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SEDIMENTATION BEHAVIOUR OF PHOSPHORIBOSYLADENOSINE TRIPHOSPHATE SYNTHETASE

EFFECTS OF SUBSTRATES AND MODIFIERS

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SUMMARY

We have studied the sedimentation properties of purified phosphoribosyladenosine triphosphate:pyrophosphate phosphoribosyltransferase (phosphoribosyladenosine triphosphate synthetase) from *Escherichia coli* as influenced by the ligands phosphoribosyladenosine triphosphate, AMP and histidine.

1. In dilute imidazole buffer the enzyme had an $s_{20,w}$ of 12.6 S, while in higher ionic strength buffer with 0.4 mM histidine the $s_{20,w}$ was 8.9 S.

2. An 8.9-S species was stabilized by the product of the synthetase reaction, phosphoribosyladenosine triphosphate, and AMP had a similar effect. High concentrations of ATP also stabilized the 8.9-S species.

3. The area under the 8.9-S peak gives a rough estimate of the ligand effect. When histidine was acting alone or in the presence of low concentrations of ATP, cooperative behaviour by histidine was apparent. When AMP was added, the enzyme responded to histidine at lower ligand concentrations and apparently with less cooperativity.

4. A hypothetical model is discussed in the light of earlier and new results.

INTRODUCTION

The enzyme phosphoribosyladenosine triphosphate:pyrophosphate phosphoribosyltransferase (phosphoribosyladenosine triphosphate synthetase) was first purified from *Salmonella typhimurium* by VOLL *et al.*[1]. More recently the phosphoribosyladenosine triphosphate synthetase from *E. coli* was isolated by KLUNGSÖYR AND ATKINSON[2]. The two enzymes were of similar size, but displayed somewhat different properties.

In a buffer containing 400 μM of the feedback inhibitor histidine, the *E. coli* enzyme sedimented at a rate corresponding to an $s_{20,w}$ of 8.9 S. However, the enzyme was considerably retarded on a Sephadex G-200 column, whereas one would expect it to be excluded by the gel[2]. It was suspected that the retardation was caused by dissociation–reaggregation processes. Phosphoribosyladenosine triphosphate or AMP

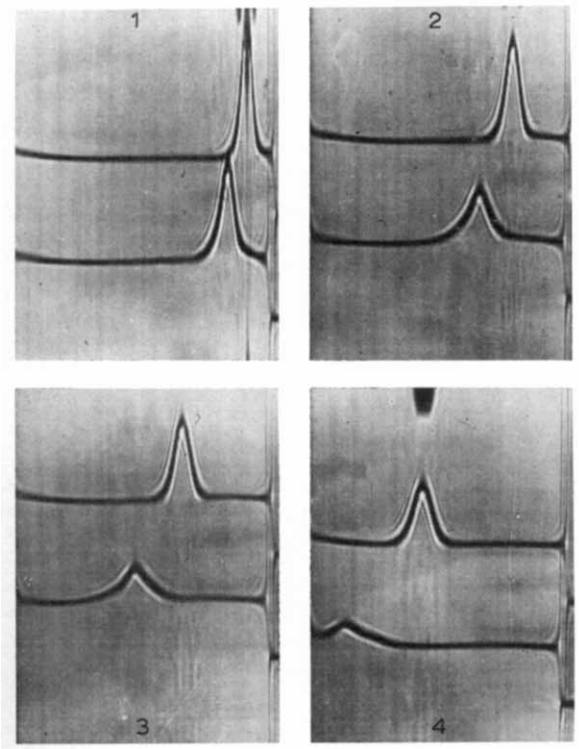


Fig. 1. Sedimentation of phosphoribosyladenosine triphosphate synthetase in two different buffers. Upper trace, Buffer II + 200 μM AMP (Basal + 0.1 M NaCl + 400 μM histidine + 200 μM AMP). Lower trace, Buffer I (Basal). The protein concentration was approx. 5 mg/ml. Sedimentation from right to left. Centrifuge speed, 59 780 rev./min. The exposures shown were made after (1) 8, (2) 20, (3) 32 and (4) 52 min centrifugation at full speed.

and histidine inhibit the enzyme synergistically. The binding of histidine by the enzyme is enhanced by phosphoribosyladenosine triphosphate or AMP[2].

We have now studied the effects of the specific ligands on the sedimentation properties of the enzyme in the ultracentrifuge.

