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Short communication

Facile method for the preparation of lyso-GM1 and lyso-GM2

Takayuki Ando [a], Su-Chen Li [a], Makoto Ito [b], Yu-Teh Li [a,*]

[a] *Department of Biochemistry, Tulane University Health Sciences Center School of Medicine, New Orleans, LA 70112, USA*
[b] *Department of Bioscience and Biotechnology, Graduate School Kyushu University, Fukuoka 812-8581, Japan*

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Abstract

This paper reports a facile method for the preparation of lyso-GM1 [Galβ1 → 3GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-sphingosine] and lyso-GM2 [GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-sphingosine], respectively, from GM1 [Galβ1 → 3GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-Cer] and GM2 [GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-Cer], using sphingolipid ceramide deacylase and high performance anion-exchange chromatography (HPAEC). The enzymatically released lyso-GM1 and/or lyso-GM2 was effectively separated from its parent ganglioside by HPAEC using a Mono Q HR 5/5 column with an Amersham Biosciences fast protein liquid chromatography system. The yield was almost quantitative and the separation completed in approximately 3 h. This method is more convenient and effective than the conventional method using alkaline hydrolysis and silicic acid chromatography to generate and purify lyso-gangliosides.

Keywords: Ganglioside; Lyso-ganglioside; Lyso-GM1; Lyso-GM2; Sphingolipid ceramide deacylase

1. Introduction

Gangliosides, sialic acid containing glycosphingolipids, are highly enriched in neural tissues. They are also important components of eukaryotic cell membranes [1]. The biological importance of gangliosides has been firmly established through the recent studies of engineered mouse models [2]. Lyso-GM1 [Galβ1 → 3GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-sphingosine] and lyso-GM2 [GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-sphingosine] are the ganglioside derivatives of GM1 [Galβ1 → 3GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-Cer] and GM2 [GalNAcβ1 → 4(Neu5Acα2 → 3)Galβ1 → 4Glcβ1 → 1′-Cer] without a fatty acyl moiety attached to the sphingosine. These two ganglioside derivatives have been detected in the tissues of patients with GM1-gangliosidosis and GM2-gangliosidosis (Tay-Sachs disease), respectively [3]. In addition to being cytotoxic, lyso-gangliosides and their metabolites have also been suggested to be potential pharmacological agents to modulate protein kinase C activities in different cell systems [4]. Such chemical methods as alkaline hydrolysis and hydrazinolysis [5–8] have been commonly used to prepare lyso-gangliosides by removing the fatty acyl moiety from gangliosides. However, in addition to removing fatty acyl moiety from ceramide, these methods also indiscriminately remove acetyl groups from *N*-acetylhexosamine and/or *N*-acetylneuraminic acid. Unlike chemical methods, the enzymatic conversion of a ganglioside to a lyso-ganglioside by a specific glycosphingolipid-cleaving enzyme, sphingolipid ceramide deacylase (SCDase), does not cause the undesired *N*-deacylation of *N*-acetylhexosamine and/or *N*-acetylneuraminic acid in the sugar chain [9]. Traditionally, the lyso-gangliosides generated by alkaline hydrolysis or hydrazinolysis were purified by silicic acid column chromatography [10]. We found that enzymatically generated lyso-GM1 or lyso-GM2 could be conveniently and effectively separated from its parent ganglioside by high performance anion-exchange chromatography (HPAEC) using a Mono Q HR 5/5 column attached to an Amersham Biosciences fast protein liquid chromatography (FPLC)

* Corresponding author. Tel.: +1 504 988 2451; fax: +1 504 988 2739.
E-mail address: yli1@tulane.edu (Y.-T. Li).

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system [11]. This report describes a facile method for preparing lyso-GM1 and lyso-GM2 from GM1 and GM2, respectively, using SCDase followed by HPAEC purification.

