THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED: THE KINETIC PROPERTIES OF PURIFIED

PHOSPHORIBOSYLPYRUVOSPHATE SYNTHETASE FROM ESCHERICHIA COLI

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY

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THE KINETIC PROPERTIES
OF
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THESIS
for the
DEGREE OF BACHELOR OF SCIENCE
IN
BIOCHEMISTRY

College of Liberal Arts and Sciences
University of Illinois
Urbana, Illinois
1984
I would like to thank Dr. Robert Switzer for introducing me to the proper way to conduct research and supervising my project. I would also like to thank all the members of the Switzer group for answering all my questions, providing a friendly atmosphere to work in and for having birthdays. A special thanks is given to Ken Harlow for his help in planning experiments and for introducing me to my favorite assay.
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A. Roles of PRPP Synthetase in Biosynthesis

Phosphoribosylpyrophosphate (PRPP) synthetase (E.C.2.7.6.1.) catalyzes the formation of PRPP from ATP and ribose - 5 - phosphate. This reaction is an important junction between the catabolic metabolism of the cell and the biosynthesis of new cellular material. PRPP is a biosynthetic precursor of purine and pyrimidine nucleotides, pyridine nucleotide, and histidine and tryptophan. The intermediate products of these pyrimidine and purine synthetic pathways are UMP and IMP, respectively. These are converted to uracil, cytosine, adenine and guanine nucleotides and their 2' deoxy derivatives.

B. Mechanism and Regulation of PRPP Synthetase from S. typhimurium

Although reactions of ATP at each of its three phosphorus atoms are essential to the metabolism of all organisms, PRPP synthetase is one of the few enzymes that catalyzes a reaction at the β - phosphate of ATP. Khorana et al. (1958) postulated that the reaction proceeded by nucleophilic attack of the anomeric hydroxyl group of R5P on the β - phosphate of ATP with the elimination of AMP.
Mechanistic properties of the enzyme were also revealed by steady state kinetics (Switzer, 1971). PRPP synthetase requires two activators: $P_i$ and a divalent cation, $Mg^{2+}$ or $Mn^{2+}$. The requirement for phosphate is kinetically complex and seems to involve 2 modes of action. A concentration of 25 to 30 mM $P_i$...
synthetase has been shown to require Mg\(^{2+}\) as the substrate, and in addition, to require free Mg\(^{2+}\).

A variety of experiments have been performed to investigate the metabolic regulation of PRPP synthetase. Sadler and Switzer (1977) studied intracellular PRPP pools in *S. typhimurium* by starvation and supplementation experiments. Small variations of PRPP pools were found during growth on different carbon sources. A rapid depletion of PRPP pools resulted from C, N or P starvation. Histidine supplementation resulted in a slight increase in PRPP pools; hypoxanthine caused a small decrease. Cells given adenine had very low PRPP pools, perhaps due to PRPP utilization by adenine phosphoribosyltransferase; uracil had no effect.

A second set of experiments was performed to investigate purine and pyrimidine starvation (Sadler and Switzer, 1977). When a pyrimidine auxotroph was starved for uracil, PRPP pools declined rapidly to less than half their levels during exponential phase. UTP and CTP levels were depleted within the first hour, whereas ATP and GTP levels increased approximately 3-fold.
that the pathway is (in some cases) less sensitive.
pathway showed a sharp contrast to purine nucleotide pools.
Upon starvation, purine pools dropped sharply; pyrimidine pools also declined. These changes were accompanied by a massive accumulation of PRPP. These results suggest that the purine nucleotide pool contains an inhibitor of PRPP synthetase.

To identify this inhibitor, strains that could be starved specifically for adenine or guanine were used (Sadler and Switzer, 1977). Guanine starvation resulted in a depletion of PRPP pools, whereas adenine starvation resulted in much smaller changes in PRPP pools. This suggest inhibition of PRPP synthetase by an adenine nucleotide, presumably AMP or ADP, since ATP is a substrate.

