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MOLECULAR DEFECT IN PROCESSING α-FUCOSIDASE IN FUCOSIDOSIS

KARL JOHNSON AND GLYN DAWSON

Departments of Biochemistry and Molecular Biology[1] and Pediatrics[2], J.P. Kennedy Jr. Mental Retardation Research Center, University of Chicago, Chicago, IL

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In normal human skin fibroblasts, an enzymatically active 53,000-dalton form of α-fucosidase is processed to a 50,000-dalton mature form. Endoglycosidase-H treatment of [^{35}S]methionine pulse-chase labelled material immunoprecipated with a polyclonal antibody to α-L-fucosidase (Andrews-Smith & Alhadeff, Biochim. Biophys. Acta 715: 90-96 (1982)) indicated the removal of a single N-linked oligosaccharide unit from both precursor and mature form of α-L-fucosidase. Tunicamycin pre-treatment of normal fibroblasts indicated that no other N-linked oligosaccharide units were present. Studies on fibroblasts from patients with less than 5% of normal α-L-fucosidase activity (fucosidosis) showed 8 of 11 patients synthesized no detectable α-fucosidase protein whereas 2 synthesized normal amounts of 53,000 dalton precursor, none of the mature 50,000 dalton form was detectable and one contained small amounts of cross-reacting material. This is the first evidence for processing of α-L-fucosidase in cells and the first precise evidence of a molecular defect in fucosidosis.

Fucosidosis was first described as a new mucopolysaccharide lipid storage disease in 2 brothers by Durand et al. (1). The disease is characterized by progressive mental deterioration and hepatosplenomegaly and the storage of oligosaccharides and glycolipids throughout the body (2). Variable phenotypic expression exists within a single kindred and it has been postulated that this may relate to differences in blood group type or secretor status. A deficiency of α-L-fucosidase gives rise to this autosomal recessive disease, the gene for α-fucosidase having been localized to chromosome 1 (3). The enzyme has been purified from a variety of tissues (3-5) and is believed to have a subunit molecular weight of approximately 50,000 daltons. Based on this, Fukushima et al, (6) isolated a cDNA clone (AF3) which coded for 347 amino acids and concluded that this represented 80% of the mature processed enzyme (α-L-fucosidase). Four potential glycosylation sites were identified

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which could add a potential 6 - 10,000 daltons to the actual size of the processed enzyme. Andrews-Smith & Alhadeff (7) have used a polyclonal antibody against α-fucosidase which can detect 1-2 ng of enzyme protein to show that liver from a patient with fucosidosis contained less than 1% of normal enzyme protein. We report evidence for processing and glycosylation of human α-L-fucosidase and the first molecular evidence for heterogeneity among patients with fucosidosis.

MATERIALS AND METHODS

Materials. Goat antisera to human placental α-L-fucosidase (7) was generously donated by Dr. J. Alhadeff, Lehigh University, Bethelehem, PA. Endoglycosidase-H was obtained from Miles, Inc., Elk Grove, IL, tunicamycin from Eli Lilly Corp., Indianapolis, IN.

Cell Culture. Fibroblast cultures were generously donated by Dr. P. Durand (Genoa, Italy), Dr. J. Spranger (Mainz, GFR), Dr. Kunze (W. Berlin, GFR), Dr. D.A. Wenger (Denver, CO), Dr. W. Zeman (Indianapolis, IN), Dr. F. Van Hoof (Amsterdam, Netherlands), Dr. J.S. O'Brien (UCSD), La Jolla, CA) and the Human Genetic Mutant Cell Repository (Camden, NJ). Fibroblasts were grown in monolayer culture as described previously (8) and used when near to confluency.

Labelling and immunoprecipitation of α-L-fucosidase. Cultures were labelled for 8h with 250 μCi/5 ml of [^{3}H]leucine, the label chased with leucine for 20h, and the cells harvested by trypsinization in buffered saline (lacking calcium and magnesium ions but containing 1% trypsin) for 15 min (9).

