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Note

Negative-ion fast-atom-bombardment mass spectrometry of native gangliosides using a high-polar matrix system

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Negative-ion fast-atom-bombardment mass spectrometry (FABMS) is an advantageous tool for molecular-weight determination, as well as for the structural elucidation of the carbohydrate component of native gangliosides. However, so far as we are aware, the negative-ion FABMS of a ganglioside having more than four sialic acid units has not yet been reported. To develop a method for the determination of the negative-ion FABMS of the poly(sialo)gangliosides, a search for a new matrix system was conducted.

In a preliminary study employing the mono(sialo)ganglioside GM1, it was shown[1] that the high polarity of a solvent as an electron-pair donor[2] and the neutrality of a matrix are significant factors for a negative-ion FABMS showing a high *s*-to-*n* ratio. Thus, we devised a method to analyze GM1 by use of hexamethylphosphoric triamide as the solvent and glycerol as the matrix[1]. This method was effective for the analysis of mono(sialo)gangliosides[3], but for tri- and tetra(sialo)gangliosides, no noteworthy effect was observed as compared to the conventional method[4] using triethanolamine as the matrix. However, a noticeable improvement of the spectra was observed when triethylene glycol[5] was the matrix instead of glycerol. The results showed that both neutrality and high polarity[6] are the significant factors of the matrix for negative-ion FABMS of native poly(sialo)gangliosides.

Triethylene glycol was the high-polar neutral matrix and triethanolamine, which possesses a high ability to capture protons, was the conventional matrix[4]. The total component (except the samples remaining on the FAB target) is generally called the "matrix system". We express, herein, the matrix system as solvent–matrix, e.g., hexamethylphosphoric triamide–glycerol. When the MeOH–$CHCl_3$ solution (stock solution) was directly analyzed with a matrix, only the name of the matrix is given.

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TABLE I

Enhancement of the absolute intensities of $[M-H]^-$ ion at m/z 1863 in negative-ion FABMS of GD1b when a different matrix system is used, as compared with a glycerol matrix

Matrix system	Enhancement (mean, n 3)
Hexamethylphosphoric triamide–triethylene glycol	30.7
Triethylene glycol alone	23.7
Hexamethylphosphoric triamide– glycerol	20.0
Triethanolamine alone	13.2
Glycerol alone	1.0

To examine the effect of the hexamethylphosphoric triamide–glycerol system used in the previous study[2], the negative-ion FAB-mass spectra of di(sialo)ganglioside GD1b with several other matrix systems were examined. Table I shows the mean values of enhancement of the absolute intensity of the $[M-H]^-$ ion at m/z 1863 derived from three indivual experiments. Though hexamethylphosphoric triamide–glycerol seemed to be more effective than triethanolamine, the role of triethylene glycol was noticeable. In particular, the use of hexamethylphosphoric triamide–triethylene glycol resulted in a greater than two-fold increase in the $[M-H]^-$ ion intensity, as compared with that obtained with triethanolamine. This result was attributed to the high potential of hexamethylphosphoric triamide as an electron-pair donor, and the physical properties of triethylene glycol. The value for hexamethylphosphoric triamide–triethanolamine is not reported because no remarkable effect was observed when compared with the use of triethanolamine alone. It was considered that the ability of triethanolamine to capture protons might inhibit the function of hexamethylphosphoric triamide as an electron-pair donor to the GD1b molecules, and the present results suggest that not only the neutrality but also the high polarity are the significant factors played by the matrix in the negative-ion FABMS of poly(sialo)gangliosides.

The hexamethylphosphoric triamide–triethylene glycol system was applied to the tri- and tetra-(sialo)gangliosides, the spectra of the tri(sialo)ganglioside GT1b being recorded in a manner similar to that for GD1b (see Diagram 1 and Fig. 1). The total intensity of the molecular-ion species at m/z 2192 (or 2164), $[M+K-2H]^-$, reflects the s-to-n value of the spectrum. In spite of the presence of Na and K salts, the molecular-ion species appeared clearly when triethylene glycol was used as a matrix (Figs. 1a and 1b). The absolute intensity of the ion at m/z 2192 (or 2164) obtained with hexamethylphosphoric triamide–triethylene glycol (Fig. 1a) was superior to the intensities of the spectra obtained with other matrix systems. On the other hand, both the hexamethylphosphoric triamine–glycerol system (Fig. 2c) and the conventional method (Fig. 1d) were not suitable for the analysis of such a ganglioside sample.

