MASS SPECTROMETRIC SEQUENCE STUDY OF THE OLIGOSACCHARIDE OF HUMAN TRANSFERRIN

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1. Introduction

By combined use of new derivatives it has been possible to sequenate rather larger lipid-linked oligosaccharides by mass spectrometry [1,2]. $LiAlH_4$ reduction of amide groups in permethylated derivatives [1] affords stabilization of high molecular weight fragments, thus far allowing a conclusive composition and sequence analysis of as large a molecule as a nonaglycosylceramide [2–4]. We therefore judged it of interest to test if the same procedure was successful on glycopeptides of the type liberated from glycoproteins by extensive proteolytic digestion (see [5]). In this paper the sialoglycopeptide and the asialoglycopeptide of human transferrin will be briefly discussed.

2. Materials and methods

The glycopeptides were prepared from commercial transferrin (grade II, Sigma). The denatured glycoprotein was extensively digested with pronase [6]. The glycopeptides obtained were purified by gel filtration on Sephadex G-25 and fractionated on a concanavalin A–Sepharose column as in [7]. Part of the pure glycopeptide was treated with neuraminidase [5]. Salts and enzyme were removed on a Sephadex G-50 column. The two glycopeptide fractions were *N*-acetylated [8] and methylated [9,10]. The reduction with $LiAlH_4$ and silylation were done as in [11]. The mass spectrometer used was MS 902 (AEI Ltd.) and the direct inlet system was equipped with a separate probe heater. The conditions of analysis are given in the figure legends.

3. Results

As is usually the case for sialic acid-containing glycolipids [2] three derivatives were analysed: methylated, methylated-reduced, and methylated-reduced-silylated compounds. The reduction converts amide to amine (loss of 14 mass units), and methyl ester (of sialic acid and amino acid) to alcohol (loss of 29 mass units). By silylation 72 mass units are added to each alcohol group produced. Only spectra of two types of derivatives are reproduced (fig.1–4). Although the amino acid composition was not confirmed, the formulae at the spectra are shown with Ser–Asn. The sugar part is drawn simplified from the structure recently confirmed by NMR spectroscopy [12].

Spectra and formulae of methylated and methylated-reduced derivatives of the sialic acid-containing glycopeptide are shown in fig.1 and 2, respectively.

The presence of a terminal sialic acid is shown by *m/e* 376 and 344 (loss of methanol), see fig.1, and 318 and 302 (fig.2). The ions at *m/e* 142 (fig.1) and 128, 232 and 248 (fig.2) are also derived from sialic acid [2,11,13]. The peak at *m/e* 128 (fig.2) is specific for *N*-acetylneuraminic acid. In case of *N*-glycoloylneuraminic acid *m/e* 158 was found as the base peak [14].

The terminal disaccharide is indicated by fragments at *m/e* 580 and 536 (fig.1), and the trisaccharide

