Human Adenine Phosphoribosyltransferase

PURIFICATION, SUBUNIT STRUCTURE, AND SUBSTRATE SPECIFICITY*

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SUMMARY

Human adenine phosphoribosyltransferase has been purified 33,000-fold from erythrocytes to a specific activity of 9.58 μmoles of AMP formed per mg of protein per min. The native enzyme has a molecular weight of 34,000 and is composed of three subunits of equal molecular weight which appear to be associated by noncovalent forces.

The highly purified enzyme is maximally active over a broad pH range from 7.4 to 9.5 and has an isoelectric point of 4.78. The compounds 4-amino-5-imidazolecarboxamide and 2,6-diaminopurine were found to be substrates for the highly purified enzyme. At 0° in the absence of Mg++, but in the presence of substrates the human enzyme catalyzed a rapid and limited synthesis of AMP.

Adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) catalyzes the magnesium-dependent formation of adenosine 5'-monophosphate (AMP) from adenine and 5-phosphoribosyl 1-pyrophosphate. In 1955, Kornberg et al. first described the enzyme and partially purified it from yeast (1). Since then adenine phosphoribosyltransferase has been purified and studied kinetically by Horii and Henderson from Ehrlich ascites tumor cells (2, 3), by Berlin from Bacillus subtilis (4), by Ozer from Escherichia coli (5), and by Young et al. from rat liver (6). Interest in the human enzyme has been stimulated by the description of elevated activity of adenine phosphoribosyltransferase in erythrocytes from patients with the Lesch-Nyhan syndrome (7, 8) and by the occurrence of a genetically determined deficiency of adenine phosphoribosyltransferase in two families (9, 10). The present study was undertaken to provide further information on the properties and subunit structure of a highly purified preparation of the normal human adenine phosphoribosyltransferase.

MATERIALS AND METHODS

Materials

Tetrasodium PP-ribose-1-P, cytochrome c, α-chymotrypsinogen, sodium dodecyl sulfate, barium 6-mercaptopurine ribo-

* This work was supported by United States Public Health Service Research Grant AM 14362 and Training Grant AM 08620, and Cerebral Palsy Foundation Grant R-247-71.

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mononucleotide product, [8-14C]AMP, was separated from the substrate, [8-14C]adenine, by high voltage electrophoresis at 250 mA and 4000 to 6000 volts for 13.5 min in a 50 mM sodium borate buffer, pH 8.5, containing 1 mM EDTA. The mononucleotide spot was visualized under ultraviolet light (254 nm) and cut out, and the material was analyzed for radioactivity at 63% efficiency in a Packard Tri-Carb scintillation counter. Under these conditions, [8-14C]AMP production was linear with respect to time and protein.

The activity of highly purified adenine phosphoribosyltransferase toward [8-14C]hypoxanthine, [8-14C]guanine, [8-14C]adenosine, 2,6-diamino[2-14C]purine, 6-mercapto[8-14C]purine, and 4-amino-5-[2-14C]imidazolecarboxamide was assayed similarly except for separation of products. Aliquots containing 20 μg of IMP, GMP, or AMP were used as carriers for the reaction mixtures containing [8-14C]hypoxanthine, [8-14C]guanine, or [8-14C]adenosine, respectively. Reactants and products were separated by high voltage electrophoresis. For assessing activity toward 2,6-diamino[2-14C]purine, 6-mercapto[8-14C]purine, and 4-amino-5-[2-14C]imidazolecarboxamide, 20-μl aliquots of the reaction mixture were spotted on Whatman No. 1 chromatography paper with approximately 20 μg of the appropriate ribonucleotide as carrier. After 40 hours of ascending chromatography in 3:1 1-propanol-0.2 N NH₄OH, the paper was dried and then filtered on a Buchner funnel. The cellulose was resuspended in the original succinate-magnesium buffer to a total volume of 300 ml. The suspension was placed in a graduated cylinder and the fines were allowed to settle for 10 minutes (centimeters) of the slurry. At the proper time, the supernatant was removed to leave a slurry volume equal to 120% of the wet settled volume. The sucinate-Mg buffer was added to bring total volume up to 150% of wet settled volume. The slurry was made 0.1 mM in PP-ribose-P, poured into a column (1.7 x 31.1 cm), and equilibrated with sodium succinate-magnesium-PP-ribose-P buffer, pH 5.18, containing 5 mM MgCl₂. The suspension was adjusted to pH 5.18 with a solution of 10 mM succinic acid-5 mM MgCl₂ and then filtered on a Buchner funnel. The cellulose was suspended in the original succinate-magnesium buffer to a total volume of 300 ml. The suspension was placed in a graduated cylinder and the fines were allowed to settle for a predetermined length of time (minutes) equal to twice the height (centimeters) of the slurry. At the proper time, the supernatant was removed to leave a slurry volume equal to 120% of the wet settled volume. The succinate-Mg buffer was added to bring total volume up to 150% of wet settled volume. The slurry was made 0.1 mM in PP-ribose-P, poured into a column (1.7 x 31.1 cm), and equilibrated with sodium succinate-magnesium-PP-ribose-P buffer, pH 5.18, containing 5 mM MgCl₂, until buffer and effluent gave identical pH and absorbance at 215 nm. After dialysis against this buffer for 4 hours, the sample was applied to the column and eluted with equilibrating buffer at a flow rate of 30 ml per hour. Fractions of 2 ml each were collected. Fractions were assayed for adenine phosphoribosyltransferase activity and those containing peak activity were pooled and concentrated.

