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Properties of Phosphoribulokinase from *Thiobacillus neapolitanus*

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Partially purified preparations of ribulose-5-phosphate kinase (specific activity, 50 to 125 μmoles per min per mg of protein) were employed in a series of kinetic experiments in the presence of several concentrations of H^+, Mg^{2+}, adenosine triphosphate (ATP), and phosphoenolpyruvate (PEP). The *p*H optimum of the enzyme was found to be 7.9; at this *p*H and above, response of the enzyme to variations in ATP concentration was hyperbolic, exhibiting a K_m of 7×10^{-4} M ATP. At *p*H values below the optimum the response to ATP was sigmoidal, as it was throughout the entire *p*H range in the presence of PEP at a concentration greater than 5×10^{-4} M. In the presence of PEP the *p*H optimum shifted to *p*H 8.4. In contrast, phosphoribulokinase from spinach exhibited hyperbolic responses throughout its *p*H range with no inhibition caused by PEP. *Thiobacillus neapolitanus* phosphoribulokinase was inhibited by PEP in a sigmoidal manner; however, in the presence of suboptimal concentrations of Mg^{2+} the addition of PEP caused significant stimulation of activity. It is postulated that the enzyme consists of interacting subunits with several sites on the enzyme for binding ATP and with several separate sites binding PEP. It is suggested that PEP functions as a regulator of CO_2 fixation when the organism is under conditions of unlimited concentrations of substrate and CO_2.

Carbon dioxide fixation in autotrophic bacteria appears to occur primarily by way of ribulose diphosphate carboxylase (6, 18). Regulation of the process was first demonstrated in cell-free extracts of *Thiobacillus thioparus* by Johnson and Peck (12), who observed that adenosine monophosphate (AMP) inhibited the adenosine triphosphate (ATP)-dependent incorporation of CO_2. The site of inhibition was identified as ribulose-5-phosphate kinase (phosphoribulokinase [PRK], ATP:D-ribulose-5-phosphate 1-phosphotransferase [EC 2.7.1.19]) (12, 16).

In view of the significant role played by ribulose-5-phosphate kinase in the regulation of carbon dioxide fixation in a variety of autotrophic bacteria (7, 8, 11, 16, 17, 23), we have examined several aspects of the enzyme from *Thiobacillus neapolitanus*, using partially purified preparations, with the goal of elucidating some of the mechanisms which control its activity and, hence, the larger process of carbon dioxide fixation.

MATERIALS AND METHODS

Preparation of cell extracts. *T. neapolitanus* (obtained from W. Vishniac) was grown and harvested as previously described (15). A 10% (wet weight/volume) suspension of cells in buffer (0.05 M potassium phosphate, *p*H 6.5) was passed through a French pressure cell at 20,000 psi, and the resulting homogenate was centrifuged at 20,000 × *g* for 30 min. The supernatant fraction was treated with ammonium sulfate at 2 C, first to 45% saturation and then to 70% saturation. The first precipitate was discarded; the second precipitate was dissolved in buffer and passed through a column (2.5 by 35 cm) of Sephadex G-25 (coarse) equilibrated with buffer. The volume of the ammonium sulfate-free protein was reduced, when necessary, by membrane filtration on a PM-30 filter (Amicon Corp., Bedford, Mass.) at 2 C.

Desalted protein (up to 100 mg in a volume of 20 ml) was adsorbed onto a column (2.0 by 45 cm) of diethylaminoethyl (DEAE)-Sephadex that had been washed and equilibrated with buffer. The column was then washed with 100 to 160 ml of buffer, and developed with a linear gradient of potassium phosphate ranging from 0.05 to 0.5 M (*p*H 6.5), total volume, 400 ml. Fractions of about 3.5 ml were collected at 2 C. Fractions containing ribulose-5-phosphate kinase were pooled and passed through a column (2.0 by 45 cm) of G-200 Sephadex equilibrated with buffer. Fractions containing PRK activity were again pooled and concentrated by mem-

brane filtration. The concentrated enzyme was either used directly or chromatographed a second time, under the same conditions, on DEAE-Sephadex A-25.

