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Effects of inherited mutations on catalytic activity and structural stability of human glucose-6-phosphate isomerase expressed in *Escherichia coli*

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ABSTRACT

Glucose-6-phosphate isomerase (GPI), a homodimeric enzyme, catalyzes the interconversion between glucose-6-phosphate and fructose-6-phosphate. In mammals, it can also act as an autocrine motility factor, neuroleukin, and maturation factor. Deficiency of the enzymatic activity in red blood cells causes nonspherocytic hemolytic anemia in human. To gain a more complete understanding of the molecular basis for the hemolytic anemia due to the GPI-deficiency, the wild-type enzyme and sixteen genetic variants were expressed in *Escherichia coli* and functionally characterized. Conclusions are as follows: (1) mutations usually have negative influences on catalytic parameters, particularly k_{cat}, as well as structure stability; (2) mutations at or close to the active site, including R273H, H389R, and S278L, cause great damage to the catalytic function, yet those at distance can still reduce the magnitude of k_{cat}, despite lesser extents; (3) mutations decrease the enzyme tolerance to heat or SDS by mechanisms of decreasing packing efficiency (V101M, T195I, S278L, L487F, L339P, T375R, I525T), weakening network bonding (R75G, R347C, R347H, R472H, E495K), increasing water-accessible hydrophobic surface (R83W), and destabilizing the ternary structure (T195I, R347C, R347H, and I525T); (4) A300P, L339P, and E495K mutations may also negatively affect the protein folding efficiency.

1. Introduction

Glucose-6-phosphate isomerase (GPI) (EC 5.3.1.9), present ubiquitously in most organisms, catalyzes the interconversion of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P), the second step of the Embden–Meyerhof glycolytic pathway. In mammals, GPI has a number of other physiological implications when it is secreted outside via unknown secretary pathways. For example, it is identical to autocrine motility factor (AMF) [1], which was originally identified in culture medium of A2058 melanoma [2]. The elevated AMF in serum or urine renders the protein a tumor marker in gastrointestinal, kidney, breast, colorectal, and lung cancer [3–6]. The metastasis-associated function of AMF is through a protein interaction with a glycosylated AMF receptor [7], by which RhoA and Rac1 will be activated, leading to a rearrangement of actin fiber [8]. Other probable mechanism such as through an increase of matrix metalloproteinases-3 was established in GPI-overexpressed Hup7 and HepG2 hepatoma [9]. GPI, also a neuroleukin secreted by lectin-stimulated T cells, is able to support the survival of cultured sensory and specific embryonic spinal neurons [10]. As a maturation factor, GPI can also mediate the differentiation of human myeloid leukemic HL-60 cells to terminal monocytic cells [11]. Anti-GPI autoantibody found in K/B×N T cell receptor-transgenic mice was the culprit for the rheumatoid arthritis developed in the mouse [12]. In addition, injection of GPI into some mouse strains, e.g. DBA/1, and C3H.NB, would trigger the onset of arthritis [13,14]. Although still in debate, a couple of literatures have also suggested a positive correlation between anti-GPI autoantibody and the arthritis disease in human [15–19].

Inherited deficiency of the enzymatic activity of human GPI (hGPI) affects mostly erythrocytes, causing hereditary nonspherocytic hemolytic anemia (HNSHA) in human. A severe deficiency can be associated with hydrops fetalis, immediate neonatal death and neurological impairment [20]. Due to the limitation of GPI, it is believed that the elevated glucose-6-phosphate would exert a feed-back inhibition on hexokinase activity, and consequently decrease the flux of glucose catabolism. Constraint on the isomerization step also diverts the catabolism to pentose phosphate shunt to recover fructose-6-phosphate. With the accumulation of erythrose-4-phosphate and 6-phosphogluconate, the inhibition of hexokinase is additionally enhanced. In the end, ATP production and glutathione reduction would decrease, causing the shortage of life span of erythrocytes. Approximately 50 clinical cases of GPI deficiency-causing HNSHA have been reported in a variety of ethnic groups and populations [20], since the first report of this hereditary disease in 1968 [21]. At the DNA level at least 29 mutations have been documented, including 24 missense, 3 nonsense and 2 splice site mutations. As an autosomal recessive inherited disorder, the anemia arises only from homozygote or compound heterozygote. To address the mechanistic basis of the anemia caused by GPI mutations, the protein from patient's erythrocytes has been characterized with