Step 6: Concentration—The sample was concentrated to a 1-ml volume by dialysis against a hyperosmolar slurry of polyethylene glycol containing 25 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, and 0.1 mM PP-ribose-P.

Step 7: Gel Filtration—The sample was applied to a Sephadex G-75 column (1.7 x 70 cm) and eluted at 15 ml per hour in 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM MgCl₂ and 0.1 mM PP-ribose-P. Fractions of 1.5 ml were collected and those containing the activity peak were pooled.

Polyacrylamide Gel Electrophoresis

All electrophoretic procedures were performed in a Buchler electrophoresis apparatus with a Beckman Duostat power source. Gels were polymerized in 7.5 x 0.5-cm glass tubes fitted with rubber caps and allowed to stand for 1 hour before use. Bromphenol blue was used as the tracking dye. Gels were stained for 2 hours with Coomassie brilliant blue as described by Weber and Osborn (15), and destained electrophoretically for 2 hours at 50 mA per gel in 5% (v/v) acetic acid 7.5% (v/v) methanol.

The abbreviation used is: PP-ribose-P, 5-phosphoribosyl 1-pyrophosphate.
Disc Gel Electrophoresis—A discontinuous Tris-glycine buffer, pH 8.9, was used as described by Davis (16). The gels were either stained for protein or assayed for adenine phosphoribosyltransferase activity. Those gels to be assayed were sliced into 1.2-mm sections and allowed to stand for 18 hours in 1 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM MgCl₂ and 0.1 mM PP-ribose-P. Aliquots (25 μl) of the eluate were assayed for adenine phosphoribosyltransferase activity as described above.

Sodium Dodecyl Sulfate Electrophoresis—Enzyme from Step 7 was dialyzed against 4000 volumes of 10 mM sodium phosphate buffer, pH 7.0, at 25°C for 2 hours. Sodium dodecyl sulfate and β-mercaptoethanol were then added to the sample to a final concentration of 1% each, and the solution was incubated for 2 hours at 37°C. Protein standards (cytochrome c, α-chymotrypsinogen, ovalbumin, and bovine serum albumin) were prepared similarly. Electrophoresis was performed according to the method of Weber and Osborn (15). Standards and enzyme were also prepared in the unreduced form by omission of β-mercaptoethanol from this procedure.

Polyacrylamide Gel Scanning—Gels stained with Coomassie brilliant blue were scanned for optical density at 562 nm in a Beckman DU spectrophotometer equipped with a Gilson gel scanner and automatic recorder.

