Isolation of three novel cholinergic neuron-specific gangliosides from bovine brain and their in vitro syntheses

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In the present study, three extremely minor but novel Chol-1 antigens, termed X1, X2, and X3 have been isolated from bovine brain gangliosides. Based on the results of sialidase degradation, TLC-immunostaining with anti-Chol-1 antibody and fast atom bombardment mass spectrometry, their chemical structures were identified as:

\[ \text{X1: } \text{GM}_{1}\alpha \]
\[ \text{X2: } \text{GD}_{1}\alpha \alpha \]
\[ \text{X3: } \text{GT}_{1}\beta \alpha \]

The yields of GM\(_{1}\alpha\), GD\(_{1}\alpha\alpha\), and GT\(_{1}\beta\alpha\) were approximately 150, 20, and 10 \( \mu \)g, respectively, from 10 g of the bovine brain ganglioside mixture. In conjunction with our previous observations, all gangliosides with anti-Chol-1 reactivity were found to contain a common sialyl a2-6 N-acetylgalactosamine residue, indicating that this unique sialyl linkage is the specific antigenic determinant. We subsequently examined the biosyntheses of the three novel Chol-1 gangliosides using rat liver Golgi fraction as an enzyme source. The results showed that GM\(_{1}\alpha\), GD\(_{1}\alpha\alpha\), and GT\(_{1}\beta\alpha\) were synthesized from asialo-GM\(_{1}\), GM\(_{1}\alpha\), and GD\(_{1}\beta\) respectively, by the action of a GalNAc a2-6sialyltransferase.

Keywords: ganglioside, cholinergic neuron, ganglioside biosynthesis, sialyltransferase

Abbreviations: the nomenclature used for gangliosides is based on the system of Svennerholm [37] and Hirabayashi et al. [8, 12]. Cer, ceramide; Gal, galactose; Glc, glucose; GalNAc, N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid; NeuGc, N-glycolylneuraminic acid; Hex, hexose; HexNAc, N-acetylhexosamine; TLC, thin layer chromatography; HPTLC, high performance thin layer chromatography; ELISA, enzyme-linked immunosorbent assay; FAB-MS, fast atom bombardment-mass spectrometry; HD, Hanganutziu-Deicher

Introduction

In order to find cholinergic neuron-specific markers, Whittaker and his colleagues have raised antisera by immunizing sheep with cholinergic synaptosomes prepared from the electric organ of Torpedo marmorata [1–4]. One of these antisera, anti-Chol-1, elicited the selective complement-mediated lysis of a cholinergic fraction of mammalian brain synaptosomes [3]. Immunohistochemically anti-Chol-1 stained cell bodies and nerve terminals of cholinergic neurons in the rat central and peripheral nerve systems [5]. Cytotoxicity tests and immunohistochemistry demonstrated that the binding of anti-Chol-1 to nerve
terinals was completely inhibited by gangliosides derived from the mammalian brain and Torpedo electric organ [3]. In addition, immunoreactive structures in rat brain with anti-Chol-1 disappeared after pre-treatment with chloroform:methanol (2:1, v/v) [3]. Thus, the chemical nature of the antigens, generally termed Chol-1, was supposed to be gangliosides. Anti-Chol-1 was found to recognize at least three gangliosides designated as Chol-1α, Chol-1β, and Chol-1γ [3].

Chol-1 gangliosides are believed to have functional importance since they are expressed in the restricted regions of the nervous systems and their expression is conserved from Torpedo to human. In spite of their importance, the chemical structures of Chol-1 antigens have remained obscure since they are extremely minor components of mammalian brains. Giuliani et al. isolated 100 μg as sialic acid of Chol-1β ganglioside from 100 kg of pig brain and characterized the partial structure of the novel antigen [6].

