6, 567-572.
Nieto, M., Perkins H. R., Frère, J. M., and Ghuysen, J. M. (1973), *Biochem. J. 135*, 493-505.
Peanasky, R. J., Gratecos, D., Baratti, J., and Rovery, M. (1969), *Biochim. Biophys. Acta 181*, 82-91.
Puigserver, A. (1976), *Biochim. Biophys. Acta 438*, 514-521.
Puigserver, A., and Desnuelle, P. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 2442-2445.
Puigserver, A., Vaugoyeau, G., and Desnuelle, P. (1972), *Biochim. Biophys. Acta 276*, 519-530.
Reeck, G. R., and Neurath, H. (1972), *Biochemistry 11*, 3947-3955.
Teale, F. W. J. (1960), *Biochem. J. 76*, 381-388.
Uren, J. R., and Neurath, H. (1972), *Biochemistry 11*, 4483-4492.
Van Holde, K. E., and Baldwin, R. L. (1958), *J. Phys. Chem. 62*, 734-743.
Wood, H. G., Ahmad, F., Jacobson, B., Chuang, M., and Brattin, W. (1975), *J. Biol. Chem. 250*, 918-926.
Yamasaki, M., Brown, J. R., Cox, D. J., Greenshields, R. N., Wade, R. D., and Neurath, H. (1963), *Biochemistry 2*, 859-866.
Yphantis, D. A. (1964), *Biochemistry 3*, 297-317.

Human Hypoxanthine Phosphoribosyltransferase. Purification and Properties†

Anne S. Olsen‡ and Gregory Milman*,§

ABSTRACT: Hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) from human erythrocytes has been purified 13 000-fold to apparent homogeneity. The native enzyme has a sedimentation coefficient of 5.9 S, determined by analytical ultracentrifugation, and a molecular weight of 81 000–83 000, determined by sedimentation equilibrium centrifugation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates a subunit molecular weight of 26 000, suggesting that the enzyme is a trimer. Isoelectric focusing resolves three peaks of enzyme activity at pH 5.6, 5.7, and 5.9. The amino acid composition of hypoxanthine phosphoribosyltransferase is 17 Lys, 5 His, 12 Arg, 0 Trp, 31 Asx, 12 Thr, 14 Ser, 16 Glx, 14 Pro, 19 Gly, 12 Ala, 5 Cys, 18 Val, 5 Met, 11 Ile, 20 Leu, 10 Tyr, and 9 Phe. The enzyme appears to have a blocked N terminus.

Hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) is a purine salvage enzyme which catalyzes the PRPP[1]-dependent conversion of the purine bases hypoxanthine and guanine to the corresponding nucleotides IMP and GMP. This enzyme is of clinical interest since an absence of hypoxanthine phosphoribosyltransferase activity results in a severe neurological disorder, the Lesch-Nyhan syndrome (Seegmiller et al., 1967). Hypoxanthine phosphoribosyltransferase is also an important enzyme marker for the study of somatic cell genetics, because the gene is X-linked and because efficient selection systems for cells either lacking or possessing the enzyme have been developed (Szybalski et al., 1962; Thompson and Baker, 1973).

Our previous work on Chinese hamster hypoxanthine phosphoribosyltransferase (Olsen and Milman, 1974a) has been extended to the human enzyme because our current genetic studies of HeLa hypoxanthine phosphoribosyltransferase (Milman et al., 1976, 1977) and Lesch-Nyhan erythrocytes (Ghangas and Milman, 1975) require a knowledge of the characteristics of the normal human enzyme. We have adapted our procedures for purification of hypoxanthine phosphoribosyltransferase from Chinese hamster brain and liver to the purification of the enzyme from human erythrocytes. Our results indicate that human and Chinese hamster hypoxanthine phosphoribosyltransferase have similar structural properties. In addition, this procedure has yielded sufficient quantities of the purified human enzyme to enable us to perform many biochemical studies, such as amino acid analysis, N-terminal analysis, and analytical ultracentrifugation, which were not feasible with the limited amounts of enzyme which could be obtained from Chinese hamsters.

Experimental Procedures

Enzyme Purification. The procedure used was a modification of the procedure previously described for the purification of the Chinese hamster enzyme (Olsen and Milman, 1974a). All steps were performed at 0–5 °C unless otherwise specified. Protein concentration was determined by the method of Lowry (Lowry et al., 1951). Enzyme activity was measured as previously described (Olsen and Milman, 1974a). The volumes and protein concentrations at each step of the purification are given in Table I.

