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A novel binding of GTP stabilizes the structure and modulates the activities of human phosphoglucose isomerase/autocrine motility factor

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ABSTRACT

Phosphoglucose isomerase (PGI) catalyzes the interconversion between glucose 6-phosphate and fructose 6-phosphate in the glycolysis pathway. In mammals, the enzyme is also identical to the extracellular proteins neuroleukin, tumor-secreted autocrine motility factor (AMF) and differentiation and maturation mediator for myeloid leukemia. Hereditary deficiency of the enzyme causes non-spherocytic hemolytic anemia in human. In the present study, a novel interaction between GTP and human PGI was corroborated by UV-induced crosslinking, affinity purification and kinetic study. GTP not only inhibits the isomerization activity but also compromises the AMF function of the enzyme. Kinetic studies, including the Yonetani-Theorell method, suggest that GTP is a competitive inhibitor with a K_i value of 63 μM and the GTP-binding site partially overlaps with the catalytic site. In addition, GTP stabilizes the structure of human PGI against heat- and detergent-induced denaturation. Molecular modelling and dynamic simulation suggest that GTP is bound in a *syn*-conformation with the γ-phosphate group located near the phosphate-binding loop and the ribose moiety positioned away from the active-site residues.

1. Introduction

Phosphoglucose isomerase (PGI, EC 5.3.1.9), ubiquitously present in cytoplasm of most organisms, catalyzes the interconversion between glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P) (Fig. 1) in the glycolysis and gluconeogenesis pathways. In mammal, PGI exhibits diverse functions. It is secreted as neuroleukin by lectin-stimulated T cells to promote the survival of specific embryonic and sensory nerves [1,2]. It is also identical to the autocrine motility factor (AMF) that triggers tumor cell migration [3,4] and a differentiation and maturation mediator that is implicated in the differentiation of myeloid leukemia H-60 cells to terminal monocytic cells [5].

The crystal structures of PGI from a wide range of organisms have been determined [6–11], all folding into a similar architecture. Human PGI (hPGI), for example, is a dimer comprising two identical subunits of ~63 kDa [8]. The individual subunit contains a large and a small domain, each having a parallel β-sheet core surrounded by α-helices. The catalytic site for phosphosugar isomerization is located at a cleft between the large and small domains and is composed of residues from the two neighbouring subunits. A comparison between the native and inhibitor-bound rabbit PGI structures revealed the movement of two loops slightly closer toward the active site upon inhibitor binding [7]. The catalytic site and the region responsible for the AMF function probably overlap because substrate analogues, such as erythrose 4-phosphate and 5-phospho-D-arabinonate, inhibited not only the protein's enzymatic activity but also the AMF function [4,12]. Moreover, a mutation at active-site R273 abolished the enzymatic activity [13] as well as attenuated the cell migration-stimulating function of hPGI [14].

hPGI deficiency shortens the lifespan of red blood cells, causing hereditary non-spherocytic hemolytic anemia (HNSHA). Erythrocytes are a specific cell type due to the absence of nucleus and mitochondrion; therefore, the cells depend on anaerobic conversion of glucose by glycolysis pathway for the generation of NADH and ATP [15]. NADH can be used to reduce methemoglobin to hemoglobin by NADH-cytochrome b5 reductase. ATP provides

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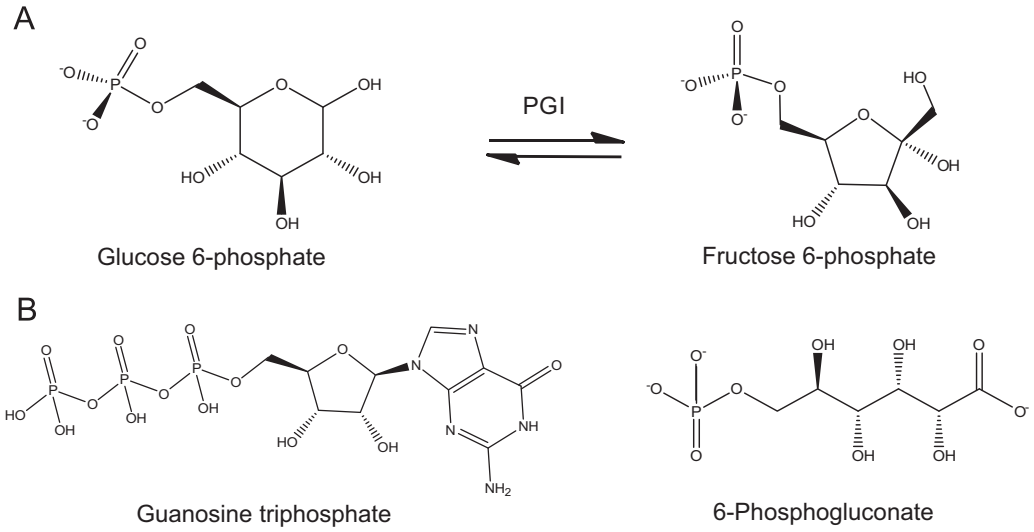

Fig. 1. The isomerization reaction catalyzed by PGI and structures of GTP and 6P-GA.

energy for membrane ion pumps to maintain the electrolyte gradient between plasma and red cell cytoplasm. ATP is also needed for glutathione synthesis and plays a crucial role in nucleotide metabolism. Approximately thirty genetic mutations associated with this genetic disorder have been identified [16,17]; they cause either unstable proteins or proteins impaired in isomerization activity [13,18]. Without the continuous supply of PGI, due to the absence of nucleus, the red blood cells carrying the deficient versions of hPGI would have shorter lifespans, leading to haemolytic anemia.

