Hypoxanthine-Guanine Phosphoribosyltransferase

CHARACTERIZATION OF A MUTANT IN A PATIENT WITH GOUT

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ABSTRACT The mutation in a young gouty male with a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase has been evaluated. The serum uric acid was 11.8 mg/100 ml, and the urinary uric acid excretion was 1,279 mg/24 h. Erythrocyte hypoxanthine-guanine phosphoribosyltransferase was 34.2 nmol/h/mg, adenine phosphoribosyltransferase was 36.5 nmol/h/mg and phosphoribosylpyrophosphate was 2.6 μM. Hypoxanthine-guanine phosphoribosyltransferase from peripheral leukocytes and cultured diploid skin fibroblasts was within the normal range, but enzyme activity in rectal mucosa was below the normal range.

Initial velocity studies of the normal enzyme and the mutant enzyme from erythrocytes with the substrates hypoxanthine, guanine, or phosphoribosylpyrophosphate showed that the Michaelis constants were similar. Product inhibition studies distinguished the mutant enzyme from the normal enzyme. Hyperbolic kinetics with increasing phosphoribosylpyrophosphate were converted to sigmoid kinetics by 0.2 mM GMP with the mutant enzyme but not with the normal enzyme.

The mutant erythrocyte hypoxanthine-guanine phosphoribosyltransferase was inactivated normally at 80°C and had a normal half-life in the peripheral circulation. The mol wt of 48,000 was similar to the normal enzyme mol wt of 47,000. With isoelectric focusing, the mutant erythrocyte enzyme had two major peaks with isoelectric pH's of 5.50 and 5.70, in contrast to the isoelectric pH's of 5.76, 5.82, and 6.02 of the normal isozymes. Isoelectric focusing of leukocyte extracts from the patient revealed the presence of the mutant enzyme.

Cultured diploid fibroblasts from the propositus appeared to function normally, as shown by the inability to grow in 50–100 μM azaguanine and by the normal incorporation of [^{14}C]hypoxanthine into nucleic acid. In contrast, erythrocytes from the patient displayed abnormal properties, including the increased synthesis of phosphoribosylphyrophosphate and elevated functional activity of orotate phosphoribosyltransferase and orotidylic decarboxylase.

These unique kinetic, physical, and functional properties provide support for heterogeneous structural gene mutations in partial deficiencies of hypoxanthine-guanine phosphoribosyltransferase.

INTRODUCTION

Studies of the metabolic basis of the primary hyperuricemias have revealed many regulatory properties of the purine pathways and provided a model for the molecular pathology associated with inherited abnormalities in man (1, 2). In particular, the demonstration of an X-linked deficiency of hypoxanthine-guanine phosphoribosyltransferase in the Lesch-Nyhan syndrome and in some patients with gout has contributed essential information on the control of purine biosynthesis *de novo* and on the critical role of this reutilization pathway in central nervous system function (3–8). Structural enzyme alterations, reflecting genetic heterogeneity in these disorders, are expressed by abnormalities of enzyme kinetic properties and physical properties (9–17).

Our studies of a patient with gout and uric acid overexcretion have recently delineated a structural disorder of hypoxanthine-guanine phosphoribosyltransfer-

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ase manifested by both abnormal kinetic properties and an altered isozyme pattern of the erythrocyte enzyme.

CASE SUMMARY

A 34-yr-old male schoolteacher of Macedonian origin first developed episodes of acute monarticular arthritis at age 22. He had a history of renal colic and hematuria. The maternal grandfather was reported to have had tophaceous gout. At the time of diagnosis the serum uric acid was 11.4 mg/100 ml, and the urine uric acid was 1,200 mg/24 h. He was treated with 300 mg allopurinol/day for 10 yr and became free of acute attacks of arthritis. Physical examination showed a normal man without tophi or chronic joint changes. Blood pressure was 150/100. Central nervous system evaluation was entirely normal.

Laboratory studies revealed a hemoglobin of 15.0 g/100 ml, hematocrit of 48%, normal erythrocyte morphology, sodium 145 meq/liter, fasting blood sugar 95 mg/100 ml, creatinine 1.2 mg/100 ml, and creatinine clearance 105 ml/min. On a purine-free diet his serum uric acid was 11.7 mg/100 ml, urine uric acid was 1,279 mg/24 h, and uric acid clearance was 7.6 ml/min. On 300 mg allopurinol/day, serum uric acid was 5.1 mg/100 ml, urine uric acid was 305 mg/24 h, and urine oxypurine was 4,408 μmol/24 h. Urine protein was 0.6 g/24 h. Roentgenograms of the chest, hands, and feet were unremarkable.