Spectrophotometric assay (standard conditions). The purification procedure removed reduced nicotinamide adenine dinucleotide (NADH) oxidase and ATP phosphohydrolase activities and allowed the use of a spectrophotometric assay of PRK. This assay was a modification of the procedure of Hurwitz (9) and depended upon the measurement of adenosine diphosphate produced by the phosphorylation of ribulose-5-phosphate by ATP. An auxiliary enzyme system consisting of phosphoenolpyruvate (PEP), pyruvate kinase, and lactate dehydrogenase was used to promote the oxidation of NADH stoichiometric with ribulose-5-phosphate phosphorylation. NADH oxidation was followed at 340 nm with a Gilford spectrophotometer with a range of 0 to 0.200 absorbancy units full scale.

Assay of PRK was carried out in a volume of 1.00 ml with the following components: sodium ATP, 5.0 mM; $MgCl_2$, 5 mM; tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 92 mM; KCl, 35 mM; potassium ribulose-5-phosphate, 0.2 mM; NADH, 0.3 mM; pyruvate kinase, 10 units; lactate dehydrogenase, 15 units; sodium PEP, 0.2 mM. Assays were conducted at 23 or 30 C. The amount of PRK added could be varied between 0.005 to 0.5 units (1 unit = 1 μmole NADH oxidized/min at 30 C) with linear response to enzyme concentration.

$^{14}CO_2$ incorporation assay. Because of interfering enzymes in crude preparations (ATP phosphohydrolase, NADH oxidase); or because of the addition of compounds interfering with the spectrophotometric assay, a $^{14}CO_2$-incorporation reaction was occasionally used to estimate the activity of phosphoribulokinase. This assay, previously described (15), required the presence of the auxiliary enzymes ribose-5-phosphate isomerase and ribulose diphosphate carboxylase. The reaction depended upon the phosphorylation of ribulose-5-phosphate, produced by the isomerase, to ribulose diphosphate; $^{14}CO_2$ incorporated into the ribulose diphosphate by the carboxylase became acid stable and was counted after the elimination of excess $^{14}CO_2$ by the addition of acid. Preparation of both ribulose-5-phosphate isomerase (R. D. MacElroy and M. K. Johnson, *manuscript in preparation*) and ribulose diphosphate carboxylase (4) have been described.

Protein determinations. Protein was estimated by the procedure of Lowry et al. (14).

Chemicals. ATP, NADH, PEP, ribose-5-phosphate, and Tris were obtained from Sigma Chemical Co., St. Louis, Mo. Ribulose-5-phosphate was either obtained from Sigma or prepared by the method of Pontremoli and Mangiarotti (21). Lactate dehydrogenase and pyruvate kinase were obtained from Boehringer, New York. Spinach phosphoribulokinase was obtained from Sigma or prepared by the procedure of Racker (22). Other chemicals were of reagent grade and obtained from commercial sources.

RESULTS

The specific activity of phosphoribulokinase in the cell homogenate was 0.067 unit/mg; after a complete purification procedure the specific activity attained was as high as 450 units/mg. Preparations with high specific activity (above 300 units/mg) were very labile and lost up to 50% of their activity per day. Stabilizing agents, such as dithiothreitol, 2-mercaptoethanol, glutathione, glycerine, or ethylene glycol were ineffective in stabilizing the enzyme; however, in the presence of bovine serum albumin the rate of decay of the enzyme was decreased slightly.

For the purpose of the experiments to be described, the final purification step, rechromatography on DEAE-Sephadex, was omitted and enzyme preparations having a stable specific activity of 50 to 125 units/mg of protein were used. Such preparations lost less than 10% of their activity when kept at 2 C for periods of 1 year or longer.

Elution of phosphoribulokinase from DEAE-Sephadex occurred at a phosphate concentration between 0.20 and 0.27 M (Fig. 1). PRK was eluted just prior to the elution of ribulose diphosphate carboxylase (0.27 to 0.32 M phosphate); ribose-5-phosphate isomerase was washed from the column with buffer before the gradient was applied. The pooled fractions containing PRK were concentrated and chromatographed on G-200 Sephadex; the fractions containing PRK emerged shortly after Dextran-2000.