ATP participates in the activity regulation of a couple of key enzymes in sugar metabolic pathways. It exerts an allosteric inhibition on the activity of phosphofructokinase [19] and pyruvate kinase [20], thus reducing the flux of glycolysis when the cellular ATP concentration becomes ample. The activity of glucose 6-phosphate dehydrogenase (G6PDH) in the pentose phosphate pathway is also inhibited by ATP [21,22] and, to a lesser extent, CTP and GTP [22]. In this study, GTP was found, for the first time, to bind to hPGI. Effects of the GTP binding on the structural stability and catalytic function of hPGI were investigated. The possible GTP-binding mode on hPGI was modelled with the docking software and molecular dynamic simulation.

2. Materials and methods

2.1. Protein preparation

The cDNA of hPGI was obtained by PCR from a human liver cDNA library (Stratagene) as described previously [13]. The cDNA was inserted into plasmid pETDuet (Novagen), and the recombinant plasmid was transformed into *E. coli* BL21(DE3). To express the N-terminally His-tagged hPGI, IPTG (final 10 μM) was added into a 500 ml LB culture when the OD_{600} reached ~1.0; the incubation was continued at 18 °C for 16 h. After recovery by centrifugation, the cell pellet was suspended in 20 ml lysis buffer (40 mM TRICINE, pH 7.5) and homogenized by sonication at 4 °C. The clarified supernatant was loaded onto a 5 ml Ni^{2+}-NTA column, followed by wash and elution with 20 mM and 500 mM imidazole-containing lysis buffer, respectively. The collected protein solution was passed through a DEAE-Sepharose column (12 × 2.5 cm), and the flow-through was loaded onto a Sephacryl S-300 gel filtration column (60 × 1.6 cm) for the further purification of hPGI. The protein concentrations were determined using a Coomassie protein assay kit (Pierce) with bovine serum albumin as the standard.

2.2. Activity assay for isomerization

The enzyme-coupled assay using G6PDH is commonly used to measure the activity of PGI for converting F6P to G6P [13]. However, this method is not applicable to study the inhibition effect of GTP or 6-phosphogluconate (6P-GA) on hPGI, because GTP inhibits G6PDH [22], and so does 6P-GA (data not shown). In this study, the catalytic rate of converting F6P to G6P was determined by an end-point assay. hPGI was first incubated with the indicated amounts of F6P and inhibitors in 1 ml 20 mM HEPES buffer, pH 7.5, at 30 °C for 2–3 min. The reaction was stopped by incubation at 100 °C for 10 min, followed by centrifugation at 13000 × *g*. The amount of G6P produced in the isomerization step was then determined by incubating an aliquot of the reaction solution with 10 units of *Leuconostoc mesenteroids* G6PDH (Sigma-Aldrich) and 2 mM β-NAD^+ in 1 ml HEPES buffer. The increase in the OD_{340} was monitored until it reached a plateau, and the change in the OD_{340} was used to calculate the G6P concentration according to the extinction coefficient of NADH (ε_{340} = 6220 $M^{-1}cm^{-1}$). The amount of hPGI used in the isomerization step was subjected to change to assure that the production of G6P was in the linear range within the incubation period. The apparent kinetic parameters, K_m and V_{max}, were determined according to the dependence of the conversion rate on the F6P concentration using Grafit software (Erithacus Software Ltd., UK). Since both GTP and 6P-GA behaved as competitive inhibitors, their K_i values were calculated based on the plot of the inhibitor concentration versus the apparent K_m. The dependence of the binding sites for GTP and 6P-GA was investigated by the Yonetani – Theorell graphical method [23].

2.3. GTP binding assays

To perform UV-induced crosslinking assay, hPGI (0.7 μg) was mixed with 1 μCi [α-^{32}P]GTP (3000 Ci/mmol/) in an 18 μl solution and placed on ice for 20 min. The reaction mixture was irradiated with UV light (254 nm) for 2 min at a distance of 8 cm using CL1000 ultraviolet crosslinker (UVP, Upland, California). The irradiated product was separated on a 12% SDS-polyacrylamide gel, followed by autoradiography using BAS-2500 Phosphorimager (Fujifilm, Tokyo, Japan).

For GTP-Sepharose binding assay, hPGI (~1 mg) was load onto a 1 ml GTP-Sepharose column (Jena Bioscience, Jena, Germany) that had been equilibrated with 40 mM TRICINE buffer, pH 7.5. After extensive wash with the equilibrium buffer, the protein bound to the column was eluted with the equilibrium buffer that