Molecular Basis of Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency in a Patient with the Lesch-Nyhan Syndrome

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ABSTRACT We have investigated the molecular basis of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency in a patient who presented with the Lesch-Nyhan syndrome. A catalytically incompetent form of HPRT has been isolated from this patient's erythrocytes and lymphoblasts. This enzyme variant, which we have termed $HPRT_{Kinston}$, is indistinguishable from the normal enzyme in terms of its intracellular concentration and maximal velocity, but differs with respect to its isoelectric point (more basic) and Michaelis constants for both substrates (markedly elevated). The tryptic peptides of $HPRT_{Kinston}$ were mapped by reverse-phase high pressure liquid chromatography in an attempt to define the precise abnormality in its primary structure. Sequence analysis of the single aberrant tryptic peptide in $HPRT_{Kinston}$ revealed an aspartic acid to asparagine amino acid substitution at position 193. Electrophoretic analysis of the CNBr peptides of $HPRT_{Kinston}$ confirmed the location of the proposed mutation. This amino acid substitution can be explained by a single nucleotide change in the codon for aspartic acid 193 (GAC → AAC). This is the first specific mutation described at the molecular level in a patient with the Lesch-Nyhan syndrome.

INTRODUCTION

Progress in defining the molecular basis of inherited diseases in man has largely been limited to the study of mutations at the globin loci. While these studies have been useful in defining the molecular pathology of the hemoglobinopathies and thalassemia syndromes, their direct relevance to other inborn errors of metabolism, especially diseases that result from defects in intracellular metabolic enzymes, has yet to be defined.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT)[1] is a nonabundant intracellular enzyme that plays a key role in the regulation of purine metabolism in man. A partial deficiency of HPRT activity has been described in some male patients who present with hyperuricemia and an early onset of gout (1). A virtually complete absence of this enzyme is found in patients with the Lesch-Nyhan syndrome (2). This X-linked disease is characterized by overproduction of uric acid and a central nervous system disorder consisting of mental retardation, spasticity, choreoathetosis, and a compulsive form of self-mutilation. The extremely low levels of HPRT in human tissues has, until recently, precluded detailed studies of normal and mutant gene products.

Considerable progress in our understanding of human HPRT has occurred in the recent past. The entire amino acid sequence of HPRT purified from human erythrocytes was defined (3). In addition, a full-length cDNA for human HPRT has now been cloned and sequenced (4). These and other studies indicate that the human erythrocyte enzyme undergoes several posttranslational modifications including (*a*) the removal of the NH_2 terminal methionine followed by acetylation of the newly formed free NH_2-terminus (3), and (*b*) the partial deamidation of asparagine 106 (5). Finally, we have developed a sensitive peptide mapping procedure for studying the primary structure of human HPRT variants from limited quantities of enzyme (5). This method has been used to identify an amino acid substitution in a variant enzyme isolated

Received for publication 7 December 1982 and in revised form 18 January 1983.

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[1] *Abbreviations used in this paper*: HPLC, high pressure liquid chromatography; HPRT, hypoxanthine-guanine phosphoribosyltransferase.

from a patient who presented with a partial deficiency of HPRT activity and an early onset of gout (6).

The majority of HPRT-deficient patients who present with the Lesch-Nyhan syndrome have essentially undetectable levels of HPRT enzyme. Of the 25 unrelated Lesch-Nyhan patients studied to date, only two had >5% of normal HPRT enzyme levels in erythrocytes (7–10 and unpublished results). The enzyme from one of these patients was purified to near homogeneity from erythrocytes (11) and cultured lymphoblasts (10). This mutant enzyme, which we have termed $HPRT_{Kinston}$ (11), is expressed at normal levels (7, 8) but is essentially nonfunctional in vivo due to markedly elevated Michaelis constants for both substrates (12).

In this study we have attempted to define the precise molecular abnormality in $HPRT_{Kinston}$ by comparative analysis of CNBr and tryptic peptide fragments. These studies indicate that $HPRT_{Kinston}$ differs from the normal enzyme by a single amino acid substitution, aspartic acid to asparagine, at position 193.

METHODS

Enzyme purification. HPRT was purified from erythrocytes obtained from normal subjects and from the Lesch-Nyhan patient with the structurally altered enzyme $HPRT_{Kinston}$. The enzyme purifications were performed according to previously published procedures (11, 13). Prior to subsequent analyses, the purified enzymes were denatured, and the sulfhydryls were reduced and *S*-pyridylethylated (3).