An analysis of end product inhibition of purified S. typhimurium PRPP synthetase in vitro was undertaken to gain information about the mechanism of regulation (Switzer and Sogin, 1973). These studies showed that ADP was the most effective inhibitor of PRPP synthetase. PRPP synthetase was found to be weakly inhibited by most other purine nucleotides; this inhibition was competitive with respect to ATP. Pyrimidine nucleotides were much less effective inhibitors. Histidine and tryptophan did not inhibit PRPP synthetase under any condition examined.
ADP inhibition has been found to be complex (Switzer and Sogin, 1973). At low levels of B5P, ADP inhibition appeared to result from ADP competition with ATP at the active site. At high levels of B5P however, parabolic curvature was observed in the slope and intercept replots of the double reciprocal plots of ADP inhibition with respect to ATP. Such a result suggests either the occurrence of cooperative ADP binding at the active site or the existence of multiple ADP sites. Since there was no evidence from ATP saturation curves for active site cooperativity, the suggestion was made that PRPP synthetase possesses an allosteric site. ADP - induced substrate inhibition was not overcome by increasing the ADP concentration. This is further evidence for an allosteric site.

Ligand binding studies (Gibson et al., 1982) were performed to elucidate the complex nature of inhibition by ADP. These studies showed that S. typhimurium PRPP synthetase possesses distinct binding sites for B5P, ATP and ADP. The active site contains a nucleotide binding region to which ATP, β,γ- methylene ATP and ADP can bind and a separate region which binds B5P. In addition, there is an allosteric site which is distinct from the active site and is highly specific for ADP. ADP binds at this site only when the active site is occupied by B5P and an adenine nucleotide.
The binding of ATP to PRPP synthetase is inhibited (Gibson et al., 1982). Further evidence for nonidenticality of the ATP sites were given by the observation that ADP or \( \alpha,\beta\)-methylene ATP stimulated ATP binding at submaximal concentration of ATP. Stimulation of substrate binding by inhibitory analogues is strong evidence for binding of both substrate analogues and inhibitor to the same set of cooperative sites. At higher concentrations, both compounds inhibited ATP binding, as expected if they compete with ATP for the same set of sites.

Increasing the concentration of R5P alters the binding of ADP to PRPP synthetase. (Gibson et al., 1982). When saturating R5P was present the ADP binding curves became biphasic and reached twice the limiting saturation values observed for ATP, \( \alpha,\beta\)-methylene ATP or ADP alone. This indicates that ADP is bound to two different classes of sites on PRPP synthetase when R5P was present. ADP binding was measured in the presence of both \( \alpha,\beta\)-methylene ATP and R5P. ADP binding at the tighter class of sites was eliminated by the \( \alpha,\beta\)-methylene ATP, whereas ADP binding at the weaker class of sites was unaffected. This shows that the former sites were the active site, whereas the latter weaker sites were ADP-specific allosteric sites.

C. Cloning of \( E.\ coli \) PRPP Synthetase

Recently the gene (pro) encoding PRPP synthetase
in *E. coli* has been identified and mapped (Hove-Jensen, 1983). The *prs* gene has been isolated from a library of *E. coli* DNA fragments cloned in bacteriophage lambda D69. A strain harboring a temperature-sensitive mutation in PRPP synthetase (prs-2) was used as the recipient, and cloning was performed by lysogenic complementation. The *prs* gene was contained on a 6000 base pair DNA fragment generated by digestion with *Eco* RI endonuclease. The *prs* gene was subcloned on the plasmid pBR322, and a restriction map was constructed. The pBR322 plasmid was digested with endonucleases and religated to decrease the plasmid size. A resultant plasmid, pHOU1, was isolated and found to carry the *prs* gene. The cloning of the *prs* gene into a high copy number plasmid has enabled rapid purification of the *E. coli* PRPP synthetase to homogeneity using methods established with *S. typhimurium* PRPP synthetase, but in a 30-50 fold greater yield. Such a readily available source of *E. coli* PRPP synthetase has led to comparison of the *E. coli* enzyme with the *S. typhimurium* PRPP synthetase.

