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PURIFICATION, SOME CATALYTIC AND MOLECULAR PROPERTIES OF PHOSPHORIBULOKINASE FROM *ALCALIGENES EUTROPHUS*

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Summary

A key enzyme of the reductive pentose phosphate cycle, phosphoribulokinase (ATP: D-ribulose-5-phosphate 1-phosphotransferase, EC 2.7.1.19) was purified from the hydrogen bacterium *Alcaligenes eutrophus* to apparent homogeneity. The purification procedure involved affinity chromatography on Cibacron Blue-agarose and AMP-agarose as the most effective method.

Initial-velocity studies showed that the enzyme has a pH optimum of 8.6. Divalent cations were essential for its activity, with Mg^{2+} supporting maximal reaction rates. Mn^{2+}, Ca^{2+}, or Co^{2+} did partially substitute for Mg^{2+} in the reaction. The enzyme exhibited a high degree of substrate specificity with respect to the sugar phosphate, while the specificity towards the nucleoside triphosphate was less pronounced.

Saturation curves for both substrates, ribulose 5-phosphate and ATP, did not follow normal Michaelis-Menten kinetics and the enzyme was activated by NADH. Activation by NADH affected the affinity of the enzyme for its substrates. An apparent activation constant for NADH of $K_a = 0.19$ mM was obtained.

The molecular weight of the native enzyme was determined by sedimentation equilibrium centrifugation to be $M_r^{c=0} = 256\,000$. Sedimentation velocity studies indicated a sedimentation coefficient of $s^0_{20,\,w} = 10.9$ S. Dissociation and subsequent polyacrylamide gel electrophoresis of the enzyme in the presence of sodium dodecyl sulfate (SDS) revealed only one type of subunit of molecular weight 33 000. It is concluded that the enzyme is an oligomer consisting of probably eight subunits of identical size.

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Introduction

Most chemo- and photoautotrophic organisms assimilate CO_2 via the reductive pentose phosphate cycle. Phosphoribulokinase (ATP: D-ribulose-5-phosphate 1-phosphotransferase, EC 2.7.1.19) and ribulosebisphosphate carboxylase (3-phospho-D-glycerate carboxy-lyase(dimerizing), EC 4.1.1.39) are the two enzymes unique to this cycle and, therefore, have a central position in autotrophic carbon metabolism.

The regulatory properties of phosphoribulokinase suggest that the enzyme is a major point of control in CO_2 fixation [1,2]. Although the regulation of the enzyme varies among organisms, NADH, AMP and phospho*enol*pyruvate seem to be the most important effectors. This is consistent with the view that CO_2 fixation is under the control of availability of reducing power and energy charge [1]. Phosphoribulokinase from the hydrogen bacteria, *Alcaligenes eutrophus* [3] and *Pseudomonas facilis* [4,5], is activated by NADH and inhibited by both AMP and phospho*enol*pyruvate. Inhibition by the latter two compounds has also been reported for the enzyme from the sulfur bacterium *Thiobacillus neapolitanus* [6] and the purple sulfur bacterium *Chromatium* D [7]. NADH also stimulates the enzyme from the purple bacteria *Rhodospirillum rubrum* [8—10] and *Rhodopseudomonas sphaeroides* [10], whereas AMP acts as an inhibitor. Spinach chloroplast phosphoribulokinase is insensitive to NADH [11] but is also susceptible to AMP inhibition [12]. Moreover, the latter enzyme is reductively activated in the light by the ferredoxin/thioredoxin system [13,14].

All studies so far done on phosphoribulokinase were performed either with crude extracts or partially purified enzyme preparations. Thus, very little is known about the molecular characteristics of this enzyme and their relation to its function. We have initiated investigations to obtain such information. A purification procedure for the phosphoribulokinase from *A. eutrophus* has been developed which involves affinity chromatography and yields apparently homogeneous enzyme. In addition, we also report on some of its catalytic properties, data on the molecular weight as well as the subunit structure of the enzyme.