MATERIALS AND METHODS

Phosphoribosyladenosine triphosphate synthetase was prepared from our *E. coli* mutant strain X-1 as described previously, including dialysis and re-precipitation[2].

The buffer referred to in this paper as Basal is identical with standard Buffer I (10 mM imidazole–HCl (pH 7.2) + 0.5 ml 2-mercaptoethanol per l)[2].

The sedimentation experiments were carried out in a Spinco Model E analytical ultracentrifuge. The temperature was 20°, and the phase plate angle was 60°.

Tris, imidazole, 2-mercaptoethanol and histidine were obtained from E. Merck AG, Darmstad. AMP, ATP, phosphoribosyl pyrophosphate and purified pyrophosphatase were purchased from Sigma Chemical Co., St. Louis.

Phosphoribosyladenosine triphosphate was prepared as described by AMES

et al.[3] using purified phosphoriboxyladenosine triphosphate synthetase and pyrophosphatase. The reaction mixture was freeze dried, dissolved in a small volume of water and applied to a Sephadex G-25 medium column, 2 cm × 120 cm. The phosphoribosyladenosine triphosphate eluted from this column was of satisfactory quality.

Protein was determined according to KLUNGSÖYR[4].

Areas under the 8.9-S peak in Schlieren patterns were determined by cutting from photographic enlargements and weighing. When necessary a curve resolver was used to separate overlapping peaks, and the areas were then determined with the electronic integrator.

RESULTS

The solubility of the phosphoribosyladenosine triphosphate synthetase of *E. coli* in $(NH_4)_2SO_4$ solutions depends upon the composition of the solvent from which it is precipitated[2], and the difference in solubility is correlated with a difference in sedimentation rate in the ultracentrifuge (Fig. 1). When dissolved in dilute imidazole buffer (Basal), the enzyme was precipitated by 22 g of $(NH_4)_2SO_4$ per 100 ml, and its $s_{20,w}$

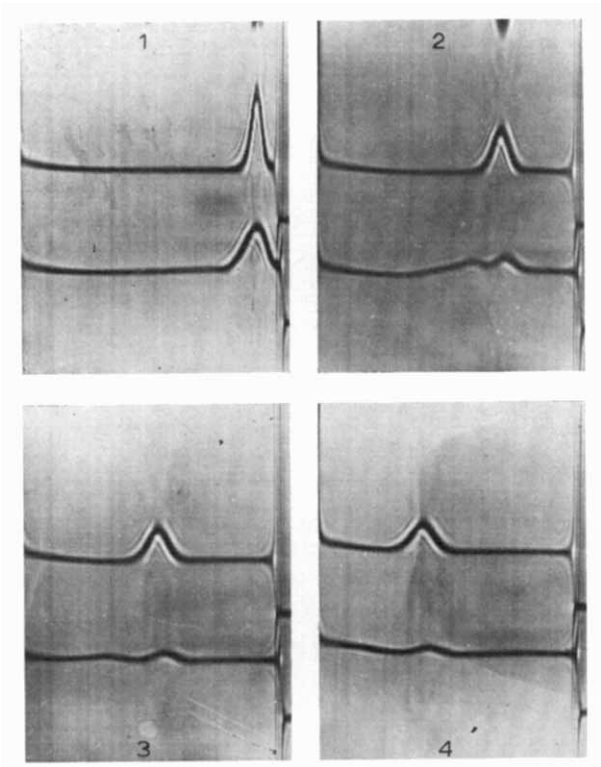


Fig. 2. Effect of 50 μM AMP as a co-ligand with histidine in the stabilization of the 8.9-S component of phosphoribosyladenosine triphosphate synthetase. The enzyme solution was dialyzed overnight against the following buffers. Upper trace, Basal + 0.1 M NaCl + 100 μM histidine + 50 μM AMP. Lower trace, Basal + 0.1 M NaCl + 100 μM histidine. Protein concentration, approx. 5 mg/ml. Centrifuge speed, 59 780 rev./min. The exposure shown were made after (1) 4, (2) 24, (3) 40 and (4) 52 min centrifugation at full speed.

was 12.6 S. In Basal buffer containing 0.1 M NaCl and 400 μM histidine, the enzyme was precipitated by 34.5 g of $(NH_4)_2SO_4$ per 100 ml, and its $s_{20,w}$ was 8.9 S.

