Human Hypoxanthine-Guanine Phosphoribosyltransferase

STRUCTURAL ALTERATION IN A DY DysfunctionAL ENZYME VARIANT (HPRTMunich) ISOLATED FROM A PATIENT WITH GOUT*

James M. Wilson† and William N. Kelley
From the Departments of Internal Medicine and Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109

Gout is a heterogeneous metabolic disease characterized by elevated serum uric acid and recurrent attacks of arthritis. In 1967, Kelley et al. described a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase in some patients who presented with a severe form of gout at an early age (1). Subsequent studies indicated that hypoxanthine-guanine phosphoribosyltransferase deficiency underlies the clinical syndrome in approximately 1% of all gouty patients (2). In an attempt to define the molecular pathology of this inborn error of purine metabolism, we have begun a detailed investigation of the functional and structural properties of hypoxanthine-guanine phosphoribosyltransferase isolated from enzyme-deficient patients.

Four unique structural variants of human hypoxanthine-guanine phosphoribosyltransferase have now been isolated from unrelated patients who presented with gout and a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase activity (3, 4). These mutant enzymes have been termed HPRTLondon, HPRTToronto, HPRTAnnArbor, and HPRTMunich. Unique amino acid substitutions have been demonstrated in the former two variants (5, 6). The enzyme deficiency states associated with the London, Toronto, and Ann Arbor alleles are caused primarily by decreased concentrations of hypoxanthine-guanine phosphoribosyltransferase protein. The deficiency of hypoxanthine-guanine phosphoribosyltransferase activity associated with the Munich mutation, in contrast, results entirely from abnormalities in enzyme function.

HPRTMunich has been purified from erythrocytes and cultured lymphoblasts of a male patient of Yugoslavian descent (3, 4). The intracellular concentration of this variant enzyme is normal in all tissues studied (3, 4). Kinetic analyses documented profound abnormalities in the catalytic function of HPRTMunich; the maximal velocity is decreased 20-fold, while the \( K_m \) for hypoxanthine is markedly increased (108-fold) and the \( K_m \) for 5-phosphoribosyl-1-pyrophosphate is normal (3, 4, 7). In this report we have defined the structural abnormality in HPRTMunich. Possible relationships between this structural alteration and associated functional abnormalities are discussed.

EXPERIMENTAL PROCEDURES

Enzyme Purification and Pyridylethylation—The purification of normal hypoxanthine-guanine phosphoribosyltransferase from control erythrocytes (8) and HPRTMunich from erythrocytes of patient I. V. (3) has been described in detail. Prior to trypsin digestion, both enzyme preparations were denatured in guanidine HCl and the sulfhydryl groups were reduced and pyridylethylated (9).

Analysis of Tryptic Peptides—The pyridylethylated enzymes (60 nmol of normal hypoxanthine-guanine phosphoribosyltransferase and 22 nmol of HPRTMunich) were digested with tosylphenylalanyl chloromethyl ketone-treated trypsin (Worthington), and the tryptic digests were fractionated by reverse phase high pressure liquid chromatography with an RP-P Synchropak column (0.41 x 25 cm; SynChrom Inc.). Complex peaks in the original chromatogram were resolved by reverse phase high pressure liquid chromatography with a different column. A detailed description of these methods has been reported elsewhere (9).

RESULTS

Enzyme Purification—Normal hypoxanthine-guanine phosphoribosyltransferase was purified from hemolsate of a single male control and from two pooled hemolsate mixtures, each containing 330 units of individual packed red cells. We have shown previously that these enzyme preparations are indistinguishable in terms of subunit molecular weight (3), isoelectric points (3), and tryptic peptide fingerprint (5). Enzyme purified from pooled hemolsate was subjected to isoelectric focusing in denaturing gels to screen for the presence of a basic electrophoretic variant in the normal population. Basic hypoxanthine-guanine phosphoribosyltransferase subunits, of the kind associated with the HPRTMunich mutation, were undetectable in heavily overloaded gels (data not shown). Scanning densitometry indicated that we would have been able to detect a basic electrophoretic variant at a frequency of 1% or greater in the normal population.

HPRTMunich was purified 16,000-fold from 200 ml of erythrocytes with a 23% recovery of enzyme activity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated

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that the enzyme preparation was greater than 96% pure.