D. Physical and Chemical Properties of PRPP Synthetase

A comparison of the physical and chemical properties of PRPP synthetase from *E. coli* and *S. typhimurium* is currently in progress (Hove-Jensen, and Harlow, unpublished). The molecular weight of PRPP synthetase from both enzymes sources was estimated from SDS polyacrylamide-gel
electrophoresis to be about 31,000. The carboxyl terminal sequences of the two enzymes are identical for the final two amino acid residues: - Glu - His - COOH. The amino terminal sequence of the two enzymes, determined by automated Edman degradation, shows a remarkable similarity: 19 out of 22 amino acids are identical. The other 3 may also be identical, but weren't identified clearly in the E coli enzyme.

This amino acid analysis of both enzymes shows a strong similarity between the two. The analysis indicates that both enzymes contain four cysteine residues, which was confirmed by sulphydryl group titration with dithiobisnitrobenzoic acid. Another interesting finding is that neither enzyme contains tryptophan.

These studies have shown strong similarities between the physical properties of PRPP synthetases isolated from E coli and from S. typhimurium. In order to compare the enzymes further, kinetic studies on the E coli PRPP synthetase were undertaken. The results of these studies has contributed to our comparison of the PRPP synthetases.
A. Purification of PRPP Synthetase

The purification of PRPP synthetase from *E. coli* used a procedure that was very similar to the purification of PRPP synthetase from *S. typhimurium* (Switzer and Gibson, 1978). A summary of this method with the alterations for the *E. coli* enzyme follows.

**Cell Extraction** - 260 grams of H0561 cells were homogenized in 1600 ml of 50 mM KP<sub>4</sub> buffer, pH 7.5. The suspension was passed three times through a Manton-Gaulin pressure mill at 3000-9900 psi at 30°C. The extract was centrifuged at 23,000 x g for 1 hour.

**Streptomycin Sulfate - Heat Treatment** - The streptomycin sulfate (10 g of powder/liter of extract) was dissolved in 200 ml of water and poured into the crude extract, which immediately became cloudier. Additional cloudiness developed during heating. After 4.5 minutes in a 59°C bath, the flask was removed, and its contents were poured into ice-cold steel cans. The mixture was centrifuged at 23,000 x g for 1 hour.

**Ammonium Sulfate I (AS I)** - Solid ammonium sulfate was added to 35% saturation. The extract was allowed to stand on ice for 1/2 hour, then the extract was centrifuged at 23,000 x g for 1-1.5 hours. The precipitate
was collected in 200 ml of 50 mM KP₄ buffer. This solution was slowly frozen at -20°C. Slow freezing often results in denaturation and precipitation of contaminants with little loss of PRPP activity.

**First Acid Precipitation (AC I)** - AS I was thawed by placing the flask in a water bath at room temperature. The debris was removed by centrifuging at 17,000 x g for 10 minutes. Acetic acid addition was performed at 4°C; acetic acid was added until the pH reached 4.8. The mixture was centrifuged at 17,000 x g for 10 minutes. The precipitate was resuspended in 100 ml of 50 mM KP₄ buffer, and the pH was adjusted to 7.5, if necessary, by addition of K₂HPO₄. This solution was frozen as before.

**Ammonium Sulfate II (AS II)** - AC I was thawed and centrifuged for 10 minutes at 17,000 x g to remove debris. To the usually hazy solution was added one-half volume of 50 mM KP₄ buffer saturated with ammonium sulfate. After centrifugation at 17,000 x g for 20 minutes, the entire precipitate was resuspended in 50 mM KP₄ buffer that was 12.5% saturated ammonium sulfate and stirred on ice for about 1 hour until it was completely dispersed. After centrifugation at 17,000 x g, the precipitate was dissolved in 50 mM KP₄ buffer and slowly frozen at -20°C.