Even the most highly purified preparations of PRK (450 units/mg) were found by electrophoresis on polyacrylamide gel to have at least two contaminating proteins. Isoelectric focusing (LKB Instruments, Bromma, Sweden) indicated an isoelectric point of 4.7, with a single peak of activity in the eluted fractions. The temperature optimum of phosphoribulokinase was found to be 38 C, with a Q_{10} of 2.5 between 20 and 30 C and 1.6 between 30 and 40 C.

Kinetic responses of phosphoribulokinase under standard conditions. The response of phosphoribulokinase to variations in ribulose-5-phosphate concentration was hyperbolic as reported previously (15). The K_m for ribulose-5-phosphate at saturating concentrations of ATP and Mg^{2+} was 2.4×10^{-5} M and was not appreciably affected by *p*H or by ATP or Mg^{2+} concentrations. The saturation of PRK by ATP at saturating concentrations of Mg^{2+} and ribulose-5-phosphate was also hyperbolic under standard conditions (Fig. 2). The K_m for ATP was calculated as 7.1×10^{-4} M. The activity of the enzyme increased sharply between *p*H 7.0 and the *p*H optimum of 7.95, and the enzyme was found to be inactive below *p*H 7.0 and above *p*H 9.2 (Fig. 3, curve A).

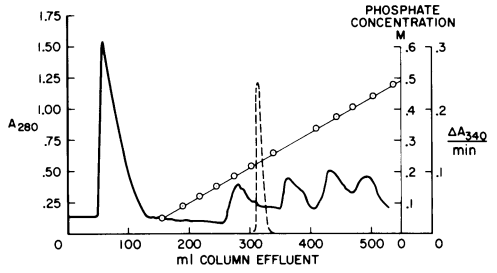

FIG. 1. *Chromatography of phosphoribulokinase on DEAE-Sephadex.* ——, *Absorbancy at 280 nm* (A_{280}); – – –, *phosphoribulokinase activity as change in absorbancy per min at 340 nm* (ΔA_{340}/*min*); (-⊙-), *concentration of potassium phosphate, pH 6.5. DEAE-Sephadex was prepared by washing with 0.5* N *KOH until all* Cl^- *had been removed. After two washes with water, the gel was washed twice with 0.5* N H_3PO_4, *three times with water, and neutralized with 0.5* N *KOH. It was equilibrated over 24 hr with buffer (0.05* M *phosphate, pH 6.5), poured into a column (2.0 by 45 cm), and cooled at 2 C. Protein (100 mg), containing about 550 units of phosphoribulokinase activity, was placed on the column in a volume of 20 ml. The column was washed with 160 ml of buffer, and elution of the remaining protein was begun with a linear gradient of potassium phosphate (pH 6.5), 0.05* M *to 0.5* M. *Phosphoribulokinase was assayed spectrophotometrically as described;* A_{280} *was measured with a Gilford spectrophotometer.*

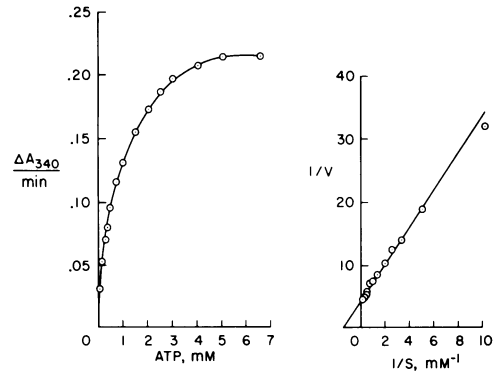

FIG. 2. *Saturation of phosphoribulokinase (PRK) with ATP at pH 7.95. To each cuvette was added 5 μliters of partially purified PRK containing 4 μg of protein. Standard assay conditions were used; incubation temperature was 30 C. The* K_m *was calculated as* 7.1×10^{-4} M *ATP.*

The effect of a variety of compounds on the activity of PRK revealed several which were inhibitory or stimulatory (Table 1, column 1). Of these, PEP and AMP were most inhibitory, and glucose-6-phosphate and glycine were slightly stimulatory.

