OCCURRENCE OF DISIALOSYL GROUPS IN GLYCOPROTEINS

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SUMMARY

The occurrence of disialosyl (α-N-acetylneuraminyl-(2→8)-N--acetylneuraminyl) groups in glycoproteins was studied. It was found by methylation analysis that 8.5 % of N-acetylneuraminic acid in brain glycoproteins was substituted at C-8. The corresponding value was 16.6 % in brain gangliosides. The substituent was identified as neuraminic acid from its lability to neuraminidase treatment. The results demonstrate that not only gangliosides contain disialosyl groups, but these are also found in glycoproteins. The group is present in several types of carbohydrate units, with the highest proportion in N-glycosidic chains of large molecular size.

INTRODUCTION

Sialic acids are common constituents of animal gangliosides and glycoproteins, in which they usually occupy a terminal position in the carbohydrate chains. In addition, two sialic acid residues may be joined together to form a disialosyl group, a characteristic feature of many gangliosides (1). Such disialosyl groups have not been known to occur in glycoproteins. Recent development in the methylation analysis has made possible the reliable analysis of the site of substituents on sialic acid moieties (2). In our studies on brain gangliosides and glycoproteins we found that not only gangliosides but also glycoproteins contain disialosyl groups. This is to our knowledge the first case in which glycoproteins are reported to contain this carbohydrate sequence.

METHODS

Chloroform-methanol extraction of whole rat brains and the preparation of the total glycopeptide fraction were performed as described previously (3). Glycopeptides from calf fetuin (type II, Sigma) and human transferrin (grade II, Sigma) were prepared with the same method. Fractionation of brain glycopeptides on Concanavalin A-Sepharose (4) and gel filtration of NaOH-$NaBH_4$ treated glycopeptides (5) were done as previously. A mixture of total rat brain

gangliosides was prepared from the combined chloroform--methanol extracts by Folch partition (6).

Neuraminidase treatment was carried out in 0.2 ml of 10 mM Tris-acetate buffer (pH 6.8) containing 2 mM $CaCl_2$ and 2.5 U Vibrio cholerae neuraminidase (grade B, Calbiochem) at 37 °C for 24 h, after which 2.5 U of neuraminidase was added and the incubation was continued for another 24 h. Control samples were treated identically but without neuraminidase.

Methylation analysis of neuraminic acid and the identification of the products by gas chromatography-mass spectrometry were carried out as previously (2). A di-N-acetylneuraminyl-lactosylceramide, which contains an equimolar amount of terminal and 8-O-substituted N-acetylneuraminic acid (2), was used as a standard. An Altema AL 5 multiple ion detector was used for mass fragmentographic detection.

Periodate oxidation and $NaBH_4$ reduction of glycopeptides were performed as described previously (5). The sugar composition of the oxidized and nonoxidized glycopeptides were determined by gas chromatography (7).

Long chain (sphingosine) bases were analyzed by gas chromatography-mass spectrometry (8). Thin-layer chromatography was performed on Silica Gel G plates with n-propanol--water (6:3, v/v) as the solvent. The sialic acid containing substances were detected with resorcinol reagent (9).

RESULTS

Total rat brain gangliosides were subjected to methylation analysis, and the methylated neuraminic acid derivatives were identified by gas chromatography (Fig. 1A) and mass spectrometry. Two neuraminic acid derivatives were observed. The major product was identified as the permethylated derivative of N-acetylneuraminic acid, indicating that most of this sugar has a nonreducing terminal position in the carbohydrate chains. The second product, which arises from 8-O--substituted N-acetylneuraminic acid residues, accounted for 16.6 % of the total neuraminic acid. This is in agreement with the known occurrence of disialosyl groups in brain gangliosides.

The same two products were obtained from total rat brain glycopeptides, with the only difference that the 8-O-substituted derivative accounted for 8.5 % of total neuraminic acid (Fig. 1B). The methyl substitution pattern of this derivative was confirmed by mass spectrometry (characteristic ions at m/e 45, 129, 161, 274 and 376 (2)).

The presence of a substituent at C-8 was also ascertained with an independent method. Substituents at C-8 are expected to make neuraminic acid residues resistant to periodate

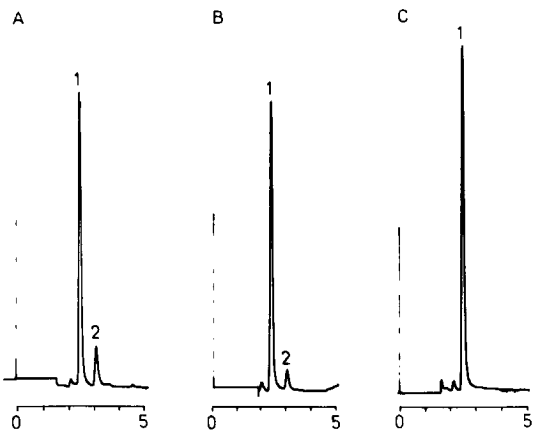

Figure 1. Gas chromatography of methylated neuraminic acids. A, total rat brain gangliosides; B, total rat brain glycopeptides; C, B after incubation with neuraminidase. Peak 1, permethylated methyl glycoside methyl ester of N-acetylneuraminic acid; peak 2, 8-O-acetyl-4,7,9-tri-O-methyl derivate of the methyl glycoside methyl ester of N-acetyl-neuraminic acid. Abscissa: retention time in minutes. Conditions: 2.2 % SE-30, 240 °C. Detection by mass fragmentography at m/e 274.

