BRAIN GLYCOPROTEINS IN GM_1-GANGLIOSIDOSIS: ISOLATION AND CARBOHYDRATE COMPOSITION OF GLYCOPEPTIDES

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Received 7 May 1973

1. Introduction

GM_1-gangliosidosis is a genetically linked disorder characterized by the accumulation in neurons of a specific ganglioside, GM_1, due to a deficiency of β-galactosidase [1]. This enzyme is active in the catabolism of gangliosides since it removes the terminal galactose of GM_1. The heteropolysaccharide chains of glycoproteins also contain galactose and it appears likely that the β-galactosidase is active in degrading these substances as well. Suzuki et al. [2] reported an accumulation of galactose-containing glycopeptides (keratan sulfate and sialomucopolysaccharides) in visceral organs, but not in brain tissue. Wolfe et al. [3] also failed to find an accumulation of galactose-containing glycopeptides in brain tissue, although excess urinary excretion of keratan-sulfate was noted. The majority of the heteropolysaccharide chains associated with brain glycoproteins appear to consist of N-acetylglucosamine—galactose—NANA branches which are attached to an internal mannose-N-acetylglucosamine core which in turn is linked to the protein by an alkali-stable β-aspartylglycosaminylamine linkage. The glycopeptides, which are obtained by treatment of the glycoprotein with papain, are partially dialyzable [4]. Catabolism would further reduce the size of these structures since enzymatic removal of terminal NANA and fucose produces glycopeptides with terminal galactose residues. These would accumulate in GM_1-gangliosidosis. The isolation procedures of Suzuki et al. [2] and Wolfe et al. [3] included a dialysis step and it seems likely that these workers consequently lost the accumulated galactose-rich glycopeptides during the procedure. The present work reveals that galactose-rich glycopeptides accumulate in neural tissue derived from patients with GM_1-gangliosidosis, suggesting that the missing β-galactosidase is active in the catabolism of the heteropolysaccharide chains of glycoproteins as well as gangliosides.

2. Methods

The preparation and isolation of non-dialyzable and dialyzable glycopeptides from brain tissue glycoproteins and the analytical methods have been previously described [4, 5].

3. Results and discussion

The patient was a 15-months old female. Autopsy findings and the results of an histochemical examination have been published [6]. The gray matter contained 4.83 μmoles of gangliosidic NANA per gram fresh brain. Normal values range from 2.05—2.57 μmoles. 69% Of the gangliosidic NANA was associated with ganglioside GM_1. The normal values range from 11—17%.

The dialyzable glycopeptide preparation showed an over 2-fold elevation in galactose content (table 1) when compared to material isolated from controls of a similar age. An increase in mannose and hexosamine content was also noted. Presumably, the accumulated glycopeptides are degradation products derived from

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Table 1
Carbohydrate composition of glycopeptides isolated from glycoproteins in the defatted tissue residue obtained from cerebral gray matter.

	NANA	Fucose	Hexosamine	Mannose	Galactose
Non-dialyzable glycopeptides					
Control (3 yr-old)	0.64	0.54	1.50	0.69	1.00
Control (8 yr-old)	0.72	0.55	1.94	1.13	1.04
Krabbe's Disease (20 months-old)	0.89	0.41	1.81	1.08	1.13
GM_1-gangliosidosis (15 months-old)	0.75	0.52	2.69	1.43	1.90
Dialyzable glycopeptides					
Control (3 yr-old)	0.11	0.26	0.60	0.72	0.32
Control (8 yr-old)	0.21	0.22	1.03	0.56	0.43
Krabbe's Disease (20 months-old)	0.10	0.14	0.58	0.44	0.21
GM_1-gangliosidosis (15 months-old)	0.10	0.15	1.36	1.07	0.88

All values are reported in terms of μmoles/g fresh tissue.

normal glycopeptides the degradation of which proceeded until terminal galactose groups became exposed. Failure to cleave the exposed galactose groups prevented α-mannosidase and β-hexosaminidases from attacking the more internally located mannose and hexosamine residues [7].