Figure 4 shows the effect of increasing concentrations of PEP on the activity of PRK. It

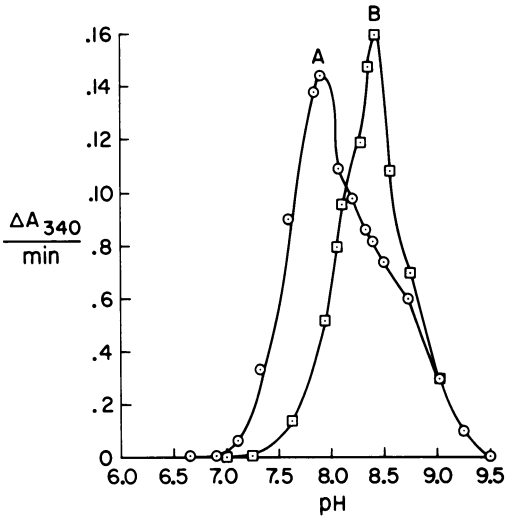

FIG. 3. *Effect of pH on the activity of phosphoribulokinase under standard conditions, and in the presence of 2.3* mM *phosphoenolpyruvate. A, Each cuvette contained reaction components at standard concentrations including Tris-hydrochloride which had been adjusted to the pH used. Immediately before the addition of 3 μg of extract protein the pH was determined. B, Conditions were as described above except that each cuvette also contained 2.3* mM *phosphoenolpyruvate, and the reaction was initiated with 6 μg of extract protein. At the pH extremes, the auxiliary enzyme system functioned at a rate at least 15 times that of the maximal activity of the phosphoribulokinase under optimal conditions.*

can be seen that at low concentrations the compound was ineffective as an inhibitor, or stimulated slightly. As the concentration increased, however, inhibition became stronger, although inhibition of more than 80% was never observed even at inhibitor concentrations equal to that of the substrate. At *p*H 8.4 the $I_{0.5}$ (50% of maximal inhibition) was calculated to be about 2.4 mM.

Saturation of phosphoribulokinase with ATP was strongly *p*H-dependent (Fig. 5). In the *p*H range between 7.0 and 7.8, saturation curves were sigmoidal, whereas at the *p*H optimum and at higher *p*H the response to ATP was hyperbolic.

Kinetic responses of PRK in the presence of high concentrations of PEP. The addition of PEP at a concentration of 2.3 mM changed the *p*H optimum of PRK activity from *p*H 7.9 to *p*H 8.4 (Fig. 3, curve B). The response of the enzyme to saturation by ATP at several *p*H values in the presence of 2.3 mM PEP is shown in Fig. 6. It can be seen that in all cases the response was sigmoidal, with interaction coef-

TABLE 1. *Effect of several compounds on the activity of phosphoribulokinase*[a]

Compound added[b]	Per cent inhibition	Per cent inhibition (plus 2.3 mM PEP)
None	0[c]	39
AMP	15	83
Acetylphosphate	10	65
3-Phosphoglycerate	5	46
Pyruvate	0	67
Glyoxalate	0	63
Fructose-6-phosphate	0	40
NADH	−10	46
α-Glycerophosphate	−13	32
Glyceraldhyde-3-phosphate	−13	61
Glycine	−16	41
Glucose-6-phosphate	−25	54

[a] The conditions of the assay have been described previously (15). The incorporation of $^{14}CO_2$ into acid-stable material in the presence of ribose-5-phosphate, added ribose-5-phosphate isomerase and ribulose diphosphate carboxylase, and ATP, was determined by liquid scintillation radioactivity counting. The reaction was initiated by the addition of 1.2 μg of extract protein in a volume of 5 μliters.

[b] Each of the added compounds was present at a concentration of 0.7 mM except for AMP, NADH, and pyruvate, each of which was present at a concentration of 3.5 mM. None of the compounds added was found to affect the activities of ribulose-5-phosphate isomerase or ribulose diphosphate carboxylase when the enzymes were tested separately.

