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α-Glucosidase Deficiency (Pompe's Disease)

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Abstract. α-Glucosidase is deficient (< 30% of control) in Pompe's disease, but the extent of the deficiency does not always correlate with the severity of the clinical symptoms. The defects that lead to a deficiency of α-glucosidase include synthesis of catalytically inactive protein, absence of mRNA for the enzyme, decreased synthesis of the precursor, lack of phosphorylation of the precursor, impaired conversion of the precursor to the mature enzyme and synthesis of unstable precursor. A single type of defect can lead to different clinical phenotypes. The precursor of α-glucosidase is present in the brush border of the polarized epithelial cells of small intestine and kidney and is secreted into urine.

Introduction

In Pompe's disease (glycogenosis type II) a deficiency of the lysosomal enzyme α-glucosidase leads to accumulation of glycogen in the lysosomes [1]. The disease is clinically heterogeneous [2]. In the infantile form, there is a generalized accumulation of glycogen and the disease is rapidly progressive; the patients usually die within the first 2 years of life [2]. In the late-onset (juvenile and adult) forms of the disease, glycogen accumulation is usually restricted to skeletal muscle and the patients may live for many years after the onset of the disease [3–6].

Initially, α-glucosidase was thought to be completely deficient in Pompe's disease, particularly in patients with the infantile form. However, beginning in 1973, reports began to appear on the presence of residual α-glucosidase activity (5–20% of control values) in patients with the late-onset forms of the disease [7–11]. This led to the view [see e.g. ref. 12] that the infantile form of Pompe's disease is caused by a virtually complete deficiency of α-glucosidase and that the presence of residual enzyme activity is responsible for the milder form of the disease in late-onset patients. Subsequent studies have emphasized that the severity of the

clinical symptoms in Pompe's disease does not always correlate with the amount of residual α-glucosidase activity in the patients [13, 14]. Thus other factors must play a role in determining the clinical course of the disease.

In the meantime our knowledge of the biosynthesis and transport to the lysosomes of α-glucosidase and other lysosomal enzymes has progressed considerably thanks to the pioneering work of Hasilik and Neufeld [15, 16] and subsequent studies by other investigators. Several laboratories [12, 15, 16, 18–21] including one in Japan [18, 20] have recently turned their attention towards investigating the biosynthesis and maturation of α-glucosidase in cultured skin fibroblasts with a view to obtain information on the molecular basis for the clinical heterogeneity observed in Pompe's disease. The aim of this paper is to review these recent studies.

Table I. α-Glucosidase activity in fibroblasts from control subjects and patients with Pompe's disease

Fibroblasts from	Number of cell lines	α-Glucosidase	
		nmol/min per mg protein	percent of control
Control subjects	3	15.4–23.2	
Pompe's patients			
Infantile	11	0.08–0.06	0.4–3.2
Juvenile	3	0.24–1.03	1.3–5.4
Adult	13	0.30–5.50	1.6–29.1

Glycogen was used as substrate. Data are taken from reference 21.

Residual α-Glucosidase Activity in Pompe's Disease

Reuser et al. [21] have recently carried out an extensive study of α-glucosidase in fibroblasts from 30 patients with different phenotypes of Pompe's disease. The α-glucosidase activity, measured with glycogen as substrate, ranged from 0.4 to 29.1% of the mean value in fibroblasts from control subjects. As indicated in table I, the residual activity tended to be higher in patients with the juvenile phenotype than in those with the infantile phenotype, and higher in patients with the adult phenotype than in those with the juvenile form of the disease. Nevertheless, there was considerable overlap, as also found earlier by others [13, 14]. The α-glucosidase activity in some of the adult phenotype patients fell within the range found for patients with the infantile phenotype.

Biosynthesis of α-Glucosidase in Cultured Skin Fibroblasts

α-Glucosidase, like other lysosomal enzymes, is a glycoprotein [22]. Hasilik and Neufeld [15, 16] were the first to show by means of metabolic labelling studies with radioactively labelled amino acids, mannose and phosphate that the enzyme is synthesized as a large precursor containing phosphorylated mannose residues and that the precursor is proteolytically converted to the mature form of the enzyme. The precursor has a molecular weight of 110 kilodaltons and is converted via intermediate forms of 105 and 95 kilodaltons to the 76-kilodalton mature protein and, after longer periods, to a 70-kilodalton form [11, 19, 21, 23]. Oude

Elferink et al. [23] have shown that the proteolytic processing steps occur in an intracellular compartment containing lysosomal enzymes; this compartment can be considered to consist of primary lysosomes. Transport of newly synthesized precursor to the primary lysosomes is completed within 8 h [23]. The half-life of the mature forms of α-glucosidase in fibroblasts has been estimated to be 5–8 days [17].