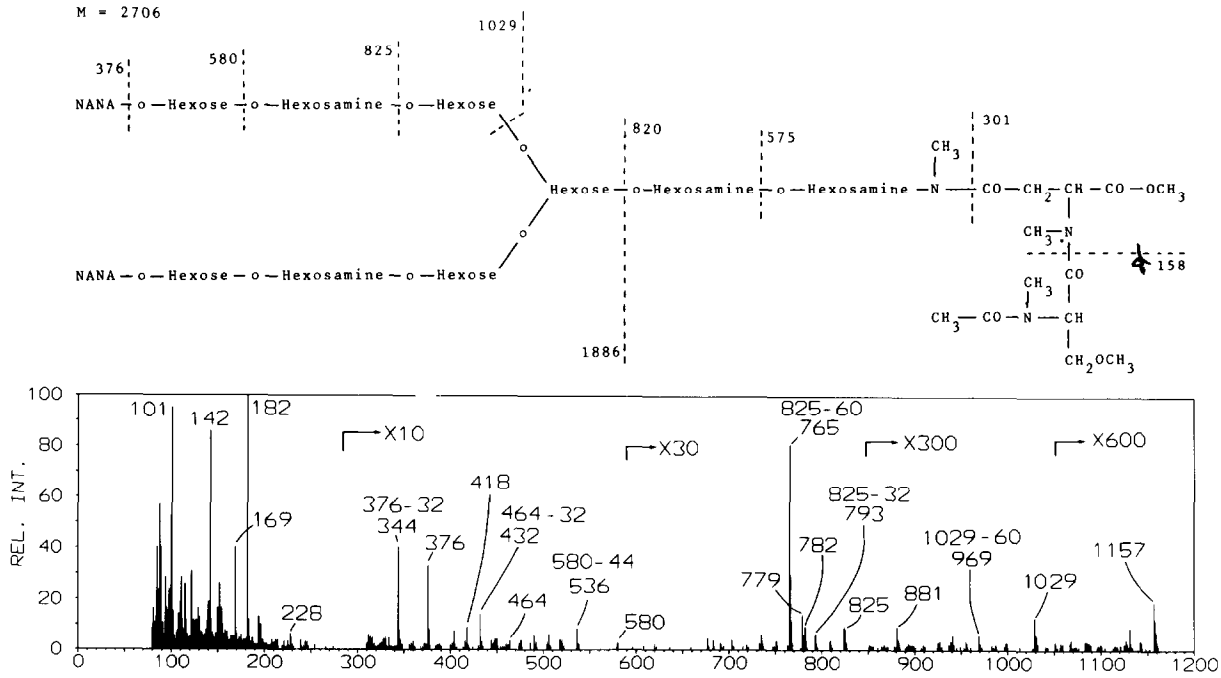
M = 2706
NANA – o – Hexose – o – Hexosamine – o – Hexose
NANA – o – Hexose – o – Hexosamine – o – Hexose
Hexose – o – Hexosamine – o – Hexosamine – N
CO – CH2 – CH – CO – OCH3
CH3 – CO – N – CH
CH2OCH3
REL. INT.
m/e

Fig.1

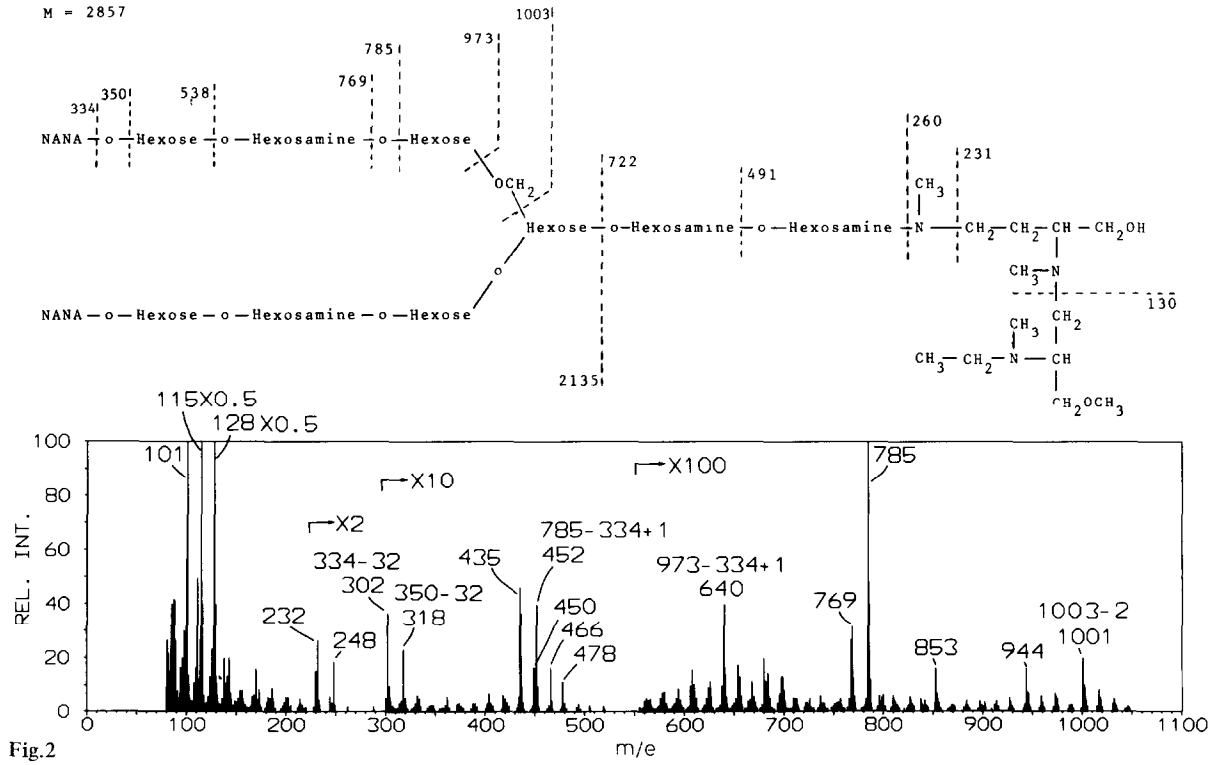
M = 2857
NANA – o – Hexose – o – Hexosamine – o – Hexose
NANA – o – Hexose – o – Hexosamine – o – Hexose
Hexose – o – Hexosamine – o – Hexosamine – N
CH2 – CH2 – CH – CH2OH
CH3 – CH2 – N – CH
CH2OCH3
REL. INT.
m/e