Materials and Methods

Materials. All chemicals used in this study were of analytical grade. AMP-agarose (agarose-hexane-adenosine 5′-phosphate, AGAMP™ type 2) was purchased from P-L Biochemicals, Milwaukee, WI, U.S.A. Cibacron Blue-agarose (Matrex™ Gel Blue A) was the product of Amicon, Lexington, MA, U.S.A. Ribulose 5-phosphate, ribulose 1,5-bisphosphate and sedoheptulose 7-phosphate were obtained from Sigma. St. Louis, MO, U.S.A. Alternatively ribulose 5-phosphate was also prepared by the method described by Pontremoli and Mangiarotti [15]. The other biochemicals and enzymes were purchased from Boehringer, Mannheim, F.R.G. The Radiochemical Centre, Amersham U.K., was the source of $NaH^{14}CO_3$. All reagents used for polyacrylamide gel electrophoresis were obtained from Serva, Heidelberg, F.R.G.

Growth of organisms. Autotrophic cultivation of *A. eutrophus* strain H16,

DSM 428, has been described previously [16]. Cells from the exponential phase of growth were harvested, washed twice with 20 mM imidazole-HCl buffer (pH 7.0)/10 mM $MgCl_2$/1 mM EDTA/1 mM dithioerythritol (buffer 1) and then stored at −20° C until used.

Enzyme assays. All assays were performed at 30° C. Assays involving NADH oxidation were followed at 365 nm using a Zeiss PL-4 spectrophotometer connected to a recorder. Enzyme activity is expressed in units (1 unit = 1 μmol product formed or substrate consumed/min).

Protein was measured according to Lowry et al. [17] using bovine serum albumin as standard. The activity of phosphoribulokinase was determined by two methods.

(i) Activity was determined in a two-stage assay, by the ribulose 5-phosphate-dependent incorporation of $^{14}CO_2$ into an acid-stable product when the phosphoribulokinase reaction was coupled to ribulosebisphosphate carboxylase purified from *A. eutrophus* [16]. The standard primary reaction mixture contained in a total volume of 0.25 ml, 100 mM Tris-HCl buffer (pH 8.6)/10 mM $MgCl_2$/5 mM ATP/7.5 mM ribulose 5-phosphate/an appropriate amount of enzyme. The reaction was initiated by adding the ribulose 5-phosphate, and was terminated 1 min later by heating in a boiling water bath for 1 min. After cooling, the mixture was incubated with 0.1 ml 100 mM Tris-HCl buffer (pH 8.0)/10 mM $MgCl_2$/250 mM $NaH^{14}CO_3$ (0.2 Ci/mol)/0.02 units ribulosebisphosphate carboxylase. After 20 min, 0.15 ml 7 M H_3PO_4 was added and the mixture was shaken for 30 min to completely liberate excess $^{14}CO_2$. Radioactivity of 0.1 ml-samples of the mixture was measured in a Packard liquid-scintillation spectrometer (model 3375). It was shown that phosphorylation of ribulose 5-phosphate proceeded linearly for at least 90 s.

(ii) Activity was also measured in a spectrophotometric assay by coupling the ribulose 5-phosphate-dependent formation of ADP to the oxidation of NADH in the presence of pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27). The standard reaction mixture contained in a total volume of 0.6 ml, 100 mM Tris-HCl buffer (pH 8.6)/10 mM $MgCl_2$/5 mM ATP/0.2 mM phospho*enol*pyruvate/0.6 mM NADH/30 μg pyruvate kinase/10 μg lactate dehydrogenase/1 mM ribulose 5-phosphate/an appropriate amount of enzyme. This assay was used for routine determinations during purification and for testing the sugar phosphate specificity (Table II) of the enzyme. Ribulosebisphosphate carboxylase was assayed by the procedure described previously [16].

