Regulatory Properties of Phosphoribosyladenosine Triphosphate Synthetase. Synergism between Adenosine Monophosphate, Phosphoribosyladenosine Triphosphate, and Histidine*

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ABSTRACT: Phosphoribosyladenosine triphosphate synthetase, which catalyzes the first step in histidine biosynthesis, was purified to apparent homogeneity from *Escherichia coli*. The enzyme is inhibited synergistically by the end product of the sequence, histidine, and the immediate product of the reaction, phosphoribosyladenosine triphosphate. Histidine inhibits the synthetic reaction much more strongly than the reverse (pyrophosphorolytic) reaction. Histidine and adenosine monophosphate also inhibit the reaction synergistically. The molecular weight of the enzyme was estimated as about 200,000 on the basis of sedimentation velocity measurements. Adenosine monophosphate was shown to bind to the enzyme both in the presence and absence of histidine. The binding of histidine was enhanced by adenosine monophosphate and phosphoribosyladenosine triphosphate, and binding of 2.5–3.0 moles of histidine/200,000 g of enzyme was observed.

Regulation by end-product feedback inhibition, first reported by Umbarger (1956) and Yates and Pardee (1956), seems to be a general property of biosynthetic sequences. The energy charge of the cell should also control biosynthetic pathways in general (Atkinson and Fall, 1967; Atkinson, 1968). Interaction between energy charge and end-product concentration has been directly demonstrated in studies on three biosynthetic enzymes *in vitro* (Klungsøyr *et al.*, 1968). We have now purified one of these enzymes, PRibATP synthetase, from *Escherichia coli*. This paper reports kinetic and ligand binding studies on the interactions of this enzyme with its substrates and modifiers.

Voll *et al.* (1967) previously reported the purification and some properties of PRibATP synthetase from *Salmonella typhimurium*.

Materials and Methods

Bacterial Strains. Strain X-1 was prepared from *E. coli* C-600-1 as described previously for strain X-9 (Klungsøyr *et al.*, 1968). Neither strain will grow on minimal media, but both grow on histidine or histidinol. Strain X-1 has a fairly high frequency of reversion to, presumably, the wild type, but is sufficiently stable under the adapted growth conditions. This strain produces much higher yields of PRibATP[1] synthase than does strain X-9.

The assay procedure was that of Voll *et al.* (1967), with the modifications described previously (Klungsøyr *et al.*, 1968). Protein was determined by a method developed in this laboratory (Klungsøyr, 1969).

PRibATP was prepared as described by Ames *et al.* (1961), except that purified enzyme preparations (synthetase and pyrophosphatase) were used, and higher yields were obtained. A value of 3.6×10^3 was used for the molar absorptivity of PRibATP at pH 8.5 and 290 nm (Smith and Ames, 1965).

Imidazole, Tris, PRibPP, and purified pyrophosphatase were obtained from Sigma, 2-mercaptoethanol from Eastman, and Sephadex G-200 and G-25 from Pharmacia.

Buffers for Enzyme Isolation. Standard buffer I was composed of 10 mM imidazole-HCl buffer (pH 7.2), containing 0.5 ml of 2-mercaptoethanol/l. Standard buffer II was composed of 10 mM imidazole buffer (pH 7.2), containing 0.5 ml of 2-mercaptoethanol/l., 100 mM NaCl, and 0.4 mM histidine.

Results

Preparation of Extracts of Derepressed X-1 Cells. The mutant was grown in the medium of Vogel and Bonner (1956) with 0.5% glucose, 50 μM histidinol, and 0.4 mM adenine as described by Margolies and Goldberger (1966). Trace amounts of Ca, Co, Fe, and Mo were added to the medium. A fresh culture on nutrient agar was used to inoculate 250 ml of inoculum medium, in which 0.1 mM histidine replaced the adenine and histidinol of the standard medium. After 24-hr incubation with shaking at 37°, this culture was used to inoculate another 250-ml portion of histidine medium, which in turn was used after 8–9 hr to inoculate 10 l. of the adenine–histidinol medium. After 15-hr growth at 37° with vigorous aeration, the cells were harvested in a Sharples centrifuge, washed once with cold standard buffer I, and

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PRibATP, *N*-1-(5-phosphoribosyl)adenosine 5′-triphosphate; PRibPP, 5-phospho-D-ribosyl α-pyrophosphate; Bicine, *N,N*-bis(2-hydroxyethyl)glycine.

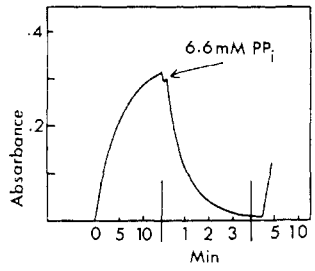

FIGURE 1: Product inhibition and reversibility of the PRibATP synthetase reaction. The reaction mixture contained: 100 mM Tris, 150 mM KCl, 5 mM Mg^{2+}, 2.5 mM ATP, about 15 units (μmoles/min) of yeast pyrophosphatase, and 56 μg of enzyme (specific activity approximately 1.2 μmoles/mg per min). The reaction was started by adding PRibPP to a concentration of 0.25 mM; final volume, 0.3 ml.