On the basis of the result described above, we examined the tetra(sialo)ganglioside GQ1b using the hexamethylphosphoric triamide–triethylene glycol matrix

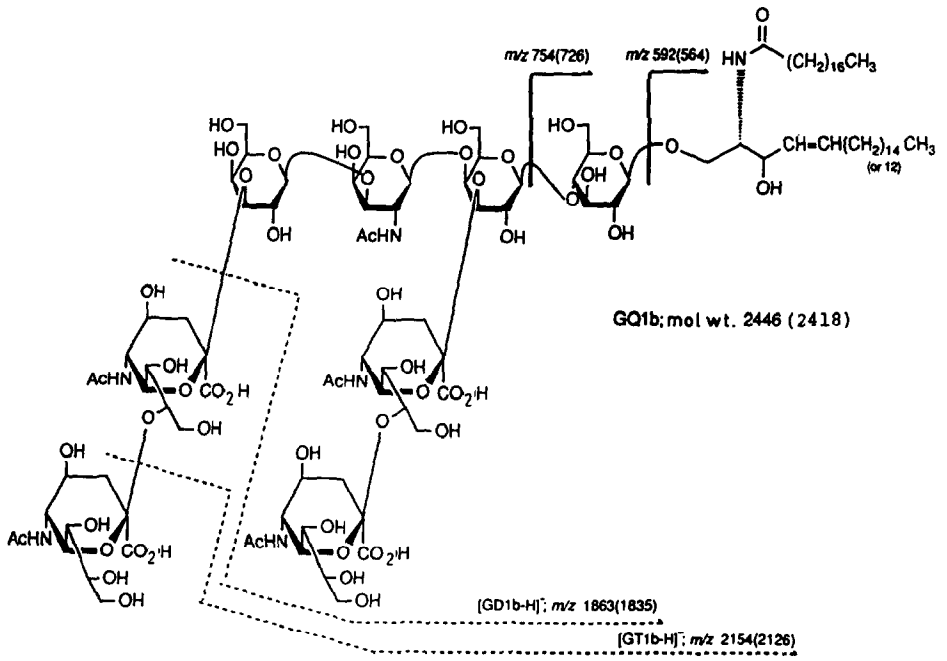

Diagram 1. Fragmentation pattern of tetra(sialo)globoside GQ1b. The fragment ion at m/z 592 (564) and 754 (726) are assigned as deprotonated ions of the ceramide (Cer) and monohexosyl ceramide (CMH) residues, respectively. The structures of the deprotonated ions of GD1b and GT1b are shown by the broken line.

system to obtain the FAB-mass spectrum of GQ1b (Fig. 2). The intense molecular-ion species at m/z 2467 $[M + Na - 2H]^-$ and 2483 $[M + K - 2H]^-$ are clearly shown in the spectrum. Thus, the use of this matrix system has made easy the molecular-weight determination of poly(sialo)gangliosides. The advantage of this system may be attributed not only to the high potential of hexamethylphosphoric triamide but also to the high polarity of triethylene glycol.

EXPERIMENTAL

Materials.—Gangliosides GD1b, GT1b, and GQ1b were purchased from Funakoshi Co. Ltd. (Tokyo, Japan) and were the crude extract from bovine brain. Hexamethylphosphoric triamide, glycerol, triethylene glycol, and triethanolamine were purchased from Nacalai Tesque Co. Ltd. (Kyoto, Japan) and were used without further purification. All other chemicals were of analytical or reagent grade.

Measurement of negative-ion FABMS.—The ganglioside stock solutions were prepared in 1:1 MeOH–$CHCl_3$ (v/v) at a concentration of 1.0 $\mu g/\mu L$. The experiments were performed under the following conditions: (*a*) the stock solution (1.0 μL) was directly subjected to negative-ion FABMS with the matrix ($\sim$ 1.0