Following pyridylethylation, a portion of each purified enzyme (5%) was hydrolyzed in 6 N HCl and analyzed for amino acid composition (Table I); serine and threonine were not accurately quantified because of variable oxidation during the acid hydrolysis. This analysis indicated a final recovery of 22 nmol of HPRT<sub>Munich</sub> and 60 nmol of normal hypoxanthine-guanine phosphoribosyltransferase. Differences in composition between the normal and mutant enzyme were less than 0.9 residue/subunit for all amino acids except alanine, arginine, and phenylalanine.

**Tryptic Peptide Analysis**—Tryptic digests of the normal and mutant enzyme were fractionated by reverse phase high pressure liquid chromatography (Fig. 1). This approach to mapping the tryptic peptides of human hypoxanthine-guanine phosphoribosyltransferase has been described in detail elsewhere (9). An important feature of this technique is that each peptide predicted from the amino acid sequence is recovered in high yield, thereby assuring a comparative analysis of the entire molecule. Differences between the peptide map of normal hypoxanthine-guanine phosphoribosyltransferase described in Ref. 9 and the normal map presented in Fig. 1 are due to variability in the extent of trypsin digestion and in the performance of the individual reverse phase column.

The peptide map of normal hypoxanthine-guanine phosphoribosyltransferase was indistinguishable from that of HPRT<sub>Munich</sub> except that peptides 14 and 14<sub>d</sub> from HPRT<sub>Munich</sub> eluted at shorter retention times. These aberrant peptides are called 14<sup>'</sup> and 14<sup>'d</sup>. Each peak eluting before 28 min was rechromatographed under different conditions to screen for alterations in HPRT<sub>Munich</sub> that affected resolution of the component peptides (Fig. 2).

**TABLE I**

| Amino acids | Normal hypoxanthine-guanine phosphoribosyltransferase | HPRT<sub>Munich</sub> |
|-------------|--------------------------------------------------------|-----------------------|
|             | Residues/subunit                                       |                       |
| Ala         | 10.6 ± 0.4                                             | 11.8                  |
| Arg         | 12.4 ± 0.5                                             | 13.3                  |
| Asx         | 28.6 ± 0.9                                             | 28.0                  |
| Cys         | 5.3 ± 0.6                                              | 5.1                   |
| Glx         | 13.0 ± 1.2                                             | 13.6                  |
| Gly         | 16.4 ± 0.4                                             | 17.2                  |
| His         | 4.8 ± 0.5                                              | 4.6                   |
| Ile         | 12.9 ± 0.8                                             | 12.1                  |
| Leu         | 23.3 ± 0.8                                             | 24.0                  |
| Lys         | 17.0 ± 1.4                                             | 16.7                  |
| Met         | 5.5 ± 0.2                                              | 4.7                   |
| Phe         | 8.5 ± 0.5                                              | 7.5                   |
| Pro         | 9.2 ± 0.5                                              | 9.1                   |
| Ser         | NQ<sup>*</sup>                                          | NQ                    |
| Thr         | NQ<sup>*</sup>                                          | NQ                    |
| Tyr         | 9.4 ± 0.4                                              | 9.9                   |
| Val         | 17.6 ± 0.8                                             | 17.3                  |

* Mean ± 1 S.D. of duplicate analyses of two hydrolysates of normal hypoxanthine-guanine phosphoribosyltransferase (8 and 14 nmol/hydrolysate).

<sup>*</sup> Mean of duplicate analyses of a single hydrolysis of HPRT<sub>Munich</sub> (2 nmol/hydrolysate).