**Second Acid Precipitation (AC II)** - AS II was thawed
and centrifuged for 10 minutes at 17,000 x g. The second acid precipitation serves to remove impurities which are even less soluble at low pH than PRPP synthetase. Acetic acid was added dropwise with stirring until the pH reached 5.8. The solution usually became cloudy during this period. The sample was centrifuged at 17,000 x g for 10 minutes. The supernatant solution was adjusted to pH 4.8 to precipitate the bulk of the enzyme, and the precipitate was redissolved in 100 ml of 50 mM KP\(_4\). The overall yield of PRPP synthetase was very high. One can obtain 500 mg of pure enzyme from 250 grams of cells. The specific activity was generally about 110 \(\mu\)moles/min/mg, and the enzyme was usually 98% homogeneous as judged by SDS polyacrylamide gel electrophoresis.

B. \textsuperscript{32}P - Transfer Assay for PRPP Synthetase Activity

This assay measured the conversion of radioactivity from \(\textsuperscript{32}P\) ATP to a form which does not absorb to charcoal (that is, PRPP and its acid breakdown products). The standard assay buffer contained 50 ml 1.0 M trithanolamine, 30 ml 1.0 M K\(_2\)HPO\(_4\), 20 ml 1.0 M KH\(_2\)PO\(_4\), 3.75 ml 0.1 M EGTA, pH 7, and H\(_2\)O to a final volume of 500 ml. The standard assay contained 5 mM R5P, 2 mM ATP, 5 mM MgCl\(_2\), and the standard buffer solution. An assay mixture was normally prepared
as follows (on a per tube basis): 0.25 ml standard assay buffer, 25 μl 0.1 M R5P, 10 μl 0.1 M ATP, 10 μl 0.1 M EGTA, pH 7, 25 μl 0.1 M MgCl₂ and about 10⁵ cpm γ-³²P ATP, whose volume was negligible. Water and enzyme were added to each reaction tube to bring the volume to 0.5 ml.

After 2 minutes of incubation of the tubes at 37°C, the reaction was started by the addition of enzyme. Enzyme solutions were diluted with standard assay buffer that contained ımg/ml bovine serum albumin. Assays were usually conducted at three enzyme volumes. The reaction was terminated by the addition of 0.5 ml 5% HClO₄. The tubes were then kept on ice, and 0.3 ml of a suspension of 20% acid-washed Norit was added with vigorous mixing, except in the case of the control tubes that were used for determination of total radioactivity added. Then 0.2 ml of carrier (5 mg per ml of bovine serum albumin in 50 mM sodium pyrophosphate, pH 7) was added to each tube. Carrier ensured complete PRPP removal from the charcoal and facilitates removal of finely divided charcoal during centrifugation, which was for 3 minutes in a clinical centrifuge at top speed. A 300 μl sample of clear supernatant liquid from each tube was placed in a scintillation vial. To each vial was added 10 ml of 0.4% diphenyloxazolone in 7:8 methanol:toluene.
C. Preparation of γ-³²P ATP

Radioactive ATP was made from ³²P, which was supplied by New England Nuclear Corp. Every shipment was checked for polyphosphate contamination by chromatography on polyethylenimine impregnated cellulose thin layer plates (Gibson et al., 1962). γ-³²P ATP was prepared by the exchange method of Glynn and Chappell (1964). The radiochemical purity was usually >95%.

D. Substrates and Inhibitors

ATP, ADP and R5P were purchased as the sodium salts from Sigma. The purity of the nucleotides was checked by comparison of ultraviolet spectral ratios to literature values. (Specifications and Criteria for Biochemical Compounds, 1967). The nucleotides were estimated to be 98% pure.

E. Analyses of Kinetic Data.

The initial velocities shown are the averages of duplicate determinations with three different volumes of PRFP synthetase. The data were fit to a rectangular hyperbola of the form

\[ v = \frac{V [s]}{K + [s]} \]  \hspace{1cm} (1)

using the HYPER computer program of Cleland (1967) and the VAX computer system. All experimental results shown in double reciprocal form are from data fitting.
to equation (1). Values for the slopes and intercepts of the lines were obtained from the program.
III. RESULTS

A. Enzyme Stability

The stability of E. coli PRPP synthetase over a 1000 fold dilution range was examined. The specific activity of the enzyme showed a slight increase (15%) at the highest dilutions. Because of this effect all studies were performed at the same enzyme dilution to provide accurate comparisons. A similar effect was reported for the S. typhimurium PRPP synthetase by Schubert (Ph.D. Thesis, 1975).