Precursor forms of α-L-fucosidase were isolated from the culture medium, following treatment of cells with 50 mM NH_4CL as described previously (9-11) by precipitation with 80% saturated $(NH_4)_2SO_4$. Both sonicated cell pellets and $(NH_4)_2SO_4$-precipitated medium extracts were dissolved in 10 mM Tris HCl buffer containing 0.125M NaCl, 0.02% Nonidet P-40, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 0.1% sodium deoxycholate and 0.02% leucine (11,12) and treated with a mixture of 4% normal goat serum and 1% goat antihuman fibronectin antibody overnight at 4°C. Following Pansorbin treatment as described prevously (9), the suspensions were treated with the goat anti-human α-fucosidase antibody overnight at 4°C and the immunoprecipitated material subjected to 9% polyacrylamide gel electrophoresis in SDS (9-11). β-Hex immunoprecipitation was performed with goat polyclonal antibodies to β-hexosaminidase B (βHex) (generously provided by Drs. E.F. Neufeld and R.L. Proia and prepared by A. Hasilik). Gels were Coomassie blue stained, enhanced, dried and exposed to Kodax XAR-5 film for up to 3 weeks. [^{14}C]Methyl-labeled protein molecular weight standards (Amersham Radiochemical Center) used were phosphorylase B (92,000), bovine serum albumin (69,000), ovalbumin (46,000) and carbonic anhydrase (30,000).

Modification of oligosaccharide content of α-L-fucosidase - Fibroblast cultures were pretreated with tunicamycin (2.5 μg/ml) for 3h prior to labelling with [^{35}S]methionine for 16h. Endoglycosidase H (50mU) digestion was carried out on [^{35}S]

immunoprecipitated material from 5 x 10^6 cells in 0.01 M sodium phosphate - 0.1 M NaCl (pH 6.0) for 2 x 16h at 37°.

Hydrolase assays - Lysosomal hydrolase activities were determined using the 4-methylumbelliferyl derivatives as described by Dawson & Tsay (8, 12).

RESULTS

α-Fucosidase synthesis and processing in normal skin fibroblasts

A single, major, slightly diffuse band of immunoprecipitated α-fucosidase was observed in all normal human skin fibroblasts studied thus far. This corresponded to a molecular weight of 50,000-daltons. Culture medium from cells contained virtually no α-fucosidase activity and no immunoprecipitable α-L-fucosidase. In contrast, cells treated with NH_4Cl did not contain any immunoprecipitable material but the medium from NH_4Cl treated cells contained enzymatically active material with an average size of 53,000 daltons, as judged by SDS polyacrylamide gel electrophoresis. In Fig. 1, lanes 1 and 2, it can be clearly seen that the precursor (53,000) and mature (50,000) form of α-fucosidase are different in size.

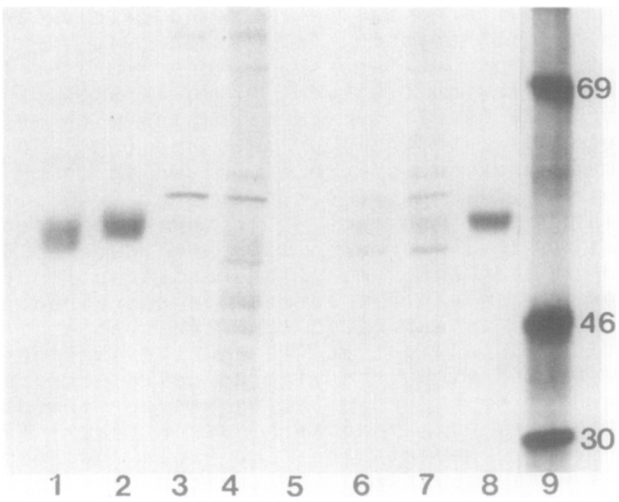

Fig. 1 Fluorography of 9% polyacrylamide gel electrophoresis separation of $NaDodSO_4$ extracts of immunoprecipitated [^{3}H]Leu-labelled α-L-fucosidase. Lane 1, normal fibroblasts; lane 2 normal fibroblast medium after NH_4Cl treatment; lane 3, fucosidosis (GM801) fibroblasts; lane 4, GM801 fibroblasts after NH_4Cl; lane 5, GM801 medium after NH_4Cl; lane 6, fucosidosis (GM802) fibroblasts; lane 7, GM802 fibroblasts after NH_4Cl; lane 8, GM802 media after NH_4Cl. Molecular weights identified from standards, lane 9.

