Pigeon Liver Amidophosphoribosyltransferase

LIGAND-INDUCED ALTERATIONS IN MOLECULAR AND KINETIC PROPERTIES

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Amidophosphoribosyltransferase (EC 2.4.2.14) has been partially purified from pigeon liver, and ligand-induced alterations in molecular and kinetic properties have been studied. In Tris-HCl buffer the predominant form of the enzyme has an $s_{20,w}$ of 5.9 ± 0.7, Stokes radius of 42 Å, and estimated molecular weight of 102,000. Incubation with phosphoribosylpyrophosphate (PP-ribose-P) results in an increase in the $s_{20,w}$ to 7.9 ± 0.6, Stokes radius to 53 Å, and estimated molecular weight to 172,000. Incubation of this larger form with purine ribonucleotides leads to a decrease in the molecular weight of amidophosphoribosyltransferase that is proportional to the concentration of purine ribonucleotide. Purine ribonucleotides produce sigmoidal kinetics with respect to the substrate PP-ribose-P, with Hill coefficients of 1.4 to 1.6 and 1.8 to 2.0 in the presence of AMP and GMP, respectively. Incubation with 0.6 M KCl leads to sigmoidal kinetics, Hill coefficient of 1.8, and dissociation of the larger form of amidophosphoribosyltransferase.

Inorganic phosphate has complex effects upon the enzyme. In 25 mM potassium phosphate buffer the enzyme aggregates to a large form with an $s_{20,w}$ of 8.3 ± 0.2, Stokes radius of 53 Å, and estimated molecular weight of 181,000. Inorganic phosphate and PP-ribose-P both stabilize the enzyme to storage in vitro at 4°C. However, inorganic phosphate is 4 times more effective than PP-ribose-P in preventing inactivation of the enzyme by sodium dodecyl sulfate. Inorganic phosphate produces sigmoidal kinetics with respect to PP-ribose-P, Hill coefficient of 1.5. The interaction coefficients for AMP and GMP are reduced from 1.8 to 1.2 and 2.2 to 1.4, respectively, in the presence of 25 mM potassium phosphate.

It is concluded that pigeon liver amidophosphoribosyltransferase is a complex allosteric protein whose activity is regulated by a series of conformational changes induced by a number of ligands.

Amidophosphoribosyltransferase (EC 2.4.2.14, ribosylamine-5-phosphate:pyrophosphate phosphoribosyltransferase) catalyzes the first committed reaction in purine biosynthesis, the $\beta$ substitution of the amido group of glutamine on C-1 of phosphoribosylpyrophosphate. This enzyme has been purified from pigeon liver (1) and its regulatory properties have been studied (2-4). The demonstration of inhibition by purine ribonucleotides (2), positive cooperativity between the binding of 6-hydroxy- and 6-aminopurine ribonucleotides (3), binding of purine ribonucleotides at sites topologically distinct from the catalytic site (2-4), and conformational heterogeneity induced by substrates and purine ribonucleotides (4) have established that pigeon liver amidophosphoribosyltransferase is an allosteric enzyme.

Purification of amidophosphoribosyltransferase from pigeon liver has disclosed three molecular species of the enzyme (1). A large form of the enzyme, $s_{20,w}$ of 10.0 and estimated molecular weight of 200,000, was identified. This form spontaneously dissociated to a protein with an $s_{20,w}$ of 6.0 and estimated molecular weight of 100,000. In the presence of thiol this smaller species of amidophosphoribosyltransferase dissociated further to a protein with an $s_{20,w}$ of 4.0 and estimated molecular weight of 50,000. Using this highly purified preparation of pigeon liver amidophosphoribosyltransferase, which was unstable and rapidly desensitized to inhibition by purine ribonucleotides, it was not possible to demonstrate ligand-induced interconversion of these molecular species (1). However, the recent demonstration that amidophosphoribosyltransferase partially purified from human placenta undergoes an association reaction when incubated with purine ribonucleotides and a dissociation reaction when incubated with PP-ribose-P(5) (5), prompted us to re-examine these properties of the pigeon liver enzyme. For these studies we have used a partially purified preparation of the pigeon liver enzyme that was stabilized by the addition of Mg$^{2+}$ phosphate. In this report we describe

* The abbreviation used is: PP-ribose-P, phosphoribosylpyrophosphate.
ligand-induced interconversions between the different molecular forms of pigeon liver amidophosphoribosyltransferase. These changes in molecular size are discussed in relationship to ligand-induced alterations in kinetic properties and stability of the enzyme.