Sucrose Gradient Ultracentrifugation
Sucrose gradient ultracentrifugation was performed with a Beckman SW 41 rotor in a Beckman model L3 ultracentrifuge. Gradients of 11.8 ml were poured at 5°C with the use of the isokinetic apparatus of McCarty et al (17), with 11.62 ml of 10% ultrapure sucrose (w:w)-5 mM Tris-HCl, pH 7.4, in the mixing chamber, and 28.2% sucrose (w:w)-5 mM Tris-HCl, pH 7.4, in the reservoir. A solution (0.1 ml) containing α-chymotrypsinogen (40 mg per ml), bovine serum albumin (40 mg per ml), and adenine phosphoribosyltransferase (Step 4, 3.6 mg per ml) was layered above the gradient. After centrifugation at 40,000 rpm for 56 hours at 5°C, 0.3-ml fractions were collected with the use of gradient tapper of McCarty et al (17). An aliquot of each fraction was assayed for adenine phosphoribosyltransferase activity and assayed for absorbancy at 280 nm.

Molecular Weight and Stokes Radius Determinations
Molecular weight was determined from the chromatographic behavior on a Sephadex G-75 column as described above or by use of ascending chromatography on a Sephadex G-100 column (2.5 × 90 cm). Buffer conditions were 50 mM Tris-HCl, pH 7.4, with or without the addition of 0.1 mM PP-ribose-P and 5 mM MgCl₂. At a flow rate of 15 ml per hour, 2.5-ml fractions were collected and assayed for enzyme activity. All columns were standardized with cytochrome c, ovalbumin, α-chymotrypsinogen, and (for the G-100 columns), bovine serum albumin.

For the estimation of Stokes radius, Kₐ, of each standard and unknown was determined by the method of Laurent and Kandler (18). The inverse error function complement of the column partition coefficient (erfc⁻¹) was derived from the Kₐ as described by Ackers (19). Molecular weight was estimated by the method of Whitaker (20).

Molecular weight and frictional ratio were also determined from the Stokes radius and erfc⁻¹ by the method of Siegel and Monty (21).

Isoelectric Focusing
The isoelectric point was determined on an LKB Uniphor 7900 column electromophoresis apparatus by the method of Vesterberg and Svensson (22) as modified by Arnold and Kelley (23). A preparation of adenine phosphoribosyltransferase purified 400-fold was produced by passing 2 ml of the enzyme from Step 2 through a Sephadex G-100 column (2.5 × 100 cm) in 50 mM Tris-HCl, pH 7.4, containing 0.1 mM PP-ribose-P and 5 mM MgCl₂. The activity peak was pooled and concentrated as described above by dialysis against polyethylene glycol. A 200-ml gradient from 40% to 1% sucrose was poured to contain 1% ampholytes, pH 4 to 6. A concentrated sample containing 14.5 mg of protein in 2.5 ml was applied to the middle of the column. After 64 hours, 1-ml fractions were collected and the pH was measured at 4°C.

pH Curve
The effect of pH on the activity of human adenine phosphoribosyltransferase was evaluated over the pH range 6 to 10, using 0.08 μg of the enzyme preparation (Step 5) in the standard assay. Substituted for the standard buffer was Tris-HCl (final ionic strength of 0.050), imidazole-HCl (ionic strength of 0.050), or glycine-KOH (0.050 M). The pH of the reaction mixtures without enzyme was determined at 37°C using a Radiometer Copenhagen pH meter equipped with a combination glass electrode.

Burst Synthesis of [¹⁴C]AMP
Burst synthesis of [¹⁴C]AMP was evaluated at 0°C in the presence of excess EDTA by the method of Groth and Young (24). The possibility of "recharging" a burst enzyme was also studied. Enzyme was subjected to burst conditions at 0°C in 50 mM Tris-HCl, pH 7.4, containing 0.025 μmol of nonradioactive adenine, 2.5 μmoles of EDTA, and 0.25 μmol of PP-ribose-P in a volume of 0.18 ml. To the solution were added 3.75 μmoles of MgCl₂ and 0.125 mg of bovine serum albumin. This preparation was incubated for 5 min at 37°C, which is sufficient time to convert essentially all of the adenine present to AMP. The reaction was stopped by addition of 2.5 μmoles of EDTA and cooled to 0°C. Subsequently the solution was made 0.1 M in [¹⁴C]adenine (total volume, 0.25 ml), initiating a new burst. At various time intervals the [¹⁴C]AMP present was determined from 20-μl aliquots as described above.

