GLYCOPEPTIDE STORAGE IN FIBROBLASTS FROM PATIENTS WITH INBORN ERRORS OF GLYCOPROTEIN AND GLYCOSPHINGOLIPID CATABOLISM

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SUMMARY

A glycopeptide fragment, undetectable in normal cells, was isolated from cultured skin fibroblasts obtained from patients with four lysosomal storage disorders. The glycopeptide isolated from G_{M1}-gangliosidosis Type I (β-galactosidase deficiency) cells contained Gal, Man and GlcNAc in the ratio 1.8:2.8:4.0; in G_{M2}-gangliosidosis Type II (Sandhoff's disease:β-N-acetylhexosaminidase deficiency) cells the ratio was Man:GlcNAc, 2.7:4.0; in mannosidosis (α-mannosidase deficiency) cells, the ratio was 3.4:1.0 and in fucosidosis (α-fucosidase deficiency) cells the ratio was Fuc:GlcNAc, 1.0:1.0. A scheme for glycoprotein catabolism can be envisioned in which sugars are sequentially removed and these four disorders are caused by enzyme deficiencies at different locations in the pathway. The relative homogeneity of the storage material confirms that the same oligosaccharide unit is common to most of the glycoproteins synthesized by the cell.

INTRODUCTION

In G_{M1}- and G_{M2}-gangliosidosis (1, 2) glycolipid storage is mainly confined to the brain, although storage of asialo-G_{M2} and globoside can be demonstrated in liver (3) and fibroblasts (4) from patients with the Sandhoff variant of G_{M2}-gangliosidosis. In fucosidosis there is minimal storage of fucoglycolipids in brain, massive accumulation in liver (5) but no glycolipid accumulation in cultured fibroblasts (4). Suzuki et al (6) and MacBrinn et al (7) reported the existence of "keratan sulfate-like" material in G_{M1}-gangliosidosis liver, and we have identified this material (8) as an octaglycopeptide corresponding to the desulfated linkage region of skeletal keratan sulfate (9) or blood group glycoprotein (10). Wolfe et al recently characterized two oligosaccharides in liver from patients with G_{M1}-gangliosidosis; Gal-GlcNAc-Man[Gal-GlcNAc-Man]Man-GlcNAc and suggested that their origin was the erythrocyte

MN glycoprotein (11). We have isolated a similar oligosaccharide from the brain of G_{M1}-gangliosidosis Type I and II patients and a related oligosaccharide fragment from Sandhoff brain (12), recently elegantly characterized by Wolfe et al (13) as GlcNAc-Man[GlcNAc-Man]Man-GlcNAc. Öckerman (14) reported the storage of a mannosaccharide in brain of a patient with mannosidosis and material in the urine has been characterized as Man-Man-GlcNAc (15) Man-Man-Man-GlcNAc (16) and, in our laboratory, as Man-Man[Man]Man-GlcNAc (17). Recent work in this laboratory (12) indicated that the major storage material in fucosidosis brain was an oligosaccharide with the structure; Fuc-Gal-GlcNAc-Man[Fuc-Gal-GlcNAc-Man]-Man-GlcNAc together with a Fuc-GlcNAc disaccharide. Since all these oligosaccharides were structurally related, it seemed to suggest considerable structural similarity amongst human glycoproteins which are actively metabolized, unless one major glycoprotein, say the MN-antigen, is the primary source.

To investigate the origin of the storage material in these inborn errors of glycoprotein catabolism, skin fibroblasts from each type of patient were previously assayed to ensure that they manifested the appropriate enzyme defect and cultured in the presence of appropriate labelled sugars to detect endogenous synthesis. The storage material was characterized and compared to that isolated from the patient's tissues and urine.

MATERIALS AND METHODS

Biopsy specimens or cultured cells were obtained from Dr. R. Matalon, University of Chicago; Dr. J.S. O'Brien, UCSD, La Jolla; Dr. J.M. Opitz, University of Wisconsin, Madison; Dr. J.W. Spranger, University of Mainz, Germany; Dr. W. Zeman, Indiana University, Indianapolis and Dr. M. Buhler, Basle Kinderspital, Switzerland. Fibroblasts were cultured according to the method of Matalon and Dorfman (18) in which the modified Eagle's medium is supplemented with fetal calf serum (10%) and calf serum (10%), and lysosomal hydrolase activities assayed with synthetic substrates (4).