Nature of the Defects Leading to a Deficiency of α-Glucosidase in Pompe's Disease

It is now clear that a number of different mechanisms can lead to a deficiency of a lysosomal enzyme [24–26]. The different defects leading to a deficiency of α-glucosidase are summarized in table II. Eight different types of defects have been observed and they may be classified as follows.

(1) Defects leading to the absence of mRNA for α-glucosidase or to the formation of mRNA reduced in amount and of aberrant size. Such mutants have recently been identified by Martiniuk et al. [27] who have cloned a cDNA for α-glucosidase.

(2) Defects leading to the synthesis of a catalytically inactive protein, an unstable precursor or a mature protein of aberrant size.

(3) Defects leading to a partial or complete impairment in the conversion of the precursor to the mature protein.

Of particular interest are the defects in category 3. Reuser et al. [19] have shown that the formation of phosphorylated mannose residues is impaired in two cell lines, one from an infantile and one from an adult patient. In a parallel study Oude Elferink et

Table II. Nature of the defect in different phenotypes of Pompe's disease

Clinical phenotype and nature of defect	Authors
Infantile	
Enzymically inactive protein synthesized	Beratis et al. [13,14] Ninomiya et al. [18]
Precursor formed but reduced amount of mature protein present	Reuser et al. [19]
Precursor formed and phosphorylated but conversion to mature protein impaired	Reuser et al. [19]
Precursor formed; no phosphorylation, and conversion to mature protein impaired	Reuser et al. [19] Iwamasa et al. [20]
Precursor very rapidly degraded in prelysosomal compartment	Steckel et al. [12] Hasilik and Neufeld [15]
No precursor or very little formed	Martiniuk et al.[1] [27]
Juvenile	
Precursor formed but reduced amount of mature protein present	Reuser et al. [19]
Precursor formed and phosphorylated but conversion to mature protein impaired	Reuser et al. [19]
Adult	
Precursor formed but reduced amount of mature protein present	Reuser et al. [19]
Precursor formed and phosphorylated but conversion to mature protein impaired	Reuser et al. [19]
Precursor formed, no phosphorylation, and conversion to mature protein impaired	Reuser et al. [19]
Precursor formed; products of proteolytic processing aberrant	Reuser et al. [21]
Reduced amount and size of mRNA	Martiniuk et al. [27]

[1] No mRNA detectable.

al. [23] demonstrated that the transport of newly synthesized α-glucosidase to the lysosomes[1] is impaired in these two cell lines, in agreement with earlier findings [see e.g. ref. 16] on the requirement for mannose-6-phosphate residues for efficient routing of α-glucosidase and other soluble lysosomal enzymes to the lysosomes in fibroblasts. In immunoelectronmicroscopic studies with these two cell lines very little cross-reactive material was observed in the lysosomes, whereas the Golgi apparatus was very strongly labelled in the cell line from the adult patient [GM 1935; see fig. 5c of ref. 21].

In another cell line from an adult patient (84RD390), in which phosporylation of mannose residues is not impaired [Reuser and Kroos, unpubl. observ.], very little newly synthesized labelled α-glucosidase could be detected in lysosomes [23]. Thus, in this cell line impaired transport of α-glucosidase to the lysosomes must be due to reasons other than the absence of mannose-6-phosphate residues.

Finally, examination of table II indicates that in those cases where the defect leads to a partial or complete conversion of the precursor of α-glucosidase to the mature protein it is not possible to predict what the clinical phenotype will be. In this category of defects all three clinical phenotypes are found.

[1] Lysosomes were defined in this study as organelles containing cathepsin C. They were identified by incubating fibroblast homogenates with glycyl-*L*-phenylalanine 2-naphthylamide, a substrate for cathepsin C [28]. The products of the hydrolysis of the substrate accumulate in the lysosomes, causing osmotic lysis. Thus, after centrifugations labelled α-glucosidase originally present in lysosomes is recovered in the supernatant and enzyme present in nonlysosomal compartments is found in the pellet.