RESULTS

Enzyme Purification—The results of the enzyme purification are summarized in Table I. Fig. 1 shows that adenine phosphoribosyltransferase activity was eluted from DEAE-cellulose as a single peak. Less than 1% of the applied activity was removed by the preliminary phosphate buffer washes, indicating that the 55% step recovery is not due to failure of the enzyme to bind to the cellulose. Replacement of the 0 to 0.3 M KC1 gradient with 0 to 0.5 M KC1 removed no additional activity peaks. While the ammonium sulfate precipitation step resulted in a loss of total enzyme activity with no increase in specific activity, it proved to be necessary to the integrity of the purification. When it was modified to yield better recovery, later steps (especially Step 5) no longer worked properly. The eluate from the CM-cellulose column yielded a single peak of protein nearly coincident with the activity peak (Fig. 2). Sephadex G-75 chromatography (Fig. 3) resulted in an additional 2-fold protein purification, but because of enzyme instability under these conditions there was a decrease in specific activity.

Properties of Enzyme—A 137-fold purified preparation was assayed for stability in 50 mM Tris-HCl, pH 7.4, at 4°C in the presence of various reagents (Table II). Ammonium sulfate
**TABLE I**

**Purification of adenine phosphoribosyltransferase from human erythrocytes**

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Specific activity</th>
<th>Total protein</th>
<th>Recovery</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemolysate</td>
<td>562</td>
<td>0.000287</td>
<td>360,000</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2. DEAE eluate</td>
<td>307</td>
<td>0.0980</td>
<td>1,500</td>
<td>55</td>
<td>130</td>
</tr>
<tr>
<td>3. Ammonium sulfate precipitation</td>
<td>9.3</td>
<td>0.0075</td>
<td>460</td>
<td>17</td>
<td>130</td>
</tr>
<tr>
<td>4. Ethanol precipitation</td>
<td>8.5</td>
<td>0.0790</td>
<td>180</td>
<td>14</td>
<td>280</td>
</tr>
<tr>
<td>5. CM-cellulose column</td>
<td>7.3</td>
<td>9.58</td>
<td>1.0</td>
<td>9.5</td>
<td>33,000</td>
</tr>
<tr>
<td>6. Osmotic concentration</td>
<td>1.0</td>
<td></td>
<td></td>
<td>7.8</td>
<td>18,000</td>
</tr>
<tr>
<td>7. Sephadex G-75</td>
<td>7.1</td>
<td>5.08</td>
<td>0.54</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Elution profile of adenine phosphoribosyltransferase from DEAE-cellulose. The ordinate represents AMP production catalyzed by a 25-µl aliquot of each fraction. Fractions were pooled from 862 to 1258 ml.

**Fig. 2.** CM-cellulose chromatography of adenine phosphoribosyltransferase. The ordinate represents AMP production catalyzed by a 25-µl aliquot of each fraction.

(1 mM), dithiothreitol (10 mM), or dimethylsulfoxide (5%) were effective in partially protecting the enzyme from inactivation during storage. More effective, though, was PP-ribose-P which gave complete protection at a concentration of 1 mM. In the presence of 5 mM MgCl₂, 0.1 mM PP-ribose-P was sufficient for complete stabilization at 4°C for up to 14 days. With 5 mM MgCl₂ and 0.1 mM PP-ribose-P, the Step 7 enzyme preparation lost no activity after storage at −70°C for 2 months.

When subjected to gel filtration, adenine phosphoribosyltransferase was usually eluted in a single peak of activity (Fig. 3). The plots of elution volume against log molecular weight of the standards gave an enzyme molecular weight of 34,000 ± 3,000 (mean ± S.D. of four determinations). Two plots of erfc−σ versus Stokes radius gave values of 24.6 Å and 25.2 Å (mean 24.9) (Fig. 4).

On two occasions enzyme activity was eluted from Sephadex G-100 columns in two peaks (Fig. 5, A and B). In one experiment these two peaks corresponded to molecular weights of 69,000 and 31,000, respectively (Fig. 5A). On a second occasion molecular weights of 34,500 and 21,000 were observed for the enzyme (Fig. 5B).

**Fig. 3.** Chromatography of adenine phosphoribosyltransferase on Sephadex G-75. The ordinate represents AMP production catalyzed by a 25-µl aliquot of each fraction. Eluate from 68 to 76 ml was pooled.