Crude Lysate. Ten pints of outdated human blood were obtained from the Alameda County Blood Bank. The eryth-

† From the Department of Biochemistry, University of California Berkeley, California 94720. *Received December 27, 1976.* This work was supported by United States Public Health Service Grant CA 21650 from the National Cancer Institute.

‡ Present address: Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.
§ Present address: Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205.

[1] Abbreviations used: PRPP, 5-phosphoribosyl 1-pyrophosphate; DTT, dithiothreitol; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

TABLE I: Purification of Hypoxanthine Phosphoribosyltransferase from Human Erythrocytes.

Step	Volume	Units[a]	Protein (mg)	Spec act. (units/mg)	Cumulative recovery (%)	Purification (-fold)
Crude lysate	10 125	700	527 000	0.00134	100	1.0
65 °C supernatant	8 575	491	394 000	0.00125	70	0.9
DEAE-Cellulose	105	371	336	1.11	53	830
40–70% ammonium sulfate	25	389	195	2.00	56	1 500
First 85 °C supernatant	26	346	77.5	4.50	49	3 300
DEAE-Sephadex	7.5	158	12.7	12.5	23	9 300
Second 85 °C supernatant	7.5	118	6.7	17.5	17	13 000

[a] One unit is defined as the amount of enzyme which catalyzes the formation of 1 μmol of IMP per min at 37 °C.

rocytes were sedimented by centrifugation at 4000*g* for 10 min, and the serum was discarded. The erythrocytes were washed twice with an equal volume of 0.9% NaCl and then frozen at −20 °C. The cells were lysed by thawing at room temperature and were diluted fivefold with enzyme buffer (20 mM Tris-HCl (pH 7.8)–20 mM KCl–6 mM $MgCl_2$–0.1 mM EDTA–0.5 mM DTT) to give the crude lysate.

65 °C Supernatant. The crude lysate, in 200-mL portions, was placed in a 65 °C water bath and heated to 62 °C (about 15 min). It was maintained at 62 to 65 °C for 10 min, quickly cooled, and centrifuged at 4000*g*.

DEAE-Cellulose Fraction. DEAE-cellulose (Bio-Rad Cellex-D, capacity 0.6 mequiv/g) was purified by washing with 0.25 M NaOH containing 0.25 M NaCl, rinsing with distilled water, then washing with 0.25 M HCl, followed by extensive washing with distilled water. The DEAE-cellulose was then suspended in enzyme buffer and allowed to settle, and the supernatant containing fine DEAE-cellulose particles was decanted. This procedure was repeated several times, and the suspension was then poured into a column (6.5 × 45 cm). The 65 °C supernatant (about 8.5 L) was applied to the column which was then washed with 3 L of enzyme buffer. The enzyme was eluted with a linear KCl gradient prepared from 4 L of enzyme buffer and 4 L of enzyme buffer containing 0.15 M KCl. Fractions containing the enzyme peak (about 2 L) were pooled and concentrated to 105 mL in an Amicon ultrafiltration cell with a PM-10 membrane.

Forty to Seventy Percent Ammonium Sulfate Fraction. The DEAE-cellulose fraction was brought to 40% saturation in ammonium sulfate by addition of solid ammonium sulfate (22.6 g/100 mL). After 30 min the sample was centrifuged at 12 000*g* for 15 min. The 40% supernatant was brought to 70% saturation by addition of solid ammonium sulfate (18.2 g/100 mL). After 1 h the suspension was centrifuged for 15 min at 12 000*g*. The pellet was resuspended in enzyme buffer and dialyzed for 18 h against enzyme buffer (three changes of 1 L each).

First 85 °C Supernatant. PRPP in enzyme buffer was added to the dialyzed ammonium sulfate fraction to a final concentration of 1 mM. The sample was placed in a 37 °C water bath for 15 min and then heated in an 85 °C water bath for 10 min. It was quickly cooled and centrifuged at 12 000*g* for 15 min.

DEAE-Sephadex Fraction. The 85 °C supernatant was applied to a column (2.5 × 40 cm) of DEAE-Sephadex A-50 equilibrated with enzyme buffer. The column was washed with 400 mL of enzyme buffer, and the enzyme was eluted with a linear KCl gradient prepared from 2 L of enzyme buffer and 2 L of enzyme buffer containing 0.17 M KCl. Fractions containing the enzyme peak (about 800 mL) were pooled and concentrated to 7.5 mL in an Amicon ultrafiltration cell with a PM-10 membrane.