TABLE I: Purification of PRibATP Synthetase.

	Protein Concn (mg/ml)	Vol (ml)	Sp Act.[a] (units/mg)	Total Units[a]
Crude extract	24.0	48.0	57	65,000
Ammonium sulfate 1	15.0	18.5	184	51,000
Concentrated gel eluate	0.79	70.0	695	39,500
Ammonium sulfate 2	16.2	2.05	1,300	43,000

[a] The units used in this table are those of Voll *et al.* (1967). To convert into micromoles per minute these units should be divided by 600.

resuspended in the same buffer for enzyme extraction. The yield was usually between 18 and 20 g of cell paste.

Purification of PRibATP Synthetase. A suspension of 18 g of cell paste in standard buffer I, with a final volume of 55 ml, was cooled in ice and treated for two 3-min periods in a Branson Sonifier (sonic oscillator). The extract was centrifuged at 35,000*g* in a Servall centrifuge for 30 min, and then for 30 min at 144,000*g* in a Spinco Model L ultracentrifuge. The extract was then dialyzed against three 1-l. changes of buffer I in a special arrangement for rapid dialysis. Dialysis tubing (30 mm diameter) was pulled over a piece of glass tubing (40 cm long, 16 mm diameter) the end of which was blown into a bulb, which fit the dialysis tubing tightly and thus closed off the lower end. The upper end of the glass tubing (with a smaller diameter) was attached to a stirring motor. The extract occupied the cylindrical space between the glass core and the dialysis tubing, and the device was rotated inside a 1-l. cylinder which contained the buffer. The extract was then diluted to 200 ml with standard buffer I and to this mixture was added a solution of 66 g of ammonium sulfate in 100 ml of standard buffer I to which 2 ml of 1 M imidazole base had been added. After stirring for about 10 min, the precipitate was collected by centrifugation and dissolved in 15 ml of standard buffer I. The enzyme was clarified by centrifugation and added to a column of Sephadex G-200 (5 × 120 cm) in equilibrium with standard buffer II. The same buffer was used to elute the proteins from the column. The main part of the protein was eluted at the void volume (approximately 650 ml), after which several fractions with relatively low protein content followed. A second peak of protein, which eluted at a little less than twice the void volume, contained the PRibATP synthetase activity. The active fractions were pooled and concentrated by ultrafiltration through dialysis tubing from about 200 ml to less than one-half of that volume. The final volume is not critical, and in recent preparations the best results were obtained when volumes between 15 and 30 ml were used. The concentrated eluate was fractionated with ammonium sulfate. The fraction which was soluble with 22 g of salt/100 ml of concentrate, and which precipitated on further addition of 12.5 g of salt, contained the enzyme in a relatively pure state. During addition of ammonium sulfate, 1 M imidazole base was added to prevent the pH from dropping below 7.2 (approximately 1 ml/100 ml of concentrate).

The enzyme could be further purified by a repetition of the dialysis step, after which the enzyme would precipitate with 22 g of ammonium sulfate/100 ml of solution. To prevent denaturation at this stage, the dialysis should be carried out with a concentrated enzyme solution, then diluted to 10 ml with standard buffer I, and precipitated with 3.3 g of ammonium sulfate in 5 ml of standard buffer I (neutralized with 0.1 ml of 1 M imidazole base).

All steps were carried out below 4°, and whenever possible all containers were kept in ice. A survey of a typical purification experiment is given in Table I.

Product Inhibition and Reversibility. The PRibATP synthetase reaction favors the pyrophosphorolysis of PRibATP, and addition of pyrophosphatase is necessary to pull the reaction in the direction of PRibATP formation. Even in the presence of pyrophosphatase, the reaction deviates from linearity, and seems to be product inhibited (Figure 1). The decrease in rate is not due to loss of enzyme activity or removal of substrate. Addition of pyrophosphate pushed the reaction in the reverse direction, and after some time, when all the added pyrophosphate had been hydrolyzed, the reaction again proceeded in the synthetase direction, and apparently with a rate similar to the initial one.

The feedback inhibitor, histidine, was reported (Voll *et al.*, 1967) to give half-maximal inhibition of the *Salmonella* enzyme at about 80 μM. Histidine inhibited the *E. coli* enzyme to about this same extent. However, the degree of inhibition was found to depend upon other factors as well, including the amount of product accumulated, as demonstrated in Figure 2. Histidine added shortly after the reaction was started had little immediate effect, while histidine added after some PRibATP had accumulated was much more effective.

