CARBOHYDRATE COMPOSITION AND SEQUENCE ANALYSIS OF CELL SURFACE COMPONENTS BY MASS SPECTROMETRY. CHARACTERIZATION OF THE MAJOR MONOSIALOGANGLIOSIDE OF BRAIN

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1. Introduction

Glycosphingolipids include the minor blood group substances of erythrocytes [1] and the classical Forsmann glycolipid haptene [2]. In recent time evidence has been obtained that these substances may be connected with differences in the dynamic behaviour of normal and tumour cells (for a critical review, see [3]). The possibility that oligoglycosylceramides (and glycoproteins) are involved in communication between cells has been developed in several laboratories (e.g. [4, 5]). The very high specificity [5] and probable surface location [6] of glycosyltransferases, and a much more pronounced structural variability of carbohydrate (e.g. branching) compared to peptide are additional evidence that cell surface information may be coded primarily in carbohydrate structure.

Surface glycolipids of low concentration are often strongly antigenic and therefore possible to study with very sensitive immunological methods. However, for structural characterization on the same scale no method has yet been presented. Mass spectrometry is a first candidate, but so far specific carbohydrate information has been lacking in spectra of lipids with more than one carbohydrate (assuming a demand for type and ratio of carbohydrates). Although fragments with the complete glycose were obtained for acetylated triglycosylceramides, pyrolysis occurred for higher oligoglycosylceramides [7]. However, concerning the lipophilic part (fatty acids and long-chain bases) a detailed interpretation of structure was possible, using acetates or trimethylsilyl ethers [7–9].

The present paper illustrates a newly developed method, applied on the major monosialoganglioside of brain. In other communications the analysis of other lipids will be considered, like the Forsmann glycolipid haptene, a tumour ganglioside and human kidney glycolipids. Methyl polyethers were used for the analysis, because of i) lowest possible mass of the lipid derivative (increasing the range of analysis), ii) stability in further handling of the lipid like partial hydrolysis of glycosidic bonds, and iii) as these derivatives are used for binding position analysis after degradation, by gas chromatography and mass spectrometry [10]. In a second step the methyl polyethers were reduced at the amides (of the lipophilic part and of the amino sugars) to 1) increase the volatility, 2) stabilize the molecular ions by removal of oxygens, and 3) improve subsequent analysis of amino sugars after degradation (stabilization of *N*-substituents and of glycosidic bonds close to the nitrogens, to be published).

2. Experimental

The major monosialoganglioside of bovine brain was methylated according to Hakomori [11]. The product was purified by silicic acid chromatography giving a single distinct component on thin-layer chromatography. Reduction was performed with $LiAlH_4$ in diethylether for 3 hr at room temp. The product was purified from reagents on silicic acid and was homogeneous on thin-layer chromatography. Silylation was done in hexamethyldisilazane–

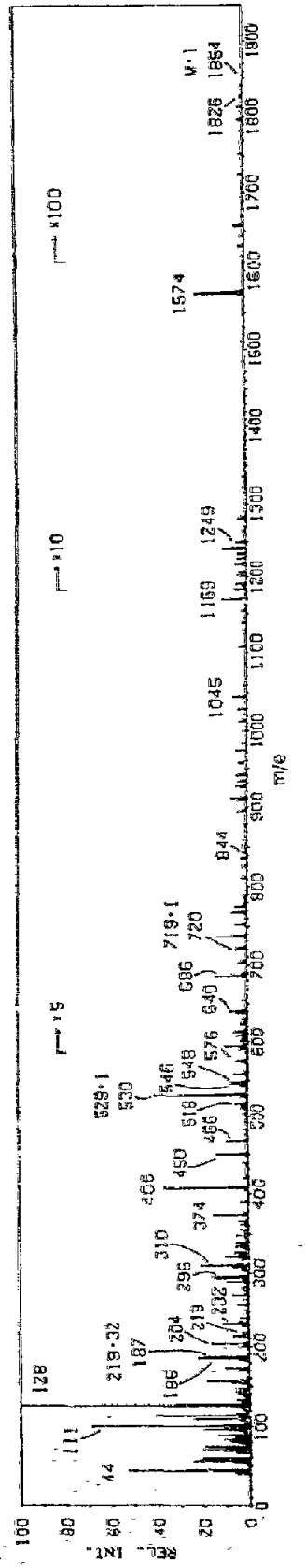

Fig. 1. Mass spectrum of methylated, reduced and trimethylsilylated monosialoganglioside of brain.

trimethylchlorosilane—pyridine 2:1:10 (by vol) for 1 hr at room temp. [7]. Suitable amounts (10–30 μg lipid) were transferred to a pyrex cuvette containing a plug of glasswool. The reagents were removed by evaporation in a stream of hot air and in the prepump chamber of the vacuum lock. The apparatus was MS 902 (AEI Ltd., Manchester, England) and the conditions of analysis as follows: electron energy 22.5 eV, filament current 500 μA, acceleration voltage 4.2 kV, ion source temperature 290°, sample evaporation temperature 320°.

