Human Hypoxanthine-Guanine Phosphoribosyltransferase

PURIFICATION AND SUBUNIT STRUCTURE

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SUMMARY

Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) has been purified to homogeneity from human erythrocytes obtained from one male donor. The normal human enzyme has a Stokes radius of 36 Å with a molecular weight of 68,000 and is composed of two subunits which have identical molecular weight and net charge.

Three isoenzymes were reproducibly distinguished by preparative isoelectric focusing. This electrophoretic heterogeneity appears to result from a nongenetic, post-transcriptional alteration of one or both subunits.

Hypoxanthine-guanine phosphoribosyltransferase catalyzes the conversion of the natural purine bases guanine and hypoxanthine to the mononucleotides, guanosine 5'-phosphate and inosine 5'-phosphate, respectively. In bacteria, these reactions are catalyzed by two distinct enzymes (1); however, numerous studies indicate that a single enzyme is responsible for both functions in mammalian cells. The reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase was originally assigned simply a "salvage function" in purine metabolism. However, the recent discovery that a functional absence of this enzyme in man leads to the development of a bizarre neurological disorder characterized by choreoathetosis, self-mutilation, mental retardation, and an accelerated rate of purine biosynthesis de novo (the Lesch-Nyhan syndrome) (2) has led to a re-evaluation of its importance in the regulation of purine biosynthesis as well as in central nervous system function. The mutations leading to the deficiency of this enzyme in man appear to be on a structural gene within the X chromosome in many if not all instances (3, 4). In addition, studies in cultured diploid cells derived from patients with deficiencies of this enzyme have provided evidence that these mutations may involve many different loci (4). In at least one case, the mutation has resulted in the production of an altered form of the enzyme which exhibits sigmoid kinetics (5); this represents a striking contrast to the normal enzyme which under most conditions obeys Michaelis-Menten kinetics (6, 7).

Elucidation of the structure of the normal enzyme would provide a basis not only for understanding the genetic lesion(s) producing altered or absent enzyme activity but also knowledge essential to the eventual study of the regulation of its synthesis in cultured human cells. Despite the importance of this enzyme and recent interest in it, no previous studies have been published on the structural characteristics of a highly purified preparation. This has been delayed because of the marked instability of the enzyme upon partial purification. However, recent studies with impure preparations have provided substantial information on the kinetic mechanism (6, 7), substrate specificity (8), and have allowed an estimate of molecular weight (8). This study describes the purification of the normal hypoxanthine-guanine phosphoribosyltransferase from human erythrocytes obtained from one male donor, defines its subunit structure, and presents evidence that post-transcriptional nongenetic alterations are responsible for the electrophoretic heterogeneity observed.

MATERIALS AND METHODS

Materials

[8-14C]Guanine (6.05 mCi per mmole), [8-14C]hypoxanthine (3.07 mCi per mmole), and [8-14C]adenine (4.95 mCi per mmole) were obtained from New England Nuclear. MgPP-ribose-P was purchased from P-L Biochemicals. NaPP-ribose-P, cytochrome c, α-chymotrypsinogen A, bovine serum albumin, and sodium dodecyl sulfate come from Sigma. Dithiothreitol was purchased from Calbiochem. Ovalbumin was purchased from Nutritional Biochemicals. Ultrapure sucrose, bromphenol blue, and Coomassie brilliant blue were from Mann. Dimethyl sulfoxide and cyclohexanone were supplied by Fisher. Acrylamide, bis-acrylamide, and N,N,N',N'-tetramethylethylenediamine were purchased from Eastman. Sephadex G-100 and blue dextran were obtained from Pharmacia. Ampholine carrier ampholytes were purchased from LKB Produkter AB, Stockholm. Highly purified neuraminidase was kindly supplied by Dr. Herbert Evans, Department of Biochemistry, Duke University Medical Center. All other reagents were of the highest purity commercially available.