**EXPERIMENTAL PROCEDURE**

**Materials**

- Uniformly labeled L-[14C]glutamine (48 mCi/mmol) was purchased from New England Nuclear Corp., and was further purified prior to use by adsorption of [14C]glutamate to an anion exchange resin AG1-X4 (CI-) purchased from Bio-Rad Laboratories. Bovine serum albumin and the sodium salts of adenosine 5'-monophosphoric acid, guanosine 5'-monophosphoric acid, and PP-ribose-P were purchased from Sigma Chemical Co. Purified powdered catalase (56 units/mg) and bovine serum albumin (50 mg/ml) were purchased from Worthington Biochemical Corp. Chicken ovalbumin was purchased from Pentex Biochemicals. Sephadex G-200 was a product of Pharmacia Fine Chemicals. Cellex D was purchased from Bio-Rad Laboratories. The concentration of all compounds used was based on data provided by the manufacturer.

**Methods**

**Enzyme Assays—**Amidophosphoribosyltransferase was assayed as previously described by determining the PP-ribose-P-dependent conversion of [14C]glutamine to [14C]glutamate (6). The standard assay was performed in a total volume of 100 μl of 25 mM Tris-HCl buffer, pH 7.4, containing 5 mM PP-ribose-P, 4 mM [14C]glutamine, 10 mM MgCl₂, and 30 mM β-mercaptoethanol.

**Glutaminase activity** was assayed as previously described (6).

**Nucleotidase activity** was assayed as previously described, using [14C]IMP as substrate (6).

**Catalase activity** was assayed according to the method described by Bein and Sizer (7).

**β-Galactosidase activity** was assayed with o-nitrophenyl-β-galactoside as substrate (6).

**Bovine serum albumin and ovalbumin,** when used as standards for calibrating sucrose density gradients or gel filtration columns, were identified by the change in absorbance at 280 nm.

**Protein** was determined by the method of Geiger and Bessman (9).

**Enzyme Purification—**Freshly excised pigeon livers (50 g) were homogenized in 2 volumes of ice-cold 25 mM potassium phosphate buffer, pH 7.4, containing 5 mM MgCl₂ and 60 mM β-mercaptoethanol (Buffer A). This and all subsequent steps were performed at 4°C. The homogenate was centrifuged at 12,000 x g for 30 min.

The supernatant fluid (90 ml) was mixed for 2 hours with 80 g of DEAE-cellulose that had been equilibrated with 5 mM MgCl₂/60 mM β-mercaptoethanol and adjusted to pH 7.4. Following this procedure, the DEAE-cellulose was washed with 7 liters of Buffer A. The DEAE-cellulose was suspended in 500 ml of Buffer A, poured into a column (6 x 35 cm), and washed with 7 liters of Buffer A containing 50 mM potassium chloride. A linear gradient, from 50 to 500 mM potassium chloride in Buffer A, was dialyzed against Buffer B and centrifuged into a sucrose gradient containing the same buffer; 0.2 enzyme preparation was dialyzed against 250 volumes of 25 mM Tris-HCl buffer for 12 hours, incubated with 1 mM PP-ribose-P and 100 μM IMP. The dialyzed enzyme preparation (10 mg of protein/ml) was added, either to an equal volume of 50 mM potassium phosphate buffer, pH 7.4, or to an equal volume of 50 mM Tris-HCl buffer, pH 7.4, containing 2 mM or 10 mM PP-ribose-P. These samples were incubated at 37°C for 15 min. This solution was then transferred to an ice bath and a 1/4 volume of ice-cold sodium dodecyl sulfate solution was added to give a final concentration of 1.4 mM sodium dodecyl sulfate. At the conclusion of the sodium dodecyl sulfate incubation period the assay for amidophosphoribosyltransferase was initiated by transferring 10 μl of enzyme-sodium dodecyl sulfate solution to 100 μl of assay mixture.