Differential Inhibition by Histidine of the Two Directions of the PRibATP Synthetase Reaction. While histidine inhibited the synthetase direction of the reaction in the presence of PRibATP, the reverse reaction under certain conditions proceeded with little effect by histidine. This is demonstrated in Figure 3, where a distinct histidine effect is seen only in the synthetic direction.

In the experiments described in Figure 3 pyrophosphate was present only when the reaction proceeded in the reverse

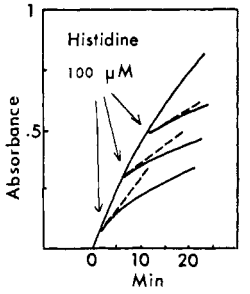

FIGURE 2: Effect of histidine and accumulation of product on the PRibATP synthetase reaction. Reaction mixtures contained: 100 mM Tris, 150 mM KCl, 20 mM Mg^{2+}, 5 mM ATP, about 15 units of yeast pyrophosphatase, and enzyme. PRibPP was added to a concentration of 0.5 mM in a final volume of 0.3 ml. Histidine, to a concentration of 100 μM, was stirred into the mixture at different times after the reaction was started. The broken lines are extrapolations of initial rates after histidine addition.

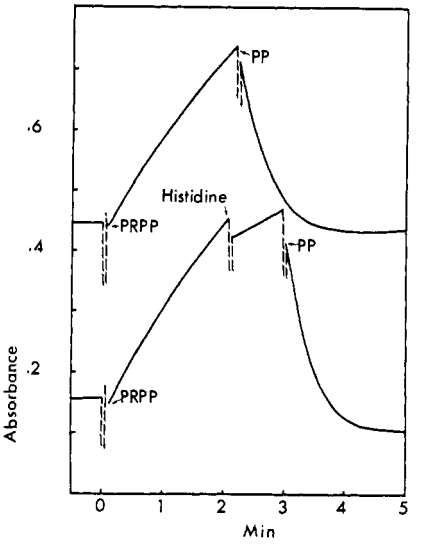

FIGURE 3: Differential inhibition by histidine of the reactions catalyzed by PRibATP synthetase. Assay mixtures contained: 100 mM Tris, 150 mM KCl, 5 mM Mg^{2+}, 5 mM ATP, 0.2 μl of PPase, 0.25 mM PRibPP, and 56 μg of enzyme (specific activity 0.6 μmole/min per mg). Pyrophosphate was added to a concentration of 5 mM, and histidine, when indicated, to a concentration of 0.1 mM.

direction. For this reason the effect of histidine on the rate of pyrophosphorolysis of PRibATP was measured at different pyrophosphate concentrations. No inhibition by histidine was found at pyrophosphate concentrations less than the concentration of magnesium in the reaction mixture (3 mM). Histidine did inhibit when pyrophosphate was present at higher concentrations than 3 mM. It would appear that pyrophosphate at high concentrations complexes the magnesium present, and that the difference in the histidine effect at different pyrophosphate concentrations may be explained by different affinities of the enzyme for PRibATP and for its magnesium complex. Thus, in the pyrophosphorylase direction of the reaction the synergistic inhibition by histidine and PRibATP is observed only when the concentration of free magnesium ions is low enough to leave PRibATP in its free form.

Differences in affinity are shown directly by the shapes of the recorder tracings describing the disappearance of PRibATP during pyrophosphorolysis with or without magnesium in excess of the added pyrophosphate. These tracings may be analyzed by means of the integrated form of the Michaelis equation (Henri equation), and approximate values for the K_m may be calculated. The K_m for PRibATP calculated this way in the presence of excess pyrophosphate was approximately 3 μM, but was five to ten times as high in the presence of excess magnesium ions.

The effect of histidine on the two directions of the reaction was further compared in the experiments illustrated in Figure 4. In order to be able to observe the histidine effect in the pyrophosphorylase reaction (curves A and B) a Mg^{2+} concentration (3 mM) was used which was less than the pyrophosphate concentration (5 mM). In the synthetase reaction (curves C and D), equal concentrations (5 mM) of Mg^{2+} and ATP were used. There was a distinct difference in the histidine effect on the two directions of the reaction when PRibATP was added to the reaction mixtures in similar concentrations (A and D). However, even lower concentrations of PRibATP seemed to be nearly saturating (C) for the comodifier role with histidine. Histidine concentrations below 50 μM were not included in this experiment, but other data show that the curve is sigmoid with a Hill coefficient of 2–3, as indicated by the broken portion of curve C.