Fig.2

at 825, 793 and 765 [11], see fig.1, and at 785, 769 and also at 452 and 435 (452–16–1), see fig.2. The sequence of the complete branch (tetrasaccharide) is finally established by the ions at *m/e* 1029 and 969 (fig.1) and possibly 1001 (cf. analogous fragment of fig.3), see fig.2.

The base peak at *m/e* 182 (fig.1,3) is diagnostic for a monosubstituted glucosamine with a 1–4 linkage [3]. Although there are still some peaks not yet identified we have so far found no fragments of value for establishing the kind of amino acids present. Some peaks which are common to fig.1,3, and fig.2,4, respectively, are sugar peaks and due to admixture of the two compounds to each other (e.g., *m/e* 825, 793, 765, 464 and 432 of fig.1,3, and 466 of fig.2,4).

Upon silylation (spectrum not reproduced) there

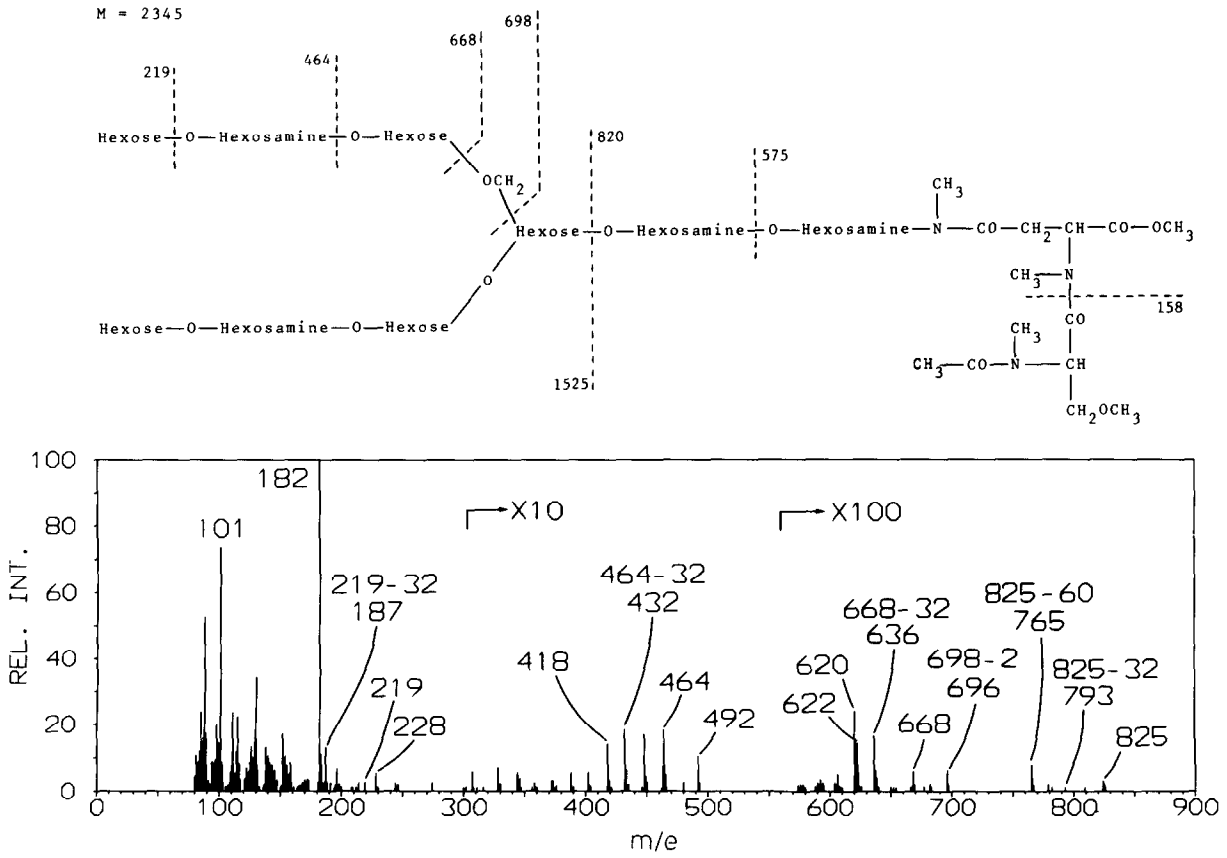

Fig.3. Mass spectrum of *N*-acetylated and permethylated asialoglycopeptide obtained from human transferrin. The conditions of analysis were: electron energy 70 eV; trap current 500 μA; acceleration voltage 6 kV; ion source temp. 255°C; probe temp. 230°C.

Fig.1. Mass spectrum of *N*-acetylated and permethylated glycopeptide obtained from human transferrin. NANA means *N*-acetylneuraminic acid. The conditions of analysis were: electron energy 70 eV; trap current 500 μA; acceleration voltage 6 kV; ion source temp. 255°C; probe temp. 250°C.

Fig.2. Mass spectrum of *N*-acetylated, permethylated and reduced glycopeptide obtained from human transferrin. NANA means *N*-acetylneuraminic acid. The conditions of analysis were: electron energy 60 eV; trap current 500 μA; acceleration voltage 6 kV; ion source temp. 260°C; probe temp. 280°C.