Purification of phosphoribulokinase. All steps were carried out at 0—4° C. Cells of *A. eutrophus* (40 g wet weight) were suspended in 80 ml buffer 1 and disrupted in a French pressure cell at 140 MPa. Deoxyribonuclease (10 μg/ml) was added to the suspension which was then centrifuged at 40 000 × *g* for 20 min. The resulting supernatant was centrifuged at 140 000 × *g* for 60 min. The final supernatant was referred to as crude extract (Table I), to which protamine sulfate was added to give a final concentration of 0.03 mg/mg protein. The precipitate was removed by centrifugation at 40 000 × *g* for 15 min. After protamine sulfate treatment, a saturated $(NH_4)_2SO_4$ solution (pH 7.0) was used to prepare a 25—40% $(NH_4)_2SO_4$ fraction from the crude extract. The enzyme solution was then subjected to sedimentation into a linear

sucrose density gradient (0.2—0.8 M in buffer 1) at 115 000 × *g* for 24 h using a SW-27 rotor (Beckman Instruments, Palo Alto, CA, U.S.A.) [16]. The fractions with highest enzyme activity (Fig. 1) were pooled and the protein precipitated with $(NH_4)_2SO_4$ (to 50% saturation).

The $(NH_4)_2SO_4$ fraction was dialyzed against buffer 1 and applied onto a Cibacron Blue-agarose column (2.6 × 13.5 cm) equilibrated with the same buffer. The column was then successively washed with 4 bed vol. of each of the following solutions: buffer supplemented with 100 mM KCl; buffer with 100 mM KCl and 2 mM NAD^+; buffer with 100 mM KCl and 3 mM ATP. Phosphoribulokinase activity was eluted with buffer containing 100 mM KCl and 15 mM ATP. Fractions with enzyme activity (70—260 ml) were combined and the protein was precipitated with $(NH_4)_2SO_4$ as above. After dialysis the enzyme was applied onto an AMP-agarose column (1.1 × 20.8 cm) equilibrated with buffer 1. The column was washed with 4 bed vol. each of buffer containing 100 mM KCl and of buffer with 100 mM KCl and 5 mM ATP. Enzyme activity was eluted with buffer containing 100 mM KCl and 15 mM ATP. Following concentration by $(NH_4)_2SO_4$ precipitation the enzyme was finally dialyzed against buffer 1 and stored at 0—4° C.

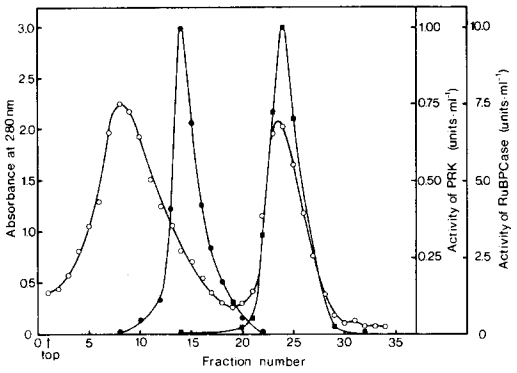
Ultracentrifuge studies. Determination of the molecular weight of phosphoribulokinase was performed by low-speed sedimentation equilibrium [18] in a Beckman-Spinco model E ultracentrifuge equipped with monochromator, photoelectric scanner and multiplexer. Double-sector cells with 12 mm light-path were used. The duration of the runs was 19—20 h at 6000 rev./min and 5° C. Molecular weight calculations were done according to Schachman [19] assuming a partial specific volume of 0.74 ml/g for the phosphoribulokinase. The sedimentation velocity experiments were carried out at 30 000 rev./min at 5° C and scanner tracings at 280 nm were taken every 8 min. Calculation of the sedimentation coefficients was done by the moving-boundary method. The values obtained were corrected for temperature, density and viscosity at standard conditions, 20° C and water. Enzyme dissolved in buffer 1 at various concentrations (0.107—0.785 mg/ml) was used throughout these experiments.

Electrophoresis. Native polyacrylamide gel electrophoresis was performed according to Davis [20]. The method of Weber et al. [21] was used for SDS-polyacrylamide gel electrophoresis.