Methods

Enzyme Assay

Hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase were assayed by previously described radiochemical techniques (9). Unless otherwise stated, the final
assay mixture contained 50 mM Tris-HCl buffer, pH 7.4, 5 mM MgCl₂, 1 mM MgPP-ribose-P, and 0.15 mM purine base ([8-¹⁴C] hypoxanthine, [8-¹³C]guanine, or [8-¹⁴C]adenine) plus enzyme in a final volume of 100 µl. Assays of highly purified enzyme preparations required bovine serum albumin at a concentration of 0.05 mg per ml or above for maximal specific activity. The reaction mixture was incubated at 37°C for 20 min, stopped by immersion in an ice water or Dry Ice acetone bath, and 5 µmoles of EDTA were added. An aliquot (20 µl) of the reaction mixture was spotted on Whatman No. 3MM chromatography paper in the presence of carrier mononucleotide (IMP, GMP, AMP). High voltage electrophoresis at 250 ma for 15 min in 50 mM sodium borate buffer, pH 8.5, separated the mononucleotide from the corresponding base. The mononucleotide spot was visualized under ultraviolet light, cut out, and counted in a Packard Tri-Carb liquid scintillation counter at 63% efficiency.

**Burst Synthesis of [C⁴GMP**

"Burst" synthesis of [C⁴GMP by hypoxanthine-guanine phosphoribosyltransferase at 0°C in the absence of magnesium was evaluated by a modification of the method of Groth and Young (10).

**Enzyme Purification**

**Preparation of Erythrocyte Hemolysate**—Single units (500 ml) of blood from male donors were obtained from the Clinical Blood Bank at Duke University Medical Center. They had been stored at 4°C for 21 to 28 days. Unless otherwise stated, all subsequent steps were performed at 4°C using erythrocytes from one subject. Serum and buffy coat were removed by centrifugation at 13,000 g for 15 min. A 220-ml, 0 to 40% linear sucrose gradient, 1% in carrier ampholytes, pH 4 to 6, was poured with the use of an LKB gradient-making device. One-third of the way through the gradient, the dialyzed enzyme sample was mixed with 2.0 ml of the gradient and carefully layered onto the column. As the remainder of the gradient was poured, the sample dispersed itself through the top one-half of the column. At this point, the voltage was increased to 600 volts. The current decreased to 1.0 ma after 36 hours. At this point, the voltage was increased to 2.3 ma of current, which decreased to 1.2 ma after 36 hours. At this point, the voltage was increased to 600 volts. The current decreased to 1.0 ma over the next 24 hours and remained there for the remainder of the electrophoresis. The temperature was maintained at 5°C throughout the run. At the end of 72 hours, the column was eluted from below with an LKB Perplex Pump at a rate of 60 ml per hour. The pH of every other fraction (1 ml) was measured at 5°C with a Radiometer Copenhagen pH meter equipped with a combination glass electrode. Absorbance at 280 nm was determined on a Zeiss Optikon spectrophotometer. The fractions comprising each isoenzyme peak were pooled and concentrated by pressure ultrafiltration as described above.

**Sephadex G-100 Chromatography**—Samples (1.5 ml) of each enzyme preparation were applied to a Sephadex G-100 column with a V(total of 125 ml and a Vₑ of 48.5 ml (blue dextran) previously equilibrated with 50 mM Tris-HCl, pH 7.4, 0.5 mM dithiothreitol, and 5% (v/v) dimethyl sulfoxide in order to stabilize the enzyme. Elution rate was 10 ml per hour and 1.85-ml fractions were collected. Fractions containing hypoxanthine-guanine phosphoribosyltransferase were pooled, concentrated by pressure ultrafiltration as described above, and stored at -70°C.

**Polyacrylamide Disc Gel Electrophoresis**

All polyacrylamide disc gel procedures were performed on a Buchner electrophoresis apparatus with a Beckman Duostat power source. Glass tubes (7.5 x 0.6 x 0.6 cm) with fitted rubber caps (Buchler) were used, and 1 hour was allowed for complete polymerization before the gels were used. Staining was done with 0.3% Coomassie brilliant blue prepared according to Weber and Osborn (13). Gels were stained from 6 to 18 hours and electrophoretic destaining was done at 4 ml per gel in 5% (v/v) acetic acid-7.5% (v/v) methanol over 2 to 3 hours. Gels were stored and photographed in destaining solution.