[c] The control (0% inhibition) incorporated 6,320 counts per min per μg of protein.

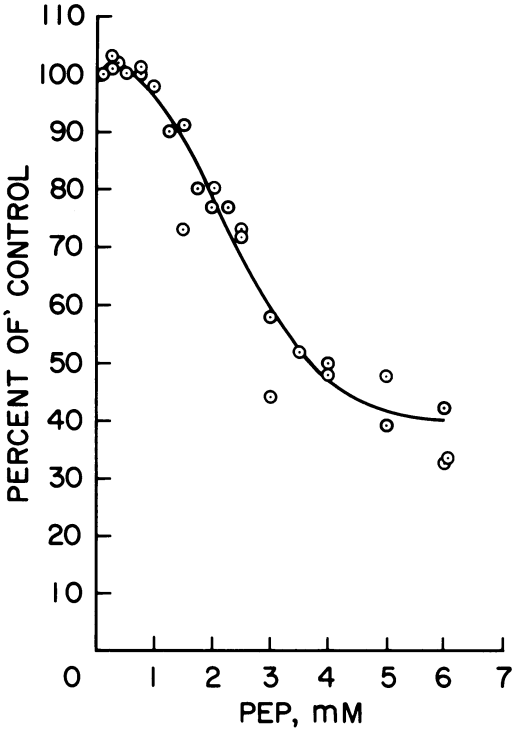

FIG. 4. *Effect of phosphoenolpyruvate on the activity of phosphoribulokinase. The assay used was that described in Table 1; the compound had no effect on the auxiliary enzymes at the concentrations used. The pH of the assay mixture was 8.4. The* $I_{0.5}$ *(50% of maximal inhibition) for PEP was calculated as 2.4 mM. (The interaction coefficient [n], calculated according to the relationship* $S_{0.9}/S_{0.1} = 81^{1/n}$ *[24], was 3.5 at pH 8.4).*

ficients decreasing from 3.9 at pH values below the optimum to 2.3 near the pH optimum.

To eliminate the possibility that the sigmoidal responses of the PRK to increased ATP concentration could be caused by effects on the auxiliary enzyme system, commercial spinach PRK was tested for response to ATP (Fig. 7). Both commercial preparations, and those prepared from fresh extracts, were found to have hyperbolic saturation curves at all pH values tested, and to have pH optima at 7.8. The spinach enzyme was unaffected by PEP.

The effect of excess PEP on PRK activity was pH-dependent (Table 2, line 4) compared to standard conditions (line 3). A stimulation of activity was observed both at standard (line 4) and low Mg^{2+} concentrations. However, at standard Mg^{2+} concentrations, excess PEP strongly inhibited at the pH optimum and below.

Several compounds affected the activity of PRK (Table 1). Some caused significant stimulation (e.g., glucose-6-phosphate), others inhibited (AMP, acetyl phosphate). The addition of PEP in the presence of these compounds generally caused inhibition greater than that of PEP alone, an effect also observed with PRK from *Pseudomonas facilis* (4).

DISCUSSION

The data we have presented emphasize the effect of pH on the activity and the regulation of ribulose-5-phosphate kinase. The pH-activity curve for the enzyme is sharp, and hyperbolic response of ATP is observed at or above the pH optimum. Below the optimum the enzyme exhibits a sigmoidal response to ATP, but a hyperbolic response to ribulose-5-phosphate throughout the pH range of the enzyme. The addition of PEP to the enzyme results in a sigmoidal, concentration-dependent inhibition curve and a shift in the pH optimum of PRK. Below the optimal pH, a Mg^{2+} "sparing" effect can be observed upon the addition of more PEP. It would thus ap-