Results

Purification of phosphoribulokinase

Introductory experiments, designed to develop a selective purification method for phosphoribulokinase from *A. eutrophus*, revealed that the enzyme adsorbed to blue-dye affinity media (Blue Dextran-Sepharose or Cibacron Blue-agarose) as well as AMP-agarose. The enzyme could be eluted from the affinity columns by including appropriate concentrations of ATP in the elution buffer (see Materials and Methods). The best purification was obtained when ribulose-bisphosphate carboxylase, which also adsorbed to the blue-dye matrices, was separated from phosphoribulokinase by sucrose density gradient centrifugation (Fig. 1) before application of the enzyme fraction to the affinity columns. The sequential use of two affinity columns proved to be most successful.



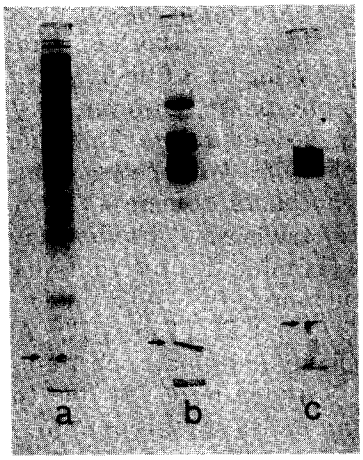


Fig. 1. Separation of phosphoribulokinase from ribulosebisphosphate carboxylase by sucrose density gradient centrifugation. A linear gradient (36 ml total volume) of 0.2—0.8 M sucrose was centrifuged in a SW-27 rotor at 115 000 × *g* for 24 h. Phosphoribulokinase activity was measured by the spectrophotometric assay. ○———○, protein content (as absorbance at 280 nm); ●———●, activity of phosphoribulokinase (PRK); ■———■, activity of ribulosebisphosphate carboxylase (RuBPCase).

Fig. 2. Analytical SDS-polyacrylamide gel electrophoresis of different phosphoribulokinase preparations. Protein was treated for 5 min at 100°C in a solution containing 1% (w/v) SDS and 100 mM dithioerythritol. Approx. 25 μg protein were electrophoresed on gels polymerized from 10% (w/v) acrylamide in the presence of SDS, until the tracking dye (marked by arrow) reached the end of the gel. (a) enzyme fraction after sucrose density gradient centrifugation; (b) enzyme fraction after affinity chromatography on Cibacron Blue-agarose; (c) final enzyme preparation after affinity chromatography on AMP-agarose.

A complete purification protocol is given in Table I. The enzyme was purified about 55-fold with an activity yield of 4.6%. SDS-polyacrylamide gel electrophoresis of the final enzyme preparation yielded a single protein band suggesting the phosphoribulokinase was apparently homogeneous (Fig. 2). The enzyme appeared to be unstable during native polyacrylamide gel electrophoresis. No formation of discrete protein bands was observed using a variety of electrophoresis systems. The enzyme showed no significant loss of activity after storage at 0—4°C for 1 month.

TABLE I

PURIFICATION OF PHOSPHORIBULOKINASE FROM *A. EUTROPHUS*

Enzyme activity was determined by the two-stage radiometric assay.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg protein)	Yield (%)	Purification (-fold)
Crude extract	3420	475	0.139	100	1.0
Protamine sulfate supernatant	3335	460	0.138	96.8	0.99
25—40% $(NH_4)_2SO_4$ precipitate	674	438	0.650	92.2	4.7
Sucrose density gradient centrifugation	111	256	2.31	53.4	16.6
Cibacron Blue-agarose chromatography	13.8	96	6.96	20.2	50.1
AMP-agarose chromatography	2.9	22	7.59	4.6	54.6

Catalytic properties of the enzyme

General properties. Phosphoribulokinase exhibited maximum activity at a pH value of 8.6 in Tris-HCl buffer. The enzyme was highly specific for ribulose 5-phosphate, however, very slow reactions occurred with fructose 6-phosphate or sedoheptulose 7-phosphate (Table II). Ribose 5-phosphate was not phosphorylated indicating that the phosphoribulokinase did not contain ribose-phosphate isomerase (EC 5.3.1.6). The specificity of the enzyme with respect to nucleoside triphosphates was less pronounced. With UTP or GTP as substrates the reaction velocity reached 37.5 or 12.6%, respectively, of that measured with ATP under identical conditions. No reaction was observed with ITP or CTP (Table III). Also, the enzyme did not function with PP_i as high-energy phosphate substrate. The observed enzyme activities with the alternative substrates were not due to small quantities of ribulose 5-phosphate or ATP, present as impurities in the compounds tested.

