B/E Constant Linked Scan Fast Atom Bombardment Analysis of a New Type of Glycosyl Phosphosphingolipid Isolated from Edible Fungi (Mushroom)

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The structures of new type of glycosyl phosphosphingolipids (1–4) isolated from *Hypsizigus marmoreus* (Bunashimeji, a mushroom) were determined by B/E constant linked scan fast atom bombardment (FAB) mass spectrometry. Each molecular species has common to a phytosphingosine (4-hydroxy- C_{18} -sphinganine) in the long-chain base, phosphodihexose group, and a fatty acid of the difference of carbon numbers. The alkyl chains of fatty acids are determined to be 2-hydroxyoctadecane, 2-hydroxyhexadecane, 2-hydroxytetradecane, and 2-hydroxydocosane for 1–4, respectively. The ions of m/z 421 and 720 in the negative ion mode analyses are assigned to be characteristic of a phosphodihexose and a phytosphingosine containing phosphodihexose, respectively.

1. Introduction

During the course of our search for functional molecules in edible fungi, we have reported¹⁾ the characterization and determination of the cerebroside in *Hypsizigus marmoreus* (Bunashimeji, a mushroom) by B/E constant linked scan fast atom bombardment (FAB) mass spectrometry, that is, the fatty acid composition and the location of double bonds in the long-chain base (C_{19} -sphingadienine).

Recently, glycosphingolipids and sphingomyelin gave rise to much interest, being postulated as modulators of cell growth and differentiation.^{2), 3)} Their characterization is usually performed by identification of the fatty acid and long-chain base components which were separated after hydrolysis. Sometimes structures of these molecules were analyzed in the past by gas chromatography/mass spectrometry (GC/MS) of derivatives such as trimethylsilyl (TMS) ethers.^{4)~6)}

Modern mass spectrometry has an original advantage in that it enables observation of molecular signals even of labile compounds, by means of "soft ionization" techniques such as FAB and of allowing characterization⁷⁾ of individual molecules present in mixtures with the use of tandem mass spectrometry (MS/MS method) or B/E constant linked scan method. In particular, these methods have been usefully applied to determination of several classes of phospholipids in crude extracts of microorganisms.⁸⁾

In this paper, we report a new type of glycosyl phosphosphingolipid which has four

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molecular species (1–4, Fig. 1) isolated from the same edible fungus, the characterization of which were carried out by B/E constant linked scan FAB mass spectrometry.

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Fig. 2. FAB mass spectra of phosphodihexose-sphingolipids (1-4): (a) negative ion mode spectra of fraction I (matrix: triethanolamine); (b) negative ion mode spectra of fraction II (matrix: triethanolamine). (Structures of peaks A, B, and C are given in Fig. 3.)

a mixture of cesium iodide, sodium iodide, and glycerol (5 : 1 : 25, w/w/w) as a mass calibrant. The experiment of linked scan (B/E constant scan) method using a JEOL HX 100 mass spectrometer fitted with a DA 5000 data system was carried out with He gas introduced into a collision chamber placed in the first field-free region of the EB geometry to obtain spectra of the daughter ions.

3. Results and Discussion

The molecular ion structures were not accessible from the positive ion mode FAB mass spectra, but were more clearly elucidated by the experiments of the negative ion mode. The negative ion FAB mass spectra of fraction I and fraction II are shown in Fig. 2. The molecular weights can be determined from intense peaks of $[M-H]^-$ in Figs. 2a and 2b, at m/z 1002 and 974, and 1086 and 1058, respectively. The difference of 28 a.m.u. suggests that these fractions are mixtures containing two molecular species. The exact mass of these molecular ions determined by the negative ion mode are listed in Table 1. The methanolysis of these fractions gave mixtures containing two FAMES with the difference of carbon numbers as indicated above.

3.1 Structural elucidation of glycosyl phosphosphingolipids 1 and 2 in FAB mass spectrum of fraction I

The peaks at m/z 924 and 896 in Fig. 2a were assigned to fragment ions due to $[M-Hex]^-$ and peaks at m/z 678 and 650 to fragment ions due to $[M-(Hex)_2]^-$.