Second 85 °C Supernatant. To the DEAE-Sephadex fraction was added 0.1 volume of 0.01 M PRPP. The sample was placed in a 37 °C water bath for 15 min and then heated in an 85 °C water bath for 10 min. The solution was quickly chilled and then centrifuged for 30 min at 41 000*g* to remove denatured contaminant proteins. This second 85 °C supernatant fraction was used in all subsequent studies, unless otherwise indicated.

Analytical Ultracentrifugation. Centrifugations were performed in a Spinco Model E analytical ultracentrifuge equipped with a rotor temperature-indicating unit and a photoelectric scanner.

Sedimentation Velocity. A solution of 0.8 mg/mL hypoxanthine phosphoribosyltransferase was dialyzed for 16 h at 4 °C against dialysis buffer consisting of enzyme buffer with 0.1 mM PRPP but without DTT. The enzyme solution and dialysis buffer (0.45 mL of each) were centrifuged in a 12-mm double sector cell with sapphire windows. The progress of the sedimenting boundary was followed by scans of the absorbance at 280 nm taken at 8-min intervals.

Sedimentation Equilibrium. High-speed sedimentation equilibrium experiments were performed following the procedure of Yphantis (1964). Hypoxanthine phosphoribosyltransferase (concentrated by ultrafiltration to 1.5 mg/mL) was dialyzed against the dialysis buffer described above and then diluted with the same buffer to give an absorbance of 0.2 (approximately 0.4 mg/mL). Immediately before centrifugation a freshly prepared solution of DTT was added to the enzyme and solvent to a final concentration of 0.5 mM. Centrifugation was performed in a 12-mm Yphantis centerpiece with sapphire windows. Equilibrium was achieved after centrifugation at 20 000 rpm for 28 h. The speed was then increased to 26 000 rpm, and centrifugation was continued for an additional 20 h. The rotor temperature was 1–2 °C. Absorbance scans were taken at 1 h intervals from 25 to 28 h and again from 46 to 48 h.

Amino Acid Analysis Following Hydrolysis with HCl. To 15 μg of hypoxanthine phosphoribosyltransferase in 15 μL of enzyme buffer was added 85 μL of distilled water and 100 μL of concentrated HCl in an acid-cleaned glass tube (Pyrex 10 × 75 mm). The tube was evacuated, sealed, and heated in a 110 °C oven for 22 h. The HCl was evaporated, and the residue was dissolved in 100 μL of water. Aliquots of 50 μL were added to the long and short columns of a Beckman 120 C amino acid analyzer, and analyses were performed according to Spackman (1967).

For cysteine determination, 30 μg of hypoxanthine phosphoribosyltransferase was dialyzed against water, lyophilized, and then treated with performic acid as described by Hirs (1967), except that the volumes were decreased tenfold. The performic acid oxidized protein was hydrolyzed with HCl and analyzed for cysteic acid and methionine sulfone. Recovery of these amino acids was determined by performic acid oxidation of standard cysteine and methionine solutions.

Amino Acid Analysis Following Hydrolysis with p-Toluenesulfonic Acid. The procedure of Liu and Chang (1971) was followed. A sample of 90 μg of hypoxanthine phosphoribosyltransferase was dialyzed for 16 h against water. The resulting suspension was divided equally among three tubes (Pyrex 10 × 75 mm) and lyophilized. To each tube was added 200 μL of 3 N *p*-toluenesulfonic acid containing 0.2% 3-(2-aminoethyl)indole. The tubes were evacuated, sealed, and heated in a 110 °C oven for 22, 49, and 72 h. After hydrolysis, 400 μL of 1 N NaOH was added to each tube. Samples of 200 to 250 μL from each tube were applied to the long and short columns of the amino acid analyzer. In order to increase the sensitivity for tryptophan detection, a larger amount of hypoxanthine phosphoribosyltransferase (115 μg) was treated as above, and 400 μL was applied to the short column of the analyzer. A sample of hypoxanthine phosphoribosyltransferase with 5 nmol of added tryptophan was hydrolyzed by the same procedure to determine the recovery of tryptophan.