Cyanogen bromide and trypsin digestion. Cleavage at methionine residues was affected by incubating the intact enzymes (2–3 nmol of each) in 70% formic acid containing CNBr (150 μg) for 24 h. The reaction was terminated by dilution with 10 vol of H_2O, and the peptides were recovered by lyophilization. CNBr digests of the normal and mutant enzymes were subjected to isoelectric focusing in the presence of 8 M urea as described (11). Peptides were visualized by staining with Coomassie Brilliant Blue.

The pyridylethylated enzymes (17 nmol of $HPRT_{Kinston}$ and 27 nmol of normal HPRT) were also digested with tosylphenylchloroketone-treated trypsin (Millipore Corp., Freehold, NJ) as described (5). Tryptic digests were fractionated by reverse-phase high pressure liquid chromatography (HPLC) using a Synchropak RP-P (0.41 × 25 cm) column (Synchrom Inc., Linden, IN). A detailed description of this peptide mapping procedure can be found elsewhere (5). Selected tryptic peptides were sequenced to the COOH-terminus by manual Edman degradation (3).

RESULTS

Enzyme purification. Normal HPRT was purified from hemolysate obtained from a single normal male subject, and from two pooled hemolysate mixtures, each containing individual units of blood from 330 different subjects. The HPRT from each of these preparations was indistinguishable in terms of subunit molecular weight (11), isoelectric points (11), and tryptic peptide map (6). The enzyme purified from pooled normal hemolysate was focused in denaturing isoelectric focusing gels as described (11) to screen for electrophoretic heterogeneity in the normal population. There was no evidence of a more basic electrophoretic variant in this normal enzyme mixture, even when the gels were heavily overloaded with enzyme (data not shown). Based on densitometer scans of these gels, we would have been able to detect the occurrence of a basic electrophoretic variant at a frequency of 1% or greater in the normal population.

$HPRT_{Kinston}$ was purified from erythrocytes (445 ml) of patient E.S. with an 18% recovery of enzyme activity. SDS-polyacrylamide gel electrophoresis indicated that the mutant enzyme was greater than 95% pure (11). Amino acid analysis of a portion (6%) of the pyridylethylated form of $HPRT_{Kinston}$ documented a final quantity of 19 nmol of enzyme for further study.

CNBr peptide maps. The mutation in $HPRT_{Kinston}$ was localized to a specific section of the enzyme by comparative analysis of the CNBr peptides. Portions of the normal and mutant enzymes were digested with CNBr and the digests were fractionated in analytical isoelectric focusing gels (Fig. 1). The only detectable difference was in the COOH terminal CNBr peptide (CB7). This CNBr peptide, which spans amino acids 157 to 217 in the normal enzyme (3), consistently demonstrated charge heterogeneity; CB7 from normal HPRT focused at two pH values, 8.8 and 9.2. Human HPRT normally exhibits isoelectric heterogeneity due to at least two posttranslational modifications (5). Only one of these modifications has been defined, the partial deamidation of asparagine 106 (5). The microheterogeneity in CB7 may be caused by a previously undescribed posttranslational modification of similar nature. As shown in Fig. 1, the COOH terminal CNBr peptide species in $HPRT_{Kinston}$ are both apparently shifted to higher pH values; the most basic of these peptides focused at the end of the gradient at the NaOH wick.

Comparative tryptic peptide mapping. The pyridylethylated enzymes ($HPRT_{Kinston}$-17 nmol and normal HPRT-27 nmol) were also digested with trypsin and the digests were separated by reverse-phase HPLC. Representative analytical separations are presented in Fig. 2. The only apparent difference between the normal and mutant peptide maps was that peptide 24b from $HPRT_{Kinston}$ consistently eluted at a slightly shorter retention time. This chromatographic abnormality was more clearly illustrated by cochromatographing equal quantities of the normal and mutant tryptic digests (Fig. 2). The aberrant peptide from $HPRT_{Kinston}$ is called 24b′.