The B/E constant linked scan spectra of the molecular ions m/z 1002 and 974 indicated that fragmentations occurred at the amide position to generate fragment A at m/z 720, and at the polar groups to generate fragment B at m/z 421. Subsequent loss of a hexose from fragment B generated fragment C at m/z 259, and then loss of a

Table 1. Determination of the Exact Mass of Molecular Ions and Fragment Ions of Compounds 1–4

Observed mass (error, m.m.u.)	Expected mass	Elemental formula	FAB mode
1002.6110 (–2.0)	1002.6130	C ₄₈ H ₉₃ O ₁₈ NP	Negative ion
974.5806 (–1.1)	974.5817	C ₄₆ H ₈₉ O ₁₈ NP	Negative ion
720.3572 (0.0)	720.3572	C ₃₀ H ₅₉ O ₁₆ NP	Negative ion
421.0724 (–2.3)	421.0747	C ₁₂ H ₂₂ O ₁₄ P	Negative ion
1086.7090 (2.0)	1086.7070	C ₅₄ H ₁₀₅ O ₁₈ NP	Negative ion
1058.6780 (2.3)	1058.6757	C ₅₂ H ₁₀₁ O ₁₈ NP	Negative ion

phosphate group took place giving rise to ions of m/z 97 and 79, as shown in Fig. 3. The results of high-resolution mass spectra of peaks A and B at m/z 720 and 421, respectively, produced in the negative ion mode FAB mass spectra (Fig. 2a), are summarized in Table 1. The possible elemental formula calculated on the basis of the exact masses are also listed in Table 1. From the results of high-resolution mass spectra, the fragment ions in Fig. 3 at m/z 259, 97 and 79 are assigned to C₆H₁₂O₉P[–], H₂O₄P[–], and O₃P[–], respectively. The characteristic fragment ion at m/z 259 closely resembled the phosphoinositol part of phosphoinositol *N*-stearoyl-sphinganine which had been previously isolated from *Leishmania* by Singh *et al.*^{10, 11)} Moreover, the long-chain base can be elucidated as a phytosphingosine with a eighteen carbon chain (4-hydroxy-C₁₈-sphinganine) on the basis of a characteristic fragment ion at m/z 720. Consequently, the fatty acid carbon numbers can be calculated from the difference between the molecular ions and the characteristic ion at m/z 720, which indicated the presence of 2-hydroxyoctadecanoic acid for the compound of m/z 1002 and 2-hydroxyhexadecanoic acid for the compound of m/z 974. The structures of these compounds having peaks at m/z 1002 and 974, respectively, in the negative ion spectrum (Fig. 2a) were thus found to be phosphodihexose *N*-(2-hydroxyoctadecanoyl)-4-hydroxy-C₁₈-sphinganine (1) and phosphodihexose *N*-(2-hydroxyhexadecanoyl)-4-hydroxy-C₁₈-sphinganine (2) as shown in Fig. 1.

3.2 Structural elucidation of glycosyl phosphosphingolipids 3 and 4 in FAB mass spectrum of fraction II

The structures of 3 and 4 were similarly determined as those of 1 and 2, as follows. The two peaks at m/z 421 and 720 in the negative ion spectrum (Fig. 2b) indicated the presence of a phosphodihexose and a phytosphingosine containing phosphodihexose, respectively. The ions at m/z 259, 97, and 79 also support the structures of the phosphodihexoses. Consequently, the structures of fatty acids are determined to be 2-hydroxytetracosanoic acid for the compound of m/z 1086 and 2-hydroxydocosanoic acid for the compound of m/z 1058 from the characteristic fragment ion at m/z 720. The B/E constant linked scan spectra of the molecular ions m/z 1086 and 1058 (Fig. 4) supported the above-mentioned postulation. The structures of these compounds having peaks at m/z 1086 and 1058, respectively, in the negative ion spectrum (Fig. 2b) were thus found to be phosphodihexose *N*-(2-hydroxytetracosanoyl)-4-hydroxy-C₁₈-sphinganine (3) and phosphodihexose *N*-(2-hydroxydocosanoyl)-4-hydroxy-C₁₈-sphinganine (4) as shown in Fig. 1.

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Fig. 3. B/E constant linked scan spectra of [M-H]⁻ ions at m/z 1002 and 974 in the negative ion mode FAB mass spectrum of fraction I.

Fig. 4. B/E constant linked scan spectra of [M-H]⁻ ions at m/z 1086 and 1058 in the negative ion mode FAB mass spectrum of fraction II.

Thus, the ions of m/z 421 and 720 in the negative ion mode are found to be very useful for the structural determination of glycosyl phosphosphingolipids (phosphodihexose-sphingolipids) in the analyses by FAB mass spectrometry, because these ion peaks are characteristic of a phosphodihexose and a phytosphingosine (4-hydroxy- C_{18} -sphinganine) containing phosphodihexose, respectively.

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Keywords

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