TABLE I

The P. Family: Serum Uric Acid and Erythrocyte Hypoxanthine-Guanine Phorphoribosyltransferase Activity (HGPRT)

Relationship	Erythrocyte HGPRT	Serum uric acid	Urine uric acid/creatinine
	nmol/h/mg	*mg/100 ml*	
Normal	87.3±16.6‡	<6.0 (females)§ <7.0 (males)§	<0.75‖
Propositus IV-1	34.2±6.1 (5)	11.8	0.67
Brother IV-2	78.6	7.1	
Mother III-1	51.3, 51.5	5.1	0.49
Grandmother II-1	72.2	6.4*	
Cousin III-4	76.4	4.3	0.47
Cousin IV-5	66.4	5.3	0.32
Cousin III-6	82.8	3.5	0.52
Cousin IV-6	77.9	4.0	0.56
Cousin IV-7	77.8	6.6	0.42
Cousin III-7	73.7	5.4	0.55
Cousin IV-8	72.6	7.3	0.40
Cousin III-9	84.4	5.4	0.47
Cousin IV-11	96.1	5.4	
Cousin IV-12	101.4	3.8	0.54
Cousin III-11	85.9	7.4	0.45
Cousin IV-14	96.5	5.3	
Cousin IV-15	96.2	4.7	
Cousin III-14	70.9	5.3	
Cousin IV-17	109.9	4.2	
Cousin IV-18	70.3	6.0	
Cousin IV-19	84.3	4.0	
Cousin IV-20	74.5	6.2	
Cousin III-15	95.8	6.2	

* Patient has Waldenström's macroglobulinemia.
‡ Normal value based on 265 patients.
§ Wyngaarden et al. (1).
‖ From Kaufman et al. (59).

The patient's family was studied with reference to serum urate, urinary uric acid, and erythrocyte hypoxanthine-guanine phosphoribosyltransferase. The results are given in Table I and Fig. 1.

METHODS

Radioactive isotopes, including [8-^{14}C]hypoxanthine (57 μCi/μmol or adjusted to 3.0 μCi/μmol with hypoxanthine), [8-^{14}C]adenine (54 μCi/μmol or 6.5 μCi/μmol), [carboxyl-^{14}C]orotic acid (10 μCi/μmol), and [8-^{14}C]guanine (60 μCi/μmol or adjusted to 6 μCi/μmol with guanine), were purchased from New England Nuclear, Boston, Mass. Tetrasodium phosphoribosylpyrophosphate (PP-ribose-P),[1] disodium AMP, disodium GMP, disodium IMP, disodium ribose-5-P, α-chymotrypsinogen A, ovalbumin, cytochrome *c* (type III), and disodium ATP were obtained from Sigma Chemical Company, St. Louis, Mo. XMP, dithiothreitol, disodium *p*-nitrophenyl-P, and crystalline bovine serum albumin were obtained from Calbiochem, San Diego, Calif. Methylphthalate and di-*n*-butylphathalate came from J. T. Baker Chemical Co., Phillipsburg, N. J. Ampholine, pH 3.5–7, was obtained from LKB Instruments Ltd., Stockholm, Sweden. Sephadex G-100 and blue dextran were obtained from Pharmacia (Canada) Ltd., Montreal, Quebec. All other regents were of the highest quality commercially available.

Peripheral circulating leukocytes were obtained by sedimentation in 6% dextran in 0.87% saline, lysis of erythrocytes in 0.58% ammonium chloride, and washing in 0.87% saline. Peripheral circulating erythrocytes were washed twice in 0.87% saline. The cells were stored at −70°C. Cultured diploid fibroblasts were harvested by trypsinization, washed in phosphate-buffered saline, and lysed by freezing and thawing twice in acetone and dry ice. Rectal mucosa was obtained by suction biopsy, washed in 0.87% saline, and frozen at −70°C. Before assay, the tissue was manually homogenized in 1 ml of 10 mM Tris-HCl pH 7.4.

Enzyme assays were performed on extracts dialyzed for 2 h at 4°C against 1 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA. Hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase were assayed in hemolysates by a previously described radiochemical technique (5). With leukocyte and fibroblast extracts, enzyme specific activity was calculated from the sum of the nucleotide and nucleoside products. PP-ribose-P synthetase was assayed by a two-step radiochemical method (18). Erythrocyte acid phosphatase was measured by the rate of *p*-nitrophenol formation at optical density 410 nm from 3 mM *p*-nitrophenyl-P in 50 mM sodium acetate buffer, pH 5.0, in a total volume of 300 μl. The latter reaction was allowed to proceed for 60 min at 37°C and was terminated by the addition of 700 μl of 0.2 N sodium hydroxide.

Kinetic studies with hemolysate were performed with initial velocity and product inhibition experiments. These experiments were repeated on at least four occasions with a different normal hemolysate for comparison. Up to 10% of the substrate was utilized, except for the lowest concentrations of hypoxanthine and guanine, where up to 25% substrate was consumed. Assays were found to be linear with time at the concentrations of substrates and inhibitors used in the kinetic studies. The apparent K_m's of substrates were determined from double reciprocal plots. The apparent K_i's of inhibitors were determined from plots of

[1] *Abbreviations used in this paper:* OMP, orotidine-5'-phosphate; PP-ribose-P, phosphoribosylpyrophosphate.

