STRUCTURE OF THE "KERATOSULFATE-LIKE" MATERIAL IN LIVER FROM A PATIENT WITH G_{M1}-GANGLIOSIDOSIS (β-D-GALACTOSIDASE DEFICIENCY)

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Summary

Large amounts of a glycopeptide containing galactose, N-acetylglucosamine, N-acetylgalactosamine and threonine in the ratio 4:3:1:1, together with smaller amounts of mannose, fucose, sialic acid, sulfate, serine, and other amino acids were isolated from the liver of a patient with G_{M1}-gangliosidosis. Treatment with mild alkali and sodium borohydride indicated an O-glycosidic linkage between N-acetylgalactosamine and threonine. All the hexosamine residues were resistant to sodium metaperiodate whereas 2 out of 4 D-galactose residues were destroyed. Further studies indicated that one of the galactose residues was 1→3 linked to N-acetylgalactosamine (as in G_{M1}) and the other 1→4 linked to N-acetylglucosamine as found in skeletal keratosulfate.

The two major forms of G_{M1}-gangliosidosis are characterized by a deficiency of β-D-galactosidase, the intraneuronal accumulation of G_{M1}-ganglioside and the visceral storage and excretion of a "keratosulfate-like" material (1-4). Although G_{M1} has been shown to have a terminal galactosyl β-(1→3)N-acetylgalactosaminyl linkage (5,6), the nature of the keratosulfate-like material is uncertain despite many reports which show it to contain primarily galactose and N-acetylglucosamine (1-4,7,8). This paper described the isolation of the accumulating glycopeptide from the liver of a patient (C.B.) exhibiting the G_{M1}-gangliosidosis phenotype (cherry red spot, mental retardation, hyperacusis, hepatosplenomegaly, progressive skeletal changes and death at 18 months of age), with total deficiency of β-D-galactosidase activity.

Materials and Methods

The post-mortem sample of G_{M1}-gangliosidosis liver (30 g; generously supplied by Dr. H. Müller, Basel Children's Hospital, Basel, Switzerland),

which had been stored at -20° for six months, was homogenized and digested with papain. Mucopolysaccharides were extracted as described previously (9). Since conventional treatment with cetylpyridinium chloride followed by Dowex 1(Cl^- form) column chromatography gave anomalous results, all fractions were pooled, dialyzed, lyophilized and digested exhaustively with Protease (Sigma Chemical Co.) at pH 7.5 for 48 hrs. The digest was further fractionated by exclusion chromatography on Sephadex G-50 (180 cm x 1 cm). The major carbohydrate-containing fraction (Peak I) (Fig. 1) appeared relatively homogeneous whereas fractions II and III were more complex. All fractions were pooled, lyophilized and final purification was achieved by paper chromatography in $\underline{n}C_4H_9OH$-CH_3COOH-H_2O (12:3:5) (10).

Carbohydrates were estimated by gas-liquid chromatography of the trimethylsilyl ethers of their O-methyl glycosides (11). Periodate oxidation and mild alkaline hydrolysis were carried out as described previously (10) and the changes in carbohydrate composition were monitored by gas-liquid chromatography.

Chromogen formation was carried out according to the procedure of Reissig *et al.* (12), as modified by Kühn and Kruger (13). The polysaccharide chromogen was fractionated on Sephadex G-25 (100 x 2.5 cm. diam) and the composition of each fraction was determined by gas-liquid chromatography. Sulfate was determined by the methods of Terho and Hartiala (14) using Rhodizonate reagents.

