

Molecular Abnormality of a Phosphoglycerate Kinase Variant (PGK-Alabama)

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ABSTRACT: The molecular abnormality of a phosphoglycerate kinase variant associated with severe red cell enzyme deficiency (about 4% of normal) and episodes of hemolysis with jaundice was examined. The Michaelis constants for the substrates and co-enzymes (1,3-diphosphoglycerate, 3-phosphoglycerate, ATP and ADP) were not grossly different from that of normal. However, the variant enzyme was very labile *in vitro*. Nucleotide sequence analysis of the variant cDNA revealed a deletion of codon AAG in exon 7. The codon deletion should result in the deletion of one of the tandem lysine residues existing at amino acid 190-191 of the enzyme protein. Based on the three dimensional structure of the protein, molecular instability could be induced by the deletion of a lysine residue.

Keywords: phosphoglycerate kinase; enzyme deficiency; codon deletion, amino acid deletion; jaundice

INTRODUCTION

Phosphoglycerate kinase (ATP: 3-phosphoglycerate 1 - phosphotransferase EC 2,7,2,3: PGK) plays an important role for ATP generation in the glycolytic pathway. This enzyme exists universally in various tissues, including red blood cells, and is encoded by a single structural gene on the X-chromosome, band q13 in humans (1). The mature enzyme consists of 416 amino acid residues with acetyl-serine at the NH₂-terminal and isoleucine at the COOH-terminal, and the monomeric enzyme (MW about 48,000 dalton) is catalytically active (2). An inherited deficiency of this enzyme is often associated with chronic hemolytic anemia and neurological disorders. We determined the molecular abnormality of a novel

PGK variant associated with severe red cell enzyme deficiency.

MATERIALS AND METHODS

Patient

The patient is a 37 year old white male school teacher who has been in excellent general health. He first came to medical attention at age 20, when he had a self-limited febrile illness associated with a high bilirubin level. He experienced no further episodes of clinical jaundice until March, 1993, when he was 36 years old. At that time he had a fever and malaise and frank jaundice with a bilirubin of 8.3 mg%. The patient was hospitalized briefly with a clinical diagnosis of "hepatitis

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versus gallbladder diseases". No definite diagnosis was made. His liver function tests at that time were fairly normal with only some elevation of the SGPT. There is no history of any jaundice in any of his siblings, and no history of transfusion requirements in the family.

In April, 1994, he again developed jaundice and had a thorough evaluation by a gastroenterologist. At this time his hemoglobin was 14.1 g/dL; hematocrit was 40%; and platelet count was normal. SGPT was slightly elevated at 65 U/L, with a normal SGOT of 30 U/L; and a bilirubin of 3.14 mg% (direct reacting= 0.59 mg%) Reticulocyte count was elevated to 6.4%. Antinuclear antibodies were positive at 1:60. Test for hepatitis A, B, and C were all negative. Hemoglobin electrophoresis was within normal limits. The liver, spleen, abdominal sonogram, and computed tomographic scan of the abdomen were also within normal limits.

Since April, 1994, the patient has continued to work regularly and has had no episodes of any clinical illness or obvious jaundice. On physical examination, he is a normal-appearing male with no noticeable icterus or lymphadenopathy, nor palpable spleen. He has no abnormalities of the heart or lungs or any joint deformities. His hematocrit is 40% with an elevated reticulocyte count and a bilirubin in the range of 3-4%.

Assay of PGK Activity

The forward (1,3-diphosphoglycerate + ADP → 3 phosphoglycerate + ATP) and backward (3-phosphoglycerate + ATP → 1,3-diphosphoglycerate + ADP) reactions of the enzyme were measured as described (3, 4).

Determination of the Mutation Site

Total cellular RNA was prepared from the patient's and control leukocytes. The entire coding region of PGK mRNA was reverse transcribed and amplified by PCR into six overlapping segments using six sets of sense and antisense primers as previously described (5). The PCR products were separated by agarose gel (2%)

electrophoresis, and the fragments were purified by the Prep-A-Gene DNA purification kit (Bio-Rad, Hercules, CA). The purified PCR products were subjected to direct PCR cycle sequencing using an automatic nucleotide sequencer. The sequences were determined in both directions.

RESULTS

Enzyme Activity and Kinetic Properties

PGK activity of the patient's red blood cells was 7 unit/gHb at 25°C, i.e., only about 4% of normal. The Michaelis constants (K_m) are: 1,3-diphosphoglycerate, 3 μ M (normal 2 ~ 3 μ M); ADP, 140 μ M (normal 100 ~ 120 μ M); 3-phosphoglycerate, 900 μ M (normal about 600 μ M); ATP, 400 μ M (normal about 300 μ M). The variant enzyme lost activity almost completely (>90%) after storing the hemolysate or partially purified preparation at pH 7.5 and 4°C for 3 days. No measurable inactivation occurred in the normal PGK under the same conditions.

Nucleotide Base Change

Nucleotide sequence analysis of the six overlapping cDNA fragments originating from the variant and control mRNAs revealed a deletion of the three nucleotides, AAG, in exon 7 of the variant gene, i.e., the cDNA sequence of the region is 5' ATGAAGAAGGAGCTG 3' (nt 568-582) in the normal and 5' ATGAAGGAGCTG 3' in the variant. No other nucleotide change was found in the coding region of the variant PGK. The nucleotide deletion should result in the deletion of one of tandem lysine residues at 190-191 of the enzyme protein.

DISCUSSION

Phosphoglycerate kinase deficiency is a rare cause of hereditary nonspherocytic hemolytic anemia (6). Despite the severe red cell PGK deficiency, no remarkable hematological problems were observed in our patient, except for occasional jaundice. The nucleotide sequence

analysis of mRNA indicated the deletion of codon AAG in exon 7. The three nucleotide deletion could arise by nonhomologous crossing over of a pair of the gene or by an erroneous DNA replication. The codon deletion should result in the deletion of a lysine residue in the variant enzyme.

The region of the amino acid deletion position is in α -Helix 7 (amino acid residue 189-202) of PGK protein (7,8). This region, ... Leu-Met-Lys-Lys-Glu-Leu ..., is well conserved in PGK in all 26 species from bacteria to humans (9). α -Helix 7 is in between the N-terminal domain and C-terminal domain of PGK, and may not be directly involved in substrate binding (8). However, the region is important for maintaining the inter-domain conformation of the enzyme, and the deletion of Lys could cause molecular instability as observed in the rapid *in vitro* inactivation of the variant PGK.

This variant has been designated as PGK-Alabama, based on the regional origin.

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