Effect of AMP. As previously reported (Klungsøyr *et al.*, 1968) the response of the PRibATP synthetase to energy charge depends upon the concentration of histidine. This indicated a possible synergistic interaction between histidine and ADP or AMP. In further experiments it was found that ADP, and also IMP, GMP, CMP, and UMP, had little effect on the degree of inhibition by histidine. However,

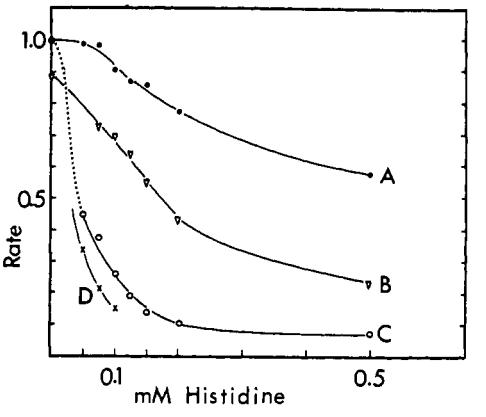

FIGURE 4: Effects of histidine and PRibATP on the reactions catalyzed by *E. coli* PRibATP synthetase. (A and B) Effect of histidine on the pyrophosphorolytic reaction. Reaction mixtures contained: 100 mM Tris, 150 mM KCl, 3 mM Mg^{2+}, 85 μM PRibATP, and 28 μg of enzyme (specific activity 0.6 μmole/mg per min). The reaction was started by the addition of pyrophosphate to 5 mM. (A) Effect of histidine only; (B) effect of histidine in the presence of 0.16 mM AMP; (C and D) effects of histidine and PRibATP on the synthetic reaction. Reaction mixtures contained: 100 mM Tris, 150 mM KCl, 5 mM Mg^{2+}, 5 mM ATP, 56 μg of enzyme, 0.2 μl of pyrophosphatase, and 90 μM PRibATP (curve D) or 30 μM PRibATP (curve C). The reaction was started by addition of PRibPP to a concentration of 0.25 mM.

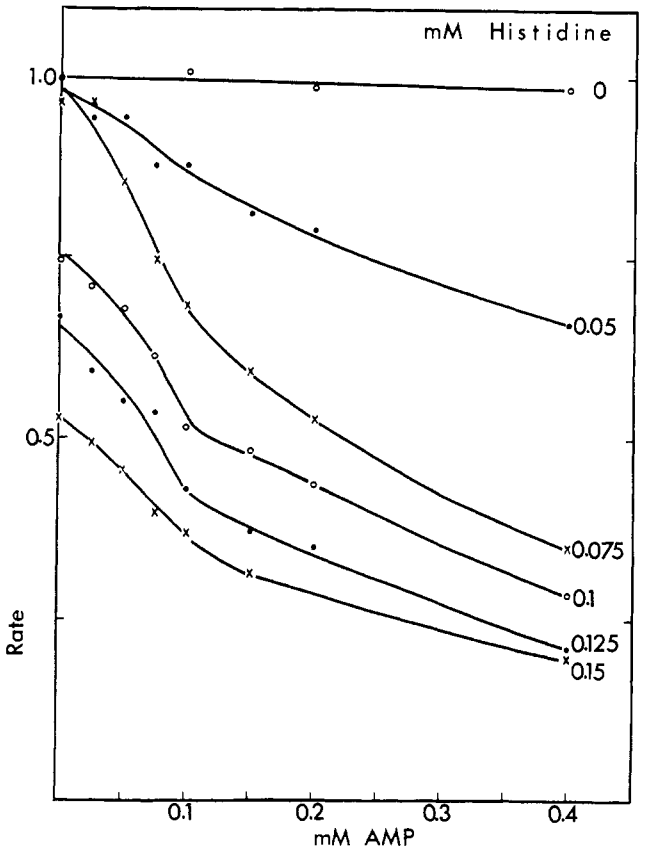

FIGURE 5: Effect of histidine and AMP on the rate of the reaction catalyzed by PRibATP synthetase. Reaction mixtures contained: 0.1 M Tris (pH 8.5), 0.15 M KCl, 5.0 mM ATP, 5 mM Mg^{2+}, approximately 0.2 μl of pyrophosphatase stabilized with bovine serum albumin, and 0.25 mM PRibPP. Enzyme (20 μl), containing 56 μg of protein, was added to a final volume of 0.3 ml. The enzyme was a partially purified preparation from a batch of poorly derepressed cells, with a specific activity of 0.6 μmole/min per mg. Numbers identifying curves indicate histidine concentration (mM).

AMP interacted strongly with histidine, as demonstrated in Figure 5. The interaction is generally similar to that between histidine and PRibATP.

The curves in Figure 5 seem to be biphasic, suggesting that the effect of low concentrations of AMP may be of a different kind or order than the effect of higher concentrations. Binding studies described below suggest that AMP at lower concentrations facilitates the binding of histidine. At higher concentrations AMP may compete with ATP in the presence of histidine during the synthetase reaction; however, competition by AMP with PRibATP is also indicated. In the experiment corresponding to curve B of Figure 4, where AMP was present in addition to the substrate, PRibATP, AMP seems to have an inhibitory effect which is not highly dependent upon histidine concentration. Competition between AMP and PRibATP is demonstrated in the recorder tracings copied in Figure 6. The tracings describe the decrease in PRibATP concentration with time in the pyrophosphorylase direction of the reaction. The concentration at which the reaction goes at half of the maximal rate is higher in the presence than in the absence of AMP.