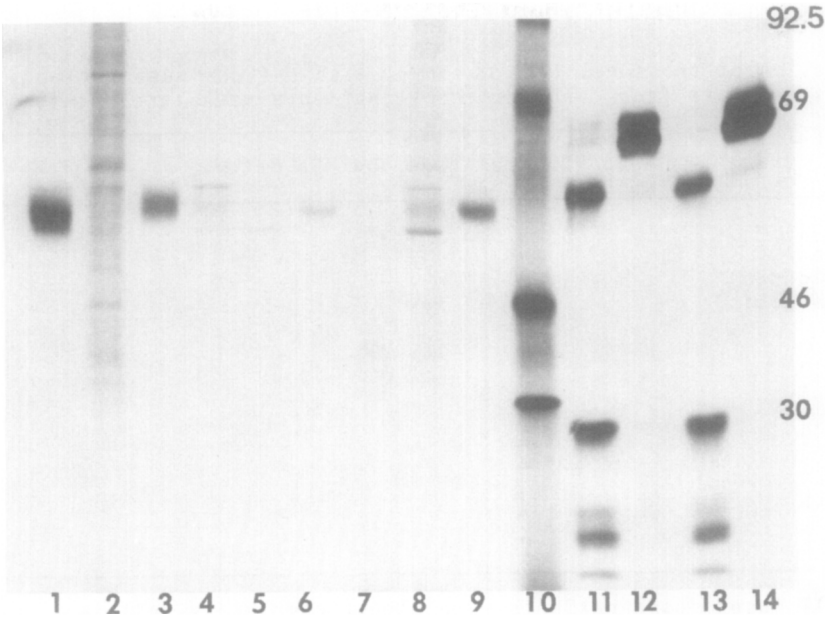

Fig. 2 Fluorograph of 9% polyacrylamide gel electrophoresis separation of NaDodSO$_4$ extracts of immunoprecipitated α-fucosidase (lanes 1-9) and β-hexosaminidase (lanes 11-14).
α-Fuc: Lane 1, normal fibroblasts; Lane 2, normal fibroblasts +NH_4Cl; lane 3, normal media +NH_4Cl; lane 4, fucosidosis (MC) fibroblasts; Lane 5, MC fibroblasts after NH_4Cl, lane 6, MC media after NH_4Cl; lane 7, fucosidoses (SS) fibroblasts; lane 8, SS fibroblasts after NH_4Cl; lane 9, SS media after NH_4Cl; lane 10, 92.5, 68, 43 and 30.5 KDa standards;β-Hex; lane 11, MC fibroblasts; lane 12, MC media after NH_4Cl; lane 13, normal fibroblasts; lane 14, normal media after NH_4Cl.

Detection of cross-reacting material in some fucosidosis patients. Immunoprecipitation studies on 8 patients with fucosidosis gave results typified by GM801 in Fig. 1. Namely, a virtual absence of cross-reacting material in either cells or in culture medium before (Fig. 1, lane 3) or after (Fig. 1, lane 4) NH_4Cl treatment. In contrast two patients (GM802 (Fig. 1, lane 9) (13) and SS (Fig. 2) (14)) showed normal amounts of α-fucosidase precursor, following NH_4Cl treatment, but little if any mature enzyme in cells (Fig. 1, lane 3 and Fig. 2, lane 7). An additional patient (MC) with a later onset form of fucosidosis and angiokeratoma (15) showed a low level of both precursor (after NH_4Cl) and mature forms of the enzyme (Fig. 2, lanes 4 and 6). The results are summarized in Table 1. No other bands on the gel were identifiable as being forms of α-L-fucosidase. As a control, equal amounts of cell extract from fucosidosis patients (e.g. MC, Fig. 2, lanes 11 and 12) and controls (e.g. JA Fig. 2,

TABLE 1

Expression of cross reacting material with α-fucosidase antibody in fibroblasts from controls and 11 patients with fucosidosis