We noted in the initial description of this peptide

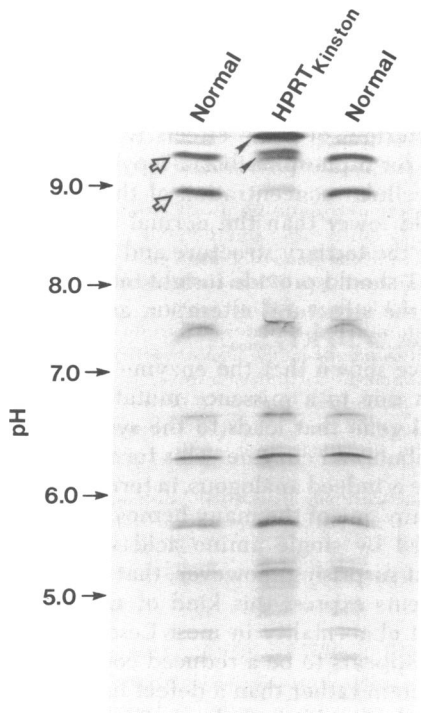

FIGURE 1 Denaturing isoelectric focusing of normal and mutant CNBr peptides. The CNBr digests of normal HPRT and $HPRT_{Kinston}$ (2 nmol) were focused in denaturing isoelectric focusing gels as described (11). The COOH terminal CNBr peptide from the normal enzyme (indicated by open arrows) was identified by focusing the purified CNBr peptides under identical conditions (data not shown). A description of the purification and characterization of the CNBr peptides from normal HPRT can be found in reference 3. These purified CNBr peptides were identical in terms of amino acid composition and NH_2-terminal sequence. The altered peptides in $HPRT_{Kinston}$ are noted by closed arrowheads.

mapping procedure that four peaks (at 4.6 min-salt peak, 6.1, 30.4, and 57.2 min in Fig. 2) contain complex mixtures of peptides. The complex peaks from the normal and mutant peptide maps were collected and rechromatographed under conditions that affected a suitable resolution of the component peptides. The specific methods used in these separations have been described (5). No additional chromatographic abnormalities were detected in these experiments (data not shown).

Amino acid sequence analysis of the aberrant peptide in $HPRT_{Kinston}$. Peptide 24b in normal HPRT spans amino acids 191 to 199 and is formed by the anomalous cleavage of Tyr_{190}-Ala_{191} and the expected cleavage of Arg_{199}-Asp_{200} (5). Peptide 24b from normal HPRT and peptide 24b' from $HPRT_{Kinston}$ were sequenced in their entirety by manual Edman degradation (Table I). These peptides differ by a single amino acid substitution at residue 193, aspartic acid in normal HPRT and asparagine in $HPRT_{Kinston}$.

DISCUSSION

The Lesch-Nyhan syndrome is a devastating inherited disease that is caused by a virtually complete deficiency of HPRT activity (2). Recent progress in defining the structure of the normal HPRT enzyme (3) and mRNA (4) has provided a basis for a detailed molecular characterization of this poorly understood disorder. In this report, we have studied the molecular basis for HPRT-deficiency in a patient with the Lesch-Nyhan syndrome.

$HPRT_{Kinston}$ is a structural variant of human HPRT that was isolated from a patient who presented with the classic manifestations of the Lesch-Nyhan syndrome (10, 11). A comparative tryptic-peptide analysis

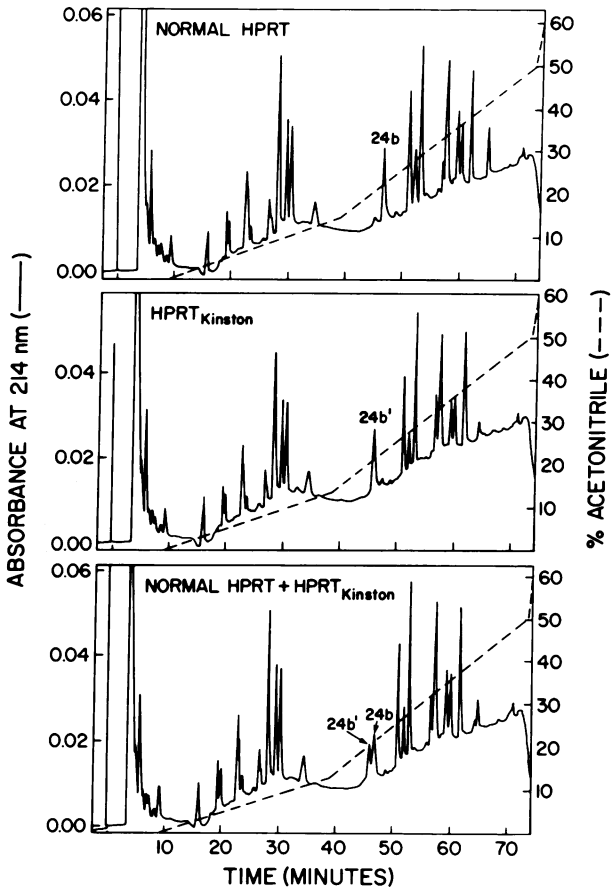

FIGURE 2 Tryptic peptide maps of normal HPRT and $HPRT_{Kinston}$. These chromatograms represent analytical separations of a portion of each digest: Normal HPRT, 200 pmol; $HPRT_{Kinston}$, 200 pmol; and a mixture, 100 pmol of each.