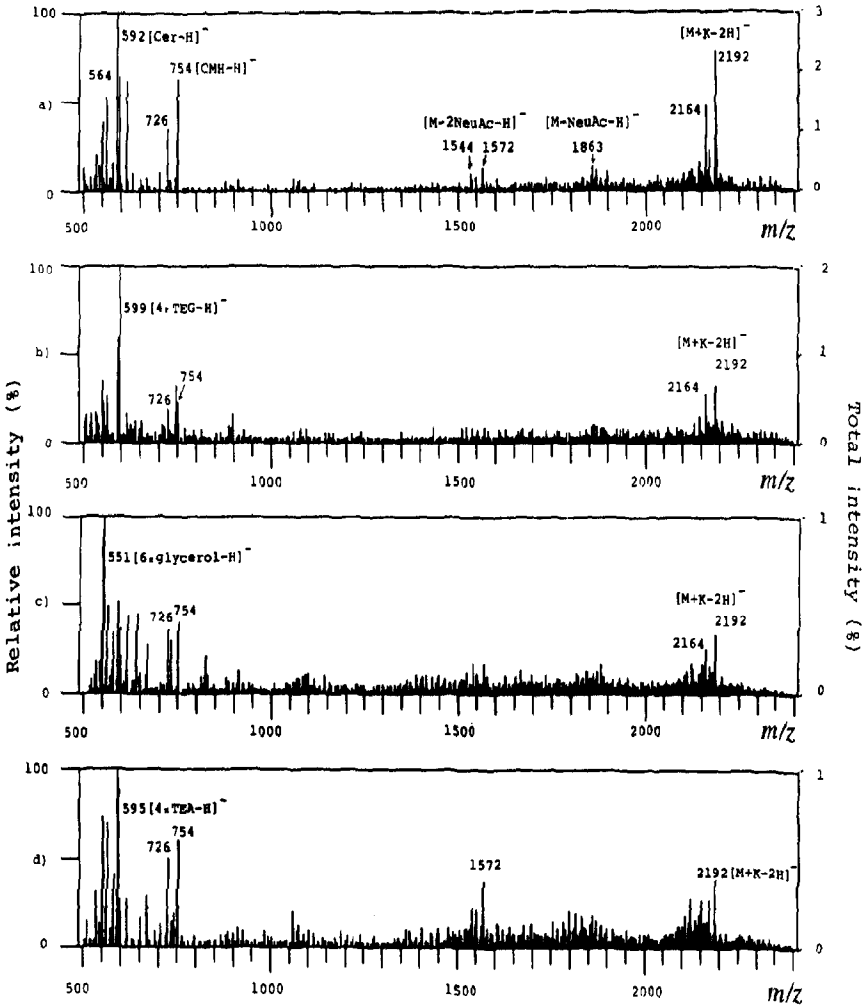

Fig. 1. Negative-ion FAB-mass spectra of GT1b obtained by use of the following matrix systems: (a) hexamethylphosphoric triamide–triethylene glycol (TEG); (b) triethylene glycol; (c) hexamethylphosphoric triamide–glycerol; and (d) triethanolamine (TEA). CMH is monohexosyl ceramide.

μL); (*b*) the stock solution (1.0 μL) was evaporated to dryness on a stainless-steel target, the residue redissolved in hexamethylphosphoric triamide (1.0 μL), mixed with the matrix ($\sim$ 1.0 μL), and subjected to analysis.

Ionization was achieved by bombardment with a neutral Xe atom beam, accelerated at 6 kV. The mass spectrometer was a JMS-DX300 (JEOL Ltd., Tokyo) interfaced to a JMA-3500 data system (JEOL Ltd., Tokyo). The mass range (m/z 100–2600) was scanned at 20-s intervals under an ion-source accelerating potential of 1 kV, and averaged intensities in decade scans were recorded. When the analysis was performed under identical conditions of the apparatus, e.g.,

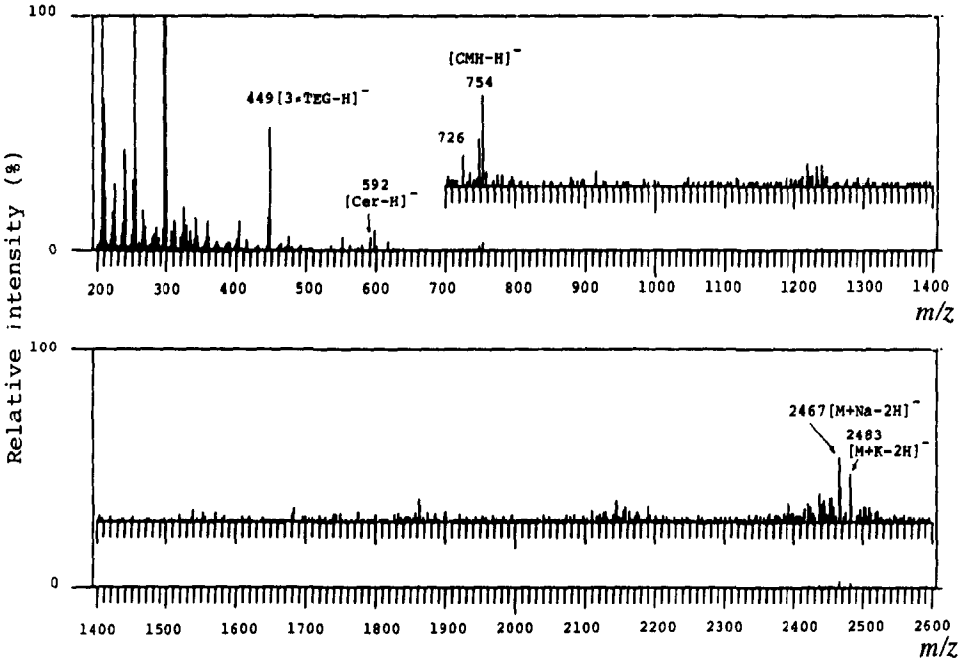

Fig. 2. Negative-ion FAB-mass spectrum of GQ1b obtained by use of the hexamethylphosphoric triamide–triethylene glycol (TEG) matrix system. CMH is monohexosyl ceramide.

ion-multiplier potential, the intensities indicated by JMA-3500 were regarded as absolute.

REFERENCES

1 R. Isobe, Y. Kawano, R. Higuchi, and T. Komori, *Anal. Biochem.*, 177 (1989) 296–299.
2 V. Gutmann, *Chem. Brit.*, 7 (1971) 102–107.
3 Y. Kawano, R. Higuchi, and T. Komori, *Liebigs Ann. Chem.*, 1990 (1990) 43–50.
4 M. Arita, M. Iwamori, T. Higuchi, and Y. Nagai, *J. Biochem. (Tokyo)*, 94 (1983) 249–256.
5 J.A. Riddick, W.B. Bunger, and T.K. Sakano, in A. Weissberger (Ed.), *Techniques of Chemistry*, Vol. 2, Wiley, New York, 1986, p. 695.
6 N. Koizumi and T. Hanai, *J, Phys. Chem.*, 60 (1956) 1496–1500.