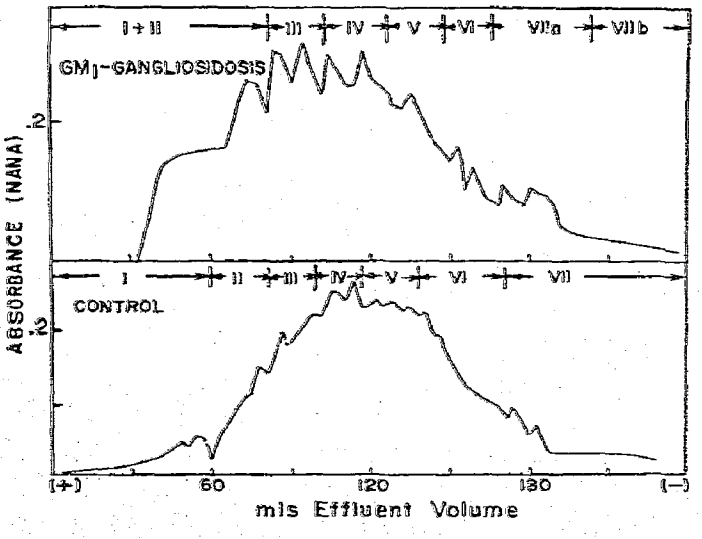

Fig. 1. Electropherograms obtained upon subjection of the non-dialyzable glycopeptide preparations from normal and GM_1-gangliosidosis cerebral gray matter on formalated cellulose using the LKB column electrophoresis apparatus. The anode is at the left. Eluate fractions were analyzed for NANA by the thiobarbituric acid method [8]. Contents of test tubes containing materials of corresponding mobilities (see top of graphs) were combined and analyzed for carbohydrate constituents (table 2).

Although the NANA and fucose content of the non-dialyzable glycopeptide preparation was similar to that of the controls, this preparation showed a nearly 2-fold elevation in galactose, mannose and hexosamine content when compared to the values obtained for a normal 3 yr-old control (table 1). This result suggests an accumulation of glycopeptides the fucose and NANA of which had been cleaved by the action of neuraminidase and α-fucosidase respectively, with the consequent accumulation of glycopeptides containing galactose, mannose and hexosamine [7]. The electrophoretic mobility of the de-sialidized glycopeptides should be considerably reduced. That the changes in carbohydrate content noted are considerably more complex than this simple explanation provides was indicated by the data obtained when the non-dialyzable glycopeptide preparations were subjected to column electrophoresis and the isolated glycopeptide fractions were analyzed for sugar content (fig. 1 and table 2). As expected, the fraction of lowest electrophoretic mobility (fraction VII) accounted for nearly twice (38%) the total non-dialyzable glycopeptide–carbohydrate material recovered from the pathological specimen as compared to that isolated from normal gray matter (16%). Furthermore, there was a marked reduction in the number of fucose and NANA residues per mannose molecule. However, the increase in the

Table 2
Carbohydrate composition of glycopeptide material recovered by column electrophoresis of the non-dialyzable glycopeptide preparation from control and GM_1-gangliosidosis cerebral gray matter (see fig. 1).

Fraction	% of Total carbohydrate in fraction	Molar Ratios of Sugar Components				
		Mannose	Galactose	Hexosamine	Fucose	NANA
Normal cerebral gray matter						
I	3	1	2.5	3.6	0.47	1.0
II	8	1	2.2	3.2	0.32	1.5
III	12	1	2.0	2.9	0.38	1.6
IV	15	1	1.7	2.4	0.48	1.4
V	20	1	1.5	2.4	0.62	1.0
VI	26	1	1.3	2.0	0.76	0.6
VII	16	1	0.9	1.5	0.58	0.4
Total	100	1	1.4	2.2	0.58	0.8
GM_1-gangliosidosis cerebral gray matter						
I + II	10	1	1.6	2.1	0.24	1.5
III	8	1	1.3	2.0	0.36	1.2
IV	15	1	1.0	1.9	0.35	0.8
V	16	1	1.2	1.8	0.44	0.5
VI	13	1	1.3	1.8	0.36	0.3
VIIa	30	1	1.4	2.0	0.19	0.1
VIIb	8	1	1.7	2.3	0.20	0.1
Total	100	1	1.3	1.9	0.30	0.4

number of galactose and hexosamine molecules per mannose molecule requires explanation. It appears likely that the glycopeptides of fractions VIIa and VIIb in the pathological material are derived from glycopeptides which are fully sialidated, possess relatively high electrophoretic mobilities, and normally appear in fractions I and II. These glycopeptides have high galactose/mannose and hexosamine/mannose ratios.

The aforementioned changes in the glycopeptide preparations are consistent with the changes expected as a consequence of the absence of β-galactosidase. On the other hand, the reduced number of galactose and hexosamine residues per mannose molecule in fraction I and the reduction in the number of NANA, galactose, and hexosamine molecules per mannose residue in fractions II through VI was unexpected. It is possible that in GM_1-gangliosidosis, a large proportion of the non-dialyzable glycopeptides are deficient in the number of N-acetylglucosamine—galactose—NANA branches that are attached to the internal mannose-rich core. Such changes cannot be ascribed to the absence of β-galactosidase and may represent secondary changes due to the effects of the disease.

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