**Analytical Gel Electrophoresis**—The procedure of Davis (14), which uses a discontinuous Tris-glycine buffer system with only the sieving gel, was employed at three different percentages of cross-linking (5, 10, and 15%) with a constant percentage of acrylamide monomer (22.2%). Electrophoresis was performed at 3 mA per gel with bromphenol blue as a tracking dye. Normally, electrophoresis was stopped when the tracking dye was four-fifths of the way down the column (60 to 90 min); however, to maximize separation in certain circumstances, electrophoresis was performed for up to 3.5 hr.
was continued for 30 to 60 min after the tracking dye came off the gel. Gels were either stained or assayed. Gels to be assayed were sliced into 4-mm sections with a scalpel, macerated, and placed in 200 µl of 50 mM Tris-HCl buffer, pH 7.4. Elution was carried out for 18 hours, and a 50-µl aliquot of the supernatant of each section was assayed for enzyme activity.

**Sodium Dodecyl Sulfate Gel Electrophoresis**—The procedure used was essentially that described by Weber and Osborn (13), except for preparation of the protein samples. Protein samples (40 to 50 µg) were dialyzed for 2 hours at room temperature against 2000 volumes of 10 mM sodium phosphate buffer, pH 7.0, with 1 mM EDTA. Sodium dodecyl sulfate and mercaptoethanol were then added to final concentrations of 1 and 3%, respectively, and the samples were incubated at 37°C for 90 min. Samples were then dialyzed for 18 hours at room temperature against 1000 volumes of 10 mM sodium phosphate buffer, pH 7.0, 0.1% sodium dodecyl sulfate, and 0.1% mercaptoethanol.

Staining of the sodium dodecyl sulfate gels for glycoprotein was done according to the procedure of Zacharius et al. (15), except that 0.5% sodium metabisulfite was used instead of the potassium salt in order to avoid precipitation with sodium dodecyl sulfate.

**Urea Gel Electrophoresis**—The procedure followed was essentially that of Reisfeld and Small (16) with only slight modification. Urea was recrystallized from absolute ethanol and vacuum-dehydrated over sulfuric acid for 18 hours prior to use. Protein samples (40 to 50 µg) were dialyzed for 18 hours against 250 volumes of 8 M urea with 3% mercaptoethanol. Electrophoresis was performed at 3 mA per gel for 90 to 120 min.

**Neuraminidase Assay**

Highly purified neuraminidase prepared according to the procedure of Cassidy, Jourdian, and Roseman (17) was used for analysis of the sialic acid content of hypoxanthine-guanine phosphoribosyltransferase, both by direct assay of hydrolyzed sialic acid and by its effect on the electrophoretic mobility of the isoenzymes. The assay system used was that of Warren (18).

**Table I**

<table>
<thead>
<tr>
<th>Step</th>
<th>Specific activity (nmol/mg protein/hr)</th>
<th>Total protein (mg)</th>
<th>Recovery (%)</th>
<th>Purity (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Homolysate</td>
<td>81</td>
<td>72,000</td>
<td>64</td>
<td>117</td>
</tr>
<tr>
<td>2. DEAE eluate</td>
<td>9,470</td>
<td>399</td>
<td>42</td>
<td>361</td>
</tr>
<tr>
<td>3. Heat-treatment</td>
<td>29,206</td>
<td>85</td>
<td>42</td>
<td>447</td>
</tr>
<tr>
<td>4. Dialysis</td>
<td>36,245</td>
<td>67</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>5. Isofocusing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5.8/11.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Sephadex G-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>229,207</td>
<td>0.56</td>
<td>3.7</td>
<td>8487*</td>
</tr>
<tr>
<td>II</td>
<td>223,883</td>
<td>1.44</td>
<td>5.3/10.0</td>
<td>8022*</td>
</tr>
<tr>
<td>III</td>
<td>95,000</td>
<td>0.47</td>
<td>1.0</td>
<td>9141*</td>
</tr>
</tbody>
</table>

* Calculations assume that homolysate contains an equal amount of each isoenzyme.

**RESULTS**

**Enzyme Purification**

Table I summarizes the results of the purification. As shown in Fig. 1, hypoxanthine-guanine phosphoribosyltransferase activity was eluted from DEAE-cellulose as a single peak nearly coincident with the major protein peak. Inclusion of 5 mM MgCl₂ or 1 mM dithiothreitol in the resin to achieve increased recovery of activity caused a decreased binding of hypoxanthine-guanine phosphoribosyltransferase and a constant loss of activity throughout the 35 mM phosphate wash. Absence of enzyme activity in the washes indicates that recovery of 60% is not due to exclusion of enzyme species which fail to bind to the resin at pH 7.4. Electrophoresis in the Tris-glycine buffer system was then performed as described above.

