Human glutamine phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase) exhibits a number of allosteric properties. One of these is interconversion between a small form, 133,000 daltons, and a large form, 270,000 daltons, following incubation with phosphoribosylpyrophosphate (PP-ribose-P) and purine ribonucleotides, respectively. The present report has examined the kinetics of this interconversion process and correlated these findings with changes in enzyme activity. At pH 7.4, virtually 100% of the small form of amidophosphoribosyltransferase was converted to the large form following incubation with purine ribonucleotides for less than 5 min at 4°C. In contrast, essentially none of the large form was converted to the small form following incubation with PP-ribose-P for 30 min at 4°C. However, incubation with PP-ribose-P for 5 min at 37°C resulted in the conversion of more than 50% of the large to the small form. Hydrogen ion concentration did not affect the apparent $K_m$ for PP-ribose-P, but the percentage inhibition produced by a given concentration of AMP or GMP was approximately twice as great at pH 7.1 as compared to pH 8.2.

In addition, it was demonstrated that human amidophosphoribosyltransferase exhibited a lag phase before it attained maximal activity when assayed at 25°C, and the duration of the lag phase was temperature-dependent. In the absence of purine ribonucleotides no lag phase was demonstrable. The lag phase could be eliminated by preincubation of the enzyme preparation with PP-ribose-P for 5 min at 37°C. However, preincubation with PP-ribose-P for 30 min at 4°C was not effective in eliminating the lag phase. The lag phase was also shown to be dependent on the hydrogen ion concentration. The duration of the lag phase was greater at pH 7.1 than pH 8.2.

It is concluded from these studies that human amidophosphoribosyltransferase is a hysteretic enzyme. It is suggested that the slow conversion of the large to the small form of the enzyme is responsible for the lag phase observed in the enzyme assay.

Glutamine phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14) catalyzes the first committed reaction of purine biosynthesis de novo and may be the rate-limiting reaction for this pathway. Recent studies have indicated that human amidophosphoribosyltransferase is an allosteric enzyme that exhibits cooperativity in the binding of its substrate, PP-ribose-P, and feedback inhibition by purine ribonucleotides at a site topologically distinct from the catalytic site (1-6). In addition, two interconvertible forms of this enzyme have been identified—a small form of 133,000 daltons and a large form of 270,000 daltons. The small form can be converted to the large form by incubation with purine ribonucleotides and the large form to the small form by incubation with PP-ribose-P. Catalytic activity is correlated with the relative amount of the enzyme present in the small form (2).

Slow transition between two enzymatic forms which have different kinetic properties has been postulated to play a role in the regulation of some enzymes (6-10). One concept has suggested that the slow transition provides a mechanism for controlling fluctuations or oscillations in metabolic pathways in response to rapid changes in ligand concentrations (6-8). Another concept states that the slow transition generates a cooperative response to alterations in substrate or ligand concentrations (9, 10). Enzymes which possess this characteristic of slow transition frequently exhibit a lag phase during the assay, and as a result they have been called hysteretic enzymes (6). In this report human amidophosphoribosyltransferase is demonstrated to have a lag phase in the enzyme assay under appropriate conditions, and this is correlated with the formation of two interconvertible enzyme forms.
with the transition from the large inactive to the small active form of the enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—Uniformly labeled L-[14C]glutamine (57.3 mCi/mmol) was purchased from Amersham/Searle. This isotope was further purified prior to use by adsorption of [14C]glutamate to the anion exchange resin AG1-X4-C1 (Bio-Rad Laboratories). L-Glutamine and the sodium salts of PP-ribose-P, adenosine 5′-monophosphate (AMP), and guanosine 5′-monophosphate (GMP) were purchased from Sigma Chemical Co. Other chemicals used were of the highest quality commercially available.

**Enzyme Purification**—Amidophosphoribosyltransferase activity was partially purified from human placental extract as previously described (2). The concentrated preparation was stable for up to 3 months when stored at −70°C, and it demonstrated no glutaminyl hydrolysis in the absence of PP-ribose-P nor nucleotidase activity when assayed at 50 mM potassium phosphate buffer at pH 7.4.