**TABLE II**

**Stability of human adenine phosphoribosyltransferase at 4°C**

All enzyme preparations contained 50 mM Tris-Cl, pH 7.4, in addition to the reagents indicated below. The enzyme sample used was 137-fold purified. Enzyme activity is expressed as per cent of control.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Concentration</th>
<th>Enzyme activity after storage at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 days</td>
</tr>
<tr>
<td>No addition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td></td>
<td>1 mM</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td></td>
<td>0.5%</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td></td>
<td>0.3%</td>
</tr>
<tr>
<td>Na₄ PP-ribose-P</td>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td>Na₄ PP-ribose-P + 5 mM MgCl₂</td>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mM</td>
</tr>
</tbody>
</table>
FIG. 4. Stokes radius of adenine phosphoribosyltransferase.

FIG. 5. Ascending chromatography of adenine phosphoribosyltransferase on Sephadex G-100. Values on the abscissa represent volume relative to the elution volume for bovine serum albumin ($V_{E,A}$). The ordinate represents AMP production catalyzed by a 25-µl aliquot of each fraction. The column illustrated in A was developed with Tris-HCl, pH 7.4. The enzyme preparation applied to the column had been eluted from DEAE-cellulose with a 0 to 0.5 M linear KCl gradient (sodium phosphate buffer, 20 to 50 mM; pH 7.0 to 7.4) and concentrated by a 30% to 60% ammonium sulfate precipitation at pH 4.8. The column illustrated in B was developed with Tris-HCl, pH 7.4, containing 5 mM MgCl₂ and 0.1 mM PP-ribose-P. This enzyme preparation had been subjected to the same DEAE-cellulose chromatography followed by a 30% to 55% ammonium sulfate precipitation at pH 4.8.

Results of sucrose density gradient ultracentrifugation are summarized in Fig. 6. Two peaks of absorbance at 280 nm and one peak of adenine phosphoribosyltransferase activity were found. The position of the peak of adenine phosphoribosyltransferase activity corresponded to an $s_{20,w}$ of 3.35. If a partial specific volume of 0.725 is assumed, the $s_{20,w}$ of 3.35 and Stokes radius of 24.9 yield a molecular weight of 34,400 and $f/f_0$ of 1.16.

The enzyme from Step 5 had maximal activity over a broad pH range from 7.4 to 9.5. The isoelectric point on two separate determinations was 4.85 and 4.70.

The enzyme from Step 7 was assessed for substrate specificity. At the concentrations used, 4-amino-5-imidazolecarboxamide and 2,6-diaminopurine were converted to their ribonucleotide derivatives at a rate which was 11.8% and 10.8%, respectively, of that observed with adenine (Table III). The other compounds tested did not function as substrates. The enzyme was not active with d-ribose-5-P, ribose-1-P, or ribose-5-P plus ATP in place of PP-ribose-P.

The typical time course of PP-ribose-P-dependent [³²P]AMP synthesis by adenine phosphoribosyltransferase at 0° in the absence of Mg²⁺ is shown in Fig. 8. By 15 s a rapid or burst synthesis of product was observed. However, the production of [³²P]AMP under these conditions was not linear with respect to protein (Fig. 9). Enzyme that had been subjected to burst synthesis could again produce a burst if it were first incubated at 37° with substrates.

DISCUSSION

Srivastava and Beutler have partially purified human adenine phosphoribosyltransferase 2,200-fold from hemolsate (27), whereas Raivio and Seegmiller allude to a 6,730-fold purification from the same source (28, 29). The purification described here resulted in a maximal specific activity of 9.58 µmoles of AMP per mg of protein per min corresponding to a 33,000-fold purifica-
FIG. 7 (left). Polyacrylamide disc gel electrophoresis of adenine phosphoribosyltransferase. The sieving gel was 10% total acrylamide and 0.27% bis. No stacking gel was used. Electrophoresis was performed with 2 µg of highly purified adenine phosphoribosyltransferase from Step 7 at 2.5 ma per gel until the tracking dye was 0.5 cm from the end of the gel (about 60 min).

FIG. 8 (center). Time course of [%]AMP synthesis at 0° in the absence of Mg++ by highly purified human adenine phosphoribosyltransferase. Values on the ordinate represent total AMP formation by 0.18 mg of the Step 4 enzyme preparation.