Confluent monolayer cultures (10^8 cells) were harvested mechanically, suspended in distilled water (1.5 ml) and sonicated as described previously (4). Cell debris was removed by centrifugation at 600g for twenty minutes. The extracts were fractionated on Bio-Gel P-10 (170 x 1 cm diam.), the carbohydrate-positive low-molecular weight fraction concentrated and re-fractionated on Bio-Gel P-2 (170 x 1 cm diam.). This material was applied to Whatman 3MM filter paper and subjected to exhaustive chromatography in n-butanol-acetic acid-water (12:3:5) to remove salts, peptides and oligosaccharides. The glycopeptide fraction was eluted with water from a strip cut around the origin (19) and assayed for both amino acid and carbohydrate content (19,20). In some cases, the molecular weight was determined by gel filtration according to the procedure of Bhatti and Clamp (21).

TABLE I. LYSOSOMAL HYDROLASE ACTIVITY IN FIBROBLASTS CULTURED FROM PATIENTS WITH INBORN ERRORS OF GLYCOPROTEIN CATABOLISM

Cell Strain	β-Galactosidase	β-N-Acetyl-hexosaminidase	α-Fucosidase	α-Mannosidase
	μmoles p-nitrophenol liberated/mg protein/hr			
Normal	0.36	6.00	0.19	0.27
G_{M1}-Gangliosidosis Type I (C.F.)	0	6.70	0.20	0.25
Type II (L.J.)	0.05	6.30	0.22	0.24
Sandhoff's disease (N.Z.)	0.42	0.10	0.20	0.25
Fucosidosis (M.S.)	0.39	5.90	0	0.31
(M.C.)	0.41	7.50	0.05	0.29
Mannosidosis (M.G.)	0.36	6.70	0.21	0.04

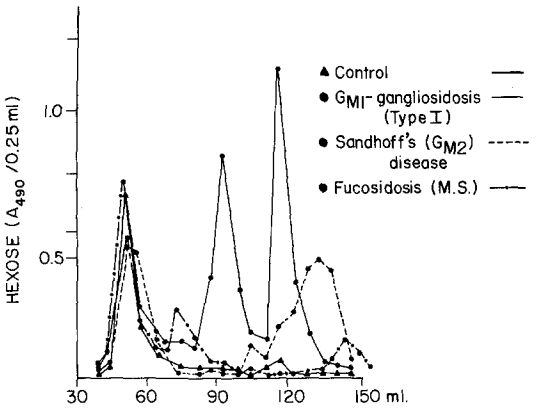

Fig. 1. BioGel P-10 column fractionation of soluble extracts cultured from patients with inborn errors of complex carbohydrate catabolism. The abnormal storage material is found in Fraction IV (110-150 ml).

RESULTS

The hydrolytic activity of cell extracts toward a variety of synthetic glycosides which are structurally related to naturally occurring complex carbohydrates is shown in Table I. Each extract exhibited the enzymic defect corresponding to that found in the patient's tissues. Soluble storage material isolated from approximately 10^8 cells was chromatographed on Bio-Gel P-10 and divided into four fractions on the basis of molecular weight (Fig. 1). Analysis of the low molecular weight fraction (110-150 ml) following Bio-Gel P-2 and paper chromatographic purification, is given in Table II. This shows that the material from G_{M1}-gangliosidosis Type I, G_{M2}-gangliosidosis Type II (Sandhoff's disease) (2 strains) fucosidosis (4 strains) and mannosidosis fibroblasts and a distinctive composition. A similar fractionation carried out on normal human skin fibroblasts together with those from patients with Hurler's disease, cystic fibrosis, G_{M2}-gangliosidosis Type I (Tay-Sachs disease) and G_{M1}-gangliosidosis Type II (juvenile form) failed to reveal the presence of any carbohydrate other than glucose in the low molecular weight fraction, i.e., there was no oligosaccharide or glycopeptide material in these cells.

Our results indicate that the storage material in each case was remarkably homogeneous. The most complex glycopeptide was stored in G_{M1}-gangliosidosis Type I fibroblasts (containing Man: Gal and GlcNAc in the ratio 2.8: 1.8: 4.0). Removal of galactose from this material would yield a glycopeptide analogous to

TABLE II. GLYCOPEPTIDE FRACTION* FROM CULTURED SKIN FIBROBLASTS OBTAINED FROM PATIENTS WITH INBORN ERRORS OF GLYCOPROTEIN CATABOLISM ISOLATED BY CHROMATOGRAPHY ON BIOGEL P-10 FOLLOWED BY REFRACTIONATION ON BIOGEL P-2 AND PREPARATIVE PAPER CHROMATOGRAPHY

Sugar	G_{M1}-Gangliosidosis Type I (C.F.)	G_{M2}-Gangliosidosis Type II Sandhoff's Disease (D.B.)	(N.Z.)	Fucosidosis (Late infantile) (M.S.)	(S.S.)	(M.Z.)	Fucosidosis (juvenile) + (M.C.)	Mannosidosis# (M.G.)
				molar ratio				
Fucose	Tr	Tr	Tr	0.8	0.8	1.0	1.2	Tr
Mannose	2.8	1.8	2.7	0.1	Tr	Tr	<0.5	3.4
Galactose	1.8	Tr	Tr	0.1	Tr	Tr	Tr	Tr
GlcNAc	4.0	4.0	4.0	1.0	1.0	1.0	1.0	1.0
NeuNAc	ND	ND	ND	ND	ND	ND	ND	ND
Total μmoles/10^8 cells	1.85	1.60	1.65	0.92	0.42	1.85	0.14	0.50

* This low-molecular weight fraction contains less than 0.1 μmoles complex carbohydrate in other normal and pathological fibroblasts.

