

Cloning and expression of the glucose-6-phosphatase catalytic subunit 1 and enzymatic characterization of active site

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ABSTRACT

Human glycogen storage disease type 1 (namely GSD-1a), also known as von Gierke disease, is a group of autosomal recessive disorders of glucose-6-phosphatase (G6PC) genes with an overall incidence of approximately 1 in 100,000. Glucose-6-phosphatase (G6PC) is a key enzyme in glucose homeostasis that catalyzes the hydrolysis of glucose-6-phosphate to glucose and phosphate in the terminal step of gluconeogenesis and glycogenolysis. G6PC was recently divided into three subunits. Mutations in the G6PC gene, located on chromosome 17q21, result in disorder of the subunit 1 of glucose-6-phosphatase. Major clinical syndromes of G6PC1 mutants are hypoglycemia, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidemia, hepatomegaly, nephromegaly, fat liver and hepatoma (G6PC1). Because there is no medicine that can treat GSD-1a disease, only relying on the future supply of nutrients to improve the relief of symptoms. If the depth of G6PC1 to be able to study if the activity location, selection of high activity G6PC1 enzymes, overexpression and purification, to produce highly active enzyme, as a treatment glycogen storage disease type I protein drug development. The molecular weight of 58 kDa, because the pQE30 expression vector that contains six Histidine (His-tag), it can be purified easy in the future, then the method using NAD to analyze the enzyme activity. The wild-type enzyme in the pH value 6.5 of phosphate buffer (100 mM) and 37 °C, and 5 mM G6P catalyzed reaction for 30 minutes, with optimum response. We observed L31 (I198F) and L21 (V318A) mutants have lower activity than wild-type k_{cat} / K_m in 8.2 fold and 10.3 fold, respectively. The L21(V318A), We also found that H4 (S196N L305P) mutant has higher activity than wild type in 8 fold, We observed H19 (S196G) and H2 (N203S) mutant has higher activity than wild type in 1.2 fold and 4 fold, respectively. We observed modeling of E.coli glucose-1-phosphatase active center is similar to G6PC1. According to modeling of structure from E.coli glucose-1-phosphatase active center, corresponding L305P was found very close to the phosphatase hydrolysis site. Other mutant I198F, V318A, N203S, S196G and S196N were on the peripheral of entrance in the active center that might change the original amino acid and influenced substrate binding for G6PC1 activity. In this study, data suggest L305P are catalytically important active site of G6PC1. We are going to add enzyme of G6PC1 into yogurt making a medicine that can be a treatment of glycogen storage disease Ia in the future.

Keywords : glycogen storage disease Ia、 von Gierke disease、 Glucose-6-phosphatase catalytic subunit 1、 Specific activity、 active site、 active center

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