Characterization of trimethylsilyl derivatives of cerebrosides by direct inlet–chemical ionization mass spectrometry

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Abstract Submicrogram quantities of trimethylsilyl derivatives of cerebrosides obtained from the spleen of a patient with Gaucher's disease and from bovine brain were analyzed by direct probe inlet–chemical ionization mass spectrometry, using isobutane as the reagent gas. Quasimolecular ions (QM^+, M + 73) and other recognizable fragment ions produced by the successive elimination of trimethylsilanol and sugar residue gave useful information about fatty acid compositions. These ions could also be utilized for qualitative analyses of the molecular species of cerebrosides. Cerebrosides with nonhydroxy and hydroxy fatty acids could be discriminated from each other by comparing the intensities of their quasimolecular ions. Cerebrosides with saturated and monounsaturated fatty acids could also be discriminated from each other, because the mass number decreased by two mass units in cerebrosides with monounsaturated fatty acids. It was concluded that structural information and molecular species determination could be obtained from small amounts of purified cerebrosides.

Supplementary key words galactocerebrosides · glucocerebrosides

In 1969, combined gas–liquid chromatography (GLC)–electron impact (EI) mass spectrometry was applied to the analyses of cerebrosides by Samuelsson and Samuelsson (1) and some information on long chain bases, fatty acid compositions, and sugar moiety was obtained. Similar studies have been performed with a number of sphingoglycolipids (2–5). However, the fragment ions in the high mass range exhibited extremely low intensities and molecular ions were not detected by conventional EI mass spectrometry.

The technique of chemical ionization (CI)–mass spectrometry has been found to be useful for structural studies of complex biological compounds which are difficult to analyze effectively by EI mass spectrometry (6, 7). Markey and Wenger (8) have recently utilized this technique for structural analyses of permethylated and peracetylated sphingoglycolipids, using methane as the reagent gas. However, the molecular ion and fragment ions in high mass range were not sufficiently intense to determine molecular species of sphingoglycolipids. Oshima, Ariga, and Murata (9) have reported the GLC–CI mass spectrometry of psychosine, ceramides, and cerebrosides from the spleen of a patient with Gaucher's disease, using methane, isobutane, and ammonia as the reagent gases. They found the technique useful for structural studies and determination of molecular species of ceramides and cerebrosides. We have analyzed the trimethylsilyl derivatives of cerebrosides from the spleen in Gaucher's disease and from the white matter of bovine brain by the direct inlet(DI)–CI mass spectrometry, using isobutane as the reagent gas. We found that the quasimolecular(QM^+) ions or recognizable ions were useful not only for the determination of molecular species of cerebrosides, but also for structural studies on long chain bases and differentiation of cerebrosides with nonhydroxy, hydroxy, saturated, and mono-unsaturated fatty acids.

This report also describes the application to qualitative analyses of molecular species of cerebrosides using DI–CI mass spectrometry.

Abbreviations: EI, electron impact; CI, chemical ionization; DI, direct inlet; GLC, gas–liquid chromatography; hM, molecular ion of cerebrosides with hydroxy fatty acid.

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MATERIALS AND METHODS

Preparation of cerebrosides

Glucocerebrosides were isolated from the spleen of a patient with Gaucher's disease as reported elsewhere (10). Galactocerebrosides were isolated from the white matter of bovine brain and purified by silicic acid and Florisil column chromatography according to the method of Rouser, Kritchevsky, and Yamamoto (11) and, finally, by preparative thin-layer chromatography with a solvent system of chloroform–methanol–water 65:25:4 (by volume). Purified cerebrosides were converted to the trimethylsilyl derivatives by the method of Carter and Gaver (12). Cerebrosides were methanolyzed with 3% HCl in methanol at 75°C for 10 hr. The solvent was then evaporated under a stream of nitrogen. The methyl esters of nonhydroxy and hydroxy fatty acids were further separated into individual components by thin-layer chromatography in *n*-hexane–ether–acetic acid 90:10:1 (by volume).

Gas–liquid chromatography

A Shimadzu gas chromatograph, Model GC-5A with dual flame ionization detectors, was used. A glass column (1 m × 3 mm ID) packed with 1% OV-17 on Gaschrom Q (80–100 mesh) was used for analyses of the trimethylsilyl derivatives of cerebrosides. The column was temperature-programmed from 280° to 350°C at a rate of 3°C/min. The temperature of the injection port and detector was maintained at 330°C. A glass column (1.5 m × 3 mm ID) packed with 10% EGSS-X on Celite 545 (80–100 mesh) was used for analyses of fatty acid methyl esters. The column temperature was maintained at 180°C, isothermally. The temperature of injection port and detector was kept at 210°C.