**Immunological Methods**

**Production of Antisera**—Hypoxanthine-guanine phosphoribosyltransferase enzyme protein used for immunizations was obtained by a similar purification which included batch extraction on DEAE-cellulose followed by a 40 to 60% ammonium sulfate precipitation. The precipitate was resuspended in borate-buffered saline, pH 8.4 (boric acid, 0.184 g; borax, 9.536 g; NaCl, 4.384 g; in a final volume of 1 liter), and could be stored at -20°C for 4 to 6 weeks with negligible loss of activity.

On the day of immunization, a 10-ml aliquot was heated at 80°C for 4 min, followed by centrifugation at 5000 × g for 30 min. Equal volumes of the slightly yellow supernatant (containing approximately 0.9 mg per ml of protein with specific activity 490 times that of homolysate) and Freund's complete adjuvant were emulsified and fully grown white male rabbits were injected in the footpads (intradermally) and in the nape of the neck (subcutaneously) with 0.5 to 1.8 mg of protein. Booster injections of antigen in Freund's complete adjuvant were given as often as every 2 weeks to maintain antibody titer. Rabbits were bled via ear vein, and the blood was allowed to sit at 4°C for 18 hours. Following centrifugation at 5000 × g for 15 min, the antiseraum was pooled, divided into 1-ml aliquots, and stored at -20°C.

**Immunoelectrophoresis**—Immunoelectrophoresis was performed in agar gel with a Gelman Immunoelectrophoresis Kit and the Deluxe Electrophoresis Chamber. Two different buffer systems were employed: Tris-barbital-sodium barbital, pH 8.8 (high resolution buffer), and 50 mM sodium barbital, pH 8.0. Electrophoresis was performed at room temperature, and antigen-antibody precipitation patterns were allowed to develop for 48 hours.

Agar slides to be assayed were electrophoresed in an identical fashion and then 3-mm sections of the gel (widthwise) were incised and placed in 200 µl of 50 mM Tris-HCl buffer, pH 7.4. Following elution for 18 hours at 4°C, a 50-µl aliquot was removed and assayed by the usual methods.

**Immunodiffusion**—Immunodiffusion was performed by the method of Ouchterlony (19) in 1% agar containing 0.9% NaCl.

**Protein Determination**

Protein was estimated by the method of Lowry et al. (20), with crystalline bovine serum albumin as a standard, or by absorbance at 280 nm.
7.0. Low ionic strength dialysis performed to reduce the ionic strength of the sample prior to isoelectric focusing resulted in an increase in specific activity. Isoelectric focusing (Fig. 2) reproducibly separated the hypoxanthine-guanine phosphoribosyltransferase activity from 500 ml of whole blood obtained from one male into three major distinct peaks, designated I, II, and III in order of their proximity to the anode. However, the adenine phosphoribosyltransferase activity coincidentally present on the same run clearly eluted as a single major peak of activity. Isofocusing for a shorter period of time (48 hours) and the use of pH 5 to 8 range ampholytes failed to separate the three peaks of activity clearly. In addition, isofofocusing with pH 5 to 7 range ampholytes for 72 hours failed to show significant enzyme activity above pH 6.0 (data not shown). The isoelectric points of the three peaks from three different runs using pH 4 to 6 range ampholytes were Peak I, pk, 5.70, 5.66, 5.58; Peak II, pk, 5.88, 5.85, 5.74; and Peak III, pk, 6.00, 6.00, 5.90. Ampholytes were removed by filtration through Sephadex G-100 as judged by the absence of diffuse background staining on analytical polyacrylamide gels.

Stability Studies

Table II contains the results of stability studies on hypoxanthine-guanine phosphoribosyltransferase at two different stages of purification. Glycerol, sucrose, and dimethyl sulfoxide were useful for preserving the activity of the 300-fold purified preparation. On the basis of these findings, Sephadex G-100 chromatography was done in the presence of 5% (v/v) dimethyl sulfoxide to increase recovery.