Intracellular Transport of α-Glucosidase in Polarized Epithelial Cells

An intriguing finding is that considerable amounts of the precursor form of α-glucosidase are present in human urine [29]. About 50% of the total α-glucosidase activity in urine represents the precursor form. The content of precursor α-glucosidase in a litre of urine, which is approximately the amount excreted daily by an adult, is 0.75 U (defined as μmol/min glucose formed at 37 °C with 4-methylumbelliferyl-α-glucoside as substrate). The total amount of precursor α-glucosidase in the kidneys of an adult is 1.1 U [Oude Elferink, unpubl. observ.]. Clearly, then, precursor α-glucosidase must be actively secreted by the kidney into the urine.

Ginsel et al. [30] have shown by means of immunocytochemical methods that in the polarized epithelial cells of human small intestine, α-glucosidase is present not only in lysosomes, endoplasmic reticulum and Golgi apparatus but also, unexpectedly, in the brush border. Using a monoclonal antibody that distinguishes between precursor and processed forms of α-glucosidase [31], Ginsel et al. [30] have established that it is the precursor of α-glucosidase that is present in the brush border. The presence of a precursor of α-glucosidase in the brush border of polarized epithelial cells has now also been demonstrated in kidney [Fransen and Oude Elferink, unpubl. observ.] and in the colon carcinoma cell lines Caco-2 and HT29 [Klumperman and Fransen, unpubl. observ.]. These findings raise the interesting possibility that in polarized epithelial cells a quantitatively important portion of newly synthesized precursor α-glucosidase is not transported to the lysosomes but to other destinations, including the secretory path-

way. Thus the presence of phosphorylated mannose residues in the precursor of α-glucosidase may not be a sufficient guarantee that the enzyme is transported preferentially to the lysosomes.

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References

1 Hers, H.G.: α-Glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). Biochem. J. *86:* 11–16 (1963).

2 Hers, H.G.; De Barsy, T.: Type II glycogenosis (acid maltose deficiency); in Hers, Van Hoof, Lysosomes and storage diseases, pp. 197–216 (Academic Press, New York 1973).

3 Smith, J.; Zellweger, J.; Afifi, A.K.: Muscular form of glycogenosis type II (Pompe); report of a case with unusual clinical features. Neurology *17:* 537–549 (1967).

4 Swaiman, K.F.; Kennedy, W.R.; Sauls, H.S.: Late infantile acid maltase deficiency. Archs Neurol. *18:* 642–648 (1968).

5 Engel, A.G.: Acid maltase deficiency in adults: studies in four cases of a syndrome which may mimic muscular dystrophy or other myopathies. Brain *93:* 599–616 (1970).

6 Angelini, C.; Engel, A.G.: Comparative study of acid maltase deficiency. Archs Neurol. *26:* 344–349 (1972).

7 Seiler, D.; Kelleter, R.; Kölmel, H.W.; Heene, R.: α-1,4-Glucosidase activity in leukocytes and lymphocytes of 2 adult patients with glycogen-storage disease type II (Pompe's disease). Experientia *29:* 972–973 (1973).

8 Mehler, M.; DiMauro, S.: Residual acid maltase activity in late onset acid maltase deficiency. Neurology *27:* 178–184 (1977).

9 Martin, J.J.; De Barry, T.; Den Tandt, W.R.: Acid maltase deficiency in non-identical adult twins. A morphological and biochemical study. J. Neurol. *213:* 105–118 (1976).

10 Reuser, A.J.J.; Koster, J.F.; Hoogeveen, A.; Galjaard, H.: Biochemical, immunological and cell genetic studies in glycogenosis type II. Am. J. hum. Genet. *30:* 132–143 (1978).

11 Schram, A.W.; Brouwer-Kelder, B.; Donker-Koopman, W.E.; Loonen, C.; Hamers, M.N.; Tager, J.M.: Use of immobilized antibodies in investigating acid α-glucosidase in urine in relation to Pompe's disease. Biochim. biophys. Acta *567:* 370–383 (1974).

12 Steckel, F.; Gieselman, V.; Waheed, A.; Hasilik, A.; Von Figura, K.; Oude Elferink, R.P.J.; Kalsbeek, R.; Tager, J.M.: Biosynthesis of acid α-glucosidase in late onset forms of glycogenosis type II (Pompe's disease). FEBS Lett. *150:* 69–76 (1982).

13 Beratis, N.G.; LaBadie, G.U.; Hirschhorn, K.: Genetic heterogeneity in acid α-glucosidase deficiency. Am. J. hum. Genet. *25:* 21–23 (1983).

14 Beratis, N.G.; LaBadie, G.U.; Hirschhorn, K.: Characterization of the molecular defect in infantile and adult acid α-glucosidase deficiency fibroblasts. J. clin. Invest. *62:* 1264–1274 (1983).