**RESULTS**

**Sedimentation Studies—**As shown in Fig. 1, the sedimentation profile of amidophosphoribosyltransferase activity was markedly affected by the composition of the buffer used in the studies. In 25 mM Tris-HCl buffer the s₂₀,₅₀ was 5.9 ± 0.7 (Table II). Essentially the same results were obtained when these studies were repeated in 50 mM Tris-HCl buffer. In Tris-HCl buffer there was a shoulder of activity with an approximate s₂₀,₅₀ of 8 (Fig. 1). In 25 mM phosphate buffer the peak of activity corresponded to an s₂₀,₅₀ of 8.3 ± 0.2 (Table II).

Although there was not a definite shoulder of activity under these conditions, there was a constituent that sedimented more slowly (Fig. 1).

**The effects of PP-ribose-P, AMP, and the combination of PP-ribose-P and AMP on the sedimentation coefficient of amidophosphoribosyltransferase in 25 mM Tris-HCl buffer (Buffer B) are summarized in Table I. When the enzyme preparation was dialyzed against 250 volumes of 25 mM Tris-HCl buffer for 12 hours, incubated with 1 mM PP-ribose-P for 15 min at 37°C, and then centrifuged into a gradient which also contained the same concentration of PP-ribose-P, the s₂₀,₅₀ increased from 5.9 - 0.7 to 7.9 ± 0.6 (Fig. 2). By contrast, when the enzyme preparation was preincubated with 5 mM AMP alone and then centrifuged into a gradient containing the same concentration of AMP, there was no significant change in the sedimentation coefficient of the major peak of enzyme activity.
enzyme was demonstrated to be sensitive to inhibition by concentrations of AMP were added during the preincubation
and centrifugation, the sedimentation coefficient of amidophosphoribosyltransferase decreased progressively (Fig. 3B).

PP-ribose-P and AMP on the sedimentation coefficient of amidophosphoribosyltransferase in 25 mM phosphate buffer, incubation with 5 mM AMP on the sedimentation coefficient of amidophosphoribosyltransferase (Table I). This preparation of the enzyme was dialyzed against Buffer B and preincubated at 37°C for 15 min with 1 mM PP-ribose-P and various concentrations of AMP. These samples were then centrifuged into gradients which contained Buffer B with the same constituents as used in the preincubation. B, enzyme preparation was dialyzed against Buffer A and preincubated at 37°C for 15 min with various concentrations of AMP. These samples were then centrifuged into gradients which contained Buffer A with the same constituents as used in the preincubation.

However, the shoulder of activity which sedimented more rapidly completely disappeared under these circumstances (Fig. 2). When increasing concentrations of AMP were added during the preincubation with 1 mM PP-ribose-P and included in the gradient, the sedimentation coefficient of amidophosphoribosyltransferase decreased progressively and approached the value obtained in the absence of PP-ribose-P (Fig. 3A).

The effects of PP-ribose-P, AMP, and the combination of PP-ribose-P and AMP on the sedimentation coefficient of amidophosphoribosyltransferase in 25 mM phosphate buffer (Buffer A) are also summarized in Table I. When increasing concentrations of AMP were added during the preincubation and centrifugation, the sedimentation coefficient of amidophosphoribosyltransferase decreased progressively (Fig. 3B). By contrast, 1 mM PP-ribose-P alone had no effect on the sedimentation coefficient of amidophosphoribosyltransferase in 25 mM phosphate buffer (Table I).