N-Terminal Analysis. Reaction of hypoxanthine phosphoribosyltransferase with dansyl (5-dimethylaminonaphthalene-1-sulfonyl) chloride in sodium dodecyl sulfate was performed according to Weiner et al. (1972). The dansylated protein (100 to 250 μg) was hydrolyzed in 6 N HCl at 110 °C for 5 to 16 h. Samples containing 10 to 50 μg were chromatographed on one side of a 7.5 × 7.5 cm polyamide sheet (Cheng Chin Trading Co.) using the solvent systems of Weiner et al. Dansylated residues were visualized under ultraviolet light and were compared with standard dansylated amino acids (Sigma) which were chromatographed on the reverse side of the same sheet. Several proteins with known N termini were dansylated and analyzed by the same procedure.

Results

Enzyme Purification. The purification of human hypoxanthine phosphoribosyltransferase from 10 pints of blood is summarized in Table I. Although the 65 °C heat step yields no net purification, it greatly improves the purification achieved by the subsequent DEAE-cellulose column chromatography step. The DEAE-cellulose column serves mainly to remove hemoglobin, which is not bound to the anion exchanger at the pH and salt concentrations used. In the procedure described hypoxanthine phosphoribosyltransferase is eluted by a KCl gradient, but a batch elution with 0.15 M KCl has also been employed. However, the purified enzyme from the batch procedure contains several minor contaminants.

Hypoxanthine phosphoribosyltransferase is precipitated between 40 and 70% saturated ammonium sulfate. Dialysis of the ammonium sulfate fraction prior to the 85 °C heat step is extremely important since the enzyme is completely inactivated at 85 °C if ammonium sulfate is present at greater than 2 to 5% saturation. The remainder of this procedure is similar to that used for the purification of the Chinese hamster enzyme (Olsen and Milman, 1974a). This procedure results in a 13 000-fold purification of human hypoxanthine phosphoribosyltransferase with a 17% recovery of activity. The yield of purified hypoxanthine phosphoribosyltransferase from 10 pints of blood is about 7 mg.

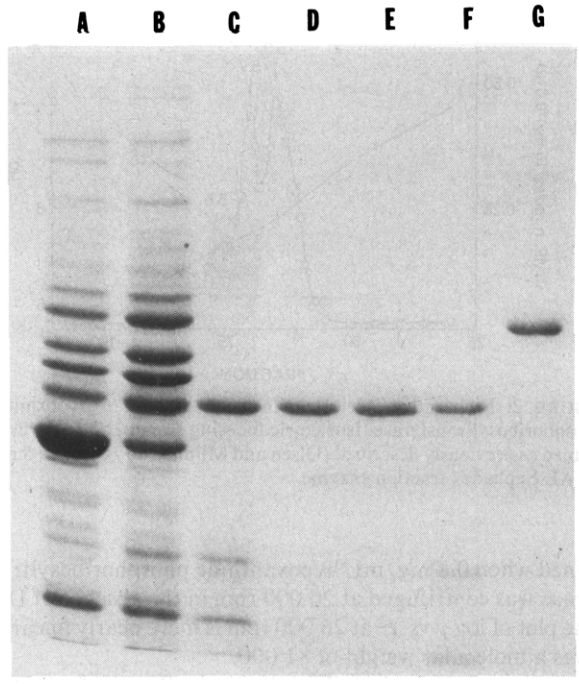

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of human hypoxanthine phosphoribosyltransferase. Electrophoresis in a 12.5% acrylamide gel slab was performed as previously described (Olsen and Milman, 1974a). Enzyme samples from successive steps in the purification procedure and amount of protein applied are: (A) DEAE-cellulose fraction, 16 μg; (B) 40–70% ammonium sulfate fraction, 16 μg; (C) 85 °C supernatant, 6 μg; (D) DEAE-Sephadex fraction, 3 μg; (E) second 85 °C supernatant, 2 μg. The molecular weight marker proteins are: (F) chymotrypsinogen (25 700), 2 μg; (G) lactate dehydrogenase (36 000), 2 μg.

Samples from each step of the purification procedure were applied to a sodium dodecyl sulfate–polyacrylamide gel, illustrated in Figure 1. A band of molecular weight approximately 26 000 is clearly visible in the DEAE-cellulose fraction and in all subsequent steps. We have previously demonstrated that this is the hypoxanthine phosphoribosyltransferase band (Olsen and Milman, 1974b), and it is the only band visible in the second 85 °C supernatant fraction, indicating an apparently homogeneous enzyme preparation.

