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Human hypoxanthine (guanine) phosphoribosyltransferase: An amino acid substitution in a mutant form of the enzyme isolated from a patient with gout

(reverse-phase HPLC/peptide mapping/mutant enzyme)

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ABSTRACT **We have investigated the molecular basis for a deficiency of the enzyme hypoxanthine (guanine) phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) in a patient with a severe form of gout. We reported in previous studies the isolation of a unique structural variant of HPRT from this patient's erythrocytes and cultured lymphoblasts. This enzyme variant, which is called $HPRT_{London}$, is characterized by a decreased concentration of HPRT protein in erythrocytes and lymphoblasts, a normal V_{max}, a 5-fold increased K_m for hypoxanthine, a normal isoelectric point, and an apparently smaller subunit molecular weight. Comparative peptide mapping experiments revealed a single abnormal tryptic peptide in $HPRT_{London}$. Edman degradation of the aberrant peptide from $HPRT_{London}$ identified a serine-to-leucine amino acid substitution at position 109. This substitution can be explained by a single nucleotide change in the codon for serine-109 (UCA → UUA). Thus a mutation at the HPRT locus has now been defined at the molecular level.**

A complete deficiency of hypoxanthine (guanine) phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) is associated with the Lesch–Nyhan syndrome (1), whereas a partial deficiency of the same enzyme leads to purine overproduction and gout (2). The precise mutational events that cause these X-linked enzyme deficiency states have remained undefined. One possible mechanism is a mutation within the coding sequences of the HPRT structural gene that leads to the synthesis of a structurally altered HPRT protein. Direct evidence in support of this hypothesis was provided by the isolation of five unique structural variants of HPRT from unrelated HPRT-deficient patients (3, 4).

Recent advances made in our understanding of the structure of the normal human HPRT enzyme have made it possible to begin a detailed investigation of the nature and consequences of mutations in its structural gene. We have recently defined the complete amino acid sequence of the normal human enzyme (5). The enzyme is 217 residues long and undergoes two post-translational modifications in erythrocytes: acetylation of the NH_2-terminal alanine (5) and partial deamidation of asparagine 106 (6). In addition, a sensitive method for mapping the tryptic peptides of the normal human enzyme has been developed (6).

In the present study, we have attempted to define the molecular abnormality in the previously described variant $HPRT_{London}$. This unique structural variant was purified from erythrocytes (3) and lymphoblasts (4) of a male patient who presented with hyperuricemia and an early onset of gout. Previous studies of the function and subunit structure of $HPRT_{London}$ demonstrated the following properties: (*i*) a decreased concentration of enzyme protein in both erythrocytes (3) and lymphoblasts (4); (*ii*) a normal V_{max}, a normal K_m for 5-phosphoribosyl-1-pyrophosphate, and a 5-fold increased K_m for hypoxanthine (unpublished data); (*iii*) a normal isoelectric point (3, 4) and migration during nondenaturing polyacrylamide gel electrophoresis (4); and (*iv*) an apparently smaller subunit molecular weight as evidenced by an increased mobility during Na$DodSO_4$/polyacrylamide gel electrophoresis (3, 4).

Our study of the tryptic peptides and amino acid composition of $HPRT_{London}$ revealed a single amino acid substitution (Ser → Leu) at position 109.

EXPERIMENTAL PROCEDURES

HPRT Purification, Trypsin Digestion, and Peptide Mapping. HPRT was purified from erythrocytes obtained from normal subjects and from a patient with the structurally altered enzyme $HPRT_{London}$. The enzyme purifications were performed according to published procedures (3, 7). In preparation for digestion with trypsin, the purified enzymes were denatured in 6 M guanidine·HCl and S-pyridylethylated (8). The pyridylethylated enzymes (20 nmol of normal HPRT and 10 nmol of $HPRT_{London}$) were digested with trypsin (1% by weight) as described (6). The trypsin digests were fractionated and the complex peaks were resolved by reverse-phase HPLC as described (6).

Amino Acid Composition and Sequence Analysis. The intact pyridylethylated enzymes were hydrolyzed in 6 M HCl for 24 hr and were analyzed for amino acid content as described (5). Peptide fragments were subjected to manual Edman degradation using the methods described by Tarr (9, 10). The resulting phenylthiohydantoin amino acids were analyzed by HPLC (11).