The effect of GMP is different from that of AMP. In phosphate buffer, incubation with 5 mM GMP did not result in a significant change in the sedimentation coefficient of amidophosphoribosyltransferase (Table I). This preparation of the enzyme was demonstrated to be sensitive to inhibition by GMP. However, in 25 mM Tris-HCl buffer, preincubation with 1 mM PP-ribose-P followed by an incubation with 5 mM GMP resulted in a decrease in the $s_{20,w}$ of amidophosphoribosyltransferase to an intermediate value of 7.0 to 7.5 (Fig. 4). Incubation of the Tris-HCl enzyme with GMP alone resulted in an increase in the sedimentation coefficient of amidophosphoribosyltransferase (Table I).

Gel Chromatography Studies—The effect of Tris-HCl and phosphate buffers on the elution pattern of amidophosphoribosyltransferase from a Sephadex G-200 column is shown in Fig. 5. In 25 mM Tris-HCl buffer the elution volume of amidophosphoribosyltransferase was greater than that observed in 25 mM phosphate buffer. The molecular Stokes radii in 25 mM phosphate and 25 mM Tris-HCl buffers were 52 Å and 42 Å, respectively (Fig. 6). Increasing the concentration of Tris-HCl buffer from 25 to 50 mM did not significantly alter the Stokes radius of amidophosphoribosyltransferase.

When the enzyme was preincubated with 5 mM AMP in 25 mM phosphate buffer for 15 min at 37°C and then applied to a Sephadex G-200 column equilibrated with the same AMP-

### Table 1

<table>
<thead>
<tr>
<th>Addition</th>
<th>$s_{10,w}$</th>
<th>Stokes radius</th>
<th>Molecular weight</th>
<th>$s_{20,w}$</th>
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</thead>
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<tr>
<td>Buffer B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5.9 ± 0.7</td>
<td>42</td>
<td>102,000</td>
<td>1.36</td>
</tr>
<tr>
<td>1 mM PP-ribose-P</td>
<td>7.9 ± 0.6</td>
<td>53</td>
<td>172,000</td>
<td>1.57</td>
</tr>
<tr>
<td>5 mM AMP</td>
<td>6.1 ± 0.7</td>
<td>49</td>
<td>123,000</td>
<td>1.49</td>
</tr>
<tr>
<td>5 mM GMP</td>
<td>6.7</td>
<td>50</td>
<td>138,000</td>
<td>1.46</td>
</tr>
<tr>
<td>Buffer A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>8.3 ± 0.2</td>
<td>53 ± 0.7</td>
<td>181,000</td>
<td>1.40</td>
</tr>
<tr>
<td>1 mM PP-ribose-P</td>
<td>8.5 ± 0.1</td>
<td>52</td>
<td>182,000</td>
<td>1.39</td>
</tr>
<tr>
<td>5 mM AMP</td>
<td>6.7 ± 0.3</td>
<td>43 ± 2.2</td>
<td>116,000</td>
<td>1.30</td>
</tr>
<tr>
<td>5 mM GMP</td>
<td>8.3</td>
<td>52</td>
<td>117,000</td>
<td>1.40</td>
</tr>
</tbody>
</table>

$^a$ The value given is the mean ± S.D. Figures in parentheses are the number of determinations.

### Fig. 2

Effects of PP-ribose-P and AMP on the sedimentation profile of amidophosphoribosyltransferase activity in Tris-HCl buffer. The enzyme preparation was dialyzed against Buffer B and preincubated at 37°C for 15 min with 1 mM PP-ribose-P (O) or 5 mM AMP (Δ). These samples were centrifuged into gradients that contained Buffer B with the same constituents as used in the preincubation. B, enzyme preparation was dialyzed against Buffer A and preincubated at 37°C for 15 min with various concentrations of AMP. These samples were then centrifuged into gradients which contained Buffer A with the same constituents as used in the preincubation.
yielded Stokes radii which were intermediate between those of AMP or GMP followed by chromatography on Sephadex G-200 in the absence of KCl resulted in the elution of a single component of the large and small form of amidophosphoribosyltransferase (Table I).

