Mini Review

Analysis of Chemical Structures of Glucosylceramides from Rice and Other Foodstuffs

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Summary Sphingolipids recently attract more attentions because of their distinctiveness on structures and expected functions. Liquid chromatography-mass spectrometry is one of the most powerful methods for the identification of chemical structures of sphingolipids. Glucosylceramides prepared from various foodstuffs including rice are generally used for functional foods and their structures are quite different from mammals. For structural analysis of glucosylceramides by LC-MS/MS, the typical signals which are characteristic for the sphingoid base moieties can be obtained as product ions. Using this method for rice and maize, glucosylceramides containing 4,8-sphingadienine (d18:2) acylated to hydroxy-fatty acids were detected as the predominant molecules. In addition, the presence of the triene type of sphingoid base (sphingatrienine, d18:3) in rice and maize was also emphasized.

Key Words sphingolipids, glucosylceramide, sphingoid bases, sphingatrienine

Sphingolipids, one of the major families of lipids, are ubiquitous in all eukaryotic organisms. There are diverse structures of sphingolipids in various foodstuffs since their structures are depend on biological species such as mammals, fish, invertebrates, higher plants, fungi etc. (1–5). Recently, physiological and nutritional functions of dietary sphingolipids are attracting attention and their effects, such as improving the barrier function of skin, lowering plasma lipids, anti-inflammatory and prevention of colon cancer, have been reported (6–15). However, the mechanisms for the effects of dietary sphingolipids, including their metabolic fates, are not fully understood. Mass spectrometry is one of the most powerful tools for detecting and identifying the chemical structures of sphingolipids. This method should be helpful for the elucidation for the role of sphingolipids as food components, because determination of their diverse structures must be important for understanding the functional and nutritional significance. We previously reported about intestinal absorption of dietary sphingolipids by LC-MS analysis (16–18). In this review, we focus on the analysis of chemical structures of glucosylceramides from foodstuffs, especially rice, by LC-MS method.

General Structures of Sphingolipids

Sphingolipids are composed of a ceramide formed by a sphingoid base bound to a fatty acid on an amide group. The molecular structure of polar heads and ceramides of sphingolipids is diverse among biological species. Sphingophospholipids have a phosphoric acid

as a headgroup. Major sphingophospholipids in mammals are sphingomyelins having a phosphocholine as a polar head. Sphingolipids having sugar residue are glycosphingolipids. Glucosylceramide is frequently found in higher plants (19).

Similar to fatty acids, diverse structures of the sphingoid base occur in nature. The most common sphingoid base of mammalian sphingolipids is sphingosine (trans-4-sphingenine, d18:1). Smaller amounts of other sphingoid bases, such as sphinganine (dihydrosphingosine, d18:0) and phytosphingosine (4-hydroxysphinganine, t18:0) are frequently encountered. The structures of the sphingoid bases in higher plants, such as 8-sphingenine (d18:1), 4,8-sphingadienine (d18:2) and 4-hydroxy-8-sphingenine (t18:1), are more complicated than those in mammals, because the sphingoid bases can be desaturated at the C8-position by a stereo-unselective $\Delta 8$ -cis/trans-sphingolipid desaturase (20). In addition, sphingolipids of marine invertebrates have unique triene types of sphingoid bases with a conjugated diene such as 2-amino-4,8,10-octatriene-1,3-diol (d18:3) and 2-amino-9-methyl-4,8,10-octatriene-1,3-diol (d19:3) (3). Thus, sphingolipids having various structures of sphingoid bases are ingested daily from food.

Analysis of Glucosylceramides from Various Sources by LC-MS

The chemical structures of glucosylceramides from maize, rice, mushroom (maitake) and sea cucumber were identified by LC-MS/MS system (21). For structural analysis of glucosylceramides, $[M+H]^+$ or $[M+H-H_2O]^+$ in the positive scan mode was used for MS/MS analysis to obtain product ions. In case of glucosylceramide mol-

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Fig. 1. Structures and characteristic product ions of sphingoid bases from various sources.

ecules consisting of 4-hydroxy-8-sphingenine (t18:1), the loss of glucose [M+H-162]⁺ was mainly detected and was used as precursor ion, because the product ions could not be detected from [M+H]⁺. The typical signals which are characteristic for the sphingoid base moieties were observed by MS/MS detection. Figure 1 shows the structures and characteristic product ions of diverse sphingoid bases contained in glucosylceramide. Pairs of these structurally specific product ions from sphingoid bases and their precursor ions are used for the identification of the glucosylceramide molecules, although it is difficult to distinguish the isomers.

Molecular structures of rice glucosylceramide were similar to those of maize and their molecular species consisted of hydroxy fatty acids with 18 to 26 carbon atoms including odd numbered fatty acid. Detection of molecules consisting of d18:2 and t18:1 in rice were separated into two peaks (cis and trans isomers) as similar as maize. The major sphingoid backbone in fungal sphingolipids is a unique 9-methyl branched sphingoid base (9-methyl-4,8-sphingadienine, d19:2). In maitake, glucosylceramides containing d19:2 acylated to hydroxy fatty acids with 14–24 carbon atoms were also identified. In addition, odd numbered hydroxyl fatty acids (C15:0 h

and C17:0 h) were uniquely predominant. It has been known that the sphingoid bases in marine invertebrates are quite different from those in mammals and plants. The product ions detected from glucosylceramides in sea cucumber were identified as d17:1, d18:1, d18:2, d18:3, d19:1, d19:2 and d19:3.

Identification of Glucosylceramides Containing Sphingatrienine in Maize and Rice

The characteristic product ion at m/z 260.2 corresponding to d18:3 was detected in maize glucosylceramide using the auto MS/MS detection mode (22). As the precursor ion of m/z 260.2, [M+H-18]⁺ ions at m/z 694.5, 722.5, 750.6, 778.6 and 806.6 were detected and identified as glucosylceramides. The acylated fatty acid moieties were hydroxy fatty acids with 16–24 carbon atoms. In the case of rice, glucosylceramide consisting of d18:3-C18:0 h and d18:3-C20:0 h were found.

Triene bases with conjugated diene, such as 2-amino-4,8,10-octatriene-1,3-diol (d18:3) and 2-amino-9-methyl-4,8,10-octatriene-1,3-diol (d19:3), are identified in marine invertebrates including ascidians, starfish, sea cucumber and squid (3, 4, 23–25). On the other hand, Sperling et al. described the presence of sphingatrienine (d19:3) in tobacco leaf by HPLC analysis of sphingoid bases (26). Several molecular species of sphingatrienine-containing glucosylceramides in maize and rice are also identified by LC-MS/MS system (22). However, the details of their structures, especially locations of double bonds, have not been identified yet. It has been reported that the composition of sphingoid bases differs between chilling sensitive and tolerant plants (27). The details of tissue distribution, synthetic pathways and functions of plant sphingatrienines remain to be elucidated.

Disclosure of State of COI

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