3. pH Optimum

The kinetic properties of the E. coli PRPP synthetase are very sensitive to small changes in pH, so care should be taken that differences in Vmax do not result from pH changes, especially when different compounds are added to the reaction mixture. The pH optimum for the E. coli PRPP synthetase was found to be 9.7 to 9.8. (Figure 1). The specific activity of the enzyme at the pH optimum was almost twice the specific activity found at the pH used for the standard assay, i.e. pH 8. The rapid decline in activity at a pH above 10.5 was probably due to denaturation. This was shown by incubating the enzyme at several high pH values for 2 minutes at 37°C and then assaying
Figure 1. pH - Activity Profile of PRPP Synthetase

All assays were conducted at 37° C with 2mM ATP, 5mM R5P and 5mM MgCl₂. Buffers contained the following components: 0 50mM KP₄; △ 50mM triethanolamine, 50mM KP₄; O 100mM glycine, 50mM KP₄; § 50mM triethylamine, 50mM KP₄.

The initial velocity units are μmoles/min/mg.
at pH 8. The specific activity of the enzyme incubated at pH values above 10 was about 10% of the specific activity of the enzyme that was incubated at pH 8.

C. Phosphate Requirement

In the absence of phosphate, the *E. coli* PRPP synthetase had no activity. As the concentration of phosphate was increased, a hyperbolic activation curve resulted; concentrations of phosphate greater than 50mM did not result in an increase in specific activity. (Figure 2). Full maximal activation occurred at 20mM phosphate.

Phosphate also seems to be required to stabilize the synthetase. Dilution of the enzyme into 50mM triethanolamine, 50mM KP4 buffer containing no phosphate resulted in a decrease in activity to about 1/3 of maximal, even though 50mM KP4 buffer, i.e. standard assay conditions, was used in the reaction assay (Figure 3). The *E. typhimurium* PRPP synthetase shows essentially no activity when diluted into buffer containing no P4 (Witzer, 1963).

Mg2+ Activation

*E. coli* PRPP synthetase was shown to require MgATP2- as a substrate and to be activated by Mg2+. This activation is illustrated by experiments in which MgCl2 and ATP were used as substrates in (a) equimolar concentrations and (b) equimolar concentrations plus 5mM excess
Figure 2. Phosphate Activation

All assays were conducted at 37° C with 2mM ATP, 5mM MgCl₂ and 5mM MgCl₂.

The initial velocity units are μmoles/min/mg.
Figure 5. Phosphate Requirement for Dilution

All assays were conducted at 37° C with 2mM ATP, 5mM R5P, 5mM MgCl₂ and 50 mM trithanolamine, 50mM KP₄ buffer. The concentration of phosphate in the diluent was varied as shown. The reaction mixture was incubated at 37° C for 2 minutes in 50mM triethanolamine buffer pH 8. The initial velocity units are μmoles/min/mg.
MgCl₂ (Figure 4). The addition of excess Mg²⁺ converted the MgATP²⁻ saturation curve from sigmoid to hyperbolic and greatly stimulated the activity of the enzyme at low MgATP²⁻ concentrations. For example, the excess of 5 mM Mg²⁺ increased the specific activity 8 fold at 0.2 mM Mg-ATP²⁻. When 2 mM ATP was present, the maximal activation by Mg²⁺ occurred at 5 mM Mg²⁺; higher concentrations of Mg²⁺ resulted in slight inhibition (Figure 5). The K_Mg was 0.64 ± 0.33 mM.

2. Mn²⁺ Activation

PRPP synthetase was also activated by Mn²⁺. At pH 8 the maximal activity with Mg²⁺ was 2 fold greater than the maximal activity with Mn²⁺. The Mn²⁺ saturation curve was hyperbolic (Figure 6). The K_Mn was 1.2 ± 0.20 mM.