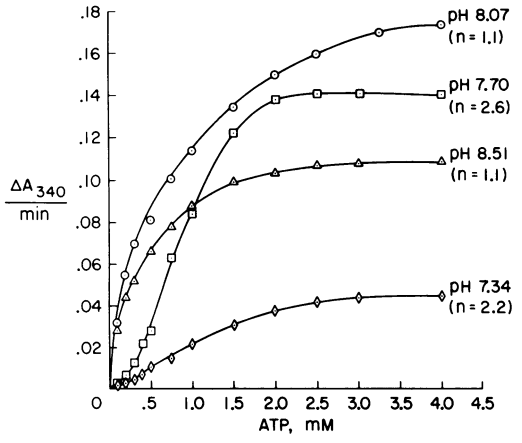

FIG. 5. *Effect of pH on the saturation of phosphoribulokinase by ATP under standard conditions. Reactions were assayed spectrophotometrically; pH was determined immediately before the reaction was initiated with the addition of 1.5 μg of extract protein. Interaction coefficients (n) were calculated as described in Fig. 4.*

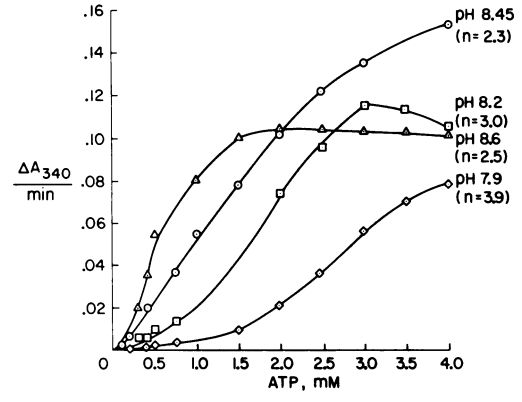

FIG. 6. *Effect of pH on the saturation of phosphoribulokinase by ATP in the presence of 2.3 mM phosphoenolpyruvate.*

pear that PEP can inhibit the enzyme under optimal conditions (*p*H 8.0, 5 mM Mg^{2+}), but while in the presence of suboptimal Mg^{2+} concentrations it can stimulate activity.

The implications of sigmoidal substrate saturation curves are that the enzyme contains multiple interacting substrate binding sites (1), and that the binding of some ligands acts to alter the enzyme configuration at distant catalytic sites, either directly, by subunit interaction (13), or indirectly, by shifting the equilibrium between active and inactive forms of an oligomeric enzyme (19). Changes in hydrogen ion concentration caused a shift in the binding constant of PRK and ATP, as reflected in altered $S_{0.5}$ values and shifts in the shape of the substrate response curves. Interpreted according to the models of allosteric enzymes requiring participation of subunits (13, 19), the data would suggest that both ATP and H^+ (or OH^-) can function as modifiers of catalytic sites. ATP could act by increasing the concentration of active forms of the enzyme or by altering the catalytic sites on a protomer distant from an initial binding site. A decrease in H^+ concentration could promote an increase in active forms of the enzyme or alter subunit interactions in such a manner that they would no longer have an effect on the configuration of

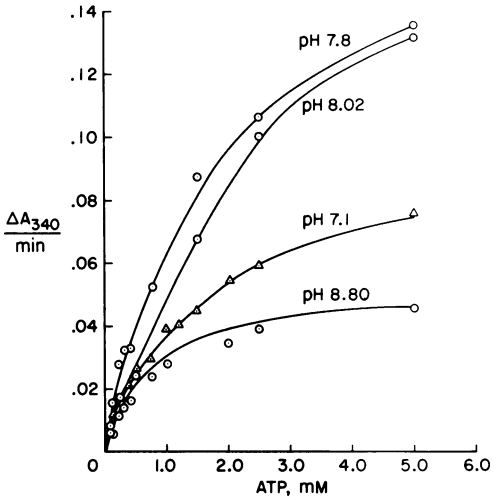

FIG. 7. *Effect of pH on the saturation of spinach phosphoribulokinase by ATP in the presence of 2.3 mM phosphoenolpyruvate. Spinach phosphoribulokinase was prepared by the procedure of Racker (22). NADH oxidase activity was eliminated by chromatography of G-200 Sephadex. Activity was assayed spectrophotometrically as described.*

TABLE 2. *Effect of variations of Mg^{2+} and phosphoenolpyruvate concentration on the activity of phosphoribulokinase*[a]

Additions (mM)		Activity[b] at *p*H:			
Mg^{2+}	PEP	7.52	8.00	8.35	8.90
2.00	0.2	0.008	0.080	0.064	0.010
2.00	2.3	0.012	0.172	0.230	0.104
5.0	0.2	0.144	0.420	0.350	0.228
5.0	2.3	0.055	0.264	0.510	0.270

[a] Conditions and concentrations were standard, except as noted. Assays were performed spectrophotometrically and were initiated with the addition of 1 μg of extract protein.