Although 20% sucrose stabilizes the highly purified isoenzymes, the presence of ampholytes with the sucrose (i.e., before desalting) is a much more effective preservative, with 80 to 90% of initial activity still present after 30 days storage at -70°C.

Under the usual assay conditions, bovine serum albumin was necessary for expression of maximal catalytic activity except when the enzyme was still in an impure form. As shown in Fig. 3, enzyme activity in dilute purified preparations was re-

**Table II**

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Conditions</th>
<th>Duration of storagea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 Days</td>
</tr>
<tr>
<td>300-foldb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) 50 mm sodium phosphate</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>(2) 1 mm dithiothreitol</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>(3) 0.5 mm dithiothreitol</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>(4) 1 mm dithiothreitol-0.01 mM MgPP-ribose-P</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td>(5) 0.5 mm dithiothreitol-0.01 mM MgPP-ribose-P</td>
<td>17</td>
<td>2.5</td>
</tr>
<tr>
<td>(6) 50% glycerol</td>
<td>108</td>
<td>99</td>
</tr>
<tr>
<td>(7) 20% sucrose</td>
<td>102</td>
<td>78</td>
</tr>
<tr>
<td>(8) 5% dimethyl sulfoxide</td>
<td>89</td>
<td>77</td>
</tr>
<tr>
<td>300-foldc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) 50% glycerol</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>(2) 20% sucrose</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>(3) 5% dimethyl sulfoxide</td>
<td>38</td>
<td>21</td>
</tr>
<tr>
<td>(4) 50 mm Tris-HCl, pH 7.4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Isoenzyme Ic**

(8487-fold)  
(1) 50% glycerol | 42 | 28 | 12 |
(2) 20% sucrose | 61 | 33 | 8 |
(3) 5% dimethyl sulfoxide | 29 | 6 | 3 |
(4) 50 mm Tris-HCl, pH 7.4 | 0 | | |

**Isoenzyme IIc**

(8022-fold)  
(1) 50% glycerol | 49 | 29 | 11 |
(2) 20% sucrose | 52 | 37 | 11 |
(3) 5% dimethyl sulfoxide | 32 | 36 | 6 |
(4) 50 mm Tris-HCl, pH 7.4 | 0 | | |

**Isoenzyme IIIc**

(3141-fold)  
(1) 50% glycerol | 49 | 29 | 11 |
(2) 20% sucrose | 52 | 37 | 11 |
(3) 5% dimethyl sulfoxide | 32 | 36 | 6 |
(4) 50 mm Tris-HCl, pH 7.4 | 0 | | |

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*a Enzyme activity expressed as percentage of initial activity remaining.
*b All enzyme preparations were buffered with 50 mm sodium phosphate, pH 7.4, in addition to the other indicated additives.
*c All enzyme preparations contained 50 mm Tris-HCl, pH 7.4, in addition to the other indicated additives.
Fig. 3. Stabilization of partially purified hypoxanthine-guanine phosphoribosyltransferase (Step 3) at 37° by bovine serum albumin. Two different dilutions of hypoxanthine-guanine phosphoribosyltransferase (--- and O---O, 14 μg per ml; △—△ and △—△, 3.5 μg per ml) were assayed at 37° for the times indicated in the presence (closed symbols) or absence (open symbols) of bovine serum albumin (0.5 mg per ml). The use of a higher (5 mg per ml) or lower (0.05 mg per ml) concentration of bovine serum albumin produced essentially identical results.

Fig. 4. Analytical polyacrylamide gel electrophoresis (5% cross-linking) of native hypoxanthine-guanine phosphoribosyltransferase isoenzymes. The anode buffer was 400 mM glycine and 40 mM Tris-HCl adjusted to pH 8.1 with 5 ml of 1 N HCl; the cathode buffer was a 1:10 dilution of the anode buffer which was pH 8.3 after dilution. Enzyme protein (10 to 20 μg in 50 to 100 μl of 60 mM Tris-HCl, pH 7.4, 5% dimethyl sulfoxide, and 0.5 mM dithiothreitol) from the ampholyte-free preparation of each isoenzyme (Step 6) was mixed with 20 μl of tracking dye (dissolved in cathode buffer) and 30 μl of glycerol before being applied to the gels. The direction of migration was downward toward the anode. A mixture of all three isoenzymes (10 μg each) was incubated at 4° for 1 hour. I, II, and III indicate isoenzymes I, II, and III. I + II + III indicates the mixture of all three.