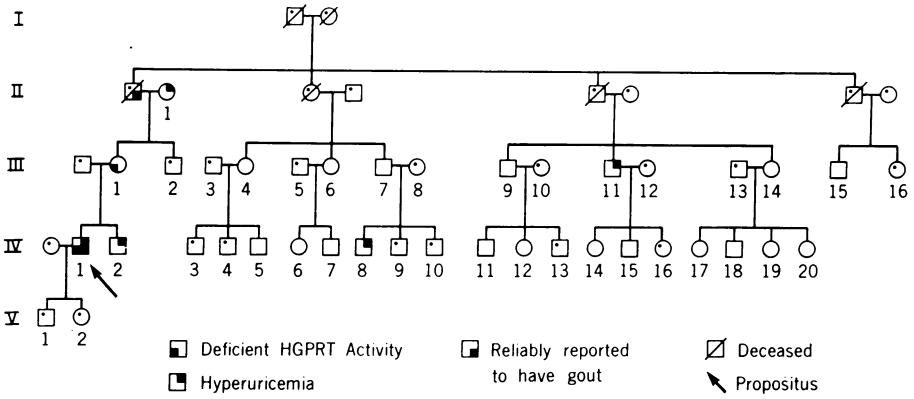

FIGURE 1 Pedigree of family with a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase. The proband is indicated by the arrow. The dot in the upper left quadrant indicates that no assay was performed.

inhibitor concentration versus the reciprocal of the velocity. Hill coefficients were determined from a log-log graph with V_{max} estimated from the plots described above. Similar kinetic studies of extracts of dialyzed cultured diploid fibroblasts were made inaccurate by the large quantities of 5′-nucleotidase. Assays included 5 mM AMP to inhibit this latter enzyme. The velocity of the reaction was calculated from the sum of the nucleotide and nucleoside products.

Hypoxanthine-guanine phosphoribosyltransferase stability was evaluated. For studies of thermal inactivation, lysed erythrocytes were diluted 1:4 with 10 mM Tris-HCl, pH 7.4. A 50-μl aliquot was heated at 80°C for 0–8 min. 950 μl of the same buffer was added, and 50 μl was removed for assay. Half-life in peripheral circulating erythrocytes was evaluated by a modification of the fractionation method of Danon and Marikovsky (19), with mixtures of dibutylphthalate and dimethylphthalate with densities of 1.084, 1.088, and 1.092.

Isoelectric focusing was performed according to the method of Vesterberg and Svensson (20), with an LKB 8100 ampholine electrofocusing column. Hemolysate 0.5 ml was dialyzed for 16 h against 1% glycine and mixed with 0% sucrose solution. Alternatively, leukocytes were isolated from 80 ml of fresh blood, lysed by freezing and thawing, and dialyzed for 4 h against 1% glycine. A 110-ml linear gradient from 0% to 40% sucrose was poured containing 1% ampholytes, pH 3.5–7. 600 V was applied for approximately 70 h at 4°C. The initial current was 3.5 mA, and this decreased to 0.8 mA by 24 h. The column was eluted from below with a Buchler multistatic pump (Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.) at the rate of 60 ml/h. The pH of every other fraction (1 ml) was measured at 4°C with a Radiometer 22 pH meter with a scale expander (Radiometer Co., Copenhagen, Denmark). Protein was quantitated by reading the absorbance at 280 nm with a Beckman DB-G spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). Enzyme activity was quantitated as described above.

Gel filtration was carried out at 4°C with Sephadex G-100 on a column 60 by 1.6 cm, previously equilibrated with 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, and 0.5 mM dithiothreitol. A 1-ml sample was applied, and 1.1-ml fractions were collected. The void volume, as quantitated by blue dextran elution, was 39.5 ml. The column was calibrated by using 5 mg/ml ovalbumin, 5 mg/ml α-chymotrypsinogen A, 2 mg/ml cytochrome *c*, and adenine phosphoribosyltransferase. 0.2 ml hemolysate was prepared for chromatography by adding 0.8 ml of the equilibrating buffer and centrifuging at 5,000 *g* for 10 min at 4°C. The supernatant protein solution was applied to the column. Elution of protein peaks was recorded by the absorbance at 280 nm by a LKB Uvicord II 8300 with an LKB 6520 recorder. The column fractions were assayed for adenine and hypoxanthine-guanine phosphoribosyltransferase activity. The Stokes radius for adenine phosphoribosyltransferase was 24.9 Å (21). The distribution coefficients (K_d) were calculated for the standard proteins and the normal and mutant hypoxanthine-guanine phosphoribosyltransferase (22). Standards separable from hemoglobin eluted from the column at the same volume whether they were applied alone or mixed with diluted hemolysate.

Sucrose gradients of 13 ml were prepared according to the method of Martin and Ames (23). Hemolysate was diluted 1:19 with 10 mM Tris-HCl, pH 7.4, and 100 μl of this solution was applied to the top of the gradient. Preparative ultracentrifugation was performed with an SW-41 rotor in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 39,000 rpm for 17 h at 4°C. The sucrose gradient was collected into fractions of 250 μl. Hemoglobin ($s_{20,w}$ 4.1) and adenine phosphoribosyltransferase ($s_{20,w}$ 3.35) (21) were used as the reference proteins and were quantitated by reading the absorbance at 420 nm and by enzyme assay, respectively.

The apparent molecular weight of hypoxanthine-guanine phosphoribosyltransferase was estimated from the observed values for the Stokes radius and the sedimentation coefficient (22).