RESULTS

Purification and Amino Acid Composition of Normal HPRT and $HPRT_{London}$. Normal HPRT was purified from pooled normal hemolysate (330 individual units of normal blood) and from hemolysate obtained from a single normal male subject. These apparently normal enzymes were indistinguishable in terms of their subunit molecular weights (3), isoelectric points (3), and tryptic peptide patterns (data not shown). All subsequent studies of normal HPRT were performed on the enzyme purified from pooled hemolysate. $HPRT_{London}$ was purified with a 23% recovery of enzyme activity from erythrocytes (500 ml) of patient G.S. (3). Na$DodSO_4$/polyacrylamide gel electrophoresis indicated that the normal and mutant enzyme preparations were greater than 95% pure.

The purified enzymes were pyridylethylated and a portion

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Abbreviation: HPRT, hypoxanthine (guanine) phosphoribosyltransferase.

of each (6%) was analyzed for amino acid composition. All residues except alanine, glycine, and tryptophan were quantitated accurately. This analysis documented a total recovery of 20 nmol of normal HPRT and 10 nmol of $HPRT_{London}$. The compositions of the normal and variant enzymes, shown in Table 1, are in good agreement. Except for leucine and serine, individual amino acids differed between the normal and mutant enzyme by less than one residue per subunit; $HPRT_{London}$ exhibited an increased value for leucine and a decreased value for serine.

Peptide Mapping of Normal HPRT and $HPRT_{London}$. In an earlier study, we described a HPLC method for mapping the tryptic peptides of normal human erythrocyte HPRT (6). We have used this method to identify the structural alteration in $HPRT_{London}$. The pyridylethylated forms of normal HPRT and $HPRT_{London}$ were digested with trypsin for 6 and 18 hr, and the digests were resolved by reverse-phase HPLC. Representative chromatograms of these separations are shown in Fig. 1.

Although most peaks remained unchanged after 6 hr of trypsin digestion, several later-eluting peaks from both enzymes exhibited reproducible, time-dependent changes in their relative peak height. This was shown in a previous study to be due to the slow hydrolysis of the Lys-Phe bond at position 185–186 and the partial cleavage of several non-Lys/Arg bonds (6).

The peptide map of normal HPRT was very similar to that of $HPRT_{London}$ after 6 and 18 hr of digestion. Of particular importance was the identity of the COOH-terminal peptide (labeled 27 in Fig. 1) in the normal and mutant enzymes. This finding effectively rules out any mutation that modifies the structure of the COOH-terminus, such as a frameshift or premature chain termination mutation. The one difference in

Table 1. Amino acid composition of normal HPRT and $HPRT_{London}$

Residue	Composition,* residues per subunit: Normal HPRT	$HPRT_{London}$
Asx	29.3	29.3
Thr	10.9	11.2
Ser	13.8	11.7
Glx	16.0	15.6
Pro	9.5	10.1
Gly	ND†	ND†
Ala	10.9	ND†
Val	17.2	17.7
Met	5.7	5.8
Ile	12.0	11.8
Leu	20.8	21.8
Tyr	9.8	9.1
Phe	7.9	7.1
His	4.3	4.0
Lys	14.5	14.3
Cys‡	2.8	3.2
Arg	11.8	11.4
Trp	ND	ND

* Values represent duplicate analyses (50% of the sample per analysis) of a single hydrolysis of normal HPRT (1.9 nmol) and of $HPRT_{London}$ (0.6 nmol). ND, not determined.

† Not determined because an inconsistent base line precluded an accurate quantitation.

‡ Quantified as pyridylethylcysteine.