Analytical Ultracentrifugation. Sedimentation velocity centrifugation of hypoxanthine phosphoribosyltransferase (0.8 mg/mL) at 60 000 rpm results in a linear plot of log x vs. time, where x is the distance of the center of the sedimenting boundary from the axis of rotation. A sedimentation coefficient, $s_{20,w}$, of 5.9 was calculated for the enzyme using a partial specific volume ($\bar{V}$) of 0.73 cm^3/g calculated from the amino acid composition (Schachman, 1957).

The native molecular weight of hypoxanthine phosphoribosyltransferase was determined by sedimentation equilibrium centrifugation using the meniscus depletion method (Yphantis, 1964). Centrifugation of samples containing 0.4 and 0.8 mg/mL hypoxanthine phosphoribosyltransferase at 20 000 rpm results in a log y vs. r^2 plot which is slightly concave upward, indicating an association or dissociation of the enzyme. A molecular weight of 83 000 was determined from the lower, linear portion of the curve using the equation of Schachman (1957):

$$M = \frac{2RT}{(1 - \bar{V}\rho)\omega^2} 2.303 \, \mathrm{d} \log y / \mathrm{d}r^2$$

This represents the molecular weight of the smallest sedimenting species (Yphantis, 1964). A similar result was ob-

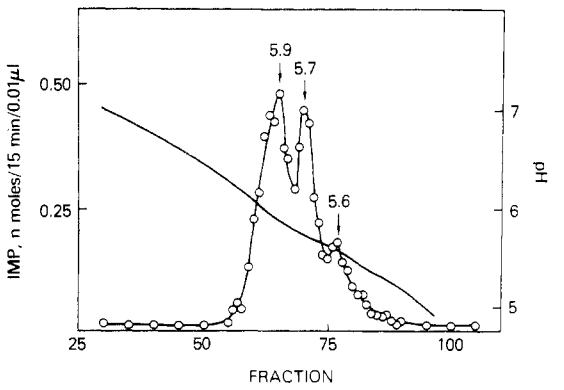

FIGURE 2: Preparative isoelectric focusing of human hypoxanthine phosphoribosyltransferase. Isoelectric focusing from pH 5 to 7 was performed as previously described (Olsen and Milman, 1974a) with 5 mg of DEAE-Sephadex fraction enzyme.

tained when 0.8 mg/mL hypoxanthine phosphoribosyltransferase was centrifuged at 20 000 rpm in the absence of DTT. The plot of log y vs. r^2 at 26 000 rpm is more nearly linear and gives a molecular weight of 81 000.

Isoelectric Focusing. Preparative isoelectric focusing of human hypoxanthine phosphoribosyltransferase results in three peaks of enzyme activity (Figure 2) with p*I*'s of 5.6, 5.7, and 5.9. Similar heterogeneity in isoelectric points was observed for the Chinese hamster enzyme (Olsen and Milman, 1974a), although the Chinese hamster isozymes were in a higher pH range (pH 6.2 to 6.6).

Amino Acid Analysis. Amino acid analysis of human hypoxanthine phosphoribosyltransferase is summarized in Table II. The values are expressed as residues per subunit of molecular weight 26 000. Enzyme samples were analyzed following hydrolysis with HCl for 22 h or with *p*-toluenesulfonic acid for 22, 49, and 72 h. The latter procedure permits determination of tryptophan (Liu and Chang, 1971). The two hydrolysis methods yield similar values for most amino acids. The values for serine and threonine are lower in the sample hydrolyzed with HCl, but in that sample the values were not extrapolated to zero time. The values for glycine and glutamate/glutamine also appear considerably lower in the HCl hydrolyzed sample. The residues present in lowest amount are histidine, cysteine, and methionine at five residues per subunit. Hypoxanthine phosphoribosyltransferase has a high aspartate/asparagine content (about 31 residues per subunit) and appears to lack tryptophan. The recovery of tryptophan in a standard sample analyzed by this procedure was 91%.

N-Terminal Analysis. Chromatography of dansylated hypoxanthine phosphoribosyltransferase following hydrolysis failed to reveal an N-terminal amino acid. The only spots visible on the polyamide plates were dansylic acid, ε-dansyllysine, *O*-dansyltyrosine, and dansylamide, even when the plates were heavily loaded (2 nmol of subunit). When 0.3 to 1 nmol of subunit of ribonuclease, myoglobin, hemoglobin, and bovine serum albumin were chromatographed by the same procedure the N-terminal amino acids were easily identified, indicating that this procedure is sensitive enough to detect a free N-terminal amino acid of the enzyme if it were present. Thus it appears that the N terminus of hypoxanthine phosphoribosyltransferase is blocked.