Effect of Temperature. O'Donovan and Ingraham (1965)

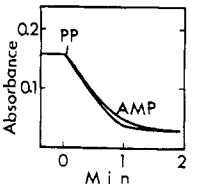

FIGURE 6: Effect of AMP on the affinity of PRibATP synthetase for PRibATP. The curves are copies of recorder tracings for experiments like these described in Figure 4A,B. The concentration of PRibATP was about 40 μM. AMP was added to a concentration of 0.16 mM in one of the experiments.

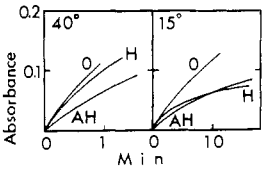

FIGURE 7: Effect of temperature on the synergistic effect of product and histidine on the reaction catalyzed by PRibATP synthetase. Experimental details are as in Figure 8. The 40° experiments are copies of recorder tracings. The 15° experiments have been redrawn with tenfold compression along the time axis. Curve identifications: H, 0.083 mM histidine added; AH, 0.083 mM histidine and 0.4 mM AMP added; 0, no additions.

have described a temperature effect on histidine sensitivity of PRibATP synthetase in extracts from *E. coli* C-600-1, and from cold-sensitive mutants of this strain. The experiments in Figure 7 show that while the effect of histidine changes little as PRibATP accumulates at 40°, histidine rapidly becomes highly inhibitory at 15°. AMP seems to counteract the effect of PRibATP accumulation, so that mixtures containing AMP yield similar results at 15 and 40°, as far as the shape of the curves are concerned. Thus, AMP decreases the apparent affinity of the enzyme for PRibATP, either as substrate (Figure 6) or as inhibitor (Figure 7). The Arrhenius plots given in Figure 8A,B illustrate the effects of histidine and AMP on the activation energy of the synthetase reaction, and the temperature dependence of PRibATP as a comodifier for the histidine effect. The curves in Figure 8A are based on the initial rates, while the data in B were obtained after the reaction had produced approximately 15 μM PRibATP. In the presence of PRibATP, histidine causes a change in activation energy of the synthetase at lower temperatures. On the other hand, with AMP as a comodifier histidine seems to cause no change in activation energy, and AMP also counteracts the high-temperature coefficient that appeared on PRibATP accumulation in the presence of histidine.

Binding Studies. Direct demonstration of binding of the ligands to the protein was attempted by the gel filtration method (Hummel and Dreyer, 1962), using Sephadex G-25 in a small-scale procedure. Histidine at 0.2 mM had no effect on the binding of 50 μM [^{14}C]AMP in the presence of 500 μM ATP, 5 μM PRibPP, and 0.7 mM Mg^{2+}. The enzyme bound 1.7 moles of AMP/200,000 g of protein.

The binding of histidine to the synthetase is illustrated in Figures 9 and 10. In Figure 9 the effect of AMP on histidine