The enzyme required divalent cations for activity. Maximum activity was reached in the presence of Mg^{2+} at concentrations above approx. 5 mM of the free ion. The concentration of free Mg^{2+} was calculated using the stability constant of 73 000 M^{-1} for the $MgATP^{2-}$ complex [22]. Mn^{2+}, Co^{2+} or Ca^{2+} could partially replace Mg^{2+}, while Ni^{2+} was ineffective. At a metal ion concconcentration of 2 mM the reaction rate with Mn^{2+}, Co^{2+} or Ca^{2+} was 58, 20 or 51%, respectively, of that measured with Mg^{2+}. An increase of Mn^{2+} above 2 mM or Ca^{2+} above 5 mM was inhibitory.

Kinetic properties. Initial-velocity studies showed that the saturation curves of phosphoribulokinase for ribulose 5-phosphate did not follow normal Michaelis-Menten kinetics (data not shown). From the Hill plot the $S_{0.5}$ value for ribulose 5-phosphate was calculated to be 2.2 mM at 10 mM ATP with a Hill coefficient, n_H, of 1.4. This indicated a weak positive cooperativity of ribulose 5-phosphate binding. Sigmoid saturation curves were observed for ATP (data not shown). However, the degree of positive cooperativity between ATP-binding sites was low as suggested by the n_H value of 1.5. The $S_{0.5}$ value for ATP was 6.8 mM at 10 mM ribulose 5-phosphate. Kinetic properties of phosphoribulokinase determined in crude extracts were similar to those observed with the homogeneous enzyme.

Activation by NADH. The enzyme was found to be activated by NADH,

TABLE II

SUGAR PHOSPHATE SPECIFICITY OF *A. EUTROPHUS* PHOSPHORIBULOKINASE

Enzyme activity was determined by the spectrophotometric assay. Concentrations of the sugar phosphates were 2 mM. The control contained no sugar phosphate.

Sugar phosphate	Activity (nmol ADP formed/min^{-1})	% Rate with ribulose 5-phosphate
Ribulose 5-phosphate	15.9	100
Ribose 5-phosphate	0	0
Fructose 6-phosphate	0.57	3.6
Sedoheptulose 7-phosphate	0.39	2.5
Control	0	0

TABLE III

NUCLEOSIDE TRIPHOSPHATE SPECIFICITY OF *A. EUTROPHUS* PHOSPHORIBULOKINASE

Enzyme activity was determined by the two-stage radiometric assay. Concentrations of the nucleotides were 10 mM. The control contained no nucleotide.

Nucleoside triphosphate	Activity (nmol ribulose bisphosphate formed/min)	% Rate with ATP
ATP	16.9	100
GTP	2.13	12.6
ITP	0	0
UTP	6.34	37.5
CTP	0	0
Control	0	0

thus confirming the earlier results of Abdelal and Schlegel [3]. This activation was very specific since other pyridine nucleotides such as NAD^+, $NADP^+$ and NADPH had no effect. The saturation curve for NADH was of the hyperbolic type showing that NADH increased the value V of the enzyme reaction (Fig. 3). By expressing the degree of activation in percent over the activity of the enzyme in the absence of NADH and plotting these data according to Lineweaver-Burk, an apparent activation constant, K_a, for NADH of 0.19 mM could be calculated (insert, Fig. 3). The value remained constant at different substrate concentrations. In the presence of 0.75 mM NADH the $S_{0.5}$ value for ribulose 5-phosphate decreased to 1.2 mM, that for ATP to 4.8 mM. Likewise, the n_H values slightly decreased from 1.4 to 1.2 for ribulose 5-phosphate and from 1.5 to 1.3 for ATP.