Chemical ionization mass spectrometry

A Shimadzu-LKB gas chromatograph–mass spectrometer, Model 9000A equipped with chemical ionization (CI) source, was used. Isobutane was used as the reagent gas. The pressure in the ion source was adjusted to 0.9 torr. The temperature of the ion source was maintained at 220°C. The mass spectra were obtained at an electron energy of 500 eV, an emission current of 500 μA, and an accelerating voltage of 1.75 kV.

Several microliters of 0.5–1% sample were injected into a Pyrex direct probe glass cell (10 mm × 1mm ID) and evaporated at room temperature in a desiccator to remove excess trimethylsilyl reagent. The temperature of the direct probe inlet was programmed from 100° to 450°C at the rate of about 50°C/min. The temperature does not indicate the temperature of the glass cell itself, which was placed about 5 cm away from the heater, so that the actual temperature of the glass cell was probably 150°–160°C lower.

RESULTS AND DISCUSSION

Chemical ionization mass spectrometry of glucocerebrosides

Fig. 1 shows isobutane CI mass spectra of the trimethylsilyl derivatives of glucocerebrosides from the spleen of a patient with Gaucher's disease,

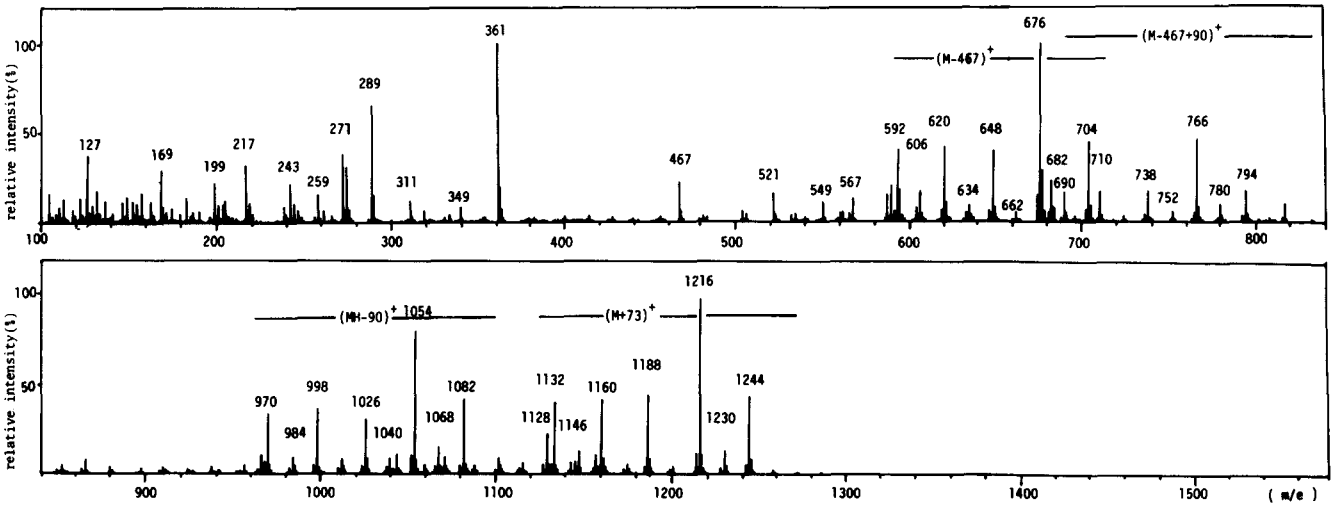

Fig. 1. Chemical ionization mass spectra of glucocerebrosides from the spleen of a patient with Gaucher's disease. The fragment ions greater than *m/e* 500 were enlarged five times.