We developed an effective method using Q-Sepharose column chromatography for the separation of minor gangliosides [7]. Using this method, we have isolated and characterized several new species of minor gangliosides such as an α-series ganglioside [8], de-N-acetylated GM1 [9], and a hybrid type of ganglioside containing GM2 epitope [10]. Recently we have also identified two species of Chol-1 gangliosides as GT1α (IV3NeuAc,III6NeuAc,II2NeuAc-GgOse4Cer) [11] and GQ1βα (IV3NeuAc,III6NeuAc,II2NeuAc-GgOse4Cer) [12]. In this study, we have isolated and characterized three additional novel Chol-1 gangliosides from minor fractions of a bovine brain ganglioside mixture. Based on the structural analysis, all Chol-1 gangliosides were shown to contain a sialyl α2-6 N-acetylgalactosamine residue as was found in GT1α and GQ1βα. The novel gangliosides were found to be synthesized by the action of a GalNAc α2-6sialyltransferase associated with a rat liver Golgi fraction.

Materials and methods

Materials

Gangliosides from bovine brain were prepared as described previously [7]. GT1α and GQ1βα, Chol-1α gangliosides [12], and GD1α [8] were purified from bovine brain ganglioside mixture. Sheep anti-Chol-1 was raised as described by Richardson et al. [3]. Sialidase L was isolated and purified from leech as described [13, 14]. Donryu rat (female, 4 weeks old) was purchased from SLC, Inc. (Hamamatsu, Japan). CMP-NeuAc and 4-methylumbelliferyl NeuAc (4MU-NeuAc) were from Sigma Chemical Company (St Louis, USA). Anti-NeuGc-containing ganglioside antibody (anti-HD antibody) was prepared and purified as described [15].

HPTLC

Analytical HPTLC was carried out on precoated HPTLC plates (E. Merck) using the following solvent systems. Chloroform:methanol:12 mM MgCl2 (5:4:1, v/v/v; solvent A) and chloroform:methanol:12 mM MgCl2:15 M NH4OH (50:40:7:3, v/v/v/v; solvent B). Gangliosides were visualized with a resorcinol/HCl reagent.

Immunological methods

TLC-immunostaining was performed by the method of Higashi et al. [16]. Gangliosides were applied on a plastic TLC plate (Polygram Sil G, Nagel, Germany) and developed with solvent B and then solvent A in the same direction. In case of two-dimensional TLC-immunostaining, a plastic plate was developed with solvent B to the left (first dimension) and then with the solvent A (second dimension). Gangliosides were visualized by immunostaining with anti-Chol-1. The enzyme-linked immunosorbent assay (ELISA) was performed by the method of Higashi et al. [17].

Isolation of Chol-1 gangliosides

Total bovine brain gangliosides (10 g) were applied onto a Q-Sepharose column (3 × 75 cm) and fractionated into 26 fractions by the method described previously [7]. By TLC-immunostaining with anti-Chol-1, X1, X2, X3, and X4 were detected in fractions 5, 17, 20 and 25, respectively (Fig. 1). In this study, X1, X2, and X3 were isolated and characterized since the content of X4 was too low to purify. Gangliosides in each fractions were dissolved in 20 ml of chloroform:methanol:water (60:25:1, v/v/v) and applied on an Aquasil SS-552N HPLC column (2 × 30 cm, Senshu Chemicals, Tokyo) which had been equilibrated with the same solvent. The column was attached to a Jasco 880-PU HPLC pump (Japan Spectroscopic Co., Ltd.) and eluted at the rate of 5 ml min⁻¹ with a linear gradient starting from the solvent system of chloroform:methanol:water (60:25:1, v/v/v) until the solvent system of chloroform:methanol:water (60:35:8, v/v/v) was attained. The elution was completed in 400 min and 15 ml fractions were collected. Five μl aliquots were analysed by TLC-immunostaining with anti-Chol-1. Fractions containing X1, X2, or X3 were collected and evaporated to dryness. The final purification of each ganglioside was achieved by a preparative TLC method as described previously [12].

Negative FAB-MS

Mass spectra were recorded on a Finnigan MAT TSQ 700 triple-stage quadrupole mass spectrometer (Finnigan MAT, Sun Jose, USA) equipped with a FAB-MS spectrometric ion source. Data were processed with a DEC Station 2100 computer. The FAB-MS spectra in the negative mode were recorded by the method of Ohashi et al. [18]. For FAB-MS