Results

Carbohydrate analysis of the major fraction (Peak I) (Fig. 1) isolated from this G_{M1}-gangliosidosis liver revealed galactose:*N*-acetylglucosamine:*N*-acetylgalactosamine in the ratio 4:3:1 per mole of threonine together with small amounts of fucose (0.1 moles), mannose (0.3 moles) and *N*-acetylneuraminic acid (0.2 moles) as shown in Table I; the ratio of sulfate to *N*-acetylglucosamine was 0.08. Analysis of Peaks II and III showed galactose and *N*-acetylglucosamine to predominate but the

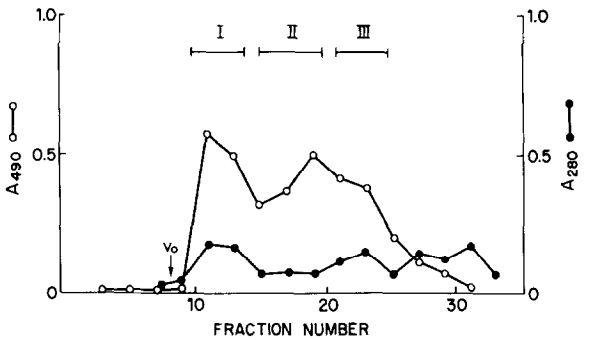


Fig. 1. Sephadex G-50 exclusion chromatography of glycoptptides isolated from a G_{M1}-gangliosidosis Type II human liver. Fractions (5.0 ml) were collected and aliquots were assayed for protein (1.0 ml) (A280; ●—●) and carbohydrate (0.1 ml) (Phenol-sulfuric acid method A490; O—O).

high mannose content (Table I) was interpreted as being indicative of contamination by other glycopeptides and all further studies were carried out on this major component, Peak I. Amino acid analysis revealed that threonine constituted about 40% of the total amino acid content and this was almost completely destroyed (0.9 moles relative to 3.0 moles of N-acetylglucosamine) by treatment with 0.5 N NaOH at room temperature overnight (Table II) (10). Following subsequent treatment with sodium borohydride, one mole of N-acetylgalactosamine was converted to N-acetylgalactosaminitol. Since no other sugar or amino acid was destroyed, this is strongly suggestive of an O-glycosidic linkage between N-acetylgalactosamine and threonine.

Incubation with 0.015 M-sodium metaperiodate overnight resulted in the loss of 2 out of 4 galactose residues but no destruction of either N-acetylglucosamine or N-acetylgalactosamine (Table III). The terminal location of these two galactose residues was confirmed enzymically by Dr. Yu Teh Li, University of Tulane, New Orleans, using an exo-β-D-galactosidase purified from Jack bean meal (15).

DISCUSSION

The presence of large amounts (1-2% of fresh wt.) of a glycopeptide

TABLE I

Carbohydrate and Amino Acid Composition of the Major Glycopeptide (Fraction I) and two Minor Fractions (II and III) Isolated from the Liver of a Patient with G_{M1}-Gangliosidosis.

Component	Sephadex G-50 Fraction		
	I	II	III
	moles/3 moles N-acetylglucosamine		
Fucose	0.1	0.1	0.1
Mannose	0.3	0.7	1.5
Galactose	3.9	3.2	2.7
N-Acetylglucosamine	3.0	3.0	3.0
N-Acetylgalactosamine	1.0	0.5	0
Sialic acid	0.2	0.2	0.2
Aspartate	0.1	0.1	0.1
Threonine	1.0	0.5	0.2
Serine	0.3	0.1	0.2
Glutamate	0.1	0.1	<0.1
Proline	0.3	<0.1	<0.1
Glycine	0.1	0.1	0.2
Alanine	0.2	0.1	0.1
Valine	0.1	TR*	TR*
Isoleucine	TR*	TR*	TR*
Leucine	TR*	TR*	TR*
Phenylalanine	TR*	TR*	TR*

* TR = Trace amounts (less than 0.1 mole/3 moles N-acetylglucosamine)

containing galactose and N-acetylglucosamine, in the liver of patients with G_{M1}-gangliosidosis, has led to the description of this material as being undersulfated "keratosulfate" (1-3). Since this is a lysosomal

TABLE II

Effect of Mild Alkali/Sodium Borohydride Treatment on the Carbohydrate and Amino Acid Composition of Fraction I

Component	Before Treatment	After Treatment
	moles/3 moles of N-Acetylglucosamine	
Fucose	0.12	0.13
Mannose	0.29	0.29
Galactose	3.93	3.95
N-Acetylglucosamine	3.00	3.00*
N-Acetylgalactosamine	0.96	0
Sialic Acid	0.21	0.20
Aspartate	0.11	0.15
Threonine	1.00	0.12
Serine	0.35	0.34
Glycine	0.14	0.18