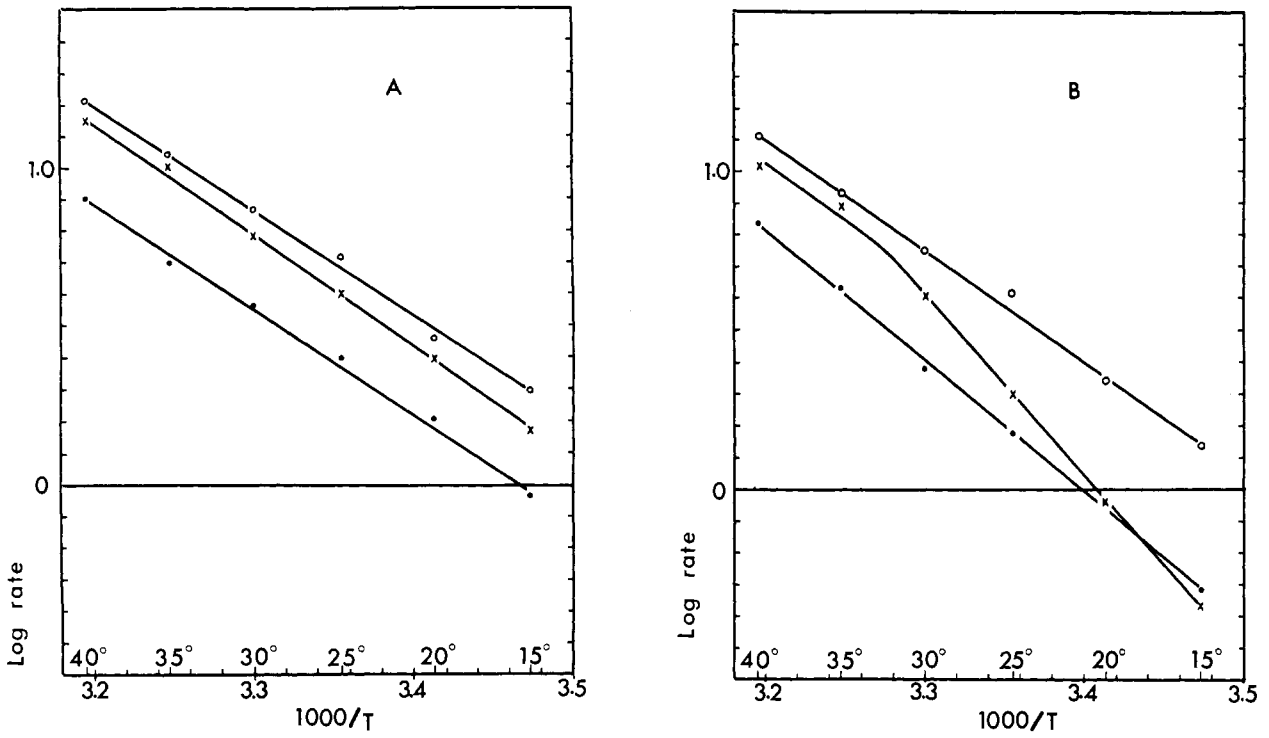

FIGURE 8: Effect of temperature on the inhibition of PRibATP synthetase by histidine in the presence of AMP or PRibATP. The reaction mixture contained: 0.1 M bicine-HCl (pH 8.5), 5 mM ATP, 5 mM Mg^{2+}, 0.15 M KCl, and 37 μg of enzyme stabilized with bovine serum albumin (specific activity 2 μmoles/min per mg). The reaction was started by adding PRibPP to a concentration of 0.25 mM. Curve identifications: (○) no additions; (×) 0.083 mM histidine added; (●) 0.4 mM AMP + 0.083 mM histidine added. (A) Initial rate of the reaction. (B) Rate measured as the tangent to the recorder tracing after an increase in absorbance of 0.05, corresponding roughly to 15 μM PRibATP.

binding is demonstrated. Addition of 50 μM AMP in the presence of 0.1 mM histidine increased the amount of histidine bound in the peak fraction from 0.7 mole/200,000 g of protein without AMP to 2.0 moles with AMP.

In the presence of PRibATP, the affinity of the enzyme for histidine is greater at 15° than at 40°, as shown in Figure 10. This figure also reports histidine binding curves obtained at 15° in the presence of AMP and in the absence of comodifiers (AMP and PRibATP). In attempts to determine the amounts of histidine bound at saturation, the values obtained were consistently between 2.5 and 3 moles of histidine per 200,000 g of protein. In one experiment, with the histidine concentration at 2 mM, and with 45 μM PRibATP as comodifier, an approximate value of 2.75 was obtained. Due to the high level of histidine used, the total radioactivity was high relative to the amount bound to the enzyme, so that the error was relatively large. In other experiments lower levels of histidine and relatively high concentrations of AMP as comodifier were used. With 250 μM histidine and 300 μM AMP, 2.5 moles of histidine/200,000 g of enzyme was found.

Six different enzyme preparations, with specific enzyme activities ranging from 2.9 to 3.1 μmoles per mg per min, were used in the binding experiments.

Sedimentation Experiments. Several observations indicated that the enzyme from *E. coli* differs from the previously purified enzyme from *S. typhimurium*. This was supported by preliminary amino acid analysis, which indicated a distinctly different but related composition. For this reason some preliminary measurements were made on the enzyme in the analytical ultracentrifuge. The preparation used was carried through the standard purification procedure, including the repeated dialysis step, and had a specific activity of 3.0 μmoles/mg per min.

The concentrated enzyme solution was dialyzed overnight against standard buffer II before use. The sedimentation experiment, which was carried out with a protein concentration of 8.3 mg/ml, indicated a high degree of homogeneity. Late in the run the peak was clearly preceded by a heavier component, and some trailing by lower molecular weight substances was present. Possibly both the heavy and light components can be explained as products of dissociation and aggregation, respectively. The main peak had an $s_{20,w}$ of 8.94 S. Voll *et al.* (1967) report values between 8.6 and 8.8 S for the *Salmonella* enzyme.

Several preparations of the enzyme were examined by disc gel electrophoresis on polyacrylamide gel containing 6 M urea (*Voll et al.*, 1967). In addition to one major band, several faint bands were visible in most preparations.

Discussion

E. coli PRibATP synthetase exhibits some unusual properties, some of which aid in its isolation. The enzyme is eluted from a Sephadex G-200 gel column after the passage of nearly two void volumes of buffer, which would suggest a molecular weight well below the size estimated from the *s* value, 200,000. One explanation for this kind of behavior might be continuous dissociation and reaggregation of the enzyme.