TABLE II: Amino Acid Composition of Human Hypoxanthine Phosphoribosyltransferase.

Amino acid	Residues per subunit: *p*-Toluenesulfonic acid hydrolysis[a]	Residues per subunit: HCl hydrolysis[b]
Lys	16.6 ± 0.6	17.6
His	5.3 ± 0.1	5.6
Arg	12.6 ± 0.4	12.4
Trp	<0.3	
Asx[c]	30.3 ± 0.7	32.4
Thr	12.8[d]	11.8
Ser	15.9[d]	11.9
Glx[c]	17.5 ± 0.3	15.0
Pro	13.3 ± 0.9	15.5
Gly	19.8 ± 0.3	17.8
Ala	13.1 ± 1.1	12.0
Cys		4.8[e]
Val	17.5[f]	17.5
Met	6.3 ± 0.2	5.4[g]
Ile	10.9[f]	12.2
Leu	20.1 ± 0.3	21.1
Tyr	10.8 ± 0.2	10.2
Phe	9.5 ± 0.2	9.4

[a] Average ± standard error of values for 22, 49, and 72 h hydrolysis. [b] Values for 22 h hydrolysis. [c] Values include the amides. [d] Extrapolated to zero hydrolysis time. [e] Determined as cysteic acid following performic acid oxidation; average of the number of residues calculated relative to leucine (4.9) and alanine (4.6). [f] Values from 72 h hydrolysis. [g] Determination of methionine sulfone in the performic acid oxidized sample also yielded 5.4 residues per subunit.

Discussion

We have described a procedure for purification of hypoxanthine phosphoribosyltransferase from human erythrocytes which yields an apparently homogeneous, 13 000-fold purified enzyme. Although the specific activity of hypoxanthine phosphoribosyltransferase in human erythrocytes (1.3 milliunits/mg) is low compared with that in Chinese hamster brain (17 milliunits/mg) or liver (4 milliunits/mg) (Olsen and Milman, 1974a), the major contaminant in the erythrocytes is hemoglobin which is easily removed by adsorption of hypoxanthine phosphoribosyltransferase to DEAE-cellulose, resulting in an 800-fold increase in specific activity. The availability of large quantities of erythrocytes makes this an excellent source for purification of milligram quantities of the human enzyme.

We have previously demonstrated that the subunit molecular weight of both human and Chinese hamster hypoxanthine phosphoribosyltransferase is 26 000 (Olsen and Milman, 1974b). Similar subunit molecular weights have now been reported for hypoxanthine phosphoribosyltransferase from rat brain (Gutensohn, 1974) and mouse liver (Hughes et al., 1975), indicating a similarity in the enzyme from these mammalian sources.

Sedimentation equilibrium experiments with purified human hypoxanthine phosphoribosyltransferase indicate a molecular weight of 81 000–83 000, but the nonideal behavior of the enzyme suggests that it is associating under our experimental conditions. A molecular weight of 81 000–83 000 is consistent with the molecular weights of 78 000–85 000 determined for the Chinese hamster enzyme by gel filtration and polyacrylamide gel electrophoresis (Olsen and Milman, 1974a). The sedimentation coefficient of 5.9 S obtained by analytical ultracentrifugation is somewhat higher than that expected for a globular protein of molecular weight 80 000.

A sedimentation coefficient of 5.9 S suggests a molecular weight of 100 000 based on the relationship $S_1/S_2 = (M_1/M_2)^{2/3}$ (Martin and Ames, 1961), using hemoglobin as a standard. Other investigators have reported native molecular weights of 63 000–64 000 (Gutensohn, 1974), 68 000 (Arnold and Kelley, 1971), and 80 000 (Hughes et al., 1975) for the enzyme from various mammalian sources. Our molecular weight data suggest that hypoxanthine phosphoribosyltransferase consists of three subunits of molecular weight 26 000.