F. Substrate Saturation Kinetics

The effects of varied concentrations of R5P on the initial rate of the PRPP synthetase are shown in double reciprocal form in Figure 7. The same data are also plotted to show the effects of varied concentrations of ATP (Figure 8). In both cases the double reciprocal plots converged to a common point to the left of the ordinate. A sequential mechanism, that is one in which both substrates must be bound before a product is released, is indicated by such a pattern. The K_M values for R5P and ATP were 0.13 ± 0.026 mM and 94 ± 1.2 μM.
Figure 4. Activation of PRPP Synthetase by Mg$^{2+}$ Ions.

All assays were conducted at 37° C with 2mM ATP, 5mM R5P and 50mM triethanolamine, 50mM KP$_1$ buffer. (●) MgCl$_2$ and ATP were added in equimolar concentrations; (O) the concentration of MgCl$_2$ exceeded the ATP concentration by 5mM. The initial velocity units are µmoles/min/mg.
Figure 5. Effect of Free Mg$^{2+}$ on the Initial Velocity

All assays were conducted at 37°C with 2mM ATP, 5mM R5P and 50mM KP$_4$ buffer. MgU$_2$ was added as shown. The initial velocity units are μmoles/min/mg.
Figure 6. Effect of Free Mn$^{2+}$ on the Initial Velocity

All assays were conducted at 37$^\circ$ C with 2mM ATP, 5mM Mg$^2+$ and 20 mM K$_2$HPO$_4$ buffer MnCl$_2$ was added as shown. The initial velocity are μmoles/min/mg.
Figure 7. Initial Velocity Pattern of PRPP Synthetase with ATP as the Varied Substrate

All assays were conducted at 37°C with 5mM MgCl₂. ATP was the varied substrate. R5P concentrations were: 1, 0.0mM; 2, 1.0mM; 3, 2.0mM; 4, 5.0mM. The units for VMAX are (μmoles/min/μg)⁻¹.
All assays were conducted at 37° C with 5mM MgCl₂. R5P was the varied substrate. ATP concentrations were: 1, 0.025mM; 2, 0.05mM; 3, 0.5mM; 4, 1.0mM. The units of \( 1/V_o \) are (µmoles/min/mg)⁻¹.
respectively.

Hove-Jønson and Nygaard (1982) recently reported Km values for E. coli PRPP synthetase in crude extracts. The assay conditions were 50mM KPi buffer and 3mM MnCl₂ (Jensen et al., 1978). The Km values determined for R5P and ATP were 45μM and 60μM respectively. The S. typhimurium PRPP synthetase Km values for R5P and ATP were 0.16 ± 0.013mM and 0.046 ± 0.003mM respectively. There is a very close agreement between the R5P Km values in both organisms. The Km value for ATP is 2 fold greater in E. coli PRPP synthetase than in S. typhimurium PRPP synthetase.

G. ADP inhibition

ADP was a very effective inhibitor of the E. coli PRPP synthetase. A study of ADP inhibitor as a function of increasing concentrations of ATP was performed (Figure 9). Inhibitor increased sharply as ADP concentration increased. The double reciprocal plots showed that inhibition was noncompetitive, that is ADP inhibition was not overcome by saturation with ATP. In fact, a replot of the intercept values at 1/ATP = 0 as a function of ADP concentration was parabolic. (Figure 10). A parabolic dependence of the inhibition of ADP concentration is consistent with two sites for ADP binding as shown in the case of S. typhimurium PRPP synthetase (Sogin and Switzer, 1973; Gibson et al., 1982).
Figure 9. Kinetics of ADP Inhibition as a Function of ATP Concentration.

All assays were conducted at 37°C with 5mM R5P, 5mM MgCl₂ and 50mM triethanolamine, 50mM KP₂₁ buffer. The double reciprocal plots show ADP inhibition with ATP as the varying substrate. The total ADP added was: 1, 0.6mM; 2, 0.4mM; 3, 0.3mM; 4, 0.2mM; 5, 0.1mM; 6, 0.05mM; 7, no ADP. The units of $1/V₀$ are (μmoles/min/mg)⁻¹.
Figure 10. Kinetics of ADP inhibition as a function of ATP concentration.