The behavior of the enzyme in ammonium sulfate solutions

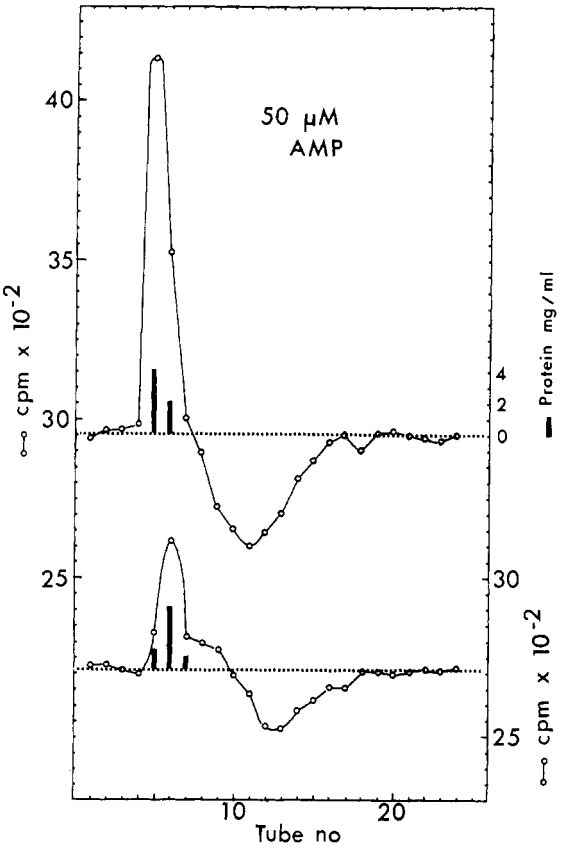

FIGURE 9: Effect of AMP on the binding of histidine to PRibATP synthetase. The column (300 × 3 mm) was packed with Sephadex G-25, and was in each case equilibrated with the appropriate buffer before the protein was added. The buffer contained: 100 mM Tris (pH 8.5), 150 mM KCl, 2 mM Mg^{2+}, 2 mM ATP, 0.05% mercaptoethanol, 15 nCi of ^{14}C-labeled histidine/ml, and 0.1 mM cold histidine. In one of the experiments, AMP was added to a concentration of 50 μM. Approximately 1.6 mg of enzyme (specific activity 3.1 μmoles/min per mg) in 0.1 ml was diluted with an equal volume of double-strength radioactive buffer and added to the column. Fractions of 3 drops (approximately 0.2 ml) were collected, of which 0.1 ml was used for counting, and 0.05 ml for protein determination. The concentration of protein, when detectable, is indicated by bars at each fraction.

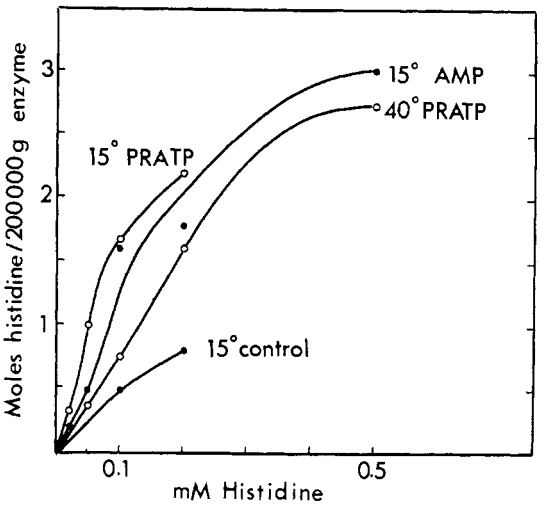

FIGURE 10: The number of moles of histidine bound per 200,000 g of enzyme, calculated for the eluate fraction containing most radioactivity and protein. The experimental conditions were the same as described in the legend to Figure 9 except that the buffer was prepared without cold histidine. The amount of histidine bound in the peak tubes is plotted as moles of histidine per 200,000 g of enzyme. Curves are labeled to indicate the temperature at which the experiment was performed and the comodifier added (50 μM AMP or 45 μM PRibATP).

also indicates that well-defined conformational changes take place as a consequence of changes in ionic strength. When the enzyme is dissolved in a histidine-free buffer with low ionic strength, it will precipitate below 37% ammonium sulfate saturation. After some time in a buffer which contains histidine and higher salt concentration, the solubility properties change and the enzyme now precipitates between 37 and 54% ammonium sulfate saturation. This conversion is slow, but it can be accelerated by increasing the temperature to 30°. In the actual purification procedure the change is accomplished during a gel filtration step. It should be noted that the ammonium sulfate solubility may not be directly related to the dissociation–aggregation phenomena, since preparations with both types of solubility behavior are similarly retarded by the gel column.