* Treatment with 0.5 N NaOH at 50° for 5 hrs resulted in the additional destruction of 0.9 μmoles of N-acetylglucosamine.

storage disease, one would expect the material to be extensively degraded (by exo- and endo-glycosidases) down to the stage where the deficient enzyme (β-D-galactosidase) would normally act. By analogy to the structure of the G_{M1}-ganglioside which accumulates in the neurons of these patients, one might anticipate the linkage involved to be Gal β(1⟶3)GalNAc. Callahan and Wolfe (16) reported the isolation of a similar glycopeptide (molecular weight 2000-10,000) from the liver of a patient with the Type I form of the disease and on the basis of periodate oxidation studies (16) concluded that the material was undersulfated keratosulfate. However, their preparations contained considerable amounts of mannose and subsequent studies (8)

TABLE III

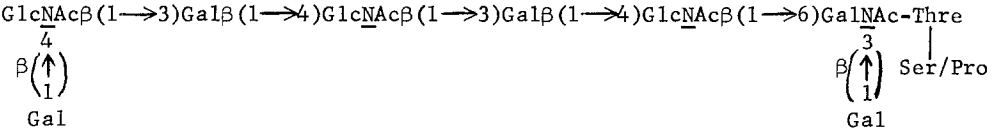
Effect of Periodate Oxidation Sodium/Borohydride on the Carbohydrate and Amino Acid Composition of Fraction I

Component	Before Treatment	After Treatment
	moles/3 moles of N-Acetylglucosamine	
Fucose	0.1	0
Mannose	0.3	0.2
Galactose	3.9	1.8
N-Acetylglucosamine	3.0	3.0
N-Acetylgalactosamine	1.0	1.0
Sialic Acid	0.2	0
Aspartate	0.1	0.1
Threonine	1.0	1.0
Serine	0.3	0.3

indicated that all the galactose residues were terminal and that mannose was part of the chain, a structure consistent with that of the MN-active glycopeptide and possibly being common to a number of glycoproteins (8). The material isolated from liver in this case of G_{M1}-gangliosidosis has a structure consistent with that of partially degraded skeletal keratosulfate and the mannose found in some of our fractions probably represents contamination by other glycopeptides or at most the residue of a mannose side chain.

The demonstration of an O-glycosidic linkage between N-acetylgalactosamine and threonine is consistent with the currently accepted concept of the linkage region of skeletal keratosulfate (17), whereas the corneal keratosulfate linkage region involves N-acetylglucosamine and asparagine (18). Serine did not appear to be involved whereas most descriptions of the skeletal keratosulfate linkage region suggest that both are involved.

This could be attributed to the greater extent of proteolysis (either lysosomally or by Papain or Protease digestion) which had taken place in this low sulfated material. We suggest the following tentative structure for the glycopeptide:



Periodate oxidation studies indicated that two out of four galactose residues were substituted at C-3 and that the other two galactose residues were probably terminal. Two galactose residues were also removed by an exo-β-D-galactosidase, which is in general agreement with the report of O'Brien (1) that liver β-D-galactosidase liberated 38.9% of the galactose from the accumulating glycopeptide. All N-acetylglucosamine and N-acetylgalactosamine residues were resistant to periodate, suggesting substitution at C-3 or C-4 or disubstitution. A glycopeptide containing N-acetylgalactosamine O-glycosidically linked to serine or threonine and specifically disubstituted at C-3 and C-6 will form a chromogen under the appropriate conditions (13,17). The formation of this chromogen was observed with Peak I and after purification on Sephadex G-25, the major chromogen peak contained galactose:N-acetylglucosamine in the ratio 3:3; the additional galactose residue must therefore be substituted at C-3 of N-acetylgalactosamine in the linkage region. The presence of a Galβ(1→3)GalNAc linkage could explain why this material specifically accumulates in G_{M1}-gangliosidosis although the Galβ(1→4)GlcNAc linkage also present in Peak I could be equally resistant in this disease.

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