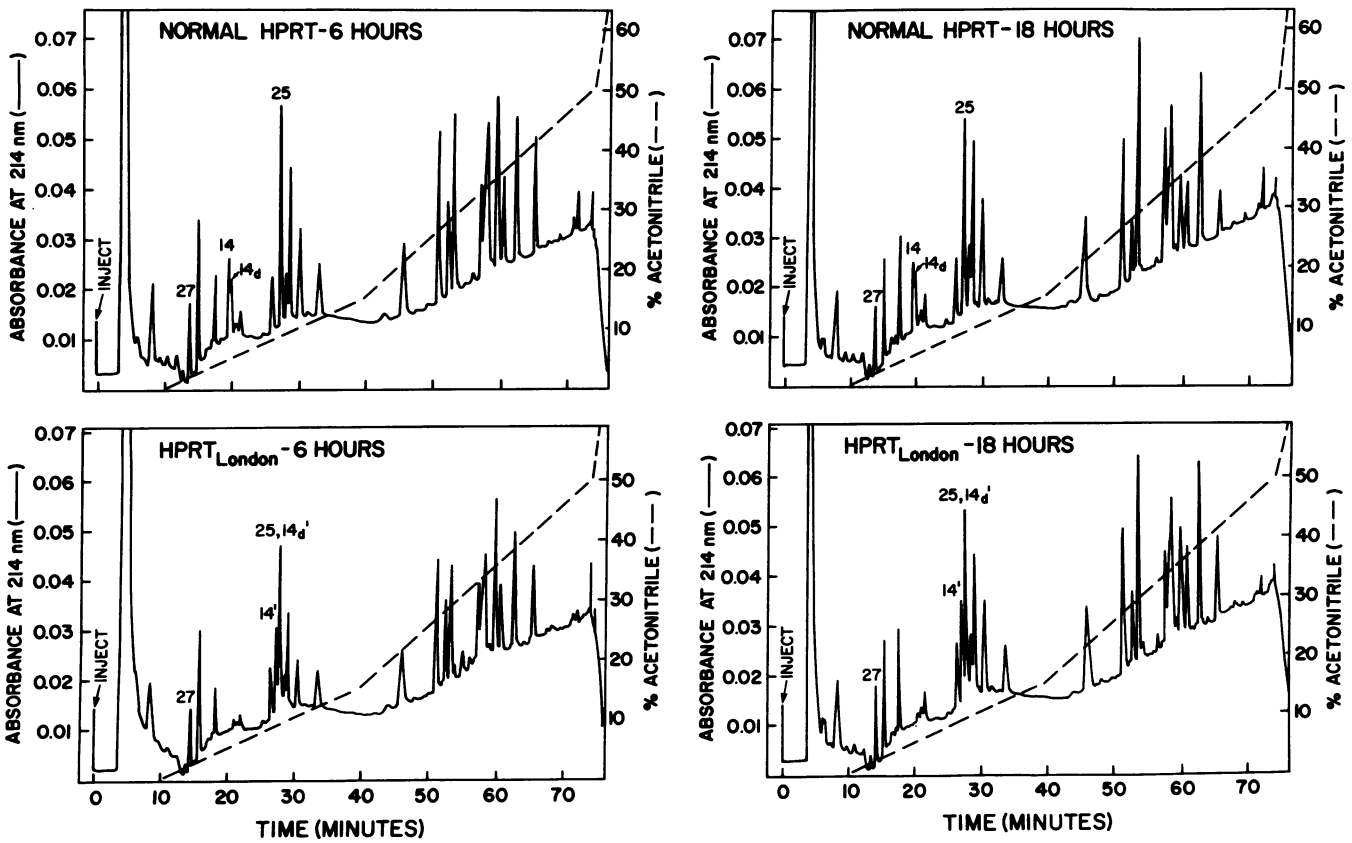

FIG. 1. Tryptic peptide maps of normal HPRT and $HPRT_{London}$. Trypsin digests were lyophilized, resuspended in 5% (vol/vol) formic acid, and fractionated by reverse-phase HPLC. These chromatograms represent analytical separations of a portion (300–400 pmol) of each digest: normal HPRT and $HPRT_{London}$ digested with trypsin for 6 hr and 18 hr. Preparative separations were performed under identical conditions with nearly identical peptide profiles.

Table 2. Manual Edman degradation of peptide 14 from normal HPRT and peptide 14′ from $HPRT_{London}$*

		Peptide 14, normal HPRT		Peptide 14′, $HPRT_{London}$	
Cycle	Position†	Residue	Yield, nmol	Residue	Yield, nmol
1	103	Ser	1.85	Ser	1.13
2	104	Tyr	1.12	Tyr	0.61
3	105	Cys	1.06	Cys	0.38
4	106	Asn	1.08	Asn	0.61
5	107	Asp	0.80	Asp	0.44
6	108	Gln	0.72	Gln	0.33
7	109	Ser	0.70	Leu	0.21
8	110	Thr	0.53	Thr	0.25
9	111	Gly	0.46	Gly	0.23
10	112	Asp	0.30	Asp	0.14
11	113	Ile	0.13	Ile	0.04
12	114	Lys	0.08	Lys	0.03

* A portion of each purified peptide (35% of 14 and 50% of 14′) was subjected to Edman degradation. Only one amino acid was released at each cycle of these degradations. We have reported 10 cycles of Edman degradation on peptide 14′ with identical results.