[b] Activity expressed as Δ absorbancy (at 340 nm) per minute.

the catalytic sites. The data are insufficient to allow a choice between these alternatives.

Maximal activity of PRK is attained, under standard conditions, when the concentration of Mg^{2+} is equimolar with ATP. In the *p*H range of enzymatic activity, the stability constant of a Mg:ATP complex is of the order of 72,000 M^{-1} (20); thus, under the conditions of assay, the substrate of the enzyme would be Mg:ATP. Simultaneous titration of Mg^{2+} and ATP gave the same results as titration with ATP alone in the presence of excess Mg^{2+}. The observation that, at limiting concentrations of Mg^{2+} and "excess" ATP, PEP caused stimulation of PRK activity (Table 2) may indicate that Mg^{2+} is required at a specific site on the enzyme, other than as part of a Mg:ATP complex at the catalytic site. If this is the case, the stimulatory effect of PEP may be due to an alteration of the Mg^{2+} binding site by PEP, obviating a requirement for Mg^{2+}. The stability constant of the Mg:PEP complex is of the order of 200 M^{-1} (20), making it unlikely that Mg^{2+} is brought into the reaction as part of a Mg:PEP chelate.

Thus far two compounds, PEP and AMP, have been found to cause significant inhibition of PRK activity. In both cases it would appear that the compounds are neither competitive nor noncompetitive inhibitors of the enzyme. In the case of AMP it has been shown (16) that its inhibitory action cannot be reversed by an excess of either substrate and that both the K_m and the V_{max} of the substrates is altered. PEP exhibits sigmoidal inhibition curves (Fig. 4) and at low concentrations (less than 0.5 mM) does not inhibit. Inhibition caused by PEP plus AMP is more than additive, a situation found also with PRK from *Hydrogenomonas facilis* (4).

Together, the data indicate that PRK is an enzyme capable of being regulated by several compounds and by H^+ ion concentration. A similar effect of H^+ concentration on allosteric properties has been demonstrated with liver pyruvate kinase (10). Since the product of PRK, ribulose diphosphate, has as its sole physiological function the acceptance of CO_2, the enzyme is in a position specifically to regulate CO_2 fixation. The regulation of the enzyme can apparently be accomplished in several ways: in response to relative AMP and ATP concentrations, i.e., in response to the energy charge (2); in response to PEP concentrations; and in response to *p*H.

A scheme involving the regulation of PRK by PEP might be stated as follows. The accumulation of PEP is a consequence of high concentrations of ATP, or of a rate-limiting step in the breakdown of PEP. In either case there is a reflection of excess energy, excess ATP production, or inability to utilize carbon in the form of PEP. Feedback regulation involving inhibition by PEP could serve to prevent the fixation of still more carbon. This situation would be the obverse of that existing under conditions of limited energy, wherein AMP levels would increase, the energy charge would decrease, and the activity of PRK would decrease, suppressing CO_2 fixation. PEP inhibition of ribulose-5-phosphate kinase has also been observed in extracts of *Chromatium* (8).

The data we have presented, together with other data (8; E. J. Johnson and R. D. MacElroy, *manuscript in preparation*), suggest that PEP plays a significant role in the regulation of autotrophic metabolism. A further demonstration of the interrelationships among the regulatory mechanisms is the thiobacilli has been provided by Cornish and Johnson (5) who have reported that the activity of *T. neapolitanus* pyruvate kinase is stimulated by ribose-5-phosphate and AMP, and is inhibited by ATP.