Included in Table I is a compilation of all experiments in which the sedimentation coefficient and Stokes radii of amidophosphoribosyltransferase were determined. These values have been used to calculate the molecular weight and frictional ratio of the different forms of pigeon liver amidophosphoribosyltransferase. As shown in Table I there are at least two forms of amidophosphoribosyltransferase. In the presence of 25 mM phosphate or 1 mM PP-ribose-P the enzyme has an $s_{20,w}$ of 8.3 to 8.5, Stokes radius of 52 to 53 Å, and estimated molecular weight of 177,000 to 182,000. In 25 mM or 50 mM Tris-HCl buffer alone, the enzyme has an $s_{20,w}$ of 3.8, Stokes radius of 42 Å, and estimated molecular weight of 102,000.

Kinetic Studies—The Michaelis-Menten plot with PP-ribose-P as the variable substrate was hyperbolic in 25 mM and 50 mM Tris-HCl buffer. The Hill plot (14) of these data yielded a straight line with a positive slope of $n = 1$ (Fig. 8A). In the absence of the negative effectors, AMP or GMP, sigmoidal kinetics were also observed in 25 mM Tris-HCl buffer when KCl was included at 0.6 and 1.0 mM. The Hill plots of these data yielded $n$ values of 1.4 and 1.6 for 5 mM and 10 mM AMP, 1.8 and 2.0 for 5 mM and 10 mM GMP, 1.8 and 2.0 for 0.6 mM and 1.0 mM KCl, respectively (Fig. 8A to C). In 25 mM and 50 mM phosphate buffer alone sigmoidal kinetics were also demonstrated. The $n$ value was 1.5 for both 25 mM and 50 mM phosphate (Fig. 8D).

When amidophosphoribosyltransferase was assayed in the presence of increasing concentrations of AMP or GMP, sigmoidal inhibition curves were obtained in 25 mM and 50 mM Tris-HCl buffer. The interaction coefficients, $n^*$ (15), for AMP and GMP were 1.8 and 2.2, respectively (Fig. 9). When these studies were repeated in the presence of 25 mM phosphate, the inhibition curves were less sigmoidal, as indicated by a decline in $n^*$ from 1.8 to 1.2 for AMP and from 2.2 to 1.4 for GMP (Fig. 9).

In the presence of 0.6 mM KCl the $n^*$ values declined to 1.3 and 1.4 for AMP and GMP, respectively (Fig. 9).

Stabilization of Amidophosphoribosyltransferase by Phospho- or PP-ribose-P—As shown in Table II, both 25 mM phosphate and 1 mM PP-ribose-P were effective in stabilizing amidophosphoribosyltransferase activity when the enzyme was stored at 4°C for 6 to 10 days. However, amidophosphoribosyl-
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Fig. 8. Effect of AMP, GMP, KCl, and phosphate on the Hill coefficient of amidophosphoribosyltransferase. All reaction mixtures contained 4 mM [14C]glutamine, 10 mM MgCl₂, 30 mM β-mercaptoethanol, and variable concentrations of PP-ribose-P. A, experiments were performed in 25 mM Tris-HCl buffer, pH 7.4, with no addition (○), with 5 mM AMP (△), or with 10 mM AMP (■). B, experiments were performed in 25 mM Tris-HCl buffer, pH 7.4, with 5 mM GMP (□) or 10 mM GMP (■). C, experiments were performed in 25 mM Tris-HCl buffer, pH 7.4, with 0.6 M KCl (△) or with 1.0 M KCl (■). D, experiments were performed in 25 mM phosphate buffer, pH 7.4, (△) or in 50 mM phosphate buffer (■).

Transfase activity was rapidly lost when the enzyme was stored in 25 mM Tris-HCl buffer alone.

Fig. 9 shows the results obtained when different preparations of amidophosphoribosyltransferase were exposed to sodium dodecyl sulfate for variable periods of time. In the presence of 25 mM phosphate over 50% of enzyme catalytic activity was retained after 40 min of incubation at 4°C with 1.5 mM sodium deoxycholate. By contrast, preincubation of the enzyme with 1 or 5 mM PP-ribose-P in 25 mM Tris-HCl buffer was relatively ineffective in stabilizing amidophosphoribosyltransferase to denaturation by sodium dodecyl sulfate (<10% residual activity).