FIG. 9 (right). Total [%]AMP formation at 0° in the absence of Mg++ by various quantities of highly purified adenine phosphoribosyltransferase (Step 5). Each point represents the mean of six determinations made at 15, 30, 45, 60, 90, and 150 s after the addition of adenine.

TABLE III
Specificity of human adenine phosphoribosyltransferase for purine bases and nucleosides

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration</th>
<th>Nucleotide formed/ µg protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>0.30</td>
<td>100</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.50</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.25</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>4-Amino-5-imidazolecarboxamide</td>
<td>2.0</td>
<td>11.8</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>1.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.40</td>
<td>0.13</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>0.77</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Henderson (2) and Shrivastava and Beutler (27) have noted the instability of partially purified adenine phosphoribosyltransferase preparations at 4°. It appears that this instability at 4° can be avoided by the use of MgCl₂ and PP-ribose-P. However, even under these conditions, the highly purified enzyme required storage at -70° to prevent loss of activity.

Previously reported molecular weights for adenine phosphoribosyltransferase are 45,000 from B. subtilis (4), 40,000 from E. coli (5), and 20,000 from rat liver (6). A value of 37,000 has been reported for the human enzyme (28). By two different techniques the data presented here assign a molecular weight of 34,000 for human adenine phosphoribosyltransferase. We have consistently found a subunit molecular weight of approximately 11,000 on sodium dodecyl sulfate polyacrylamide gels in either the presence or absence of β-mercaptoethanol. These results suggest that under most conditions adenine phosphoribosyltransferase exists as a trimer composed of subunits of equal molecular weight associated by noncovalent forces. Only a few other proteins are known to exist as noncovalently bound trimers: aldolase (30), phycocyanin (31), myosin (32), and certain human serum lipoproteins (33). The presence, in some preparations, of forms of adenine phosphoribosyltransferase with molecular weights of 21,000 or 69,000, in addition to a more prominent form at a molecular weight of approximately 34,000, suggest that this protein may also be active as the dimer and hexamer, respectively.
Flaks et al. have demonstrated phosphoribosyltransferase activity toward 4-amino-5-imidazolecarboxamide in preparations of adenine phosphoribosyltransferase partially purified from beef liver (11). Kalle and Gots (34) reported concomitant elution of adenine phosphoribosyltransferase and 2,6-diaminopurine phosphoribosyltransferase activities from Salmonella typhimurium on a DEAE-cellulose column, whereas Kornberg et al. (1) could find no activity of a partially purified yeast enzyme preparation toward 2,6-diaminopurine. Using a partially purified enzyme preparation from monkey liver, Krenitsky et al. found activity toward 4-amino-5-imidazolecarboxamide as well as hypoxanthine (35).

In the present study, we found significant nucleotide formation catalyzed by the highly purified human enzyme with either 2,6-diaminopurine boxamide as well as hypoxanthine (35). In the present study, adenine phosphoribosyltransferase and 2,6-diaminopurine phosphoribosyltransferase activities from Salmonella typhimurium on a DEAE-cellulose column, whereas Kornberg et al. (1) could find no activity of a partially purified yeast enzyme preparation toward 2,6-diaminopurine. Using a partially purified enzyme preparation from monkey liver, Krenitsky et al. found activity toward 2,6-diaminopurine and 4-amino-5-imidazolecarboxamide as well as hypoxanthine (35). In the present study, we found significant nucleotide formation catalyzed by the highly purified human enzyme with either 2,6-diaminopurine or 4-amino-5-imidazolecarboxamide but not with hypoxanthine or guanine as substrate.

Employing a homogeneous preparation of adenine phosphoribosyltransferase from rat liver, Groth and Young observed a PP-ribose-P-dependent initial burst of AMP synthesis from adenine in the absence of Mg++ (24). They interpreted this as evidence that the enzyme normally binds a phosphorylated ribose group. We found similar results with the human enzyme. In addition, we have been able to replace phosphorylated ribose groups removed either under burst conditions or during the course of enzyme purification. However, we were not able to reproduce the linear relation, reported by Groth and Young (24), between enzyme protein and amount of AMP synthesis under burst conditions with the highly purified preparation of human adenine phosphoribosyltransferase.

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