Serum uric acid and urine uric acid were measured by the uricase assay (24). Urinary total oxypurines were quantitated by an enzymatic spectrophotometric method (25). Creatinine was measured by the method of Brod and Sirota (26). Erythrocyte PP-ribose-P was quantitated in fresh cells chilled immediately to 4°C by a radiochemical method (27). Protein was estimated by the method of Lowry et al. (28) with crystalline bovine serum albumin as a standard. The concentrations of compounds used in these studies were based on the description or assay provided by the manufacturer.

Diploid fibroblasts were grown in Falcon 30-ml tissue

culture flasks (Falcon Plastics, Div. of BioQuests, Oxnard, Calif.) in alpha minus medium supplemented with 15% fetal calf serum. Cell strains were established from two patients with partial hypoxanthine-guanine phosphoribosyltransferase and nine other patients with normal hypoxanthine-guanine phosphoribosyltransferase. For studies of azaguanine resistance, cells were trypsinized and counted in a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.), and 2×10^4 cells were plated in 4-oz Sani-glas bottles (Brockway Glass Co., Inc., Brockway, Pa.) to begin growth studies. 8-Azaguanine in concentrations of 0, 5, 10, 50, or 100 μM was added after 24 h of culture. Cells were fed every 2nd day and counted in duplicate in a Coulter counter after 7 days' growth. For studies of [^{14}C]hypoxanthine incorporation into nucleic acids, cultures were innoculated in duplicate with 1×10^5 cells and incubated for 30 h in standard medium. The medium was then aspirated from each culture and replaced by standard medium containing 0.1 mM [^{14}C]hypoxanthine adjusted to 0.1 μCi/μM. At the end of 3 days, the cells were harvested by trypsinization, washed, and precipitated in 5% cold trichloroacetic acid. The precipitate was collected on a 0.45 μM Millipore filter (Millipore Corp., Bedford, Mass.), which was washed with cold 5% trichloroacetic acid and cold water, dried, and counted in a Packard 2425 liquid scintillation spectrometer system (Packard Instrument Co., Inc., Downers Grove, Ill.).

Fresh peripheral erythrocytes for in vitro studies were collected at 4°C from the propositus and 18 normal subjects and washed in 0.87% saline. 20 μl of packed erythrocytes were incubated in a total volume of 100 μl containing 5.5 mM glucose, 10 mM inorganic phosphate, 154 mM saline, and 67 mM Tris-HCl, pH 7.4. The cell suspension was incubated for 1 h at 37°C. PP-ribose-P formation was quantitated by a two-step radiochemical assay (27). Alternatively, the incorporation of [^{14}C]orotic acid into nucleotide was measured by incubation of 10 μM [^{14}C]orotic acid under the same conditions. The [^{14}C]CO_2 released by orotidylic decarboxylase was collected onto a filter paper, moistened with 200 μl of hyamine, and placed in a polyethylene well. The reaction was stopped by the addition of 200 μl of 10% trichoroacetic acid, and the mixture was incubated for 30 min longer. The filter paper was removed and counted.

RESULTS

Family study

Erythrocyte hypoxanthine-guanine phosphoribosyltransferase was decreased in two members of the three generations of the family (Fig. 1, Table I). The propositus had enzyme activity 39% of normal, while his mother (III-1) had enzyme activity 59% of normal. Her serum uric acid was 5.1 mg/100 ml, and urine uric acid was 289 mg/24 h. No other persons assayed had a deficient enzyme. Four other family members (IV-2, II-1, IV-8, and III-11) were hyperuricemic, but their values for the serum uric acid were below 7.5 mg/100 ml. Two of these family members (IV-8 and III-11) had uric acid-to-creatinine ratios of 0.40 and 0.45 on a spot urine. Cousin III-11 had a urine uric acid of 401 mg/24 h. The maternal grandmother (II-1) had a secondary hyperuricemia related to Waldenström's macroglobulinemia. The maternal grandfather had a history of tophaceous gout.

Enzyme deficiency and disordered metabolism

The propositus was found to have evidence of uric acid overproduction, with an output of 1,279 mg/24 h on a purine-free diet. The urine uric-acid-to-creatinine

TABLE II

Metabolic Parameters in the Propositus and his Mother

Parameter studied	Propositus	Mother	Normal
Urine			
Uric acid, *mg/24* h	1,279	289	<590‡
Hypoxanthine/xanthine*	1.4		<1.0§
Erythrocyte			
Adenine phosphoribosyltransferase, *nmol/h/mg*	36.5	28.8	24.4±6.8 (265)
PP-ribose-P synthetase, *nmol/h/mg*	46.5	44.9	43.8±11.3 (240)
PP-ribose-P, *μM*	2.6		4.4±1.8‖
Hypoxanthine-guanine phosphoribosyltransferase			
Leukocyte, *nmol/h/mg*	95.4	113.1	143.2±36.8 (115)
Fibroblast, *nmol/h/mg*	47.2		70.2±12.0 (9)
Rectal mucosa, *nmol/h/mg*	19.1		62.1 (36.5–89.7) (5)

Figures in parentheses indicate number of subjects.

* On allopurinol 300 mg/day.

‡ From Seegmiller et al. (60).

§ From Sweetman and Nyhan (61).

‖ From Fox and Kelley (62).

ratio was 0.67. No other family member had clinical evidence of excessive production of uric acid. In erythrocytes the propositus had normal PP-ribose-P levels of 2.6 μM, normal PP-ribose-P synthetase level of 46.5 nmol/h/mg and a modest elevation of adenine phosphoribosyltransferase of 36.5 nmol/h/mg.

