sephadex G-75 gel filtration on the extent of DTT stimulation shows that the C. fritschii phosphoribulokinase may be regulated by reduced thiols, but no evidence for a role for thioredoxin was obtained. Clearly, this possibility is not excluded, but it is of interest that Wolosiuk & Buchanan (1978) found a form of phosphoribulokinase in spinach which was not significantly regulated by thioredoxin.

Little information is available on the molecular weight and quaternary structure of phosphoribulokinase. The molecular weight of the C. fritschii enzyme compares with that of the enzymes from other autotrophic prokaryotes (Table 3), although whether a hexameric or octameric phosphoribulokinase quaternary structure predominates within these groups awaits further purifications. The high molecular weight of the bacterial phosphoribulokinase contrasts with the 90000 of the tobacco dimeric enzyme (Table 3). Although the C. fritschii enzyme more closely resembles the other prokaryotic enzymes in size, the regulatory properties of the cyanobacterial phosphoribulokinases described by Duggan & Anderson (1975), Tabita (1981) and us share characteristics with the enzymes of Chlorella and higher plants. The essential and specific role of phosphoribulokinase in the Calvin cycle suggests that comparative studies of the enzyme molecule, and gene, from different sources may contribute to the further understanding of the evolution of autotrophy.

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REFERENCES