Patient	53 KDa Precursor	50 KDa Mature	Angiokeratoma
JA (Normal)	+	+	-
DW (Batten's)	+	+	-
CM (sialidosis)	+	+	-
MC (fucosidosis)	(+)[a]	(+)	+
SS "	+	(+)	-
MZ "	-	-	+
GM 801 "	-	-	+
VA "	-	-	+
GM 802 "	+	(+)	+
MS "	-	-	-
MI "	-	-	-
ST "	-	-	-
SU "	-	-	-
CE "	-	-	+

All 11 fucosidosis patients were almost completely deficient in α-fucosidase activity (less than 5% of normal activity toward 4MU α-fuc). (+)[a] Indicates slightly positive (<10% of control).

lanes 13 and 14) were incubated with anti-Hex B. Normal amounts of mature (54,000) and precursor (67,000) α-chains and normal amounts of precursor (63,000) and mature (29,000, 25,000) β-chains were synthesized by MC.

Evidence for a single oligosaccharide unit in human fibroblast α-fucosidase. Treatment of cells with tunicamycin (Fig. 3, lane 5) resulted in the synthesis of [^{35}S] labelled enzyme with a molecular weight of around 50,000; presumably this represents unglycosylated 53,000-dalton precursor. Endo-H treatment of mature (processed) α-fucosidase (Fig. 3, lane 2) generated slightly smaller material (48,000 daltons). Similarly, endo-H treatment of precursor (Fig. 3, lane 4) α-fucosidase generated material of molecular size approximately 51,000 daltons which was clearly different from the deglycosylated mature form of the enzyme.

DISCUSSION

Previous studies on fucosidosis have indicated the presence of 1-2% of residual enzyme activity in cultured skin fibroblasts (8) and less than 1% of the normal amount of α-L-fucosidase protein in liver (7). There has been some disagreement as to whether the residual enzyme activity in fucosidosis has normal

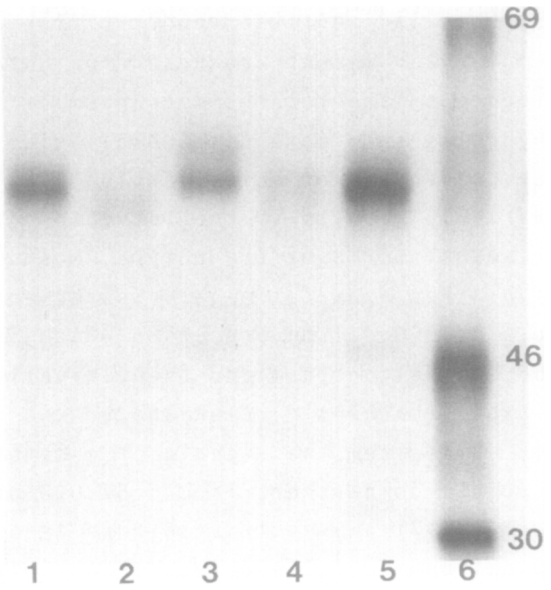

Fig. 3. Fluorograph of 9% polyacrylamide-gel electrophoresis separation of $NaDodSO_4$ extracts of immunoprecipitated α-fucosidase. Lane 1, normal fibroblasts after NH_4Cl; lane 2, normal media after NH_4Cl (53 KDa precursor); lane 3, 50 KDa mature (cell) form after endo-H; lane 4, 53 KDa precursor after endo-H; lane 5, normal fibroblasts after tunicamycin treatment; lane 6, BSA (69 KDa), Ovalbumin (46 KDa), Carbonic Anhydrase (30 KDa).

thermostability, K_m, pH optimum or isoelectric focussing profile (for a review, see 16) and some speculation as to the basis of phenotypic heterogeneity (12, 16). This report is the first evidence that some patients with fucosidosis, with no detectable lysosomal enzyme, can synthesize apparently normal amounts of α-fucosidase precursor protein whereas others do not.The results of our study suggest that 8 out of 11 patients studied (by analogy with patients with the Ashkenazi form of Tay-Sachs disease (17)) probably lack the ability to produce or translate mRNA for α-L-fucosidase. It is also possible that enzyme with normal leader sequence is synthesized but degraded prior to entering the Golgi or that insoluble enzyme is synthesized and then degraded. 2 out of 11 patients in our study synthesized apparently normal amounts of the 53,000 dalton α-fucosidase precursor, but no detectable 50KDa mature form which suggests a defect in processing, resulting in premature, complete degradation.

