Hypoxanthine-Guanine Phosphoribosyltransferase

Genetic Evidence for Identical Mutations in Two Partially Deficient Subjects

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Abstract

In past reports of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency a marked degree of molecular heterogeneity has been noted. We have previously described two apparently unrelated subjects with partial HPRT deficiency, G.S. and D.B., who have a mutant form of HPRT with remarkably similar alterations in physical and kinetic properties. The mutation in G.S. is a serine to leucine substitution at amino acid 110 as determined by amino acid sequence analysis. This mutant enzyme has been designated $HPRT_{London}$.

We have examined HPRT cDNA from D.B. using two different methods to determine if the similar properties of mutant HPRT from these two subjects are the result of a common mutation. HPRT cDNA clones were obtained by routine cloning techniques and by polymerase chain reaction amplification of single-stranded cDNA reverse transcribed from mRNA derived from subject D.B. Dideoxynucleotide sequencing revealed a single mutation, a C to T transition at bp 329 in clones generated by both methods. This mutation in D.B. predicts the identical amino acid substitution described in $HPRT_{London}$.

A C to T nucleotide transition at 329 in D.B. creates an *Hpa* I site in exon 4 of the HPRT gene. Southern blot analysis of genomic DNA isolated from lymphoblasts derived from G.S. and D.B. revealed that both have this additional *Hpa* I site, indicating that the similarly altered protein sequence is due to the identical transition in the HPRT gene.

Introduction

Hypoxanthine-guanine phosphoribosyltransferase (HPRT)[1] is a purine salvage enzyme that catalyzes the conversion of hypoxanthine and guanine to inosine monophosphate and guanosine monophosphate, respectively. Partial deficiency of HPRT results in hyperuricemia, hyperuricaciduria, uric acid nephrolithiasis, and precocious gout (1). Virtually complete deficiency of HPRT causes the Lesch-Nyhan syndrome (2). In addition to hyperuricemia and hyperuricaciduria, this disease is characterized by growth and mental retardation, spasticity, choreoathetosis, and compulsive self-mutilation (3).

Previous evidence suggests that new and independent mutations sustain these disorders in the population (4). We have previously examined this hypothesis by analyzing 24 unrelated HPRT-deficient patients with regard to HPRT enzyme activity and intracellular concentration, physical and kinetic properties, mRNA levels, and restriction fragment length polymorphisms. Substantial heterogeneity was demonstrated with 67% of cases studied representing different mutations (5).

On the basis of these observations, HPRT-deficient subjects were classified into 16 types (5). 3 of these 16 categories (types I, XI, and XVI) contain more than one mutant. Type I has recently been shown to be heterogeneous by cDNA sequencing (6, 7), and definition of the mutations in type XI at the nucleotide level should result in further subdivision of this group.

Similarities between D.B. and G.S., the two partially deficient subjects with type XI HPRT deficiency, extend beyond DNA haplotype and the presence or absence of HPRT mRNA. Analyses of HPRT from these subjects are similar with respect to enzyme activity, intracellular concentration, kinetic and catalytic parameters, and physical properties. The amino acid substitution in HPRT purified from cultured cells derived from G.S. ($HPRT_{London}$) has been determined to be a serine to leucine substitution at position 110 (8).

The phenotypic similarity of these two mutant proteins raised the question of genotypic identity. Therefore, we have cloned HPRT sequences from mRNA isolated from EBV-transformed lymphocytes derived from subject D.B. Additionally, we have examined the entire coding sequence using the polymerase chain reaction (PCR) to amplify single-stranded cDNA reverse transcribed from D.B.'s mRNA. Dideoxynucleotide sequencing of the HPRT cDNA from D.B. revealed a C to T transition at base position 329 that causes a serine to leucine substitution at amino acid 110. This point mutation creates an *Hpa* I site in the HPRT gene that allows confirmation at the genomic DNA level that both $HPRT_{London}$ and HPRT from patient D.B. have identical mutations.

Methods

Cell lines. Lymphoblastoid cell lines from patients G.S., D.B., and subjects with normal HPRT activity were established and maintained

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1. *Abbreviations used in this paper:* ds, double-stranded; HPRT, hypoxanthine-guanine phosphoribosyltransferase; PCR, polymerase chain reaction; P_t, relative probability.

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as described (9). GM1416, which is tetraploid for the X chromosome, was obtained from the Human Mutant Cell Repository, Camden, NJ.

RNA isolation, cDNA cloning, and PCR amplification. RNA was extracted from B lymphoblasts by guanidium isothiocyanate (10). Poly $(A)^+$ mRNA was purified by oligo d(T) cellulose column chromatography (11).