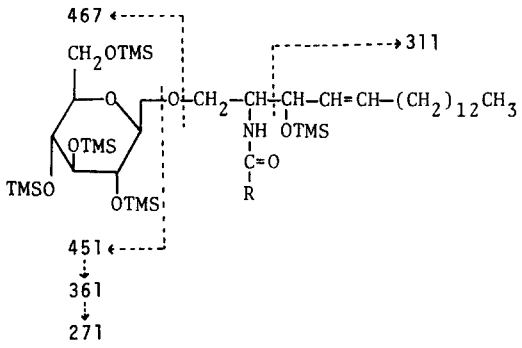

Fig. 2. Simplified formula showing the fragmentations of glucocerebrosides.

using the DI system. Four recognizable ion groups, ranging from *m/e* 1118 to 1286, from *m/e* 956 to 1182, from *m/e* 668 to 794, and from *m/e* 578 to 704 are recorded at high mass range. The detailed study of these four ion groups gives reliable determination of molecular species of glucocerebrosides. The ions ranging from *m/e* 1118 to 1286 are formed by the ion molecular reaction between the molecular ion and the trimethylsilyl radicals in the CI source. The ions ranging from *m/e* 956 to 1082 are produced by the elimination of trimethylsilanol from the protonated molecular ions (MH^+), which may be an unstable species. The ions ranging from *m/e* 578 to 704 are formed from the elimination of the sugar moiety (*m/e* 467) and indicate the molecular species of ceramides. The ions ranging from *m/e* 668 to 794 may be recombination ions, which are formed by an ion molecule reaction between ceramides and the trimethylsilanol in the CI source. In glucocerebrosides with monounsaturated fatty acids, all of these ions are recorded at two mass units less than saturated compounds. **Fig. 2** shows the fragmentation diagram of glucocerebrosides. The characteristic ions at *m/e* 467, 361, and 271 give structural information on the sugar moiety. The predominant ion at *m/e* 311 gives structural information about the long chain base, which is 1,3-dihydroxy-2-amino-4-octadecene.

Chemical ionization mass spectrometry of galactocerebrosides

Fig. 3 shows isobutane–CI mass spectra of the trimethylsilyl derivatives of galactocerebrosides from the white matter of bovine brain. Galactocerebrosides from bovine brain characteristically contain 2-hydroxy fatty acids as well as nonhydroxy fatty acids. Therefore, the CI mass spectra of galactocerebrosides contain the ions originating from galactocerebrosides with nonhydroxy fatty acids as well as those with hydroxy fatty acids. Galactocerebrosides with hydroxy and nonhydroxy fatty acids are distinguished from each other by comparing the intensities of their quasimolecular ions, because galactocerebrosides with hydroxy fatty acids are recorded at 88 mass units higher. The ions ranging from *m/e* 1160 to 1286 correspond to quasimolecular ions $(M + 73)^+$ of galactocerebrosides with nonhydroxy fatty acids and the ions ranging from *m/e* 1248 to 1374 correspond to quasimolecular ions $(hM + 73)^+$ of those with hydroxy fatty acids. In the CI mass spectra of galactocerebrosides with monounsaturated fatty acids, these quasimolecular ions have peaks that are two mass units smaller. Fragment ions originating from galactocerebrosides with hydroxy fatty acids are also recorded 88 mass units higher, so that the ions at