**Routine Enzyme Assay**—Amidophosphoribosyltransferase activity was assayed as previously described with the PP-ribose-P-dependent conversion of [14C]glutamine to [14C]glutamate, as previously described (1).

**Demonstration of Lag Phase**—In experiments designed to test for a lag phase, the stored enzyme preparation was thawed for the first and only time the morning of the study and dialyzed for 2 h at 4°C against 100 volumes of 50 mM Tris/HCl buffer, containing 5 mM MgCl₂ and 60 mM β-mercaptoethanol. The pH of the dialysis buffer was adjusted at the temperature at which the reaction was to be performed. Unless otherwise specified, the reaction mixture contained 3 to 5 mg of protein, 10 mM PP-ribose-P, 4 mM [14C]glutamine, 5 mM MgCl₂, 50 mM KCl, and 30 mM β-mercaptoethanol in a final volume of 200 µl of 50 mM potassium phosphate and 25 mM Tris/HCl buffers, pH 7.4. Other ligands were added as indicated in the text. The following flow diagram illustrates the order of addition and preincubation conditions for each substrate and ligand.

**Flow Chart**

Dialyzed enzyme preparation (100 µl)

- KCl (0.75 mg added in crystalline form)
- AMP or GMP (40 µl of 6 mM)
- PP-ribose-P (20 µl of 100 mM)
- [14C]glutamine (40 µl of 20 mM)

Enzyme assay performed at 25°C, 30°C, or 37°C

In all experiments the reaction was initiated by the addition of [14C]glutamine. A 15-µl aliquot was removed at time zero for a blank, and the reaction was stopped by adding this aliquot to 5 µl of cold 200 mM EDTA. Additional aliquots were removed at subsequent time intervals following incubation at the appropriate temperature. A 15-µl aliquot of these samples was spotted on Whatman No. 3MM chromatography paper for electrophoresis (1).

**Other Assays**—Glutaminase and nucleotidase were assayed as previously described (1).

**Sucrose Gradient Ultracentrifugation**—Isokinetic sucrose gradients were prepared as previously described (2) and centrifugation was performed with an SW41 rotor in a model L-50 Beckman ultracentrifuge for 16 h at 5°C. The sucrose was dissolved in 50 mM potassium phosphate buffer containing 5 mM MgCl₂ and 60 mM β-mercaptoethanol at the indicated pH. Purine nucleotidase was included in the gradient buffer at the concentration listed in the text. The enzyme preparation was dialyzed against 50 mM Tris/HCl buffer, as described above, and preincubated with purine ribonucleotide or PP-ribose-P, or both, for the time and temperature indicated. A 200-µl aliquot of this enzyme preparation was applied to the gradient, and following centrifugation, fractions of 300 µl were collected. Amidophosphoribosyltransferase was assayed by the routine procedure (1). Bovine serum albumin was used as a standard marker in companion gradients.

**Protein Determination**—Total protein in the crude and purified enzyme preparations was determined by the method of Lowry et al. (3) with bovine serum albumin as standard. In sucrose gradient experiments, bovine serum albumin was determined by adsorption at 280 nm.

**RESULTS**

**Characterization of Lag Phase**—The results depicted in Fig. 1 demonstrate that the lag phase was dependent upon preincubation of the enzyme with purine ribonucleotide. (The flow diagram under "Experimental Procedures" indicates the order of addition and experimental conditions used for these studies.) The duration of the lag phase was maximal when the enzyme was preincubated with AMP at 4°C for as short a period of time as technically possible (<2 min). Although not shown here, similar results were obtained with GMP.

The lag phase could be eliminated by incubation of the enzyme under the appropriate conditions with PP-ribose-P before beginning the assay (Fig. 2). While incubation with PP-ribose-P for 30 min at 4°C did not eliminate the lag phase, incubation with PP-ribose-P for 5 min at 37°C was effective in eliminating the lag phase. Incubation at 37°C in the absence of PP-ribose-P had no effect on the duration of the lag phase.