3. Results and discussion

The mass spectrum recorded is shown in fig. 1. The two spectra recorded on the methylated derivative before reduction and the methylated and reduced derivative before silylation will be discussed elsewhere. The structure of the lipid has been worked out earlier [12] The lipophilic part (ceramide) was predominantly stearic acid and equal amounts of sphingosine and its 20 carbon homologue. The chemical formulae used (figs. 2 and 3) are of the higher homologue. Part of the interpretation was based on analysis of synthetic monoglycosylceramides and on high resolution analysis (to be published).

Although of low intensity, molecular ions (M−1) were readily discernible at *m/e* 1854 (1826 for the lower homologue). A relatively abundant fragment at *m/e* 1574 contained the fatty acid plus the complete carbohydrate chain (fig. 2). A corresponding fragment at *m/e* 1169 was due to the loss of sialic acid (fig. 3). Specific sialic acid peaks were intense, at *m/e* 406 and 374 (406 minus methanol). An *N*-glycolyl derivative should have given other peaks. This together with peaks at *m/e* 1249, 1045, 466, 450, 219 and 187 (compare fig. 1 and 2), and 844, 720 and 530 (compare fig. 1 and 3) not only gave information on the ratio and type of sugars (three hexoses, one *N*-acetylated hexosamine, and one *N*-acetylated neuraminic acid) but also on their sequence. The last statement was based on the following. The terminal disaccharide was evident at *m/e* 219 and 187 (hexose) and 450 and 466 (amino sugar and hexose). Positive evidence for the sialic acid binding to the second hexose was present at *m/e* 720 and 530, and also at *m/e* 844 and 640. A binding to the first hexose should have given a strong peak at *m/e* 516 (530 minus 14).

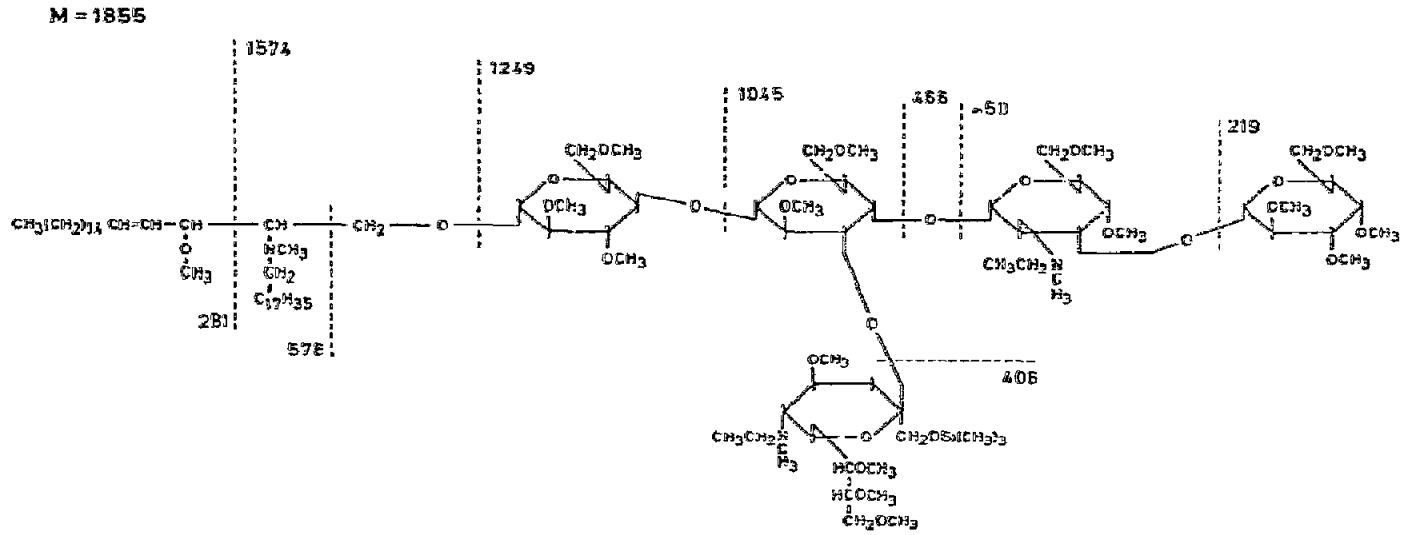

Fig. 2.

Fragments at *m/e* 576 and 546 contained the ceramide (the lower homologue at *m/e* 548 and 518), and the relatively intense peaks at *m/e* 296 (576–281 + 1) and 310 (296 + 14) were evidence for stearic acid. The base peak at *m/e* 128 was a nitrogen-containing fragment of the amino sugars ($C_7H_{14}NO$).