treatment, whereas nonsubstituted residues are oxidized. Of total glycopeptide neuraminic acid 12 % was found to be resistant to periodate, which is in agreement with the methylation analysis.

To study the nature of the substituent at C-8, the glycopeptides were incubated with neuraminidase. After neuraminidase treatment and methylation analysis, the 8-O-substituted derivative was not observed, but was present in the control samples treated similarly but without neuraminidase (Fig. 1C). The results indicate, that the 8-O-substituent of the neuraminic acid residues was another neuraminic acid residue, and confirms the presence of disialosyl groups in the glycopeptide fraction.

The possibility that gangliosides were present in the glycopeptide fraction was excluded by two independent methods. First, analysis of the glycopeptides by thin-layer chromatography revealed no components with similar mobility to those of gangliosides. Second, no long chain (sphingosine) bases (which are integral constituents of glycosphingolipids) were detected in the glycopeptide fraction by gas chromatography--mass spectrometry.

Table 1. Relative proportion of 8-O-substituted N-acetylneuraminic acid in brain gangliosides and glycoproteins. Values are given as per cent of total N-acetylneuraminic acid in each fraction.

Total ganglioside fraction	16.6
Total glycopeptide fraction	8.5
O-glycosidic glycopeptides	2.9
N-glycosidic glycopeptides	8.1
Fraction A	8.2
Fraction B	3.0

To characterize the type of carbohydrate chains in which the disialosyl groups occur, the glycopeptides were subjected to $NaOH-NaBH_4$ treatment and the O-glycosidic oligosaccharides were separated from the N-glycosidic glycopeptides by gel filtration. Both types of carbohydrate chains contained disialosyl groups as indicated by the finding of 8-O-substituted neuraminic acid by methylation analysis (Table 1), but the proportion in the N-glycosidic chains was significantly higher. The N-glycosidic glycopeptides were further fractionated by affinity chromatography on Concanavalin A-Sepharose. Most of the glycopeptides were not bound by the lectin (Fraction A). These glycopeptides are of larger molecular size than those bound by the lectin (Fraction B)(4). The relative proportion of 8-O-substituted neuraminic acid was clearly higher in glycopeptides not bound by the lectin.

For reference, two glycoproteins of known structure, human transferrin and calf fetuin (10) were also analysed. No 8-O-substituted neuraminic acid residues were observed by methylation analysis, which indicates that no disialosyl groups are present in these glycoproteins.

DISCUSSION

The occurrence of disialosyl groups in gangliosides is well known. In this study the occurrence of disialosyl (α-N-acetylneuraminyl-(2→8)-N-acetylneuraminyl) groups in

glycoproteins is reported. The determination of the substituents on neuraminic acid has been possible by the use of the methylation technique, which has recently been extended to the analysis of neuraminic acids (2). The presence of a disialosyl group was demonstrated by the finding that 8.5 % of the neuraminic acid residues in brain glycoproteins contains a substituent at C-8, which is hydrolyzable by neuraminidase. The corresponding value (16.6 %) for gangliosides is only twice as much. Since each disialosyl group contains one terminal and one 8-O-substituted internal sialic acid residue, it can be calculated that one of six neuraminic acid residues in brain glycoproteins occurs in a disialosyl group.

Analysis of the various glycopeptide fractions indicates that the disialosyl groups occur in several types of oligosaccharide chains. Both O- and N-glycosidic chains contain these groups, although the proportion in the latter is much greater. Disialosyl groups are found in two types (Fraction A and B) of N-glycosidic carbohydrate chains. These glycopeptides are probably composed of a similar pentasaccharide core, to which three or two peripheral N-acetylneuraminyl-galactosyl--N-acetylglucosamine-branches are attached (11). The presence of disialosyl groups in glycoproteins cannot therefore be attributed to one specific carbohydrate structure. Instead, the results may reflect a non-specificity of the neuraminyl transferase(s) catalyzing the biosynthesis of this carbohydrate sequence. It is even possible that the biosynthesis of the disialosyl groups in glycolipids and glycoproteins is catalyzed by the same enzymes.

Gangliosides containing disialosyl groups have been suggested to function as receptors for thyrotropin (12), serotonin and tetanus toxin (13). The presence of disialosyl groups in glycoproteins indicates that also these glycoconjugates must be taken into consideration in various biological interactions involving disialosyl groups.

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