Cloning and expression of the glucose-6-phosphatase catalytic subunit 1 and enzymatic characterization of avtive 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 ℃, 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 kcat / Km 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.coil glucose-1-phosphatase active center is similar to G6PC1. According to modeling of structure from E.coil 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 la in the future.

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

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REFERENCES

參考文獻 1.Nagasaka H, Hirano K.i, Ohtake A, et al. Improvements of hypertriglyceridemia and hyperlacticemia in Japanese children with glycogen storage disease type Ia by medium-chain triglyceride milk. Eur J Pediatr 2007;166:1009-1016 2.Bio-Rad Laboratories (1994) Bio-Rad Protein Assay, Bulletin No. 600-0005, Bio- Rad Laboratories GmbH, Munich, Germany. 3. SARA LINDBLOOM*, MICHELLE LECLUYSE*, & THOMAS SCHERMERHORN,et al. Cloning and comparative bioinformatic analysis of feline glucose-6-phosphatase catalytic subunit cDNA. DNASequence, June 2008; 19(3):256-263 4. John C. Hutton1 and Richard M. O ’ Brien2, et al. The Glucose-6-Phosphatatase Catalytic Subunit

Gene Family.JBC Paper in press 2009 5. Chen, YT. Glycogen storage diseases. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle D.; Childs, B.; Kinzler, KW.; Vogelstein, B., editors. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill; 2001. p.1521-1551. 6. Chen YT, Cornblath M, Sidbury JB. Cornstarch therapy in type I glycogen storage disease. N Engl J Med1984;310:171 – 175. 7. Chou JY, Matern D, Mansfield BC, Chen YT. Type I glycogen storage diseases: disorders of the glucose-6-phosphatase complex. Curr Mol Med 2002;2:121 – 143. 8. Chou JY, Mansfield BC. Molecular genetics of type 1 glycogen storage diseases. Trend Endocrinol Metab1999;10:104 – 113. 9. Daublin G, Schwahn B, Wendel U. Type I glycogen storage disease: favorable outcome on a strict management regimen avoiding increased lactate production during childhood and adolescence. Eur J Pediatr 2002;161:S40 – S45. 10. Feldman F, Butler LG. Protein-bound phosphoryl histidine: a probable intermediate in the microsomal glucose-6-phosphatase-inorganic pyrophosphatase reaction.Biochim Biophys Acta 1972;268:698 – 710. 11. Greene HL, Slonim AE, O'Neill JA Jr, Burr IM. Continuous nocturnal intragastric feeding for management of type 1 glycogen-storage disease. N Engl J Med 1976;294:423 – 425. 12. Hiraiwa H, Pan CJ, Lin B, Moses SW, Chou JY. Inactivation of the glucose-6-phosphate transporter causes glycogen storage disease type 1b. J Biol Chem 1999;274:5532 – 5536. 13. Lei KJ, Pan CJ, Shelly LL, Liu JL, Chou JY. Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. J Clin Invest 1994;93:1994 – 1999. 14.Martin CC, Oeser JK, Svitek CA, Hunter SI, Hutton JC, O'Brien RM. Identification and characterization of a human cDNA and gene encoding a ubiquitously expressed glucose-6-phosphatase catalytic subunit-related protein. J Mol Endocrinol 2002;29:205 – 222. 15. Martens DH, Kuijpers TW, Maianski NA, Rake JP, Smit GP, Visser G. A patient with common glycogenstorage disease type Ib mutations without neutropenia or neutrophil dysfunction. J Inherit Metab Dis2006;29:224 – 225. 16. Pan CJ, Lei KJ, Annabi B, Hemrika W, Chou JY. Transmembrane topology of glucose-6-phosphatase. J Biol Chem 1998;273:6144 – 6148. 17. Rake JP, ten Berge AM, Verlind E, Visser G, Verlind E, Niezen-Koning KE, Buy CH, Smit GPA,Scheffer H. Glycogen storage disease type 1a: recent experience with mutation analysis, a summaryof mutations reported in the literature and a newly developed diagnostic flowchart. Eur J Pediatr 2000;159:322 – 330. 18. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Glycogen storage disease type I:diagnosis, management, clinical course and outcome. Results of the European Study on GlycogenStorage Disease Type I (ESGSD I). Eur J Pediatr2002;161:S20 – S34. 19. Stukey J, Carman GM. Identification of a novel phosphatase sequence motif. Protein Sci 1997;6:469 – 472. 20. Shieh JJ, Terizioglu M, Hiraiwa H, Marsh J, Pan CJ, Chen LY, Chou JY. The molecular basis of glycogen storage disease type 1a: structure and function analysis of mutations in glucose-6-phosphatase. J Biol Chem 2002;277:5047 – 5053. 21. Weston BW, Lin JL, Muenzer J, Cameron HS, Arnold RR, Seydewitz HH, Mayatepek E, Van SchaftingenE, Veiga-da-Cunha M, Matern D, Chen YT. Glucose-6-phosphatase mutation G188R confers anatypical glycogen storage disease type 1b phenotype. Pediatr Res 2000;48:329 – 334. 22. Weinstein DA, Wolfsdorf JI. Effect of continuous glucose therapy with uncooked cornstarch on the longtermclinical course of type 1a glycogen storage disease. Eur J Pediatr 2002;161:S35 – S39.