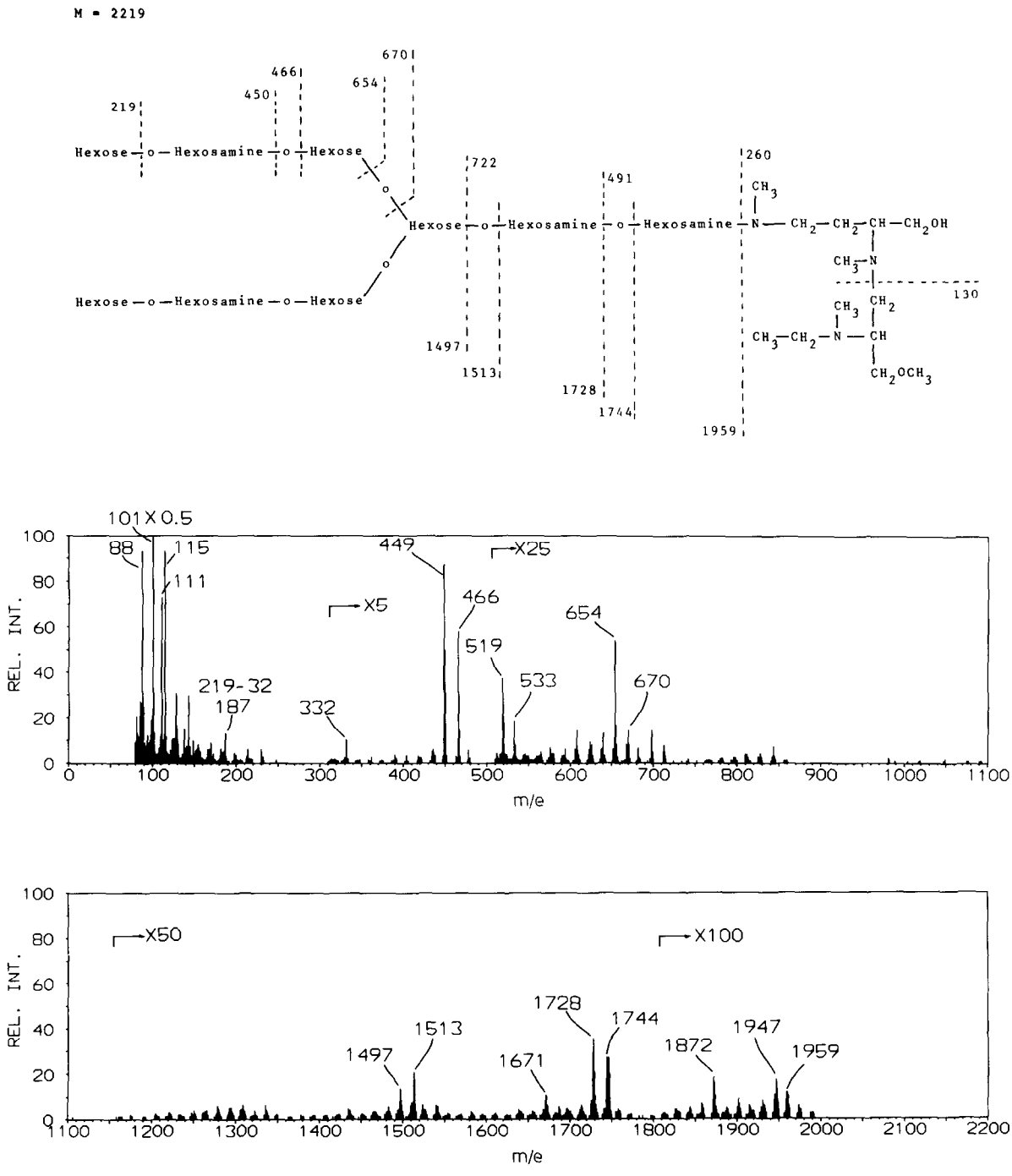

Fig.4. Mass spectrum of *N*-acetylated, permethylated and reduced asialoglycopeptide obtained from human transferrin. The conditions of analysis were: electron energy 60 eV; trap current 500 μA; acceleration voltage 6 kV; ion source temp. 260°C; probe temp. 270°C.

are some significant changes for sugar peaks but again no information concerning amino acids. Strong ions diagnostic for the trisaccharide were found at *m/e* 524 (857–406+73) and 507 (524–16–1), compare [11,13], and for the tetrasaccharide at 728 (1061–406+73) and 712 (728–16). In addition there was an even distribution of low-intensity peaks up to about *m/e* 2100, but these were not easily interpretable.

For the asialoglycopeptide (fig.3,4) sequence ions for the trisaccharide branch are found at *m/e* 187, 219, 432, 464, 636, 668 and 696 for the methylated derivative (fig.3), and at *m/e* 449 (450–1), 466, 654 and 670 for the methylated-reduced derivative (fig.4).

Of special interest is the spectrum of fig.4, which gives information for the complete nonasaccharide. The practical absence of distinct peaks between *m/e* 670 and 1497 is an indication of the branched structure. Then following is a series of peaks including the remaining trisaccharide linked to the peptide, namely *m/e* 1513, 1728, 1744 and 1959. Some rather abundant additional ions have not yet been identified (*m/e* 332, 519, 533, 1671 and 1872). It may be noted that the mass difference of 533 and 332 is the same as of 1872 and 1671, namely 201. The difference of 1872 and 533 is about the mass of the two branches, indicating that this series may come from secondary fragmentations from the peptide end.

After silylation there is practically no change of the mass spectrum (not reproduced). This is expected if the peptide part, where a trimethylsilyl group is added (cf. formula of fig.4) does not show up with primary ions in the spectrum (cf. data from the sialo-derivative).

4. Discussion

The results show that this mass spectrometric technique is as well applicable on glycopeptides as on glycolipids [1–3]. Although there is no specific information from the peptide part, in contrast to the case for the lipophilic part of glycolipids, the presence of the peptide may nevertheless be of importance for directing a primary fragmentation from the terminal sugar end. The method should be useful for a microscale fingerprinting of mixtures of glycopeptides released from glycoproteins by proteolytic digestion [5]. By a programmed temperature-dependent evaporation in the ion source one may obtain a separation according to the number of sugars. We have recently developed a computer-based technique of this kind for glycolipids [15].

Of relevance now is a detailed study of glycopeptides of less complexity and with varying structure, to learn if the specificity and unambiguity of the mass spectrometric information is as great as for glycolipids.

The present result is also a confirmation with an independent method of the oligosaccharide structure of human transferrin (discussed in [12]).

Acknowledgements

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