\+ Cells from the juvenile form stored much less of the Fuc-GlcNAc glycopeptide and may represent a partial α-fucosidase deficiency even though this could not be convincingly demonstrated using artificial substrates.

\# Gel filtration gave a molecular weight of 1100 which corresponded to a glycopeptide shown by analysis to contain - Man:GlcNAc:Asn:Glu:Gly:Ser:Ala:Thr in the ratio 3.0:1.0:1.0:1.0:1.0:0.8:0.8:0.5.

ND = not detectable

that stored in G_{M2}-gangliosidosis Type II fibroblasts (containing Man and GlcNAc in the ratio 2.7: 4.0). Similarly, removal of three GlcNAc residues would yield a glycopeptide with a composition similar to that stored in mannosidosis fibroblasts (containing Man and GlcNAc in the ratio 3: 1). In fucosidosis fibroblasts, the Fuc-GlcNAc glycopeptide is essentially free from any mannose or galactose. Because of limitations on the amount of material available, structural studies and sequential enzymic digestion was not possible. However, by analogy to material stored in solid tissue from such patients (12) one may envision a sequential catabolic scheme. Thus far we have been unable to separate the carbohydrate and amino acid moieties in the storage material and since asparagine is always a major amino acid, it is presumed that the linkage between carbohydrate and amino acid involves GlcNAc and Asn.

DISCUSSION

The study of human skin fibroblasts has been extremely rewarding in elucidating the biochemical abnormalities responsible for a number of lysosomal storage disorders. These include the glycogen storage diseases, the mucopolysaccharidoses, for example Hurler's disease (22) and the sphingolipidoses (1, 4). Although cultured human skin fibroblasts lack the ability to synthesize the glycosphingolipids (gangliosides and sulfatide) characteristic of nervous tissue or the blood group antigens, the enzymes responsible for their catabolism can be detected and deficiencies readily diagnosed. This has proved especially advantageous for prenatal diagnosis of these rapidly fatal disorders. Despite our failure to demonstrate G_{M1}, G_{M2} or H-antigen accumulation in appropriate fibroblasts (4), the fact that the enzyme in question was deficient suggested that it might be possible to demonstrate the storage of material derived from impaired glycoprotein catabolism, since glycoproteins and glycolipids share common sugars and glycosidic linkages and fibroblasts synthesize a wide variety of glycoproteins (including the lysosomal hydrolases themselves).

The results of our studies on fibroblasts cultured from patients with G_{M1}-gangliosidosis Type I (generalized gangliosidosis), G_{M2}-gangliosidosis Type II (Sandhoff's disease), fucosidosis and mannosidosis suggest that fibroblast glycoproteins contain oligosaccharide units of the $[NeuNAc\text{-}Gal\text{-}GlcNAc\text{-}Man]_{2\text{ or }3}$-Man-GlcNAc-GlcNAc-Asn type (23, 24) and that the oligosaccharide units are catabolized by the sequential action of exo-glycosidases. The storage material

in G_{M1}-gangliosidosis cultured cells appears similar to that of an oligosaccharide isolated by Wolfe et al. from G_{M1}-gangliosidosis liver (Gal β(1→4)GlcNAc β(1→2) Man α(1→3) [Gal β(1→4)GlcNAc β(1→2)Man α(1→6)Man β(1→4)GlcNAc (11) and the material stored in Sandhoff and mannosidosis fibroblasts is derived from this structure by the loss of terminal galactose and GlcNAc residues respectively. Fucosidosis fibroblasts are an exception in that only a Fuc α(1→6) GlcNAc glycopeptide was stored whereas in brain it was possible to isolate this material together with oligosaccharides of the Fuc-Gal-GlcNAc-Man type (12). A second important difference involves the ability of cultured cells to hydrolyze the GlcNAc-Asn linkage with sufficient facility to produce an oligosaccharide rather than a glycopeptide storage product. We could only demonstrate a low level of N-acetylglucosaminylasparaginase activity in vitro, and the evidence suggests that this enzyme is not active in fibroblasts in vivo.

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