There seems to be an obvious functional advantage in simultaneous control of a biosynthetic sequence both by energy charge and by end-product inhibition (Atkinson, 1968). In PRibATP synthetase the presence of the end product, histidine, is necessary for the enzyme to exhibit sensitivity to energy charge (Klungsøyr *et al.*, 1968). This behavior has been shown to result from synergistic interaction between histidine and AMP. The synergism has been demonstrated by both kinetic and binding experiments. Histidine affected the synthetase reaction weakly and AMP had almost no effect, but the two compounds were strongly inhibitory when added together. This kinetic effect seems to depend upon modulation by AMP of the enzyme's affinity for histidine, since addition of 50 μM AMP almost tripled the binding of histidine at 100 μM concentration.

In addition to the synergistic interaction between end product and energy charge, PRibATP synthetase exhibits another interesting kind of regulatory interaction: a synergistic effect between the immediate product of the first reaction and the end product of the sequence. Additive inhibition by immediate product and end product in a biosynthetic pathway has been observed before (Cordaro *et al.*, 1968), but a synergistic action of the two seems to offer a more sensitive control mechanism. Again, the synergism has been shown to be based upon the effect of PRibATP on histidine binding, and these experiments also explained an interesting temperature effect upon histidine feedback control.

Little is known about the mechanism of the PRibATP synthetase reaction. The slope of the Arrhenius plot shows that the reaction rate increases 2.2 times with a 10° temperature increase. It is interesting to note that with AMP as comodifier, histidine inhibition to the extent of 50% gives no change in slope of the Arrhenius plot, indicating perhaps that histidine affects steric and orientational arrangements, rather than enthalpy-related processes. In line with this reasoning is the finding that histidine has a differential effect

upon the two directions of the reaction. Conditions have been found under which a concentration of histidine that will lead to almost complete inhibition of the synthetase reaction will, when pyrophosphate is added, leave the pyrophosphorylase reaction practically unaffected. This seems to eliminate what is usually called the catalytic step, *i.e.*, the transfer of the phosphoribosyl moiety from pyrophosphate to ATP, as the point of action of histidine.

The $(S)_{0.5}$ for ATP is about 30 μM, and this value changes very little in the presence of histidine. The $(S)_{0.5}$ value for PRibATP has been determined by means of the integrated form of the Michaelis equation. This method is not very satisfactory since the back-reaction cannot be neglected when the reaction mixture is close to equilibrium. Direct measurement of initial rates is, however, even less satisfactory, since the molar absorbance change in connection with the reaction is approximately 3.6×10^3, and the $(S)_{0.5}$ value is quite low. Several determinations gave a value of approximately 3 μM, and this value changed little when histidine was present at inhibitory concentrations. These low $(S)_{0.5}$ values support the assumption that the rate constant for the formation of the enzyme–PRibATP complex is very much higher than the rate constant for the dissociation of the complex, and the same is also the case for ATP–enzyme interaction. It is suggested that the limiting steps may be the dissociation of the enzyme–PRibATP complex in the forward reaction, and possibly the dissociation of the enzyme–ATP complex in the back-reaction. This would allow histidine to act differentially, by influencing the two dissociation rates to different degrees. Since histidine and AMP do not change the activation energy of the reaction, it is tempting to speculate that the active site of the enzyme is located inside a cavity of such a shape that fairly rigid requirements are set for the approach of the substrates and products to the site of binding and for the departure from the site. Histidine and comodifiers then would be assumed to change the arrangements around the site in such a way that the spatial requirements for substrate approach and departure became more rigorous.

References

Ames, B. N., Martin, R. G., and Garry, B. J. (1961), *J. Biol. Chem. 236*, 2019.

Atkinson, D. E. (1968), *Biochemistry 7*, 4030.

Atkinson, D. E., and Fall, L. (1967), *J. Biol. Chem. 242*, 3241.

Cordaro, J. C., Levy, H. R., and Balbinder, E. (1968), *Biochem. Biophys. Res. Commun. 33*, 183.

Hummel, J. P., and Dreyer, W. J. (1962), *Biochim. Biophys. Acta 63*, 530.

Klungsøyr, L. (1969), *Anal. Biochem. 27*, 91.

Klungsøyr, L., Hageman, J. H., Fall, L., and Atkinson, D. E. (1968), *Biochemistry 7*, 4035.

Margolies, M. N., and Goldberger, R. F. (1966), *J. Biol. Chem. 241*, 3262.

O'Donovan, G. A., and Ingraham, J. L. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 451.

Smith, D. W., and Ames, B. N. (1965), *J. Biol. Chem. 240*, 3056.

Umbarger, H. E. (1956), *Science 123*, 848.

Vogel, H. J., and Bonner, D. M. (1956), *J. Biol. Chem. 218*, 97.

Voll, M. J., Appella, E., and Martin, R. G. (1967), *J. Biol. Chem. 242*, 1760.

Yates, R. A., and Pardee, A. B. (1956), *J. Biol. Chem. 221*, 757.