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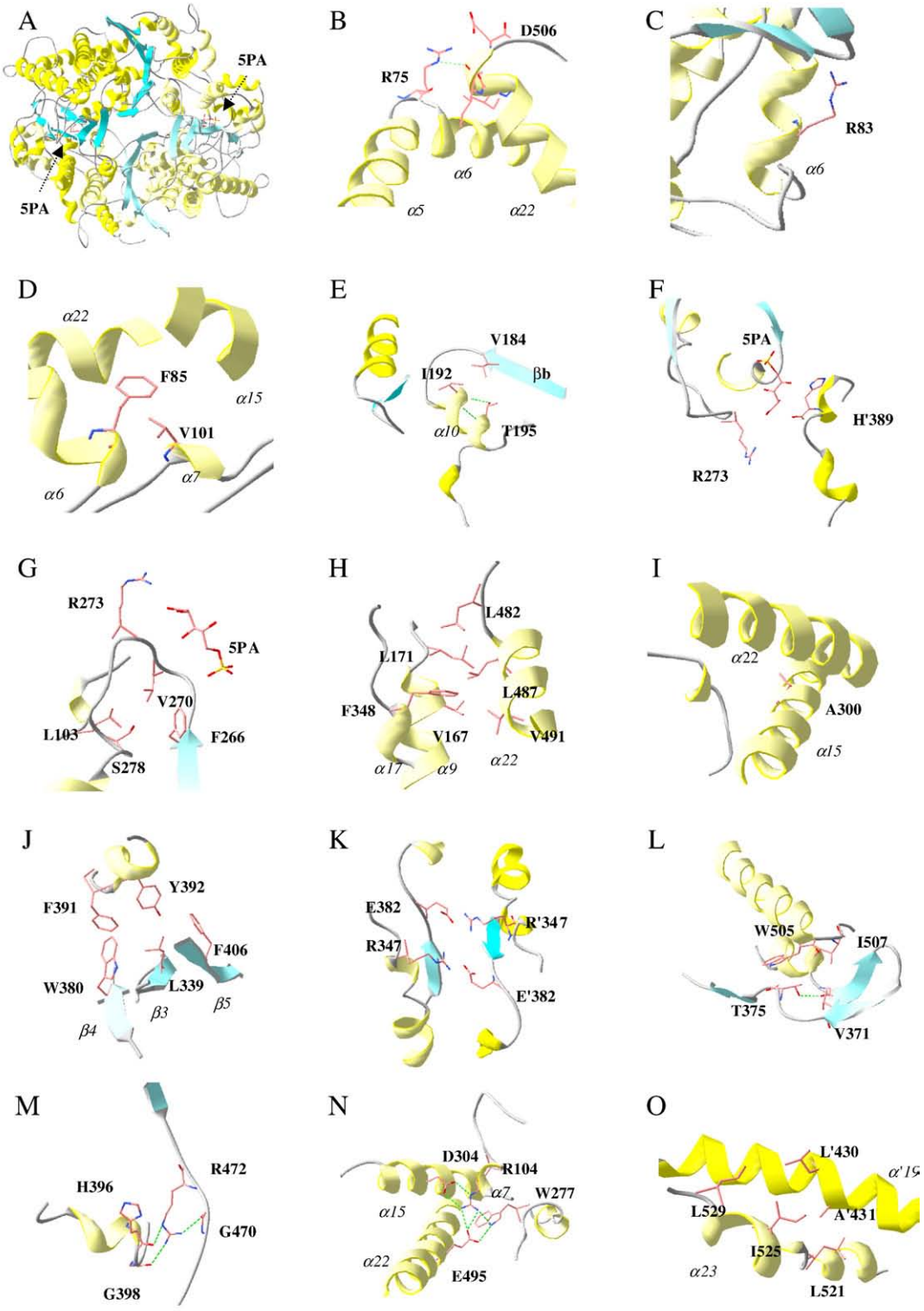

Fig. 1. Ribbon representations of the dimeric structure of human GPI (A), and the local structures around the mutation residues (B–O). The illustrations are created by Swiss-PdbViewer based on PDB ID 1NUH. The two subunits are colored in different tints. Green dot lines represent hydrogen bonds.

regard to its activity and thermostability [22–27]. In spite of providing essential information, results and interpretations from those studies might be inconclusive due to limitations such as inevitable contamination of normal blood cells in transfusion-dependent patients and inability to discern each of the mutational effects in patients with compound heterozygous genotype. The genetic background, such as the rate of erythropoiesis, can also affect the overall GPI activity. Approach of using recombinant hGPI expressed in *E. coli* has been taken as an alternative. For instance, mutational effects of T5I, T224M, Q343R, and D539N have been characterized using recombinant hGPI, on which glutathione S-transferase was fused to the N terminus to facilitate the protein purification [28].