Isoelectric focusing of human hypoxanthine phosphoribosyltransferase resolves three peaks of enzyme activity with isoelectric points of 5.6, 5.7, and 5.9. Similar isozymes have been observed in hypoxanthine phosphoribosyltransferase preparations from several mammalian sources (Olsen and Milman, 1974a; Gutensohn, 1974) including erythrocytes from a single male donor (Arnold and Kelley, 1971). When the enzyme is immunoprecipitated from fresh erythrocytes and electrophoresed on two-dimensional isoelectric focusing sodium dodecyl sulfate–polyacrylamide gels, two major spots are visible at pH 5.9 and 5.7. The enzyme immunoprecipitated from HeLa or human lymphoblast extracts displays only a single spot located at the same position as the most basic of the erythrocyte isozymes focusing at pH 5.9 (Ghangas and Milman, 1977). Genetic evidence indicates a single gene locus for hypoxanthine phosphoribosyltransferase, and it seems likely that these apparent isozymes are initially products of the same gene but differ by some type of modification, such as deamidation. Similar differences have been observed for human erythrocyte purine nucleoside phosphorylase which in older erythrocytes consists of several isozymes but in young erythrocytes consists of only a single species corresponding to the most basic isozyme (Turner et al., 1971).

The results of dansylation of hypoxanthine phosphoribosyltransferase indicate that the N terminus of the enzyme is blocked. Two types of blocked N termini occur frequently among mammalian proteins: N-acetylated amino acids occur at the N termini of many histones, ovalbumin, and several enzymes including alcohol dehydrogenase and carbonic anhydrase (Dayhoff, 1972). Pyroglutamic acid (pyrrolidonecarboxylic acid) occurs at the N terminus of many immunoglobulin heavy chains (Dayhoff, 1972). In addition, pyroglutamic acid can be generated by cyclization of N-terminal glutamate or glutamine during purification of proteins containing these N-terminal amino acids (Doolittle, 1970).

Acknowledgments

We are grateful to Ms. Alice Taylor for performing the ultracentrifugations, to Ms. Barbara Baltimore for assistance in the amino acid analyses, and to Dr. Myrtle Hsiang for advice on N-terminal analysis.

References

Arnold, W. J., and Kelley, W. N. (1971), *J. Biol. Chem. 246,* 7398–7404.

Dayhoff, M. O. (1972), Atlas of Protein Sequence and Structure, Washington, D.C., National Biomedical Research Foundation.

Doolittle, R. F. (1970), *Methods Enzymol. 19,* 555–569.

Ghangas, G. S., and Milman, G. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4147–4150.

Ghangas, G. S., and Milman, G. (1977), *Science* (in press).

Gutensohn, W. (1974), in Purine Metabolism in Man: Enzymes and Metabolic Pathways, Sperling, O., Devries, A., and Wyngaarden, J. B., Ed., New York, N.Y., Plenum Press, pp 19–22.

Hirs, C. H. W. (1967), *Methods Enzymol. 11,* 59–62.

Hughes, S. H., Wahl, G. M., and Capecchi, M. R. (1975), *J. Biol. Chem. 250,* 120–126.

Liu, T.-Y., and Chang, Y. H. (1971), *J. Biol. Chem. 246,* 2842–2848.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265–275.

Martin, R. G., and Ames, B. N. (1961), *J. Biol. Chem. 236,* 1372–1379.

Milman, G., Krauss, S. W., and Olsen, A. S. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 926–930.

Milman, G., Lee, E., Ghangas, G. S., McLaughlin, J., and George, M. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 4589–4593.

Olsen, A. S., and Milman, G. (1974a), *J. Biol. Chem. 249,* 4030–4037.

Olsen, A. S., and Milman, G. (1974b), *J. Biol. Chem. 249,* 4038–4040.

Schachman, H. K. (1957), *Methods Enzymol. 4,* 32–103.

Seegmiller, J. E., Rosenbloom, F. M., and Kelley, W. N. (1967), *Science 155,* 1682–1684.

Spackman, D. H. (1967), *Methods Enzymol. 11,* 3–15.

Szybalski, W., Szybalski, E. H., and Ragni, G. (1962), *Natl. Cancer Inst., Monogr. 7,* 75–88.

Thompson, L. H., and Baker, R. H. (1973), *Methods Cell Biol. 6,* 209–281.

Turner, B. M., Fisher, R. A., and Harris, H. (1971), *Eur. J. Biochem. 24,* 288–295.

Weiner, A. M., Platt, T., and Weber, K. (1972), *J. Biol. Chem. 247,* 3242–3251.

Yphantis, D. A. (1964), *Biochemistry 3,* 297–317.