10 μg of poly $(A)^+$ mRNA derived from D.B. was used to synthesize an oligo d(T)-primed cDNA library. The first strand was synthesized according to Okayama and Berg (12), followed by treatment with 20 U/ml RNase H, 40 U/ml *Escherichia coli* DNA ligase, 100 U/ml *E. coli* DNA polymerase holoenzyme, and 90 μM dNTPs (13). This method yielded ~ 1.0 μg of double-stranded (ds) cDNA with an average length of 1.8 kb (range 0.7 to 3.0 kb). One third of the dsDNA was tailed with 15–20 d(C) residues using terminal deoxynucleotidyltransferase (14). *Pst* I-cut, d(G)-tailed pBR322 was annealed to the poly-d(C)–tailed dscDNA. *E. coli* (strain RR1) were transformed with recombinant plasmids as described (15).

Approximately 23,000 recombinants were transferred to nitrocellulose paper disks and screened with human HPRT cDNA sequences labeled with [α-^{32}P]dCTP by the hexadeoxynucleotide priming method of Feinberg and Vogelstein (16). One positive recombinant, pHDB1, was isolated, cut with *Pst* I, mapped with *Hae* III and *Hin*d III, and recloned into M13mp18 for dideoxynucleotide sequencing (17) using HPRT-specific primers and the universal primer (6).

PCR-amplified cDNA clones of D.B. were generated by reverse transcribing (12) 1 μg total cellular RNA using an HPRT-specific reverse transcriptase primer and taking one-fifth of this reaction up in a buffer containing 40 mM KCl, 0.01% gelatin, 2 mM DTT, 1.0 μg of each PCR primer, and 1 U Taq polymerase in 50 μl total reaction volume. The sequences of the PCR primers and HPRT-specific primer for the first-strand reaction are given in Fig. 1. Each cycle of the PCR reaction, which was repeated 30 times, consisted of a 1-min denaturation step at 94°C followed by a 3-min annealing and extension step at 72°C. After 30 cycles a 10-min extension step at 72°C was done. These experiments were performed in a Perkin Elmer Cetus thermocycler (Perkin Elmer Cetus, Norwalk, CT) (18).

The PCR-amplified products were gel purified, cleaved with *Eco* RI and *Bam* HI, and cloned into M13mp18 and M13mp19. The recombinant clones were sequenced in both orientations as previously described (6).

Genomic DNA isolation and analysis. Genomic DNA was isolated from B lymphoblast cell lines G.S., D.B., GM1416 (4X), and J.R. (normal) and digested with *Hin*d III and *Hpa* I. The DNA was then fractionated by electrophoresis in 0.8% agarose gels and blotted onto nitrocellulose (19). Genomic blots were probed with ^{32}P-labeled normal HPRT cDNA sequences.

Determination of probability of β-turn occurrence. The relative probability (P_t) of β-turn occurrence from amino acids 104 to 114 was calculated as $P_t = f_i \times (f_{i+1}) \times (f_{i+2}) \times (f_{i+3})$ where f_i, f_{i+1}, f_{i+2}, and f_{i+3} are the frequencies of occurrence of a certain residue at the first, second, third, or fourth positions of a tetrapeptide sequence of a β-turn (20).

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HPRT1                    GAACTGATAG
                       975       965

HTPCR1
          CACGAATTCCTCCTCCTGAGCAGTCAGCCCGCGC
        67                                94

HTPCR2    GTGGGATCCAGATGTTTCCAAACTCAACTTGAACTCTC
        802                                771
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Figure 1. HPRT-specific PCR primers for the reverse transcriptase reaction (HTPCR3) and for PCR (HTPCR1 and HTPCR2). The bold type indicates *Eco* RI and *Bam* HI sites, added to the 5′ end of the primers for directional cloning into M13 for sequencing. The nucleotides in each primer that anneal to HPRT sequences are indicated.

The values for f_n of residues at these positions were determined by Chou and Fasman (20). The cutoff value for predicting a β-turn was chosen as $P_t = 1.0 \times 10^{-4}$ (21).

Results

pHPDB1 contains all but 198 bp of coding sequence and differs from the normal HPRT sequence at a single nucleotide. At base position 329 in exon 4, a C to T transition was found that predicts a serine to leucine substitution at amino acid 110. To obtain the remainder of the coding sequences and confirmation of the mutation in multiple independent clones of D.B., PCR amplification was done as described in Methods and Fig. 1. PCR-amplified sequences from D.B. confirmed the C to T substitution at 329 found by cDNA cloning. No other deviations from normal sequence were noted.

This transition, which creates an *Hpa* I site (GTCAAC to GTTAAC), allows confirmation at the genomic level that the serine to leucine substitutions in G.S. and D.B. are due to the same mutations. Southern blot analysis showed that a 4.9-kb *Hin*d III fragment that contains exon 4 (22, 23) is cleaved by *Hpa* I in genomic DNA from both G.S. and D.B. (Fig. 2, lanes *1* and *3*) but not from a normal cell line (lane *4*) or GM1416 (lane *2*).