Hypoxanthine-guanine phosphoribosyltransferase in extracts from peripheral circulating leukocytes and cultured diploid skin fibroblasts was 95.4 and 47.2 nmol/h/mg, respectively, at the lower limits of normal (Table II). Enzyme activity in rectal mucosa, 19.1 nmol/h/mg, was diminished as compared to the range of normal established in five patients. The heterozygote (III-1) had normal activity in leukocytes and fibroblasts.

Properties of the deficient erythrocyte enzyme

The presence of a partial deficiency of erythrocyte enzyme activity that was nevertheless easy to detect provided a unique opportunity for study. We attempted to distinguish whether or not the abnormal quantity of enzyme was the result of a structural gene mutation.

Kinetic Properties

Initial velocity studies were performed with the three enzyme substrates, PP-ribose-P, guanine, and hypoxanthine. The apparent K_m's derived from double-reciprocal plots were found to be the same for the normal and the mutant as follows: 0.2 mM and 0.1 mM for PP-ribose-P, 2 μM and 2 μM for hypoxanthine, and 2 μM and 2 μM for guanine.

The effects of inhibition by GMP was evaluated with variable PP-ribose-P concentrations (Fig. 2). With the mutant enzyme, 0.2 mM GMP converted the hyperbolic kinetics to sigmoid kinetics, while this was not the case for the normal enzyme, as has been shown previously (29, 30). The corresponding Hill plots demonstrate that GMP converted the Hill coefficient of the mutant enzyme from 1.0 to 2.2, while the Hill coefficient for the normal enzyme remained unchanged. These changes were the same when either hypoxanthine or guanine was used as a substrate. Linear inhibition kinetics with 0.2 mM GMP were observed with variable hypoxanthine or guanine and a fixed saturating concentration of PP-ribose-P. Sigmoid kinetics could not be demonstrated with 0.2 mM GMP in extracts of cultured diploid fibroblasts from the propositus.

Inhibition kinetics were then studied with varying concentrations of GMP, XMP, IMP, and AMP. These compounds differ by substitution at the 2 and 6 positions of the purine ring. The inhibitory effects of GMP were distinctly different for the mutant enzyme. Linear inhibitory kinetics were found for the normal enzyme while cooperative inhibition of the mutant enzyme was

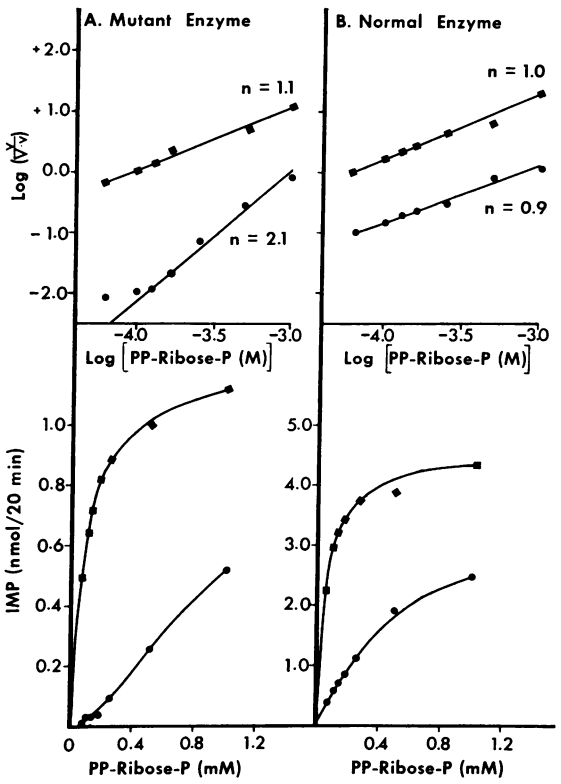

FIGURE 2 Inhibition of hypoxanthine-guanine phosphoribosyltransferase by GMP. Dialyzed mutant or normal hemolysate, 17.2 or 20.5 μg, was incubated for 20 min at 37°C with variable concentrations of PP-ribose-P without GMP (■—■) and with 0.2 mM GMP (●—●). Other conditions were as described in the Methods. The lower parts of the figure are substrate-velocity plots of the normal (A) and mutant (B) enzymes. The upper half of the figure contains the corresponding Hill plots and coefficients (n).

observed. The Hill coefficient was 2.2 for the mutant and 0.9 for the normal enzyme. The $I_{0.5}$ for the normal and mutant enzymes were 0.15 mM and 0.06 mM, respectively. An equal mixture of hemolysate from the propositus with normal hemolysate resulted in the expected intermediate level of activity and linear inhibition in the presence of varying concentrations of GMP. Under the same conditions, a mixture of normal and mutant enzyme hemolysate of equivalent activity resulted in a median level of activity and modified cooperative inhibition, as reflected by an increase of the Hill coefficient to 43% above the normal enzyme value. The hemolysate from the obligate heterozygote displayed normal linear kinetics with GMP. The effect of XMP was considerably less than the effect of GMP, although cooperative kinetics were observed for mutant as compared to the normal enzyme with the Hill coefficients being 1.3 and 0.9, respectively. With IMP the

mutant and normal enzymes were both inhibited linearly with an apparent K_i of 0.35 mM. Neither enzyme was inhibited by AMP.