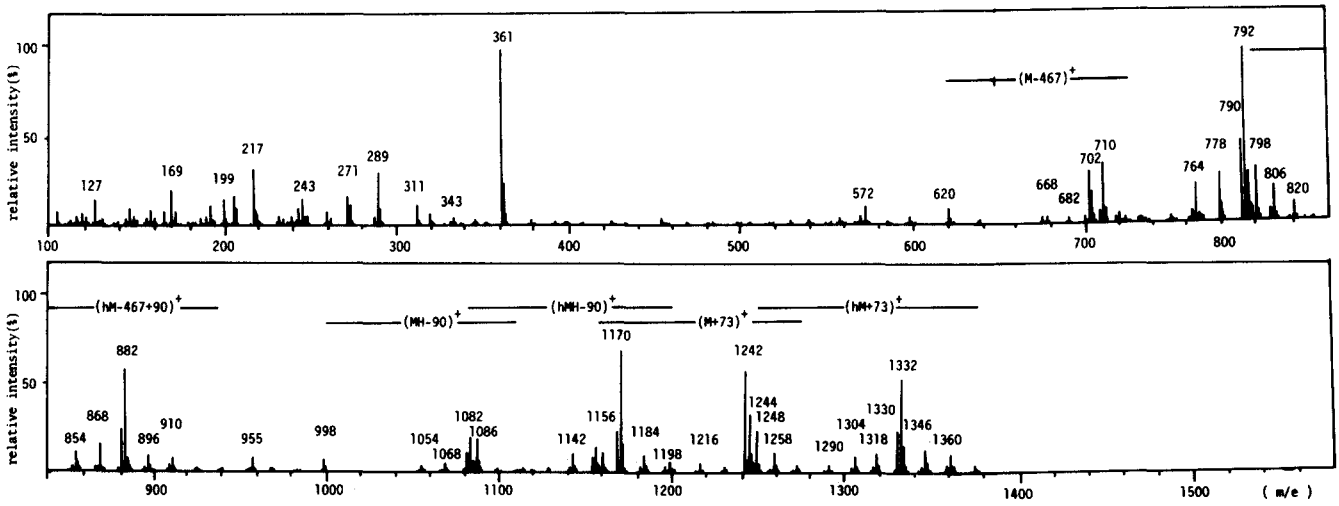

Fig. 3. Chemical ionization mass spectra of galactocerebrosides from the white matter of bovine brain. The fragment ions greater than *m/e* 500 were enlarged five times.

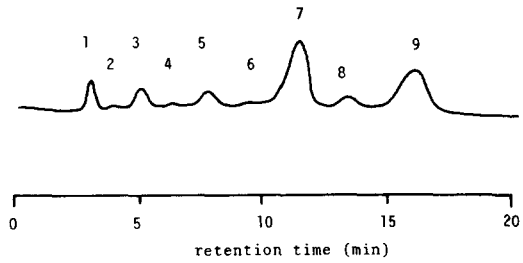

Fig. 4. Gas chromatogram of the trimethylsilyl derivatives of glucocerebrosides from the spleen of a patient with Gaucher's disease. Peaks: 1, C_{16}: 2, C_{17}, 3, C_{18}; 4, C_{19}; 5, C_{20}; 6, C_{21}; 7, C_{22}; 8, C_{23}; 9, C_{24}. The glucocerebrosides with monounsaturated fatty acids cannot be isolated from those with saturated fatty acids as a separated peak by GLC.

m/e $(hM - 467)^+$ are overlapped with the recombination ions at *m/e* $(M - 467 + 90)^+$ from galactocerebrosides with nonhydroxy and monounsaturated fatty acids. The ion groups at *m/e* $(MH - 90)^+$ and $(hMH - 90)^+$ arising from the elimination of trimethylsilanol from the protonated molecular ions are recorded as a slightly separated peak. However, the ions at *m/e* $(M - 467)^+$ and the recombination ions at *m/e* $(hM - 467 + 90)^+$ are clearly indicated.

Qualitative analyses by direct inlet mass spectrometry

Fig. 4 shows gas chromatograms of the trimethylsilyl derivatives of glucocerebrosides from the spleen of a patient with Gaucher's disease. The profile of four ion groups in Fig. 1 should be in reliable agreement with the pattern of gas chromatograms. For example, the ions at *m/e* 1132, 1160, 1188, 1216, 1230, and 1244 due to $(M + 73)^+$ correspond to 1, 3, 5, 7, 8, and 9, representing glucocerebrosides with C_{16}, C_{18}, C_{20}, C_{22}, C_{23}, and C_{24}, respectively. In GLC analyses, glucocerebrosides with monounsaturated fatty acids cannot be isolated from those with saturated fatty acids as separate peaks (9). Thus the DI–CI mass spectrometry, without the separation by GLC, may provide a powerful technique for the identification of molecular species of glucocerebrosides, including those with monounsaturated fatty acids. Similar results are obtained for the ion groups at *m/e* $(MH - 90)^+$, $(M - 467)^+$, and $(M - 467 + 90)^+$.

Table 1 shows the intensities of the ion groups at *m/e* $(M - 467)^+$ by mass spectrometry and the fatty acid composition of glucocerebrosides by GLC analyses. The large proportion of the glucocerebroside with $C_{22:0}$ is characteristic of the spleen of a patient with Gaucher's disease. There are predominantly glucocerebrosides with saturated fatty acids, the major ones being $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$ and $C_{24:0}$. The mass spectrometric data correlates with fatty acid compositions of glucocerebrosides determined by GLC analyses.