† Indicates the position of the amino acid with respect to the NH_2 terminus of the intact enzyme (5).

$HPRT_{London}$ that was noted at both time points was the absence of peptides 14 and 14_d and the appearance of a new peak at 27.1 min (labeled 14′ in Fig. 1).

Peptides that eluted as mixtures (peaks at 4.3, 8.2, 30.3, and 58.0 min in Fig. 1) were rechromatographed under different conditions to screen for alterations that may have been obscured in the original peptide map. The component peptides of these complex peaks were resolved by reverse-phase HPLC with a different column (6). These experiments detected no additional alterations in the chromatographic properties of the peptides in $HPRT_{London}$ as compared to the corresponding peptides of the normal enzyme (data not shown).

Amino Acid Sequence of Aberrant Peptides in $HPRT_{London}$. The entire sequences of peptide 14 from normal HPRT and peptide 14′ from $HPRT_{London}$ were established by manual Edman degradation. As shown in Table 2, these peptides differ by a single amino acid at position 109; a serine in normal HPRT and a leucine in $HPRT_{London}$. Peptide 14_d differs from peptide 14 by the presence of an aspartic acid in place of an asparagine at position 106, reflecting partial deamidation of the normal HPRT protein *in vivo* (6). The corresponding peptide in $HPRT_{London}$, peptide $14'_d$, eluted to the right of peptide 14′ as a mixture with peptide 25. The NH_2-terminal sequence of peptide $14'_d$ was established by subjecting the mixture to seven cycles of Edman degradation and subtracting the sequence contributed by peptide 25 (data not shown). This analysis confirmed the serine-to-leucine substitution at position 109 as well as the deamidation of asparagine-106.

These studies indicate that a serine-to-leucine substitution at position 109 has shifted the retention times of peptides 14 and 14_d from 19.5 min and 19.8 min in normal HPRT to 27.1 min and 27.7 min in $HPRT_{London}$. These results are summarized in Fig. 2.

DISCUSSION

We have defined the molecular abnormality in a structural variant of human HPRT ($HPRT_{London}$) isolated from a patient with a severe form of gout. These studies were complicated by the limited quantity of purified $HPRT_{London}$ that was available; approximately 250 μg of $HPRT_{London}$ was isolated from 500 ml of erythrocytes. A detailed analysis of the primary structure of $HPRT_{London}$, therefore, required sensitive methods of peptide mapping and protein sequence analysis. The tryptic peptides were mapped and purified by reverse-phase HPLC (6) and the sequences of peptide fragments were determined by using the micromethods of manual Edman degradation described by Tarr (9, 10).

A detailed analysis of the tryptic peptides of $HPRT_{London}$ revealed an amino acid substitution (Ser → Leu) at position 109. Evidence in support of this substitution was provided by amino acid analysis of the intact enzyme. Recent studies on the nucleotide sequence of human HPRT cDNA have established the codon for Ser-109 as UCA (12). The serine-to-leucine substitution in $HPRT_{London}$ can be explained by a single nucleotide change in this codon (UCA → UUA).

In a previous study, we demonstrated that each tryptic peptide predicted from the amino acid sequence of human HPRT is recovered in high yield from the reverse-phase HPLC separation (6). This approach to peptide mapping, therefore, assured a direct comparison of peptides that span the entire enzyme molecule. We conclude that a second site of mutation in $HPRT_{London}$ is highly unlikely because, with the exception of peptides 14 and 14_d, the tryptic peptide pattern of $HPRT_{London}$ was indistinguishable from the pattern of the normal enzyme. Further evidence against a second structural alteration in $HPRT_{London}$ was provided by rechromatographing the peaks from the original peptide map on a different reverse-phase column. No additional chromatographic abnormalities were detected in the peptides from $HPRT_{London}$ in these experiments.