The molecular weight of the enzyme was determined by sedimentation equilibrium according to the Lamm method[5] after dialysis against three different solvents: A, Basal + 0.1 M NaCl; B, Basal + 0.1 M NaCl + 100 μM histidine; C, Basal + 0.1 M NaCl + 400 μM histidine + 200 μM AMP + 1.0 mM $MgSO_4$. Both A and B yielded results indicating polydispersity, with initial and final slopes in A corresponding to a range of "molecular weights" of 162 000–302 000; in B, 174 000–224 000. C yielded linear plots throughout the cell, corresponding to a molecular weight of 206 000 (assuming a partial specific volume of 0.75). This, like the $s_{20,w}$, is close to the value determined for the *S. typhimurium* enzyme by VOLL *et al.*[1].

The subunit size of the two enzymes seems to be similar, as we have found an $s_{20,w}$ of 1.78 S for the enzyme in 6 M guanidinium chloride, as compared with 1.75 S given for the *S. typhimurium* enzyme[1]. It is therefore possible that the histidine-stabilized 8.9-S form of the *E. coli* enzyme is a hexamer, each dimer having one binding site for histidine[2].

Sedimentation and enzyme ligand interaction

As indicated by the sedimentation equilibrium experiments, the *E. coli* enzyme may behave as a homogeneous species or exhibit polydispersity, depending upon the

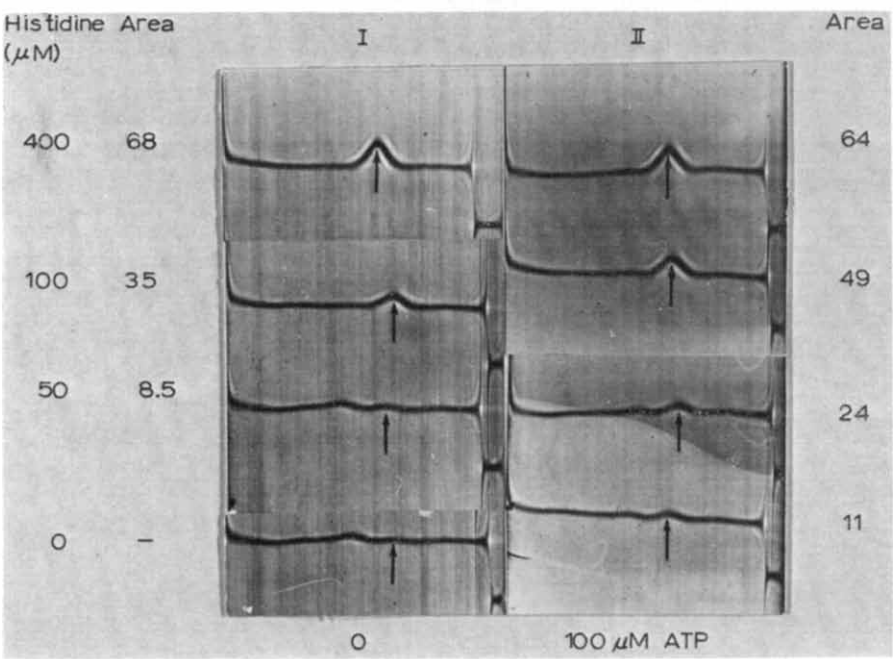


Fig. 3. Effect of histidine on the sedimentation properties of phosphoribosyladenosine triphosphate synthetase in the presence of no other ligands, or with low concentrations of ATP. The enzyme preparations were dialyzed against Basal buffer + 0.1 M NaCl. At the time of the experiments histidine and ATP were added as shown. Approximate protein concentrations: I, 2.6 mg/ml; II, 3.8 mg/ml. The exposures shown were made 32 min after full speed was reached (59 780 rev./min). The areas under the 8.9-S peak (arrows) are given in arbitrary units.

ligands added. In the sedimentation experiments, enzyme ligand interaction manifested itself by the appearance of an 8.9-S peak, the initial size of which was determined by the kinds and concentrations of the ligands (Fig. 2). As the sedimentation proceeded, the area under the peak diminished, probably because the equilibrium situation in the cell was upset by separation of species representing different degrees of aggregation. The rate of loss of area under the 8.9-S peak was also ligand dependent. Therefore, in sedimentation experiments under various conditions, the 8.9-S-peak size and stability are useful for evaluating, at least qualitatively, enzyme ligand interaction.