Fig. 5. Subunit molecular weight of hypoxanthine-guanine phosphoribosyltransferase as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein standards were prepared for electrophoresis in a fashion identical with the hypoxanthine-guanine phosphoribosyltransferase isoenzymes and simultaneously subjected to electrophoresis on separate gels. The hash mark indicates the migration of each of the isoenzymes which corresponds to a mean molecular weight of 34,500.

Polyacrylamide Disc Gel Electrophoresis

Analytical Disc Gels—Analytical polyacrylamide electrophoresis of each of the isoenzymes at three different cross-linkings (5, 10, 15%) revealed only one protein band for Peaks I and II and a minor contaminant for Peak III (Fig. 4). Hypoxanthine-guanine phosphoribosyltransferase activity assayed from companion gels was located in a single 4-mm section corresponding to the position of the major band in the stained gel.

Fig. 4 also demonstrates the distinct electrophoretic behavior of each of the isoenzymes. The isoenzymes were optimally distinguished on polyacrylamide gels with 5% cross-linking. However, a mixture of Peaks I, II, and III migrated in the 5% gel as a single band in a position identical with that of Peak II (Fig. 4). Essentially the same results were obtained when a mixture containing equal quantities of Peaks I and III was studied in this manner. A mixture of Peaks I and II or Peaks II and III revealed two bands. After storage for 2 weeks at −70°, the mobility of isoenzyme III became identical with that of II. Neuraminidase digestion failed to alter either the absolute or relative migration of the isoenzymes.

Sodium Dodecyl Sulfate Gel Electrophoresis—The molecular weight of the major band present for each isoenzyme proved to be 34,000 ± 4,000, 34,600 ± 1,700, and 34,700 ± 5,000 (mean ± S.D. from six experiments) for Peaks I, II, and III, respectively (Fig. 5). In any given experiment, no significant difference in molecular weight could be demonstrated between the subunits of each isoenzyme.

Glycoprotein staining of companion gels was negative.

Urea Gel Electrophoresis—Only a single band was seen for each isoenzyme and a mixture of the three when subjected to electrophoresis in the presence of 8 M urea (Fig. 6).

Gel Filtration

The elution profile for each of the isoenzymes and the DEAE-cellulose chromatography (117-fold purified) enzyme preparation

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were identical. The position of the single activity peak noted for each of the enzyme preparations corresponded to a Stokes radius of 36 Å and a molecular weight of 68,000, as determined the methods of Ackers (21) and Andrews (22), respectively.

**Immunological Studies**

All three isoenzymes are immunologically identical and have only a single precipitin arc on immunodiffusion and immunelectrophoresis. Hypoxanthine-guanine phosphoribosyltransferase activity was assayed only in the position in which the precipitin arcs subsequently formed. The isoenzymes could be distinguished from each other by immunelectrophoresis in 50 mM sodium barbital buffer at pH 8.0 (Fig. 7).

**Miscellaneous**

Neuraminidase Assay—Incubation with neuraminidase failed to liberate detectable sialic acid from any of the three isoenzymes.

**Burst Synthesis of GMP**—Crude hemolysate demonstrated an initial burst of [14C]GMP synthesis at 0° in the absence of magnesium which was complete within 20 sec. However, no synthesis of [14C]GMP under these conditions could be demonstrated for either the isofocusing or Sephadex G-100 chromatography isoenzyme preparations.

**Substrate Specificity and Product Inhibition Studies**—Table III summarizes attempts to demonstrate catalytic differences between the three isoenzymes. As can be seen, all have essentially the same activity with hypoxanthine or guanine as the purine substrate and each has retained sensitivity to product inhibition by GMP. Increasing the concentration of MgPP-ribose-P to 10 mM produced the same degree of substrate inhibition for each of the three isoenzymes.