TABLE 1. Molecular species of glucocerebrosides from the spleen of Gaucher's disease

Fatty Acid	Mass Spectrometry[a]	GLC[b]
16:0	10.5	12.2
16:1	1.8	1.4
17:0	1.1	0.6
18:0	10.5	11.7
19:0	0.2	—
20:0	11.1	8.3
20:1	1.8	0.6
21:0	1.1	0.5
22:0	30.5	28.7
22:1	4.7	1.7
23:0	5.3	5.1
23:1	1.4	1.0
24:0	13.5	18.0
24:1	4.0	8.2
25:0	2.3	2.0

[a] Values of the DI–CI mass spectrometry are expressed as peak intensity percent of total glucocerebrosides. Values of the DI–CI mass spectrometry were calculated from the ion groups at *m/e* $(M - 467)^+$.

[b] GLC values are expressed as weight percent of total fatty acids from glucocerebrosides.

Fig. 5 shows gas chromatograms of the trimethylsilyl derivatives of galactocerebrosides from the white matter of bovine brain. The GLC analyses of galactocerebrosides may be more complicated than those of glucocerebrosides, because galactocerebrosides contain 2-hydroxy fatty acids as well as nonhydroxy fatty acids. In addition galactocerebrosides with a fatty acid of a given chain length have the same GLC retention time whether the fatty acid is saturated, monounsaturated, or contains a hydroxyl group.

Table 2 shows the intensities of the ions at *m/e* $(M - 467)^+$ and $(hM - 467 + 90)^+$, and the fatty acid compositions of galactocerebrosides by GLC analyses.

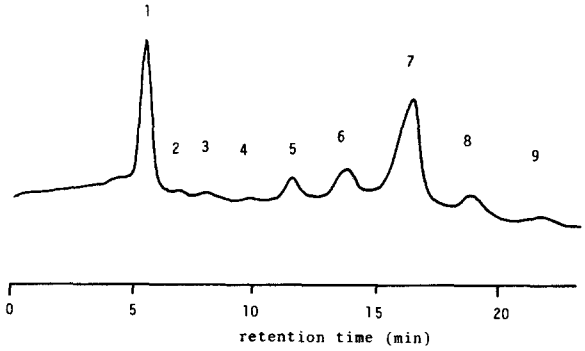

Fig. 5. Gas chromatogram of the trimethylsilyl derivatives of galactocerebrosides from the white matter of bovine brain. Peaks: 1, C_{18}; 2, C_{19}; 3, C_{20}; 4, C_{21}; 5, C_{22}; 6, C_{23}; 7, C_{24}; 8, C_{25}; 9, C_{26}. The galactocerebrosides with nonhydroxy, hydroxy, monounsaturated, and saturated fatty acids have the same GLC retention times.

TABLE 2. Molecular species of galactocerebrosides from the white matter of bovine brain

Fatty Acid	Mass Spectrometry[a]		GLC[b]	
	Normal	Hydroxy	Normal	Hydroxy
18:0	11.0	12.1	12.9	17.8
18:1	3.6	3.9	1.0	0.1
21:0	0.7	1.0		
22:0	8.5	5.9	9.4	11.2
22:1	5.0	1.8	1.8	1.8
23:0	3.6	7.3	8.0	9.0
23:1				2.4
24:0	23.4	35.3	23.7	39.6
24:1	34.0	15.2	37.4	13.4
25:0	6.0	5.8	4.0	4.0
25:1	2.0	3.0	0.6	0.9
26:0	2.2	4.5	1.2	1.0
26:1		1.5		

[a] Values of the DI–CI mass spectrometry are expressed as peak intensity percent of total galactocerebrosides. Values of the DI–CI mass spectrometry were calculated from the ion groups at *m/e* $(M - 467)^+$ for galactocerebrosides with nonhydroxy fatty acids and *m/e* $(hM - 467 + 90)^+$ for galactocerebrosides with hydroxy fatty acids.

[b] Values of gas chromatography were expressed as weight percent of total fatty acids from galactocerebrosides.

Galactocerebrosides with $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, $C_{23:0}$, $C_{24:0}$, and $C_{24:1}$ are present in large proportions. Significant differences are noted in both analyses.

CI mass spectrometry should be a suitable technique for structural studies of cerebrosides; the mode of fragmentation is quite simple and molecular ions and/or fragment ions indicating molecular species of cerebrosides, even those with hydroxy fatty acids, are clearly revealed with high intensities. However, qualitative analyses by DI–mass spectrometry could not be directly applied to the determination of cerebrosides with new sphingosine bases. Nevertheless, this method should be more informative than GLC analyses, because the proportions of cerebrosides with nonhydroxy, hydroxy, saturated and monounsaturated fatty acids from natural sources, particularly of very small amounts, could directly be determined.

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