$HPRT_{London}$ is indistinguishable from normal HPRT in terms of its isoelectric point and migration during nondenaturing polyacrylamide gel electrophoresis (3, 4). These findings are consistent with the proposed neutral-to-neutral amino acid substitution. A finding that was apparently inconsistent with an amino acid substitution was the faster migration that $HPRT_{London}$ exhibited during $NaDodSO_4$/polyacrylamide gel electrophoresis; the apparent subunit molecular weight of $HPRT_{London}$ was decreased in comparison to the normal value by the equivalent of 8–10 amino acid residues (3, 4). Our studies of the tryptic peptides and amino acid composition of $HPRT_{London}$ argue against a real difference in the length of this variant enzyme. A secondary change in the subunit molecular weight of $HPRT_{London}$ due to differential processing (i.e., glycosylation

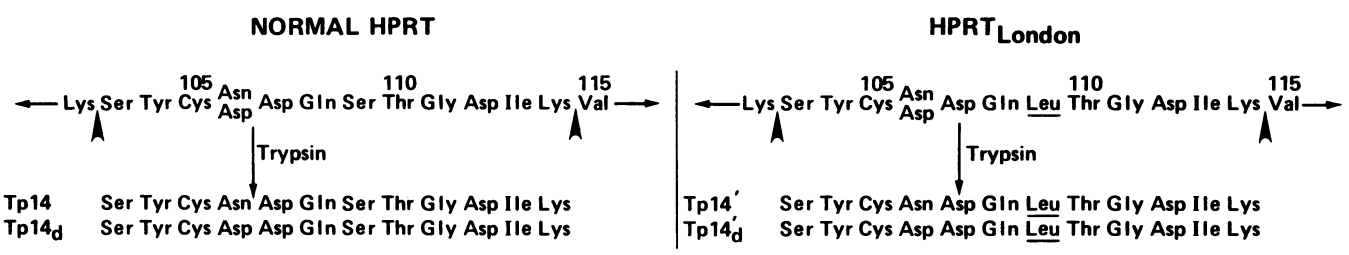

FIG. 2. Peptides 14 and 14_d from normal HPRT and the corresponding peptides from $HPRT_{London}$. Tp, tryptic peptide. The arrowheads indicate bonds that are cleaved by trypsin.

or phosphorylation) is also unlikely (3). A more likely explanation for the anomalous migration of $HPRT_{London}$ during $NaDodSO_4$/polyacrylamide gel electrophoresis is that the serine-to-leucine substitution has increased the binding of $NaDodSO_4$ to the enzyme, resulting in its faster electrophoretic mobility. There are, indeed, previous examples of single amino acid substitutions that have affected a protein's migration during $NaDodSO_4$/polyacrylamide gel electrophoresis (13, 14). de Jong *et al.* suggest that hydrophilic-to-hydrophobic amino acid substitutions, such as the serine-to-leucine substitution in $HPRT_{London}$, are more likely to increase $NaDodSO_4$ binding, giving the resultant increased electrophoretic mobility (13).

Mutant forms of HPRT have also been isolated from mutagenized mammalian somatic cells selected for HPRT deficiency (reviewed in ref. 15). Many of these abnormal enzymes exhibited altered mobilities during $NaDodSO_4$/polyacrylamide gel electrophoresis (16–20). This type of electrophoretic abnormality was used as evidence to suggest mutations that affect the length of the HPRT polypeptide (i.e., nonsense mutations, deletions, or mutations that modify proteolytic processing). Our studies on $HPRT_{London}$ provide structural evidence for a single amino acid substitution in an HPRT variant causing an artefactual decrease in its apparent subunit molecular weight. We conclude that an abnormal migration during $NaDodSO_4$/polyacrylamide gel electrophoresis does not necessarily indicate an alteration in the length of the HPRT protein. In addition, $NaDodSO_4$/polyacrylamide gel electrophoresis may prove to be an effective electrophoretic technique for detecting neutral amino acid substitutions in other structural variants of human HPRT.

In summary, we have identified the molecular abnormality in $HPRT_{London}$, a serine-to-leucine substitution at residue 109. This structural alteration results in an increased K_m for hypoxanthine and a decreased intracellular concentration of HPRT protein, both effects contributing to the deficiency of enzyme activity *in vivo*. A complete understanding of the relationship between the amino acid substitution and the kinetic abnormality and decreased intracellular enzyme concentration will require additional studies of the enzyme's intracellular turnover and three-dimensional structure.

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