DISCUSSION

Pigeon liver amidophosphoribosyltransferase has been reported to be an allosteric protein (2-4), that undergoes conformational changes in the presence of ligands such as PP-ribose-P and purine ribonucleotides (4). In this report we extend these observations and describe two forms of the enzyme which differ in molecular weight and are interconvertible upon incubation with these ligands. In 25 mM or 50 mM Tris-HCl buffer the predominant form of the enzyme has an S20,w of 5.9 ± 0.7, Stokes radius of 42 Å, and estimated molecular weight of 102,000. Incubation of this form of the enzyme with 1 mM PP-ribose-P is associated with an increase in the S20,w to 7.9 ± 0.6, Stokes radius to 50 Å, and estimated molecular weight to 172,000. Incubation of this larger form of the enzyme with purine ribonucleotides, AMP and GMP, results in a decrease in the molecular weight of amidophosphoribosyltransferase. The decrease in S20,w is proportional to the concentration of purine ribonucleotide in the incubation mixture.

In the presence of 25 mM phosphate the enzyme also aggregates to a large form with an S20,w of 8.3 ± 0.2, Stokes radius of 53 ± 0.7 Å, and estimated molecular weight of 181,000. These values are similar to those obtained for the large form of the enzyme in Tris-HCl buffer following incubation with PP-ribose-P. In addition, incubation with AMP also results in a dissociation of this large form of amidophosphoribosyltransferase.

Menine ribonucleotides are not the only compounds that lead to a dissociation of amidophosphoribosyltransferase. High

![Graphs and tables showing data from the experiments]
concentrations of KCl (0.6 M) dissociate the large to the small form of amidophosphoribosyltransferase and appear to mimic the effect of purine ribonucleotides in this regard.

There are at least two explanations for the interconversion between the small and large forms of amidophosphoribosyltransferase. One is the association of a molecule of the small form with some other protein, and the other is the association of two molecules of the small form to yield the large form. While the estimated molecular weight of the large form is somewhat less than would be expected if two molecules of the small form were to aggregate, this alternative cannot be excluded, since the activity peak obtained from sucrose gradient or column chromatography experiments may contain varying proportions of the large and small form, and under these circumstances the estimated molecular weight would be a weighted average of the two forms (16). This explanation is supported by the observation that the decrease in $s_{20,w}$ following exposure to purine ribonucleotides is proportional to the concentration of ligand used, suggesting the establishment of a rapid equilibrium between the small and large forms (17). Also to be noted is that the mean + 2 S.D. range of $s_{20,w}$ values for the enzyme in Buffers A and B extends to 8.7 and 9.1, which together with Stokes radii of 53 to 54, yields estimated molecular weights of 190,000 to 202,000, values which approach those reported for the highly purified avian enzyme in a state of insensitivity to purine ribonucleotides (3, 18).

Human amidophosphoribosyltransferase isolated from placenta is also known to undergo an association-dissociation reaction in the presence of purine ribonucleotides and PP-ribose-P (3). However, the response of the human placental enzyme to purine ribonucleotides and PP-ribose-P with respect to association-dissociation is opposite in direction to the response observed in the present study of pigeon liver enzyme. With human amidophosphoribosyltransferase, PP-ribose-P stabilizes the enzyme in a form having a molecular weight of 133,000, whereas in the presence of purine ribonucleotides the enzyme has a molecular weight of 270,000.