![Fig. 6. Polyacrylamide gel electrophoresis of a mixture of the three hypoxanthine-guanine phosphoribosyltransferase isoenzymes in 8 M urea. The anode buffer chamber contained 60.0 ml of 1 N HCl which was adjusted with crystalline Tris-HCl (13.5 g) to pH 8.3 and brought to a volume of 1000 ml with distilled water; the cathode buffer chamber contained 1000 ml of 38 mM glycine, 5 mM Tris-HCl, pH 8.5. A mixture of the urea-denatured isoenzymes as shown here resulted in only a single protein band. The dark area at the bottom (anode) of the gel is dye artifact.](http://www.jbc.org/)
DISCUSSION

Hypoxanthine-guanine phosphoribosyltransferase from one male donor has been reproducibly purified to homogeneity as indicated by the presence of a single protein band on three different cross-linkages of polyacrylamide disc gels (23). The normal human enzyme has a Stokes radius of 36 A with a molecular weight of 68,000 and is composed of two subunits which have identical molecular weight and net charge.

A surprising finding resulting from the purification of human hypoxanthine-guanine phosphoribosyltransferase was the demonstration of three electrophoretically different forms of the native enzyme. Although multiple electrophoretic forms of one enzyme may be genetic in origin, the isoenzymes of hypoxanthine-guanine phosphoribosyltransferase appear to result from post-transcriptional nongenetic alterations. Since all available genetic data indicate that the gene coding for hypoxanthine-guanine phosphoribosyltransferase resides on the X chromosome (3), the use of erythrocytes from one male donor in the present study assures that the three isoenzymes are not the product of allelic genes. In addition, the fact that the single mutational event producing the Lesch-Nyhan syndrome results in a virtual absence of hypoxanthine-guanine phosphoribosyltransferase makes the occurrence of duplicate enzymes unlikely (24). Consistent with this hypothesis are our observations that (a) the three isoenzymes had the same Stokes radius and are composed of two subunits of identical molecular weight and net charge, (b) there were no detectable immunological or catalytic differences between the three isoenzymes, and (c) isoenzyme III assumed the electrophoretic mobility of isoenzyme II after storage for 2 weeks at -70°C.

The nature of the post-transcriptional alteration responsible for the electrophoretic heterogeneity of hypoxanthine-guanine phosphoribosyltransferase remains unclear. The absence of hydrolyzed sialic acid after exposure of each of the isoenzymes to neuraminidase and the failure of neuraminidase to affect the migration of each isoenzyme make differential sialation an unlikely explanation for the hypoxanthine-guanine phosphoribosyltransferase isoenzymes. The negative glycoprotein stain supports these observations. Association of the subunits into tetramers or hexamers has been eliminated by the identical elution patterns in Sephadex G-100, thus excluding electrophoretic heterogeneity on this basis. Ampholytes have been implicated in producing artifactual electrophoretic heterogeneity by differential binding. The consistent finding of three isoenzymes and the recent reports of two to four isoenzymes of hypoxanthine-guanine phosphoribosyltransferase found by techniques other than isoelectric focusing (25, 26) suggest that this is not the case. Binding of ribose 5-phosphate has been hypothesized as being responsible for an initial burst of C9IMP synthesis seen with a crude preparation of hypoxanthine-guanine phosphoribosyltransferase (8) and could theoretically have been responsible for the isoenzymes we observed. However, the absence of an initial burst of GMP synthesis by any of the three isoenzymes renders this possibility unlikely. The observation that electrophoresis of a mixture of the three isoenzymes in polyacrylamide gels results in a single protein band which corresponds with the electrophoretic mobility of isoenzyme II may account for our inability to detect more than one peak of hypoxanthine-guanine phosphoribosyltransferase activity in preparations of the enzyme prior to isoelectric focusing and suggests an equilibrium between the subunits which favors isoenzyme II.

Several recent studies have also reported the presence of isoenzymes for hypoxanthine-guanine phosphoribosyltransferase. Rubin et al. (25) have described a purification of this enzyme from pooled blood which resulted in the recovery of two isoenzymes after DEAE-cellulose chromatography. In addition, Bakay and Nyhan (26) have reported four different areas of apparent hypoxanthine-guanine phosphoribosyltransferase activity after electrophoresis of crude hemolysate from male subjects on polyacrylamide gels. However, it is not clear if the isoenzymes reported in these two studies are similar to those reported here since we failed to observe isoenzymes after DEAE-cellulose chromatography or after electrophoresis in polyacrylamide gels without prior isoelectric focusing.

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REFERENCES

Human Hypoxanthine-Guanine Phosphoribosyltransferase: PURIFICATION AND SUBUNIT STRUCTURE
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