The results shown in Fig. 3 illustrate that the duration of the lag phase was temperature-dependent, being greater at 25°C than 30°C. When the enzyme was assayed at 37°C no lag phase could be detected.

The duration of the lag phase was also affected by salt and protein concentration. Increasing the KCl concentration from 0 to 150 mM was associated with a progressive increase in the duration of the lag phase. The lag phase was easily demonstrated at 50 mM KCl and all experiments reported here were performed at this concentration of KCl. In addition, a reduction in the protein concentration in the assay from 5.0 mg to 1.25 mg was associated with an increase in the duration of the lag phase.

**Effect of Hydrogen Ion Concentration on Duration of Lag Phase**—Hydrogen ion concentration was also shown to affect the duration of the lag phase (Fig. 4). At pH 8.2, the duration of the lag phase was shorter than that observed at pH 7.1.

**Interconversion of Molecular Forms**—Human amidophosphoribosyltransferase obtained from the routine purification procedure was demonstrated to have an s₂₀,w of 5.9, which corresponds to a molecular weight of 133,000 (Fig. 5) (2). Following incubation with 1.2 mM AMP at pH 7.4, the small form of the enzyme was converted to the large form, s₂₀,w of 10.0, which corresponds to a molecular weight of 270,000 (Fig. 5) (2). Although not shown here, incubation of the enzyme preparation with GMP yielded similar results. Following incubation with purine ribonucleotide at 4°C, the transition from the small to the large form of human amidophosphoribosyltransferase was essentially complete in less than 5 min.

After preincubation of the enzyme with AMP to generate the large form, incubation with 5 mM PP-ribose-P for 30 min at 4°C resulted in essentially no conversion of the large to the small form of human amidophosphoribosyltransferase (Fig. 6A). When the same protocol was repeated, except the incubation with 5 mM PP-ribose-P was performed at 37°C for 5 min, a significant percentage of the large form was converted to the small form (Fig. 6B). Incubation with PP-ribose-P for longer periods of time at 37°C did not result in the conversion of a greater percentage of the large to the small form. The
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Effect of purine ribonucleotide on the lag phase. The dialyzed enzyme preparation was incubated with KCl first, followed by an incubation with 0 or 6 mM AMP at 4°C for 5 min. The reaction was initiated by the addition of PP-ribose-P and [14C]glutamine (see flow diagram). The assay was performed at 25°C. ▲▲, no AMP; ●●, 1.2 mM final concentration of AMP.

Effect of preincubation with PP-ribose-P on the lag phase. The dialyzed enzyme preparation was incubated with KCl first, AMP second, and the reaction was initiated by the addition of PP-ribose-P and [14C]glutamine (flow diagram under "Experimental Procedures" indicates time and temperature of incubation as well as ligand concentration). ○○, assay performed at 30°C; ●●, assay performed at 25°C.

Effect of temperature on the lag phase. The dialyzed enzyme preparation was incubated with KCl first, AMP second, and the reaction was initiated by the addition of PP-ribose-P and [14C]glutamine (see flow diagram). The assay was performed at 25°C. ○○, pH 7.1; ●●, pH 8.2.

Effect of pH on lag phase. The enzyme preparation was dialyzed against 50 mM Tris/HCl buffer, pH 7.1 or pH 8.2, containing 5 mM MgCl₂ and 60 mM β-mercaptoethanol. All ligands and substrates were added in buffer at the appropriate pH. Following incubation with KCl and AMP, the reaction was initiated by the addition of PP-ribose-P and [14C]glutamine (see flow diagram). The assay was performed at 25°C. ●●, pH 7.1; ○○, pH 8.2.