TABLE I
Manual Edman Degradation of Peptide 23b from Normal HPRT and Peptide 23b' from $HPRT_{Kinston}$

		23b-Normal HPRT		23b'-$HPRT_{Kinston}$	
Cycle	Position*	Residue	Yield (nmol)	Residue	Yield (nmol)
1	191	Ala	1.67	Ala	0.44
2	192	Leu	1.18	Leu	0.49
3	193	Asp	1.60	Asn	0.53
4	194	Tyr	0.98	Tyr	0.29
5	195	Asn	0.98	Asn	0.26
6	196	Glu	0.76	Glu	0.34
7	197	Tyr	0.32	Tyr	0.07
8	198	Phe	0.36	Phe	0.14
9	199	Arg	0.07	Arg	0.05

* With respect to the NH_2-terminus of the intact enzyme.

identified the molecular abnormality in this variant, an aspartic acid to asparagine substitution at position 193. A single base change in the codon for aspartic acid 193 can account for this amino acid substitution (GAC → AAC). Several independent findings support the proposed structural abnormality. The previously described electrophoretic alterations in $HPRT_{Kinston}$, a basic isoelectric point (10, 11, 14) and a cathodal migration during native polyacrylamide gel electrophoresis (10), are consistent with the proposed substitution of an acidic amino acid (aspartic acid) by a neutral amino acid (asparagine). In addition, electrophoretic analysis of CNBr digests demonstrated a basic alteration in the CNBr peptide from $HPRT_{Kinston}$ that contains the site of the mutation (residue 193).

We demonstrated, in a previous study, that each tryptic peptide of HPRT is recovered in high yield from the reverse-phase HPLC separation (5). This approach to peptide mapping assured a direct comparison of peptides that span the entire enzyme molecule. A second structural alteration in $HPRT_{Kinston}$ is unlikely because, with the exception of peptide 24b, the tryptic peptide maps of the normal and mutant enzyme were indistinguishable. This is supported by the CNBr peptide analysis, which demonstrated an electrophoretic abnormality in only one CNBr peptide of $HPRT_{Kinston}$. Finally, electrophoretic studies of HPRT from a large population of normal subjects indicated that the structural alteration in $HPRT_{Kinston}$ is not a polymorphism. We conclude that the aspartic acid to asparagine substitution at position 193 of $HPRT_{Kinston}$ is the only structural alteration in this variant and that it is responsible for the abnormalities in catalytic function.

The intracellular concentration and intrinsic activity (V_{max}) of $HPRT_{Kinston}$ are normal (7, 8, 10, 12). Markedly elevated Michaelis constants (K_m) for both substrates (5-phosphoribosyl-1-pyrophosphate and the purine base) are apparently responsible for the catalytic incompetence of this enzyme in vivo (12). The most deleterious of these effects is probably the elevated K_m for 5-phosphoribosyl-1-pyrophosphate since the intracellular concentration of this substrate is 20- to 100-fold lower than the normal K_m (15). Ongoing studies of the tertiary structure and active site of normal HPRT should provide insight into the relationship between the structural alteration and functional abnormalities in $HPRT_{Kinston}$.

We have shown that the enzyme deficiency in this patient is due to a missence mutation in the HPRT structural gene that leads to the synthesis of a functionally abnormal enzyme. This form of Lesch-Nyhan syndrome is indeed analogous, in terms of its molecular basis, to any one of the many hemoglobinopathies that are caused by single amino acid substitutions. It is somewhat surprising, however, that so few Lesch-Nyhan patients express this kind of mutation; the predominant abnormality in most Lesch-Nyhan patients (~92%) appears to be a reduced concentration of enzyme protein rather than a defect in enzyme function (7–10 and unpublished data). The mechanisms responsible for these apparent defects in gene expression are unknown. However, the recent isolation of cDNA probes for the HPRT gene (4, 16) should greatly facilitate the molecular characterization of these mutations.

In summary, we have identified the molecular abnormality in a rare HPRT variant isolated from a patient with the Lesch-Nyhan syndrome. A single amino acid substitution, aspartic acid to asparagine at residue 193, has apparently rendered the HPRT enzyme from this patient virtually nonfunctional in vivo.

ACKNOWLEDGMENTS

We would like to thank L. Landa for expert technical assistance, R. Kobayashi and Dr. G. Tarr for performing the manual Edman degradations, and Dr. D. Jolly and Dr. T. Friedmann for sharing unpublished data. This work was supported by grant R01-AM1905 from the National Institutes of Health. J. Wilson was supported by the Medical Scientist Training Program.

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