Although it has been known for many years that lysosomal α-fucosidase has a basic subunit size of approximately 50,000

daltons (3-5, 16) this is the first report of the existence of a discrete higher molecular weight precursor for α-fucosidase. Considerable evidence exists for the mechanism of processing of many lysosomal hydrolases in some post-Golgi acidified compartment since prevention of acidification with chloroquine or NH_4Cl almost invariably results in the secretion of higher than normal molecular weight forms of the enzyme, most of which are enzymically active. For example, β-galactosidase is synthesized and processed to a 85,000 dalton precursor which is then further processed to a 66,000 dalton form and then, finally, by a leupeptin-inhibitable (cathepsin) cleavage to the 64,000 dalton mature form (10). Likewise, the α-chain of β-hexosaminidase is processed in an acidic compartment from a 67,000 precursor to a 54,000 mature form (9,17) and β-glucuronidase is processed from 75,000 to 72,000 daltons (18). A defect at any of these stages can result in the type of enzyme deficiency we recognize as a lysosomal storage disease and therapeutic strategies must be tailored to the precise biochemical defect. This study of α-fucosidase indicates that a peptide fragment of approximately 3000 daltons, similar in size to that removed from β-galactosidase and β-glucuronidase, is cleaved from the 53,000-dalton precursor under acidic conditions. In two patients with fucosidosis, the defect involves loss of enzyme at this stage of processing.

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REFERENCES

1. Durand, P., Borrone, C., & Della Cella, G. (1969) J. Pediat. 75 665-674.
2. Tsay, G-C., Dawson, G., and Sung, S-J. J. (1976) J. Biol. Chem. 251: 5852-5859.
3. Beratis,N.G., and Hirschhorn, K. (1978) Am. J. Med. Genet. 19: 171-180.
4. Alhadeff, J.A., Miller, A.L., Wenaas, H., Vedvick, T. and O'Brien, J.S. (1975) J. Biol. Chem. 250: 7106-7113.
5. Chien, S-F., and Dawson, G. (1980) Biochim. Biophys. Acta 614: 476-488.
6. Fukushima, H., DeWet, J.R., and O'Brien, J.S. (1985) Proc. Natl. Acad. Sci. USA 82: 1262-1265.

7. Andrews-Smith, G., and Alhadeff, J.A. (1982) Biochim. Biophys. Acta 715: 90-96.
8. Dawson, G., Matalon, R., and Dorfman, A. (1972) J. Biol. Chem. 247: 5944-5958.
9. Hasilik, A., and Neufeld, E.F. (1980) J. Biol. Chem. 255 4937-4945.
10. D'Azzo, A., Hoogeveen, A., Reuser, A.J.J., and Galjaard, H. (1982) Proc. Natl. Acad. Sci. USA 79: 4535-4539.
11. Myerowitz, R., Robbins, A.R., Proia, R.L., Sahagian, G.E., Puchalski, C.M., and Neufeld, E.F. (1983) Meth. Enzymol. 96: 729-736.
12. Dawson,G. and Tsay, G. (1977) Arch. Biochem. Biophys. 184: 12-23.
13. Loeb, H. Tondeur, M., Jonniaux, G., Mockel-Pohl, S., and Vamos-Hurwitz, E. (1969) Helv. Paed. Acta 36: 519-537.
14. Snyder, R.D., Carlow, T.J., Ledman, J., and Wenger, D.A. (1976) Birth Defects: Orig. Article Series 12: 241-251.
15. Patel, V., Watanabe, I., and Zeman, W. (1972) Science 176: 426-427.
16. Alhadeff, J.A., and O'Brien, J.S. (1977) In: Practical Enzymology of the Sphingolipidoses, Callahan, J. and Lowden, S. eds. Alan R. Liss, N.Y. pp 247-281.
17. Myerowitz, R., and Proia, R.L. (1984) Proc. Natl. Acad. Sci. USA.81: 5394-5398.
18. Erickson, A., and Bloebel, G. (1982) Biochemistry 22: 5201-5205..