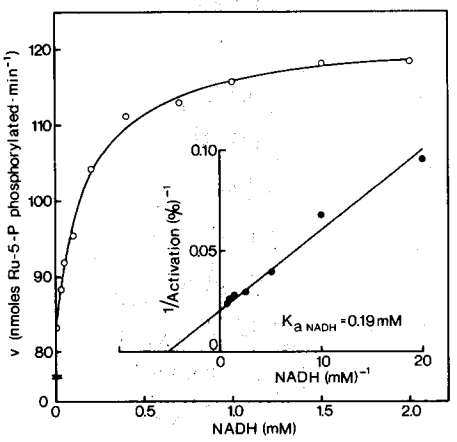


Fig. 3. Activation of phosphoribulokinase at increasing concentrations of NADH. Enzyme activity was measured by the two-stage radiometric assay. Reaction mixtures contained in a final volume of 0.25 ml, 100 mM Tris-HCl buffer (pH 8.6); 15 mM $MgCl_2$; 10 mM ATP; 10 mM ribulose 5-phosphate; 10 μg enzyme and NADH as indicated. (Insert) Analysis of the data according to Lineweaver-Burk. Activation is expressed in percent over the activity of the enzyme without NADH.

Molecular characteristics of the enzyme

Molecular weight. An apparently homogeneous preparation of phosphoribulokinase was subjected to analytical ultracentrifugation at various low protein concentrations. The molecular weight of the enzyme was determined by the low-speed sedimentation equilibrium technique. Experimental plots of r^2 (r = distance of sample from axis of rotation) vs. log c (c = protein concentration) were linear (data not shown), confirming that the enzyme was homogeneous and did not dissociate during centrifugation. The apparent molecular weights were nearly independent of the protein concentration. A molecular weight of $M_r^{c=0} = 256\,000$ at a protein concentration of zero was calculated. This value coincided very well with a $M_r = 252\,000$ estimated by gel filtration on a Sephadex G-200 column. The molecular weight did not change detectably during the purification.

From the data of velocity centrifugations the sedimentation coefficient of the enzyme at infinite dilution was determined to be $s^0_{20,w} = 10.9$ S.

Subunit structure. When the pure enzyme was treated with SDS subsequent electrophoresis in SDS-polyacrylamide gels revealed the presence of only one type of subunit (see Fig. 2c). The molecular weight of the subunit calculated from its relative mobility was 33 000 ± 1000, using catalase (M_r 58 000), ovalbumin (45 000), aldolase (40 000), chymotrypsinogen (25 700) and cytochrome c (12 500) as standards. This result, in connection with the data on the molecular weight of the native enzyme, suggests that the intact molecule of phosphoribulokinase is an oligomer consisting of probably eight subunits of identical size.

Discussion

Affinity chromatography on blue-dye substituted gel matrices such as Blue Dextran-Sepharose and Cibacron Blue-agarose has been successfully used in the purification of a number of dehydrogenases and kinases [23—26]. These enzymes require nucleotides either as coenzymes or substrates. In both of the mentioned affinity media Cibacron Blue F-3GA is the ligand which brings about the binding of the enzymes. This dye has been proposed to be a structural analogue of adenine nucleotides and to interact with enzymes apparently possessing a dinucleotide fold [27,28] or a nucleotide binding site [25]. It is, thus, not surprising that this group of enzymes also binds to immobilized adenine nucleotides such as AMP, ADP and ATP [29—31].

The results of the present study demonstrate the usefulness of Cibacron Blue-agarose and particularly of AMP-agarose in purifying phosphoribulokinase from *A. eutrophus*. However, the activity yields of the individual affinity steps were only between 23 and 38%, much lower than usually observed in affinity chromatography. The activity losses were attributable to partial inactivation rather than to incomplete elution of the enzyme. The yields might be improved by modification of the elution conditions. Nevertheless, the complete procdure allows the simultaneous purification of both key enzymes of the reductive pentose phosphate cycle, phosphoribulokinase and ribulosebisphosphate carboxylase [cf. Ref. 16]. Similar procedures could conceivably be used to purify the phosphoribulokinase from other autotrophic organisms as well. Very

recently, the isolation of homogeneous kinase from *Rhodopseudomonas capsulata* involving affinity chromatography on Cibacron Blue-agarose was reported [10].