The increase in 8.9-S-peak area as a function of histidine concentration is illustrated in Fig. 3. A concentration of histidine of 50 μM had little effect, whereas 100 μM histidine increased the 8.9-S-peak area considerably, perhaps indicating cooperativity in histidine action. In a similar experiment, 100 μM ATP and 0.5 mM $MgSO_4$ were included in the solvent buffer. This concentration of ATP had little effect upon the 8.9-S peak, and also little effect on the enzyme–histidine interaction.

In contrast to ATP, the product of the synthetase reaction, phosphoribosyladenosine triphosphate, had a powerful effect on the 8.9-S-peak size at low concentrations (22 and 55 μM) (Fig. 4); and 50 μM histidine, which had little effect in combination with 100 μM ATP, greatly increased the area of the peak when acting together with 22 μM phosphoribosyladenosine triphosphate. A combination of the substrates (50 μM phosphoribosyl pyrophosphate and 100 μM ATP) did not mimic the effect of phosphoribosyladenosine triphosphate (exposures not shown).

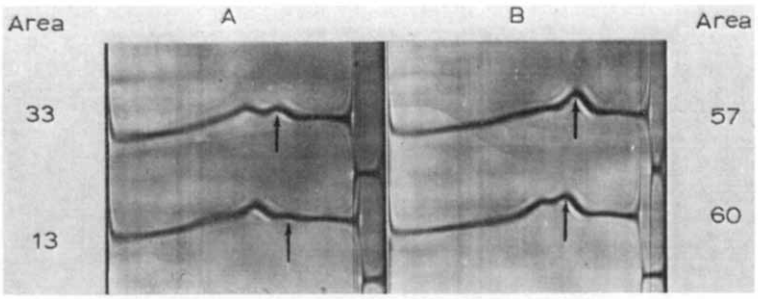


Fig. 4. Effect of phosphoribosyladenosine triphosphate and phosphoribosyladenosine triphosphate + histidine on the sedimentation properties of phosphoribosyladenosine triphosphate synthetase. The enzyme was dialyzed against Basal buffer + 0.1 M NaCl + 0.5 mM $MgSO_4$. At the time of the experiment the following additions were made: A, lower trace, none; A, upper trace, 22 μM phosphoribosyladenosine triphosphate; B, upper trace, 22 μM phosphoribosyladenosine triphosphate + 50 μM histidine; B, lower trace, 55 μM phosphoribosyladenosine triphosphate. The protein concentration was approx. 3 mg/ml. The exposures shown were made 24 min after full speed was reached (59 780 rev./min). The areas under the 8.9-S peak (arrows) are given in arbitrary units.

While ATP at a total concentration of 100 μM had little effect on the sedimentation properties of phosphoribosyladenosine triphosphate synthetase, intermediate concentrations of ATP (625 μM) created an 8.9-S species, although less efficiently than the same concentration of AMP (Fig. 5). High concentrations of ATP (2 mM ATP + 2.5 mM $MgSO_4$, exposures not shown) resulted in a practically homogeneous 8.9-S peak.

The interaction between AMP, histidine and phosphoribosyladenosine tri-

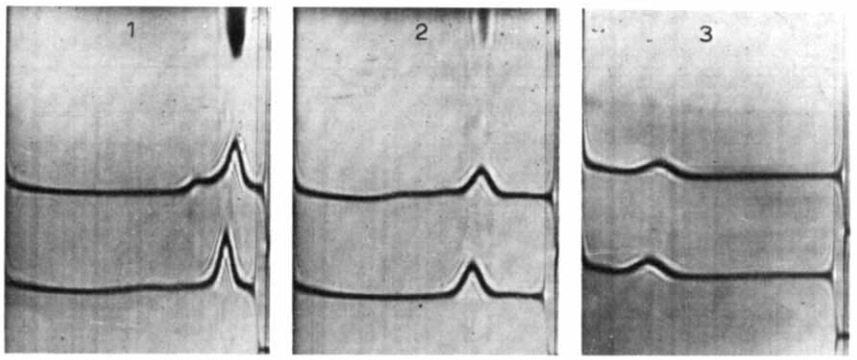


Fig. 5. Effect of AMP and ATP upon the sedimentation of phosphoribosyladenosine triphosphate synthetase. Upper trace, Basal + 0.1 M NaCl + 625 μM ATP. Lower trace, Basal + 0.1 M NaCl + 625 μM AMP. Protein concentration approx. 3.6 mg/ml. The exposures shown were made after (1) 8, (2) 24, and (3) 64 min centrifugation at 59 780 rev./min.