15 Hasilik, A.; Neufeld, E.F.: Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursor of higher molecular weight. J. biol. Chem. *255:* 4937–4945 (1980).

16 Hasilik, A.; Neufeld, E.F.: Biosynthesis of lysosomal enzymes in fibroblasts. Phosphorylation of mannose residues. J. biol. Chem. *255:* 4946–4950 (1980).

17 Reuser, A.J.J.; Kroos, M.: Adult form of glycogenosis type II: a defect in an early stage of acid α-glucosidase realization. FEBS Lett. *146:* 361–364 (1982).

18 Ninomiya, N.; Matsuda, I.; Matsuoka, T.; Iwamasa, T.; Nonaka, I.: Demonstration of acid α-glucosidase activity in different types of Pompe's disease by use of an immunochemical method. J. neurol. Sci. *66:* 129–139 (1984).

19 Reuser, A.J.J.; Kroos, M.; Oude Elferink, R.P.J.; Tager, J.M.: Defects in synthesis, phosphorylation and maturation of acid α-glucosidase. J. biol. Chem. *260:* 8336–8342 (1985).

20 Iwamasa, T.; Nashiro, K.; Ohshita, T.; Matsuda, I.: Subcellular distribution of acid α-glucosidase in fibroblasts and of antigenically cross-reactive material in Pompe's disease fibroblasts. Histochem. J. *18:* 613–624 (1986).

21 Reuser, A.J.J.; Kroos, M.; Willemse, R.; Swallow, D.; Tager, J.M.; Galjaard, H.: Biosynthesis and in situ localization of acid α-glucosidase in mutant fibroblasts. J. clin. Invest. (in press).

22 Callahan, J.W.; Lowden, J.A.: Lysosomes and lysosomal storage diseases (Raven Press, New York 1981).

23 Oude Elferink, R.P.J.; Van Doorn-Van Wakeren, J.; Strijland, A.; Reuser, A.J.J.; Tager, J.M.: Biosynthesis and intracellular transport of α-glucosidase and cathepsin D in normal and mutant fibroblasts. Eur. J. Biochem. *153:* 55–63 (1985).

24 Von Figura, K.; Hasilik, A.: Genesis of lysosomal enzyme deficiencies. Trends biochem. Sci. *9:* 29–31 (1984).

25 Tager, J.M.; Jonsson, L.M.V.; Aerts, J.M.F.G.; Oude Elferink, R.P.J.; Schram, A.W.; Erickson, A.E.; Barranger, J.A.: Metabolic consequences of genetic defects in lysosomes. Biochem. Soc. Trans. *12:* 902–905 (1984).

26 Tager, J.M.: Biosynthesis and deficiency of lysosomal enzymes. Trends biochem. Sci. *10:* 324–326 (1985).

27 Martiniuk, F.; Mehler, M.; Pellicer, A.; Tzall, S.; La Badie, G.; Hobart, C.; Ellenbogen, A.; Hirschhorn, R.: Isolation of a cDNA for human acid α-glucosidase and detection of genetic heterogeneity of mRNA in three α-glucosidase-deficient patients. Proc. natn. Acad. Sci. USA *83:* 9641–9644 (1986).

28 Jadot, M.; Colmant, C.; Wattiaux-De Coninck, S.; Wattiaux, R.: Intralysosomal hydrolysis of glycyl-*L*-phenylalanine 2-naphthylamide. Biochem. J. *219:* 695–670 (1984).

29 Oude Elferink, R.P.J.; Brouwer-Kelder, E.M.; Surya, I.; Strijland, A.; Kroos, M.; Reuser, A.J.J.; Tager, J.M.: Isolation and characterization of a precursor form of lysosomal α-glucosidase from human urine. Eur. J. Biochem. *139:* 489–495 (1984).

30 Ginsel, L.A.; Fransen, J.A.M.; Klumperman, J.; Hauri, H.P.; Hilgers, J.; Oude Elferink, R.P.J.; Tager, J.M.: Intracellular transport of brush-border and lysosomal enzymes in the human enterocyte; in Benga, Tager, Basic and medical research on biomembranes (Springer, Heidelberg, in press).

31 Oude Elferink, R.P.J.; Strijland, A.; Surya, I.; Brouwer-Kelder, E.M.; Kroos, M.; Hilkens, J.; Hilgers, J.; Reuser, A.J.J.; Tager, J.M.: Use of a monoclonal antibody to distinguish between precursor and mature forms of human lysosomal α-glucosidase. Eur. J. Biochem. *139:* 497–502 (1984).

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