Sigmoid kinetics for the normal enzyme have been previously reported with respect to PP-ribose-P when Mg was 0.05 mM (31). Studies with equimolar GMP and $MgCl_2$ (0.2 mM) were the same as the kinetics with GMP alone. In the presence of 0.05 mM $MgCl_2$, the kinetics for the normal and mutant enzymes were both sigmoidal at PP-ribose-P concentrations below 0.25 mM. With the concentration of PP-ribose-P constant at 0.25 mM, the apparent K_m for $MgCl_2$ was 0.4 mM for the mutant and normal enzymes.

Physical Properties

Stability. Enzyme stability, as assessed by thermal inactivation at 80°C and the activity in density-fractionated peripheral circulating erythrocytes, was indistinguishable from the similar studies on the normal enzyme.

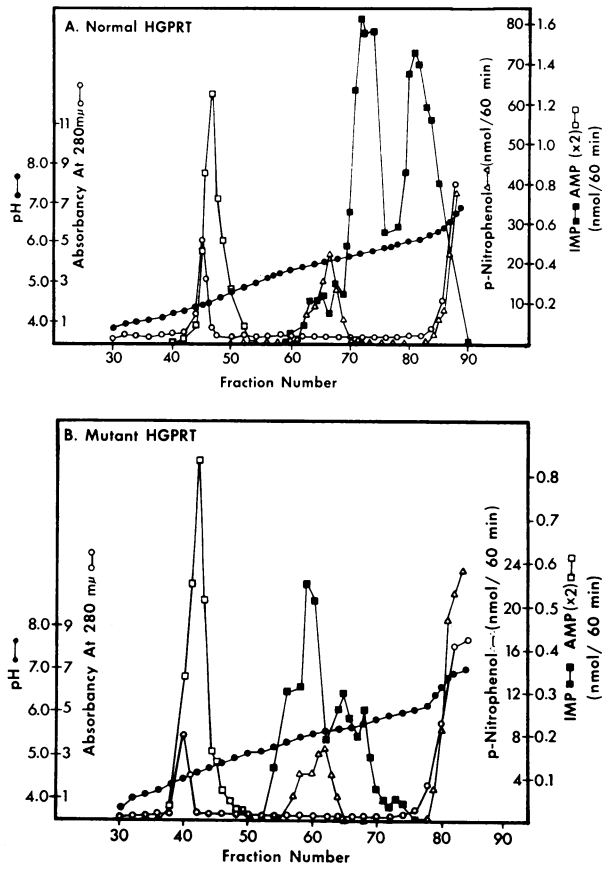

Figure 3 Isoelectric focusing of hemolysate. Hemolysate from a normal male (A) and the propositus (B) were studied as described in Methods. pH ●—●, protein ○—○, hypoxanthine-guanine phosphoribosyltransferase ■—■, adenine phosphoribosyltransferase □—□, and acid phosphatase △—△.

Electrical charge. Isoelectric focusing of hemolysate was used to assess the electrical charge properties of the mutant hypoxanthine-guanine phosphoribosyltransferase as compared to the normal enzyme. Fig. 3A demonstrates the results of a study on hemolysate from a man with normal enzyme activity. The isoelectric pH's of the hypoxanthine-guanine phosphoribosyltransferase isozymes were 5.76, 5.82, and 6.02 with a few minor components at pH 5.4–5.6. Adenine phosphoribosyltransferase had an isoelectric pH of 4.58. These were comparable to the values reported by Arnold and Kelley (15, 32) and Der Kaloustian et al. (33). One acid phosphatase peak had a pI of 5.54, and PP-ribose-P synthetase exhibited an isoelectric pH of 4.47. Hemolysate containing the mutant enzyme was isofocused twice under identical conditions (Fig. 3B). Adenine phosphoribosyltransferase, acid phosphatase, and PP-ribose-P synthetase had the same pI as normal hemolysate. Two major peaks of hypoxanthine-guanine phosphoribosyltransferase were evident with isoelectric pH's of 5.50 and 5.70 with a shoulder at pH 5.4, a distinct difference from normal. Isoelectric focusing of the hemolysate from the patient's mother gave a series of major enzyme peaks with isoelectric pH's of 5.35, 5.48, 5.58, 5.65, and 5.82. A shoulder was evident at pH 6.0. Enzyme from a normal woman had two major peaks, at pH 5.61 and pH 5.69, and minor components at pH 5.31 and pH 5.95.

Isoelectric focusing of leukocyte extract was comparable to that of the hemolysate. In leukocytes from a normal man the isoelectric pH's were 4.53 for adenine phosphoribosyltransferase and 5.88 for hypoxanthine-guanine phosphoribosyltransferase. Isoelectric pH's of leukocytes from the propositus were 4.55 for an adenine phosphoribosyltransferase and 5.40, 5.59, and 5.67 for hypoxanthine-guanine phosphoribosyltransferase.

The purified peaks of the mutant enzyme from hemolysate were pooled separately and studied in the presence of increasing GMP concentrations. The results were comparable to studies in hemolysate, with the Hill coefficient being 1.5 and 1.7 for peak I (pI 5.50) and peak II (pI 5.70), respectively.