This figure is a replot of the intercepts figure 2 versus the logADP$^{-2}$ concentration.
ADP inhibition was also studied as a function of increasing R5P concentration (Figure 11). A constant excess of ATP (2mM) and Mg (5mM) was used. ADP inhibited the enzyme at all R5P levels, but as the ADP concentrations increased, the enzyme became increasing more susceptible to pronounced R5P substrate inhibition. In other words, ADP became an increasingly effective inhibitor as the enzyme was saturated with R5P. This is precisely the behavior observed with *S. typhimurium* PRPP synthetase and presumably results from similar properties of the allosteric site for ADP.
Figure 11. Kinetics of ADP Inhibition as a Function of R5P Concentration.

All assays were conducted at $37^\circ$ C with 2mM ATP. R5P concentrations were as shown. ADP concentrations were: 1, 0.4 mM; 2, 0.2 mM; and 3, no ADP. The units of $1/V_o$ are ($\mu$moles/min/mg)$^{-1}$. 
IV. DISCUSSION

The general kinetic properties of *E. coli* and *S. typhimurium* PRPP synthetase appear to be very similar. In fact, no significant difference was found in their pH optimum, Mg$^{2+}$ activation curves inhibition by ADP or $K_m$ values for substrates.

The pH optimum of the *E. coli* PRPP synthetase was found to be 9.7 to 9.8, while the *S. typhimurium* pH optimum was approximately 9.2 (Gibson, Ph.D. Thesis, 1982). The pH-activity profiles of enzymes often reflect the pH at which important proton-donating or proton-accepting amino acid residues in the catalytic site are in their required state of ionization. Therefore, it is likely that both enzymes have a similar amino acid(s) in the active site which needs to be unprotonated for activity.

PRPP synthetase from both *E. coli* and *S. typhimurium* share with many ATP-requiring enzymes the need for Mg$^{2+}$ as a free ion and MgATP$^{2-}$ as a complex (Switzer, 1971). Both enzymes show a large activation by addition of Mg$^{2+}$ ion to mixtures containing a low concentration of MgATP$^{2-}$. *E. coli* and *S. typhimurium* PRPP synthetase also show activation by Mn$^{2+}$ when Mn$^{2+}$ is used instead of Mg$^{2+}$.

Initial studies indicate that *E. coli* PRPP syn-
The thylase follows a sequential kinetic mechanism. In *Salmonella typhimurium* an ordered kinetic mechanism has been established by kinetic analysis (Switzer, 1971) and ligand binding studies (Gibson et al., 1982). The following scheme was determined:

\[
\text{ATP} \quad \text{R}5\text{P} \quad \text{AMP} \quad \text{PRPP}
\]

Further investigation would be needed to determine if this ordered scheme is also applicable to the *E. coli* PRPP synthetase.

The inhibition by ADP of the PRPP synthetases from *S. typhimurium* and *E. coli* shows similarities. ADP became an increasing effective inhibitor as the enzyme was saturated with R5P. This behavior is exactly as seen with the *S. typhimurium* enzyme, in which case it has been shown to result from ADP binding to an allosteric site that binds well only when the active site is saturated with R5P and ATP. The double reciprocal plots also showed that ADP inhibition is noncompetitive with respect to ATP.

Considering the general similarities found between the two enteric bacteria, *E. coli* and *S. typhimurium* it is reasonable that the PRPP synthetases from both sources should have very similar physical, che-
mical and kinetic properties. Future research to elucidate further the comparison of the two enzymes may include ligand binding studies with the *E. coli* PRPP synthetase. Amino acid or DNA sequencing might also be undertaken to determine whether the enzymes have similar sequences. The data available on the N-terminal and C-terminal sequences suggests that the two enzymes will have strongly homologous structures. Furthermore, Dr. Stan Bower in our laboratory has shown that *E. typhimurium* contains discrete DNA sequences that hybridize strongly to the cloned *E. coli* prs gene, which also requires extensive sequence homology in the prs gene from the two species.
REFERENCES