Effect of Hydrogen Ion Concentration on the Interconversion Process — The effect of pH on the interconversion between the large and small forms of human amidophosphoribosyltransferase is illustrated in Fig. 7. At pH 7.1, as at pH 7.4, the small form was readily converted to the large form following incubation with AMP for 5 min at 4°C (Fig. 7A). However, at pH 7.1, incubation with 5 mM PP-ribose-P for 30 min at 37°C resulted in the conversion of very little of the large form of amidophosphoribosyltransferase to the small form (Fig. 7A).

In contrast to the results obtained at pH 7.1 and 7.4, incubation with 1.2 mM AMP at pH 8.2 resulted in the conversion of less than 50% of the small form of amidophosphoribosyltransferase to the large form (Fig. 7B). On the other hand, incubation with 5 mM PP-ribose-P at pH 8.2 for 30 min at 37°C resulted in essentially 100% conversion of the large to the small form of the enzyme.

Effect of Hydrogen Ion Concentration on Apparent $K_m$ for PP-ribose-P and Inhibition by Nucleotides — The apparent $K_m$ for PP-ribose-P was determined at pH 7.1 and pH 8.2 from graphic analysis of Lineweaver-Burk plots. PP-ribose-P concentration was varied from 0.1 to 10 mM, and the glutamine concentration was saturating at 4 mM. The apparent Michaelis constant for PP-ribose-P was calculated to be 1.0 mM and...
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**Fig. 6.** Effect of PP-ribose-P on the conversion of the large to the small form. The crude enzyme preparation was dialyzed and preincubated with KCl and 1.2 mM AMP as described in the legend of Fig. 5. Following this, the enzyme preparation was incubated with 5 mM PP-ribose-P for 30 min at 4° (A) or for 5 min at 37° (B), and a 200-μl aliquot was applied to the sucrose gradient which contained 1.2 mM AMP. A: O-O, 1.2 mM AMP at 4° for 5 min, 30 min at 4° with no PP-ribose-P; Δ—Δ, 1.2 mM AMP at 4° for 5 min, 30 min at 4° with 5 mM PP-ribose-P. B: O-O, 1.2 mM AMP at 4° for 5 min, 5 min at 37° with no PP-ribose-P; Δ—Δ, 1.2 mM AMP at 4° for 5 min, 5 min at 37° with 5 mM PP-ribose-P.

**Fig. 7.** Effect of pH on the conversion of the large to the small form. As described in the legend to Fig. 5, the enzyme preparation was dialyzed against 50 mM Tris/HCl buffer, pH 7.1 or pH 8.2, containing 5 mM MgCl₂ and 50 mM β-mercaptoethanol before preincubation with KCl. This preparation was incubated with 1.2 mM AMP at 4° for 5 min. Following this, the enzyme sample was incubated with 0 or 5 mM PP-ribose-P for 30 min at 37° before it was applied to the sucrose gradient which contained 1.2 mM AMP. A, pH 7.1: O-O, 1.2 mM AMP at 4° for 5 min, 30 min at 37° with no PP-ribose-P; O-O, 1.2 mM AMP at 4° for 5 min, 30 min at 37° with 5 mM PP-ribose-P. B, pH 8.2: Δ—Δ, 1.2 mM AMP at 4° for 5 min, 30 min at 37° with no PP-ribose-P; □—□, 1.2 mM AMP at 4° for 5 min, 30 min at 37° with 5 mM PP-ribose-P.

**Table I**

*Effect of hydrogen ion concentration on the inhibition produced by purine ribonucleotides*

For these studies, an aliquot of the enzyme was dialyzed at 4° for 2 h against 1000 volumes of 50 mM Tris/HCl which contained 60 mM β-mercaptoethanol and 5 mM MgCl₂. The pH of the Tris/HCl buffer was determined at 37° so that the pH during the enzyme assay corresponded to the value shown in the table. The [1⁴C]glutamine concentration was 4 mM during the assay, and those of PP-ribose-P and purine ribonucleotide are indicated in the table.