Apparent molecular weight. The apparent molecular weights, as estimated from the Stokes radius and the sedimentation coefficient, were 48,000 for the mutant enzyme and 47,000 for the normal enzyme (Table III). These observations would appear to indicate that the human erythrocyte enzyme is composed of two subunits of about 25,000 daltons (34–36). In the presence of 0.5 mM $MgCl_2$ and GMP no alteration in Stokes radius could be documented with either the normal or mutant hypoxanthine-guanine phosphoribosyltransferase.

TABLE III

Estimation of the Apparent Molecular Weight of Erythrocyte Hypoxanthine-Guanine Phosphoribosyltransferase

	$s_{20,w}$*	Stokes radius‡	Apparent molecular weight
	$\times 10^{13}$ s	Å	daltons
Mutant	4.0	29.0	48,000
Control	3.9	29.0	47,000

* Estimated from sucrose gradient ultracentrifugation.
‡ Estimated from gel filtration studies.

Metabolism of intact cells

Fibroblasts. Cultured diploid fibroblasts from the propositus, two normal subjects, and one patient with a severe partial deficiency of hypoxanthine-guanine phosphoribosyltransferase were grown in different concentrations of azaguanine. The two normal cell lines and the fibroblasts from the propositus had substantially decreased growth with azaguanine 50–100 μM, while the patient with the severe deficiency was resistant to the effect of azaguanine (Table IV).

Cultured diploid fibroblasts from the propositus, four normal subjects, and one patient with a severe partial deficiency of hypoxanthine-guanine phosphoribosyltransferase were grown in the presence of [^{14}C]hypoxanthine. The normal cell lines incorporated 0.15–0.27 μmol hypoxanthine into nucleic acid/mg protein. The cell line from the propositus incorporated 0.21 μmol/mg, while those from the patient with the severe deficiency incorporated 0.05 μmol/mg (Table V).

Erythrocytes. Fresh peripheral erythrocytes from the propositus and normal subjects were studied for PP-ribose-P production and [^{14}C]orotic acid incorporation in vitro (Table VI). The erythrocytes from the propositus demonstrated substantially increased production of PP-ribose-P and elevated incorporation of [^{14}C]-orotic acid into pyrimidine nucleotides, as compared to the erythrocytes with normal hypoxanthine-guanine phosphoribosyltransferase. Increased activity of orotate phosphoribosyltransferase and OMP decarboxylase has been observed in erythrocytes from patients with the Lesch-Nyhan syndrome (37).

DISCUSSION

Hypoxanthine-guanine phosphoribosyltransferase catalyzes the transfer of the 5-phosphoribosyl moiety of PP-ribose-P to the 9 position of guanine, hypoxanthine, or xanthine to form GMP, IMP, or XMP, respectively. The enzyme is present in many tissues in man, with the highest specific activities in brain, placenta, gonads, erythrocytes, fibroblasts, and leukocytes (6). Although the actual physiological role of the enzyme is unclear, it is known to have a salvage function allowing the conservation of purine bases that might otherwise be further degraded to uric acid (38).

Deficiency of hypoxanthine-guanine phosphoribosyltransferase has profound effects in man (2–8). There is a striking overproduction of uric acid resulting in gouty arthritis, tophi, and renal calculi. Central nervous system dysfunction, including self-mutilation, choreo-athetosis, spasticity, and mental retardation, has been associated in variable degrees with this enzyme deficiency. The severest forms of these abnormalities are associated with a complete deficiency of hypoxanthine-guanine phosphoribosyltransferase in erythrocytes (5) and have been described as an X-linked, recessive syndrome by Lesch and Nyhan (3). Partial deficiencies of this enzyme with only mild or no neurological disease were later observed by Kelley et al. as an X-linked disorder (39). The patient described in this article and patients described by others (13, 40–43) fit into this latter category.

TABLE IV

Evaluation of Azaguanine Resistance in Cultured Diploid Fibroblasts

Cell line	HGPRT	Azaguanine 0 μM	5 μM	10 μM	50 μM	100 μM
		cells × 10^{-4}				
N_5	Normal	25.3	61.4	67.0	5.7	1.9
N_7	Normal	24.2	18.2	17.0	1.5	0.7
D_2	Severe deficiency	23.9	34.5	34.0	52.2	39.5
D_4*	Normal	25.6	27.0	28.7	4.1	1.0

Fibroblasts, 2 × 10^4 cells/medium, were plated and grown in increasing concentrations of azaguanine as described in methods.
* D_4 is the cell line derived from the propositus' hypoxanthine-guanine phosphoribosyltransferase (HGPRT).

TABLE V

Incorporation of [^{14}C]Hypoxanthine in.o Nucleic Acids by Fibroblast Strains

Cell strain	Hypoxanthine-guanine phosphoribosyl-transferase	Hypoxanthine incorporation
		μmol/mg protein
N_5	Normal	0.27
N_7	Normal	0.15
D_3	Normal	0.27
D_5	Normal	0.17
D_2	Severe deficiency	0.05
D_4 (Propositus)	Normal	0.21

N and *D* denote different cell lines in our laboratory.