Peptide 13 from normal hypoxanthine-guanine phosphoribosyltransferase is a 12-residue peptide spanning amino acids 103 to 114 (9). Peptide 14<sub>d</sub> from HPRT<sub>Munich</sub> differs from peptide 14 at position 106, an asparagine in 14 and an aspartic acid in 14<sub>d</sub>. We have shown previously that this Asn/Asp heterogeneity is caused by the partial deamination of normal hypoxanthine-guanine phosphoribosyltransferase in vivo. Edman degradation established the entire sequence of peptides 14<sup>'</sup> and 14<sup>'d</sup> from HPRT<sub>Munich</sub> (Table II). These peptides differ from the corresponding peptides in the normal enzyme (14 and 14<sub>d</sub>) at their NH<sub>2</sub>-termini; the NH<sub>2</sub>-terminal serine in peptides 14 and 14<sub>d</sub> is missing in peptides 14<sup>'</sup> and 14<sup>'d</sup>. The peptide alterations in HPRT<sub>Munich</sub> are summarized in Fig. 3.
FIG. 2. Repurification of tryptic peptides in the void volume. Peptides that eluted in the void volume of the initial fractionation (4, 11, 13, and 26) were collected and rechromatographed on an ultrahydro-ODS column (Altex Co.) as described (9). The individual peptides are labeled. Peptide 26 eluted at approximately 8 min of each chromatogram (not shown). Represented are analytical separations of a portion (10%) of the original peaks. An identical perturbation in baseline was consistently present when blank gradients were run at the same sensitivity. HPRT, hypoxanthine-guanine phosphoribosyltransferase.

Table II

| Cycle | Normal hypoxanthine-guanine phosphoribosyltransferase* | HPRTMunich |
|-------|-------------------------------------------------------|-------------|
|       | 14          | 14a         | 14          | 14a         |
|       | Residue     | Nanomoles   | Residue     | Nanomoles   | Residue     | Nanomoles   | Residue     | Nanomoles   |
| 1     | Ser         | 1.44        | Ser         | 5.44        | Tyr         | 0.86        | Tyr         | 0.62        |
| 2     | Tyr         | 1.41        | Tyr         | 4.11        | Cys         | 0.34        | Cys         | 0.25        |
| 3     | Cys         | 0.95        | Cys         | 1.64        | Asn         | 0.13        | Asp         | 0.20        |
| 4     | Asn         | 0.76        | Asp         | 0.98        | Asp         | 0.29        | Asp         | 0.20        |
| 5     | Asp         | 0.71        | Asp         | 2.41        | Gln         | 0.10        | Gln         | 0.09        |
| 6     | Gln         | 0.16        | Gln         | 1.60        | Ser         | 0.30        | Ser         | 0.23        |
| 7     | Ser         | 0.44        | Ser         | 2.25        | Thr         | NQ*         | Thr         | NQ*         |
| 8     | Thr         | 0.24        | Thr         | 1.26        | Gly         | NQ*         | Gly         | NQ*         |
| 9     | Gly         | 0.25        | Gly         | 0.85        | Asp         | 0.04        | Asp         | 0.02        |
| 10    | Asp         | 0.18        | Asp         | 0.62        | Ile         | 0.13        | Ile         | 0.16        |
| 11    | Ile         | 0.09        | Ile         | 0.15        | Lys         | 0.05        | Lys         | 0.04        |
| 12    | Lys         | NQ          | Lys         | 0.03        |             |             |             |             |

* Data from Ref. 9.

A portion of each purified peptide (50%) was subjected to manual Edman degradation. We have repeated 5 cycles of Edman degradation on peptides 14' and 14'a with identical results. NQ, residues were clearly identified but not accurately quantified.
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**AMINO ACID SEQUENCE OF NORMAL HPRT**

| 100 | Phe | Ile | Arg | Leu | Lys | Ser | Tyr | Cys | Asp | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys | Val | Ile | Gly |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 105 | Ser | Tyr | Cys | Asn | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys | Val | Ile | Gly |
| 110 |

**TRYPTIC PEPTIDES OF NORMAL HPRT**

| TRYPTIC PEPTIDE 14 | Ser | Tyr | Cys | Asn | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| TRYPTIC PEPTIDE 14d | Ser | Tyr | Cys | Asp | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys |

**CORRESPONDING PEPTIDES IN HPRT_MUNICH**

| TRYPTIC PEPTIDE 14' | Try | Cys | Asn | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys |
| TRYPTIC PEPTIDE 14d' | Try | Cys | Asn | Asp | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys |

**POSTULATED ABNORMALITY IN HPRT_MUNICH**

| 100 | Phe | Ile | Arg | Leu | Lys | Ser | Tyr | Cys | Asp | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys | Val | Ile | Gly |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 105 | Ser | Tyr | Cys | Asn | Asp | Gln | Ser | Thr | Gly | Asp | Ile | Lys | Val | Ile | Gly |
| 110 |

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J M Wilson and W N Kelley

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