The P_t values for normal HPRT and HPRT from D.B. and G.S. were determined and plotted for the region surrounding the serine to leucine substitution (Fig. 3). In the case of normal HPRT there are three sites for which the probability of β-turn occurrence is $> 1 \times 10^{-4}$ (*solid line*). The serine to leucine substitution dramatically affects the probability of β-turn occurrence in two of the three sites (*dashed line*).

Discussion

We previously demonstrated that $HPRT_{London}$ and HPRT from subject D.B. constitute a distinct HPRT phenotype (5, 8). In this study we show that the genetic basis for this observation

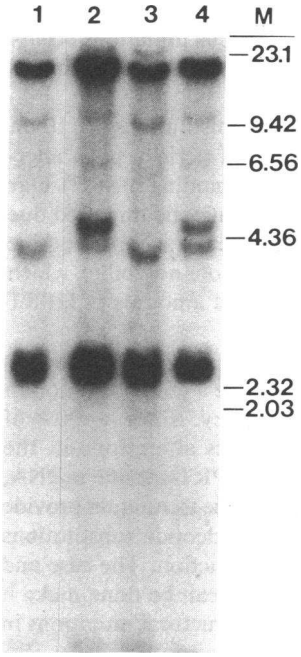

Figure 2. Southern blot of *Hin*d III/*Hpa* I–digested genomic DNA demonstrating the presence of an additional *Hpa* I site in G.S. and D.B. Lanes *1–4* correspond to DNA from G.S., GM1416 (4X), D.B., and J.R. (normal), respectively. The X-related 4.9-kb *Hin*d III fragment containing exon 4 is cleaved by *Hpa* I in G.S. and D.B. DNA only.

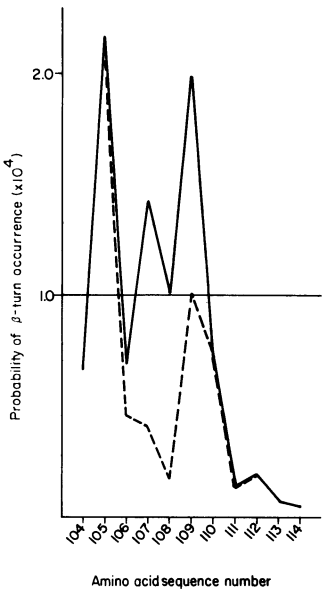

Figure 3. Probability of β-turn occurrence from amino acids 104 to 114 for normal HPRT protein (——) and HPRT from G.S. and D.B. (– – –). The probability value of a tetrapeptide composed of i–i + 3 is plotted against i, where i represents the amino acid sequence number. A cutoff value of 1×10^{-4} is represented by the horizontal line.

is an identical mutation within the HPRT coding sequence. In view of the striking heterogeneity of HPRT mutations this genotypic identity is remarkable.

There are three possible explanations for the origin of this mutation in these two individuals: (*a*) common ancestry of the affected individuals (a single mutational event); (*b*) independent events at a mutational "hot spot"; and (*c*) coincidental independent mutational events. Unfortunately we cannot determine if this mutant allele is shared by G.S. and D.B. because of common ancestry since G.S. is adopted. In the case of D.B. there is no family history of gout or kidney stones, suggesting that this mutation arose within a recent generation. Mutational hot spots are most often found in GC-rich areas, with transitions occurring in a CpG or CpC dinucleotide context (24–26). No CpC or CpG dinucleotide exists at the transitional position in $HPRT_{London}$ (CAGTCAACA). Thus the classification of codon 110 in the HPRT gene as a hot spot for transitional mutations is unlikely in the context in which nucleotide 329 is found. The frequency of spontaneous mutations occurring at this position can only be addressed by a more extensive survey.

The impact of the serine to leucine substitutions at 110 on enzymatic function is probably a result of steric or polar alterations within the putative hypoxanthine binding site (27). Our calculations predict that proper folding may be impaired due to an alteration in the β-turn structure near the hypoxanthine binding pocket. This may in turn affect protein stability, which could account for the lower than normal amounts of HPRT protein in G.S. and D.B. (5, 28).

cDNA cloning and PCR amplification of HPRT mRNA have been used in this study to determine a point mutation in a patient with partial HPRT deficiency. Since ~ 90% of HPRT-deficient subjects show no gross alterations in the HPRT gene, have normal amounts of HPRT-specific mRNA, and have low levels of HPRT protein, these techniques provide a direct means for determination of nucleotide substitutions causing altered protein structure and function. The ease and rapidity with which the PCR technique can be done make it particularly useful for further defining structural mutations in HPRT.

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