Structural alterations of hypoxanthine-guanine phosphoribosyltransferase mutants have been described in circulating erythrocytes (10–17) and cultured cells (9, 44–47). These observations provide evidence that the mutations are on the structural gene coding for the enzyme, although the possibility of a regulator gene mutation has recently been suggested (48). The mutant enzyme studied in this report had two characteristics that distinguished it from the normal enzyme. Firstly, in hemolysate it was kinetically different by its abnormal sensitivity to inhibition by GMP. GMP was an allosteric inhibitor of the mutant enzyme and converted the hyperbolic kinetics with increasing PP-ribose-P concentrations to sigmoid kinetics. Altered kinetic properties of mutant enzymes have been previously described as substrate K_m and feedback inhibitor abnormalities (10, 14, 16, 17). Secondly, these kinetic abnormalities were accompanied by a discernible charge difference of the mutant enzyme protein from erythrocytes and leukocytes. The normal enzyme has been shown to have three isozymes (32, 33) on the basis of a nongenetic alteration of the enzyme protein (15). Multiple molecular forms have also been described in mouse and human cultured cell extracts (49). In this study the mutant enzyme from the patient had an abnormal isozyme pattern by isoelectric focusing, while the enzyme from the patient's mother appeared to have a series of isozymes that included the mutant and normal forms.

TABLE VI

Selected Studies of Purine and Pyrimidine Metabolism in Intact Erythrocytes

Subject	PP-ribose-P production	Subject	Incorporation of orotic acid
	nmol/h/ml		[C^{14}]CO_2 *·nmol/h/ml*
Propositus	48.0, 45.5	Propositus	21.4, 22.0
A	27.8	I	9.6
B	20.7	J	7.9
C	11.2	K	9.0
D	30.0	L	9.7
E	22.7	M	10.3
F	10.7	N	9.9
G	26.2	O	13.2
H	27.5	P	8.4
		Q	6.6
		R	5.8
Mean of normals	22.1		9.0
±SD	±7.5		±2.1

The complete deficiency of hypoxanthine-guanine phosphoribosyltransferase has been found in the brain, liver, fibroblasts, and erythrocytes (6). A discrepency between erythrocyte and leukocyte activity in one study has been attributed to enzyme lability in the absence of protein synthesis in the former (50). This hypothesis may not completely explain the discrepancy, since no correlation has been found between enzyme heat stability in vitro and enzyme degradation rates in vivo in a series of cell lines with mutant hypoxanthine-guanine phosphoribosyltransferase (51). Molecules of mutant hypoxanthine-guanine phosphoribosyltransferase are degraded much faster than the normal enzyme in mammalian cells (51). Therefore, the level of mutant enzyme specific activity of a particular tissue may be dependent upon the relative balance of synthesis and degradation of the mutant protein. In the patient evaluated, hypoxanthine-guanine phosphoribosyltransferase was partially deficient in erythrocytes and rectal mucosa but was normal in leukocytes and cultured diploid fibroblasts. The enzyme deficiency in erythrocytes was associated with increased synthesis of PP-ribose-P and increased functional activity of orotate phosphoribosyltransferase and orotidylic decarboxylase. In spite of a normal specific activity in leukocytes, the mutant enzyme was demonstrated by its abnormal electrical charge. The normal enzyme activity in fibroblasts was associated with sensitivity to growth in increasing concentrations of azaguanine and normal incorporation of hypoxanthine into nucleic acid. In contrast, intact fibroblasts from another patient with a more severe partial deficiency of hypoxanthine-guanine phosphoribosyltransferase had reduced enzyme function, as shown by resistance to the toxic effect of azaguanine and by a diminished incorporation of hypoxanthine into nucleic acid. Azaguanine is known to select for deficiencies of this enzyme and related disorders (44, 47, 52–55).

Other biological implications of these combined structural and functional alterations of the hypoxanthine-guanine phosphoribosyltransferase should be considered. (*a*) The different inhibitory properties of purine nu-

cleoside monophosphates substituted at the 2 and 6 positions (GMP and XMP) as compared to the 6-substituted compounds (IMP and AMP) suggest that the mutant enzyme may be altered near the binding site for the former compounds. (*b*) The presence of normal intracellular PP-ribose-P levels and increased synthesis of this compound in the erythrocytes of the propositus demonstrates a more subtle abnormality of PP-ribose-P metabolism. Increased turnover of this compound has been described in the erythrocytes from gouty patients (56) and has been associated with overproduction of uric acid (57). A direct inverse relationship between enzyme deficiency and erythrocyte PP-ribose-P levels has been demonstrated (58) and accounts for the normal concentration of this compound in the erythrocytes from the propositus. (*c*) Neurological disorders and erythrocyte enzyme specific activities are associated only poorly. A spinocerebellar syndrome, mental deficiency spasticity, and choreo-athetosis have been observed with erythrocyte hypoxanthine-guanine phosphoribosyltransferase activity as high as 5–11% of normal with hypoxanthine as a substrate and 1–2% of normal with guanine as a substrate (39, 43). Neurological disease has not been reported with erythrocyte enzyme activity above these values. However, a number of patients have been described with normal central nervous system function and less than 1% of normal activity (39–43, 50). Accordingly, our patient would not be expected to have neurological abnormalities.

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