An unexpected observation was the adsorption of ribulosebisphosphate carboxylase to Cibacron Blue-agarose, since the enzyme is not nucleotide-dependent. This finding may be explained by a reported nonspecific, hydrophobic interaction of the dye with proteins [25,32].

Abdelal and Schlegel [3] determined a pH optimum of 9.0 for the phosphoribulokinase from *A. eutrophus*. We found maximal reaction rates at pH 8.6 regardless of the presence or absence of the activator NADH. The optimum of the *A. eutrophus* enzyme is considerably higher than that reported for the enzyme from *T. neapolitanus* [6], *Chromatium* D [7], spinach [33] and pea [34]. The *A. eutrophus* kinase resembles the same enzyme from other sources [7,33] in its absolute requirement for divalent metal ions. Its specificity for nucleoside triphosphates, however, appears to be less strict. UTP was not a substrate of the *Chromatium* enzyme [7] and supported only very slow reaction rates with the spinach enzyme [33].

Basic kinetic constants of phosphoribulokinase from *A. eutrophus*, as determined in the present work, are similar to those reported previously [3]. A difference was observed for the ATP saturation kinetics. We did no find a sequence of positive and negative cooperativity in ATP binding. The activation of the enzyme by NADH was confirmed. Yet, in contrast to the earlier data [3], NADH did not abolish the weak positive cooperativity in ribulose 5-phosphate binding. It lowered the $S_{0.5}$ values of the enzyme for both substrates, although the positive cooperativity of substrate binding was not greatly altered. The activation constant for NADH, of K_a = 0.19 mM, is about 10-times higher than the one measured for the enzyme from *Nitrobacter winogradskyi* [11].

The intracellular concentration of NADH in autotrophically growing cells of *A. eutrophus* was 0.83 mM [35], well above the activation constant of the enzyme for this effector. NADH regulation seems to be a common feature of phosphoribulokinase in the chemolithotrophic hydrogen bacteria. Not only the enzyme from *A. eutrophus* and *P. facilis* [4], but also that from *Paracoccus denitrificans*, *Xanthobacter autotrophicus*, *Nocardia opaca 1b*, and *Arthrobacter* 11/x is activated by NADH (Schobert, P. and Bowien, B., unpublished data). It was also found that the kinase from different species of purple bacteria was activated by NADH to varying degrees [10].

Little information is available on the molecular weight of phosphoribulokinase. This work presents for the first time a detailed study on this subject. The molecular weight of $M_r^{c=0}$ = 256 000 determined for the *A. eutrophus* enzyme is similar to the values of 240 000 and 220 000 estimated for the enzymes from *Chromatium* [7] and *R. capsulata* [10], respectively. It is tempting to speculate that the phosphoribulokinase from other organisms may fall within the same size range.

Until now, only few data were reported on the subunit structure of phosphoribulokinases. Lavergne and Bismuth [36] estimated the molecular weight of the subunit of the spinach enzyme to be 46 000, but no data on the size of the native enzyme were given. Tabita [10] found phosphoribulokinase from *R. capsulata* to presumably be a hexamer of identical subunits with a molecular

weight of 36 000. Our results suggested that the phosphoribulokinase from *A. eutrophus* contains one type of subunit with a molecular weight of 33 000. The enzyme is probably an octamer. In view of a possible evolutionary relationship between phosphoribulokinase and phosphofructokinase (EC 2.7.1.11) it is of interest to note that bacterial phosphofructokinases are commonly tetramers consisting of identical size subunits with molecular weights between 35 000 and 38 000 [37—39]. Further experiments are certainly necessary to prove the proposed quaternary structure of the *A. eutrophus* phosphoribulokinase.

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