It is therefore concluded that the spectrum presented gave conclusive information concerning the complete molecule. The carbohydrate quality and quantity were obtained as well as carbohydrate sequence. The stereochemistry or positions of binding were not possible to interprete at the present stage. Similar information was obtained for the Forsmann glycolipid hapiene (also a pentaglycosylceramide). Lipids with more than five carbohydrates are under investigation. It is assumed that a combined pyrolysis

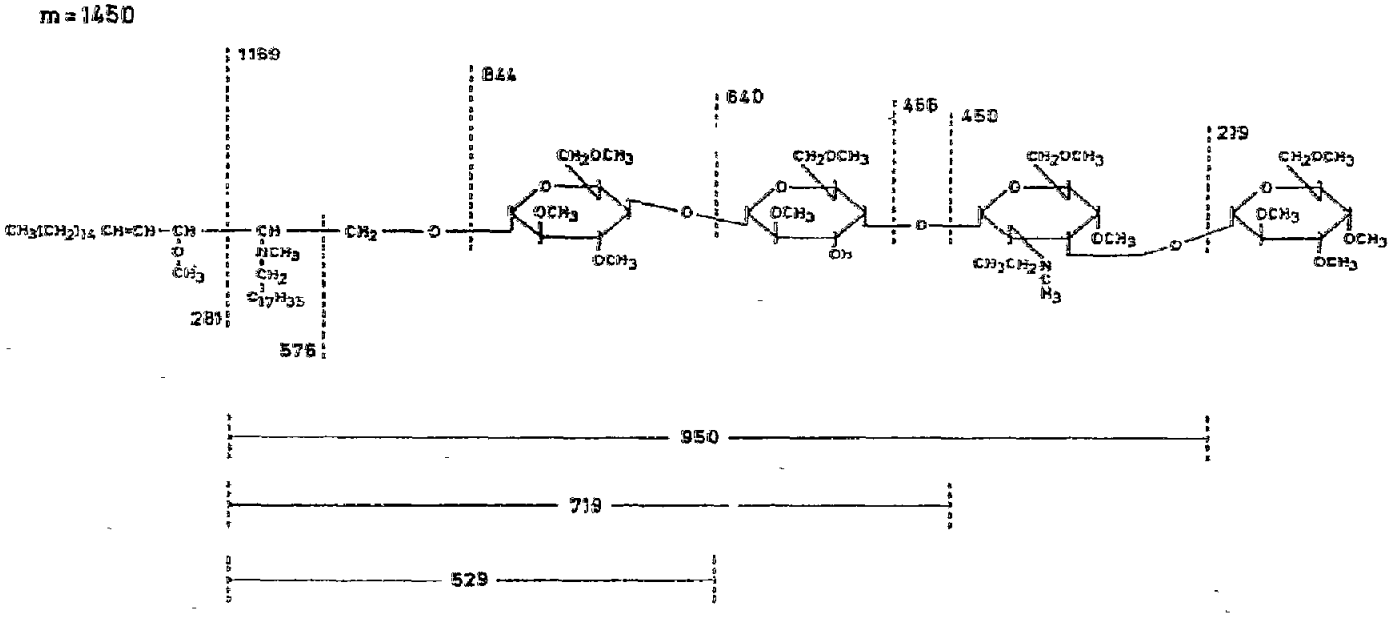

Fig. 3

and electron impact will be of value for still more complex derivatives [13].

This method in combination with the analysis of partially methylated alditol acetates after degradation [10], should be a powerful tool when adapted to minor surface components of cells.

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References

[1] S.-I. Hakomori and G.D. Strycharz, Biochemistry 7 (1968) 1279.
[2] B. Siddiqui and S.-I. Hakomori, J. Biol. Chem. 246 (1971) 5766.
[3] H.R. Herschman in: Membrane molecular biology, eds. C.F. Fox and A.D. Keith (Sinauer Ass. Publishers, Stamford, Conn. USA, 1972) p. 471.
[4] S.-I. Hakomori, Chem. Phys. Lipids 5 (1970) 96.
[5] S. Roseman, Chem. Phys. Lipids 5 (1970) 270.
[6] S. Roth, E.J. McGuire and S. Roseman, J. Cell Biol. 51 (1971) 536.
[7] K.-A. Karlsson, I. Pascher, B.E. Samuelsson and G.O. Steen, Chem. Phys. Lipids 9 (1972) 230.
[8] G. Dawson and C.C. Sweeley, J. Lipid Res. 12 (1971) 56.
[9] S. Hammarström and B. Samuelsson, J. Biol. Chem. 247 (1972) 1001.
[10] H. Björndal, C.G. Hellerquist, B. Lindberg and S. Svensson, Angew. Chem. 9 (1970) 610.
[11] S.-I. Hakomori, J. Biochem. 44 (1964) 205.
[12] H. Wiegandt, Adv. Lipid Res. 9 (1971) 249.
[13] S.-I. Hakomori, K. Stellner and K. Watanabe, Biochem. Biophys. Res. Commun. 49 (1972) 1061.