phosphate synthetase was studied (Figs. 6 and 7). In these experiments the enzyme solution was dialyzed against a buffer containing all the components except histidine. With the high concentrations of enzyme and the low concentrations of ligands that were used, any ligand binding by the enzyme may of course influence the concentration of free ligand. It appears that a concentration of free AMP of 50 μM by itself stabilizes the 8.9-S peak considerably; and more in the absence than in the presence of Mg^{2+}. In addition to the AMP effect, low concentrations of histidine created synergistic stabilization, and the histidine effect was most pronounced in the series in which Mg^{2+} was present. Distinct effects were seen with total concentrations of 15 μM histidine, which is about the same as the enzyme concentration (based on a molecular weight of 200 000).

DISCUSSION

The enzyme phosphoribosyladenosine triphosphate synthetase occurs in a number of different aggregate forms. The interconversion between the various forms is largely reversible and influenced by the natural substrates and inhibitors of the enzyme. Two characteristic aggregates are defined here by their sedimentation properties and are those existing in dilute (10 mM) imidazole buffer, and in imidazole buffer with relatively high concentration of histidine (400 μM) and NaCl (0.1 M). These species have previously been shown to have different solubilities in $(NH_4)_2SO_4$, a property routinely used for the purification of the enzyme[2].

The histidine-stabilized form of the enzyme had an $s_{20,w}$ of 8.9 S, and a molecular weight determined by sedimentation equilibrium of 206 000. These values are close to those found by VOLL *et al.*[1] for phosphoribosyladenosine triphosphate synthetase from *S. typhimurium*. The enzyme from *S. typhimurium* was excluded on Sephadex G-200 gel, while the *E. coli* enzyme was considerably retarded, and it is therefore probable that the two enzymes differ in dissociation-aggregation properties.

In dilute imidazole buffer the *E. coli* enzyme had an $s_{20,w}$ of 12.6 S. Species sedimenting with this speed were also found in buffers containing 0.1 M NaCl and Mg^{2+}, but, in the buffers with higher ionic strength, monomers and several aggregate

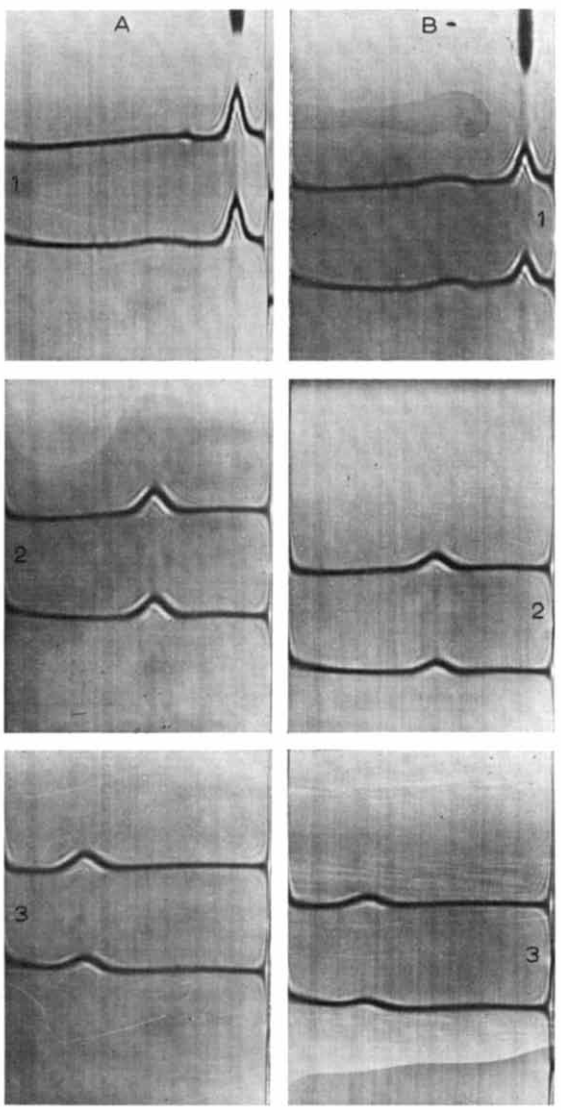


Fig. 6. Stability of the 8.9-S component of phosphoribosyladenosine triphosphate synthetase in the presence of 50 μM AMP and 1 mM Mg^{2+} as a function of histidine concentration. The enzyme was dialyzed against Basal buffer +0.1 M NaCl + 1 mM $MgSO_4$ + 50 μM AMP. At the time of the centrifugation, histidine was added to the following concentrations: A, upper trace, 100 μM; A, lower trace, 30 μM; B, upper trace, 15 μM; B, lower trace, no histidine. Protein concentration, 3 mg/ml. The exposures shown were made at (1) 8, (2) 40 and (3) 64 min after full speed was reached (59 780 rev./min).

forms were present simultaneously. The relative amounts of the 12.6-S species found in high ionic strength buffers in the absence of ligands varied somewhat from preparation to preparation.