<table>
<thead>
<tr>
<th>pH</th>
<th>% inhibition by AMP</th>
<th>% inhibition by GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>96</td>
<td>44</td>
</tr>
<tr>
<td>7.4</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>8.2</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>0.5 mM PP-ribose-P + 1 mM AMP</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>0.5 mM PP-ribose-P + 5 mM AMP</td>
<td>88</td>
<td>80</td>
</tr>
</tbody>
</table>

DISCUSSION

In the assay for human amidophosphoribosyltransferase, a lag phase can be demonstrated before the enzyme attains maximal activity. The lag phase is dependent upon prior exposure of the enzyme to purine ribonucleotides and can be eliminated by incubation of the enzyme with PP-ribose-P.

Since previous work from this laboratory has shown that incubation of human amidophosphoribosyltransferase with purine ribonucleotides results in the conversion of a small
active to a large inactive form, while incubation with PP-ribose-P results in the conversion of the large inactive to the small active form (2), it was hypothesized that the transition from the large to the small form occurred relatively slowly compared to the rate of the enzymatic reaction and this was responsible for the lag phase observed in the assay. To test this hypothesis, experiments were performed in which aliquots of the enzyme, obtained at different times during the evolution of the lag phase, were applied to sucrose gradients and the gradients then were analyzed for the relative distribution between the large and small forms of the enzyme. However, it was not possible to demonstrate slow transition in these gradient experiments because the duration of the lag phase was short (<15 min) compared to the time required for separation of the large and small forms in the ultracentrifuge (16 h). Since this experimental approach was unsuccessful, the hypothesis was tested by comparing those factors which affected the duration of the lag phase and the interconversion between the two forms of amidophosphoribosyltransferase.

Incubation with purine ribonucleotides for less than 2 min at 4°C resulted in a lag phase of maximal duration; these same conditions resulted in essentially complete conversion of the small to the large form of the enzyme. Incubation with PP-ribose-P for 30 min at 4°C did not affect the lag phase, while incubation with PP-ribose-P for 5 min at 37°C eliminated the lag phase; likewise, incubation with PP-ribose-P at 37°C, but not 4°C, resulted in conversion of the large to the small form of amidophosphoribosyltransferase.

Additional support for the association between the lag phase and interconversion of amidophosphoribosyltransferase is found in the experiments where hydrogen ion concentration was varied. These experiments made use of the fact that the inhibition produced by purine ribonucleotides was more apparent at pH 7.1 than pH 8.2. It was predicted from these results that hydrogen ion concentration should affect the distribution between the small and large form of the enzyme. At pH 7.1, the enzyme was readily converted to the large form by incubation with nucleotides, but the percentage of the large form converted to the small form by incubation with PP-ribose-P was less than that observed at pH 7.4; at pH 8.2 the reverse was obtained; i.e., the large form was readily converted to the small form by incubation with PP-ribose-P, but the small form was not completely converted to the large form by incubation with purine ribonucleotide. In conjunction with these findings, the duration of the lag phase was shown to be longer at pH 7.1 than at pH 8.2.

The data presented here establish the hysteretic behavior of human amidophosphoribosyltransferase and demonstrate a correlation between the transition from the large to the small form of the enzyme and the presence of a lag phase in the enzyme assay. These findings could be explained by the following mechanism. Incubation with PP-ribose-P leads to a slow temperature-dependent change in the conformation of the large form of amidophosphoribosyltransferase. This is responsible for the dissociation of the large form to a more catalytically active small form, and the time required for this transition determines the duration of the lag phase.

One might speculate that the hysteretic properties of amidophosphoribosyltransferase provide an additional mechanism for the control of this enzyme which catalyzes the probable rate-limiting reaction in the pathway of purine biosynthesis de novo. Since PP-ribose-P is not used exclusively for purine biosynthesis and its concentration in rodent liver fluctuates as much as 10-fold over a 30-min period in response to a number of physiological variables (12), the hysteretic response of amidophosphoribosyltransferase to PP-ribose-P provides a mechanism for modulating the activity of this enzyme and the pathway of purine biosynthesis to transient fluctuations in the concentration of this important metabolite.

However, the physiological importance of such a regulatory process remains to be determined by studies with intact cells.

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