LITERATURE CITED

1. Atkinson, D. E. 1966. Regulation of enzyme activity. Annu. Rev. Biochem. **35**:85-124.
2. Atkinson, D. E. 1968. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry **7**:4030-4034.
3. Atkinson, D. E. 1969. Regulation of enzyme function. Annu. Rev. Microbiol. **23**:43-68.
4. Ballard, R., and R. D. MacElroy. 1971. Phosphoenolpyruvate, a new inhibitor of phosphoribulokinase in *Pseudomonas facilis*. Biochem. Biophys. Res. Commun. **44**:614-618.
5. Cornish, A., and E. J. Johnson. 1971. Regulation of pyruvate kinase from *Thiobacillus neapolitanus*. Arch. Biochem. Biophys. **142**:584-590.
6. Elsden, S. R. 1962. Photosynthesis and lithotrophic carbon fixation, p. 1-40. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 3. Academic Press Inc., New York.
7. Gale, N. L., and J. V. Beck. 1966. Competitive inhibition of phosphoribulokinase by AMP. Biochem. Biophys. Res. Commun. **24**:792-796.
8. Hart, B. A., and J. Gibson, 1971. Ribulose-5-phosphate kinase from *Chromatium* sp. strain D. Arch. Biochem. Biophys. **144**:308-321.
9. Hurwitz, J. 1962. Phosphoribulokinase, p. 258-261. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 5. Academic Press Inc., New York.
10. Jiménez de Asua, L., E. Rozengurt, and H. Carminatti. 1970. Some kinetic properties of liver pyruvate kinase (type L). II. Effect of pH on its allosteric behavior. J. Biol. Chem. **244**:3142-3147.
11. Johnson, E. J. 1966. Occurence of adenosine monophosphate inhibition of carbon dioxide fixation in photosynthetic autotrophs. Arch. Biochem. Biophys. **114**: 178-183.
12. Johnson, E. J., and H. D. Peck. 1965. Coupling of phosphorylation and carbon dioxide fixation in extracts of

Thiobacillus thioparus. J. Bacteriol. **89**:1041–1050.
13. Koshland, D. E., and K. E. Neet. 1968. The catalytic and regulatory properties of enzymes. Annu. Rev. Biochem. **37**:359–410.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. MacElroy, R. D., E. J. Johnson, and M. K. Johnson. 1968. Characterization of ribulose diphosphate carboxylase from *Thiobacillus thioparus* and *Thiobacillus neapolitanus*. Arch. Biochem. Biophys. **127**:310–316.
16. MacElroy, R. D., E. J. Johnson, and M. K. Johnson. 1968. Allosteric regulation of phosphoribulokinase activity. Biochem. Biophys. Res. Commun. **30**:678–682.
17. MacElroy, R. D., E. J. Johnson, and M. K. Johnson. 1969. Control of ATP-dependent CO_2 fixation of *Hydrogenomonas facilis:* NADH regulation of phosphoribulokinase. Arch. Biochem. Biophys. **131**:272–275.
18. Milhaud, G., J. P. Aubert, and J. Millet. 1956. Metabolisme du carbone dans la chimiautotrophic cycle d'assimilation de l'anhydride carbonique. C. R. Acad. Sci. **243**:102–105.
19. Monod, J., J. Wyman, and J. P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. J. Mol. Biol. **12**:88–118.
20. O'Sullivan, W. J. 1969. Stability constants of metal complexes, p. 423–434. *In* R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones (ed.), Data for biochemical research. Oxford University Press, New York.
21. Pontremoli, S., and G. Mangiarotti. 1962. A simple method for the preparation of D-ribulose 5-phosphate. J. Biol. Chem. **237**:643–645.
22. Racker, E. 1957. The reductive pentose phosphate cycle. I. Phosphoribulokinase and ribulose diphosphate carboxylase. Arch. Biochem. Biophys. **69**:300–310.
23. Rindt, K. P., and E. Ohmann. 1969. NADH and AMP as allosteric effectors of ribulose-5-phosphate kinase in ***Rhodopseudomonas spheroides***. Biochem. Biophys. Res. Commun. **36**:357–364.
24. Segel, I. H. 1968. Biochemical calculations. J. Wiley & Sons, New York.