In analogy with the data of VOLL *et al.*[1] we assumed the 8.9-S molecule to be a hexamer. The equation given by HALSALL[6] predicts a ratio of 1.68 between the

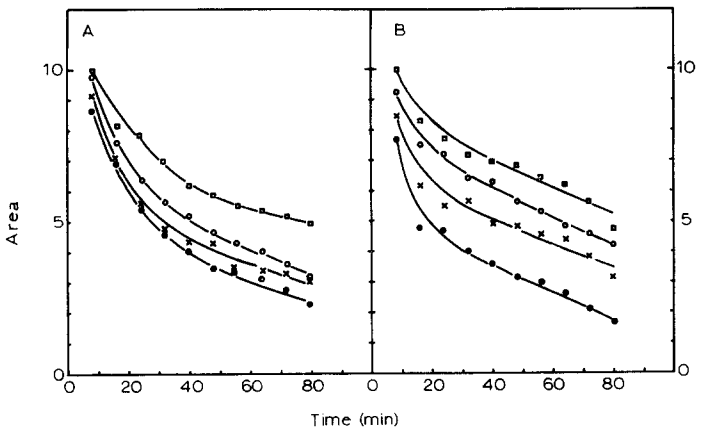


Fig. 7. Areas under the 8.9-S peak (arbitrary units) as functions of centrifugation time. A. Experiment with no Mg^{2+} added. B. Experiments shown in Fig. 6, with 1 mM $MgSO_4$ in the buffer. ●, no histidine; ×, 15 μM histidine; ○, 30 μM histidine; □, 100 μM histidine.

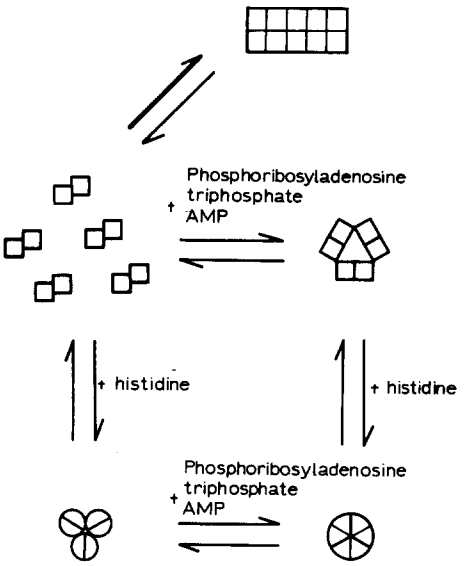
molecular weights of the 12.6- and 8.9-S species, and this 5:3 ratio corresponds to 10 and 6 subunits, respectively, for the two forms.

The 12.6-S species seems to be a member of a more or less continuous spectrum of aggregates, while the assumed hexamer is distinguished by its responses to various ligands which increase its relative concentration and stability. Phosphoribosyladenosine triphosphate and AMP are efficient stabilizers of the 8.9-S molecule at low concentrations. ATP and phosphoribosyl pyrophosphate have less effect at concentrations well above the catalytic $(S)_{0.5}$ values[2]. However, higher concentrations of ATP stabilize this species well. Apparently ATP then acts at a site that is different from the catalytic.

When histidine stabilized the 8.9-S form, the effective concentrations were in the same range as those active for inhibiting the synthetase reaction. Phosphoribosyladenosine triphosphate and AMP enhance the stabilizing action of histidine, in analogy with the synergism observed in the binding of histidine to the synthetase, and in the inhibitory function of histidine in synthetase catalysis[2].