The association-dissociation reaction of the human enzyme is correlated with a change in catalytic activity (3). Because of this correlation, the kinetic properties of pigeon liver amidophosphoribosyltransferase were evaluated after exposure of the enzyme to those compounds which affect its state of aggregation. In 25 mM and 50 mM Tris-HCl buffer the pigeon liver enzyme exhibits hyperbolic kinetics with respect to both PP-ribose-P and glutamine. Inclusion of the purine ribonucleotides, AMP or GMP, in the reaction results in a shift from hyperbolic to sigmoidal kinetics. These observations are in general agreement with previous studies of pigeon liver amidophosphoribosyltransferase (3), although the Hill coefficients were somewhat lower under the present experimental conditions. High concentrations of KCl, like purine ribonucleotides, dissociate the large form of amidophosphoribosyltransferase to the small form and shift the kinetics from a hyperbolic to a sigmoidal function. The sensitivity to inhibition by purine ribonucleotides is also enhanced by 0.6 M KCl, as shown by the decrease in the interaction coefficient for AMP or GMP. Since both purine ribonucleotides and KCl lead to dissociation as well as inhibition of the enzyme, and since PP-ribose-P leads to aggregation of the enzyme (in Tris-HCl buffer), these results might suggest that the large form of the pigeon liver amidophosphoribosyltransferase is the catalytically active species.

However, this explanation does not account for the complex effects of inorganic phosphate on pigeon liver amidophosphoribosyltransferase. On the one hand, the effect of phosphate resembles that of PP-ribose-P, i.e. the enzyme assumes the large form and the enzyme is stabilized to storage in vitro following incubation with either of these agents. On the other hand, phosphate appears to antagonize PP-ribose-P, i.e. it produces sigmoidal substrate kinetics with respect to PP-ribose-P and it increases the effectiveness of nucleotide inhibition (decrease in nucleotide interaction coefficient). These results suggest that the large form of amidophosphoribosyltransferase may assume more than one conformational state.

Support for this is found in the studies which examined the rate of inactivation of amidophosphoribosyltransferase by sodium dodecyl sulfate, a technique that has been used previously to explore differences in enzyme conformation (19). Phosphate was shown to be approximately 4 times as effective as PP-ribose-P in preventing inactivation by sodium dodecyl sulfate.

The effects of 25 mM phosphate upon the physical and kinetic properties of amidophosphoribosyltransferase cannot be explained solely by a change in the ionic strength of the buffer, since increasing the concentration of Tris-HCl from 25 mM to 50 mM did not produce a significant alteration in the sedimentation coefficient, Stokes radius, or kinetic properties of the enzyme. The experiments performed with 0.6 M KCl substantiate that the changes in physical properties of amidophosphoribosyltransferase which occur in the presence of 25 mM phosphate cannot be attributed entirely to a change in ionic strength. The mechanism by which 0.6 M KCl alters the physical and kinetic properties of amidophosphoribosyltransferase was not explored in detail. However, a plausible explanation might attribute these effects to an increase in ionic strength, since the low concentrations of potassium in the phosphate buffer and the low concentrations of chloride in the Tris-HCl buffer did not reproduce the effects of 0.6 M KCl on either the physical or kinetic properties of the enzyme.

The results presented here indicate that there is not a simple correlation between the catalytic activity of pigeon liver amidophosphoribosyltransferase and the molecular weight of the enzyme. This concept is further supported by the differential effects of 6-hydroxypurine ribonucleotides (GMP) and 6-aminopurine ribonucleotides (AMP) on the molecular prop-
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The classes of purine ribonucleotides are equally effective inhibitors of the enzyme, yet the large form of the enzyme obtained upon incubation with phosphate is not converted to the small form by GMP as it is by AMP. In addition, AMP does not alter the $s_{20,w}$ of the small form (Tris-HCl buffer), while incubation with GMP is associated with a slight increase in the $s_{20,w}$ of the small form of amidophosphoribosyltransferase. Thus, the 6-hydroxy- and 6-aminopurine ribonucleotides appear to produce different conformational alterations in pigeon liver amidophosphoribosyltransferase. These observations provide additional support for the existence of separate binding sites for these two classes of purine ribonucleotides.

Analysis of the physical and kinetic properties of pigeon liver amidophosphoribosyltransferase presented in this report leads to the conclusion that this enzyme is a complex allosteric protein regulated by conformational changes induced by a number of ligands. In particular, there appear to be important roles for PP-ribose-P and purine ribonucleotides in the control of activity of this enzyme. It is also possible that inorganic phosphate plays a heretofore unrecognized role in the regulation of pigeon liver amidophosphoribosyltransferase.

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