The interaction between phosphoribosyladenosine triphosphate synthetase and histidine may, according to conditions, obey hyperbolic or cooperative kinetics. For the stabilization of the 8.9-S peak in the ultracentrifuge with 50 μM AMP present, histidine seemed to show little cooperativity. Experiments with end-product and energy charge control of phosphoribosyladenosine triphosphate synthetase (ref. 7, and L. KLUNGSÖYR AND D. E. ATKINSON, unpublished experiments) have demonstrated that histidine inhibits much more strongly at low than at high energy charge values, and this is most pronounced at low histidine concentrations. As a consequence the histidine effect should be less cooperative at low than at high energy charge, and this deduction we have confirmed in kinetic experiments[8]. In the ultracentrifuge histidine seems to display cooperativity in 8.9-S-peak formation in the absence of AMP or phosphoribosyladenosine triphosphate, and cooperativity is also seen when ATP is present at concentrations high enough largely to saturate the catalytic site.

Scheme 1 presents a model that illustrates some facts and assumptions about



Scheme 1. Phosphoribosyl adenosine triphosphate synthetase.

the function of phosphoribosyladenosine triphosphate synthetase. In the absence of histidine, the enzyme exists in active dimers and hexamers. The synthetase reaction produces phosphoribosyladenosine triphosphate which interacts strongly with the enzyme leading to the formation of a hexamer with trapped phosphoribosyladenosine triphosphate (product inhibition). AMP also displaces the equilibrium towards the hexamer and competes with phosphoribosyladenosine triphosphate[2]. The feedback inhibitor, histidine, locks the hexamer in a rigid, inactive form, eventually with trapped phosphoribosyladenosine triphosphate or AMP. Histidine may react with the enzyme, and in the process of hexamer formation cause subunit interaction, and therefore, according to current theories, display cooperative kinetics[9,10]. If AMP or phosphoribosyladenosine triphosphate are present, these ligands cause subunit interaction, and hyperbolic kinetics of the histidine effect are observed.

A weakness in this model is the finding in the sedimentation experiments that ATP caused 8.9-S-peak formation at concentrations which, when used in kinetic experiments gave cooperative kinetics of histidine inhibition. We suspect that this is a matter of protein concentration, and we have observed decreasing specific activity with increasing protein concentration[8]. Were this a sign of increased 8.9-S-species formation at high enzyme concentration, ATP might be unable to form this species at dilute enzyme concentrations.

We see no reason to postulate separate regulatory sites for AMP and phosphoribosyladenosine triphosphate, and our hypothesis is that AMP is bound at the ribose-5-*P* end of phosphoribosyladenosine triphosphate, including the adenine site. AMP competes with phosphoribosyladenosine triphosphate both in the pyrophosphorolysis reaction and as co-ligand with histidine in phosphoribosyladenosine triphosphate synthetase inhibition. The two have similar effects in the binding of histidine[2]. AMP is effective at low concentrations in the presence of much higher ATP concentrations, indicating that ATP competes only feebly. Possibly, in the hexamer-forming function,

ATP may itself compete at the "wrong" end of the phosphoribosyladenosine triphosphate site, but with low affinity.

The choice of the dimer as the enzymically active form is more or less fortuitous, and it is not implicit that other aggregates or the monomer are inactive. Probably, the appearance of the 8.9-S species is intimately tied to the formation of phosphoribosyladenosine triphosphate as a product. Low-molecular-weight material is visible in most sedimentation experiments, and the behaviour of the enzyme on Sephadex gel shows that small species are present and easily formed from higher aggregates. Even were the decamer and higher aggregates enzymically active, dissociation before reaggregation is likely when lower aggregates are made from higher ones, so that monomers or dimers are intermediates in hexamer formation. It appears that the hexameric enzyme can bind a maximum of 3 histidine molecules[2]. This may be interpreted as experimental support for regarding the dimer as a functional unit.

Low concentrations of phosphoribosyladenosine triphosphate favour 8.9-S-species formation, while phosphoribosyl pyrophosphate and ATP lack the stabilizing effect on this species at concentrations that should give high saturation ratios at the catalytic sites. This may possibly indicate that a conformational change takes place in connection with the catalytic event.

The asymmetrical inhibition of the phosphoribosyladenosine triphosphate synthetase reaction by histidine[2] may be explained in the light of these results. Phosphoribosyladenosine triphosphate formation gives tightening of the enzyme and trapping of this product especially in the presence of histidine, resulting in inhibition of the synthetase direction of the reaction. Phosphoribosyladenosine triphosphate removal by pyrophosphorolysis has as a consequence a loosening of the structure of the enzyme, even in the presence of histidine, and therefore this direction of the reaction will be less inhibited.

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