2. Experimental

2.1. Materials and instruments

GM2 was isolated from the brain of a Tay-Sachs patient [12]. The recombinant *Shewanella alga* SCDase expressed in *Escherichia coli* was prepared by the method described previously [13]. The following chemicals and reagents were obtained from commercial sources: GM1, Trans Bussan (Geneva, Switzerland); Triton X-100, Sigma–Aldrich (St. Louis, MO, USA); silica gel coated TLC plates, Merck (Darmstadt, Germany); Mono Q column (10 cm × 1 cm) and a FPLC system, Amersham Biosciences (Piscataway, NJ, USA); Sep-Pak C_{18} cartridge (size 35 ml, 10 g), Waters (Milford, MA, USA). All other chemicals were of the highest grade from commercial sources.

2.2. TLC analysis

For the TLC analysis of gangliosides and lyso-gangliosides, the solvent system used was chloroform: methanol: 10% acetic acid (5:4:1, v/v/v) [9]. To visualize the gangliosides, the plate was sprayed with the diphenylamine–aniline–phosphoric acid reagent as previously described [14]. The quantitative estimation of ganglioside bands on a TLC plate was carried out using an EPSON Perfection 3170 photo scanner and NIH 1.63 program.

2.3. Enzyme reaction and purification of lyso-GM1 and lyso-GM2

For the generation of lyso-GM1 or lyso-GM2, 20 mg of GM1 or GM2 was incubated at 37 °C for 24 h with 1 unit of the recombinant SCDase in 20 ml of 25 mM sodium acetate buffer, pH 5.8, containing 0.8% Triton X-100 as described previously [9]. The reaction mixture was lyophilized, dissolved in 5 ml of methanol, and applied onto a Mono Q 5/5 column (10 cm × 1 cm) attached to a FPLC system. The elution was performed at a flow-rate of 1 ml/min and 1 ml fractions were collected. The column was initially eluted with methanol for 20 min followed by 60 min with a linear gradient formed by methanol and 0.25 M ammonium acetate in methanol. A 3 μl-aliquot of each fraction was spotted on a silica gel plate and analyzed by TLC as described above. The fractions containing pure lyso-GM1 or lyso-GM2 were pooled, evaporated to dryness, dissolved in 5 ml of 0.1 M KCl and applied onto a Sep-Pak C_{18} cartridge [15]. After washing with 150 ml of water, lyso-GM1 or lyso-GM2 retained by the C_{18} cartridge was quantitatively eluted with 25 ml of methanol [15] and evaporated to dryness to obtain 10.3 mg of lyso-GM1 and 8.4 mg of lyso-GM2 in pure form.

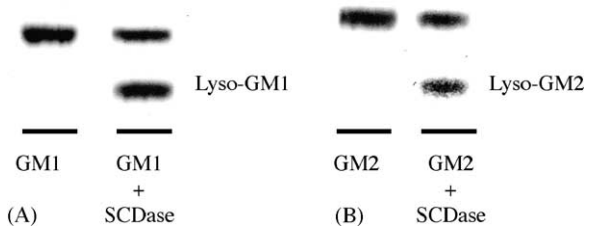

Fig. 1. TLC-analysis showing the conversion of GM1 to lyso-GM1 (A) and GM2 to lyso-GM2 (B) by the recombinant SCDase. The solvent system used was chloroform: methanol:10% acetic acid (5:4:1, v/v/v). To visualize the gangliosides, the plate was sprayed with the diphenylamine–aniline–phosphoric acid reagent [14], followed by heating for 10 min at 110 °C.

3. Results and discussion

By TLC analysis (shown in Fig. 1), the recombinant SCDase was found to convert about 63% of GM1 and 54% of GM2 into lyso-GM1 and lyso-GM2, respectively. These results indicate that the recombinant SCDase produced approximately 10.4 mg of lyso-GM1 and 8.8 mg of lyso-GM2 from 20 mg of GM1 and GM2, respectively.

Lyso-GM1 or lyso-GM2 in the reaction mixture was subsequently separated from GM1 or GM2 very effectively by HPAEC using a Mono Q/FPLC system. Those fractions

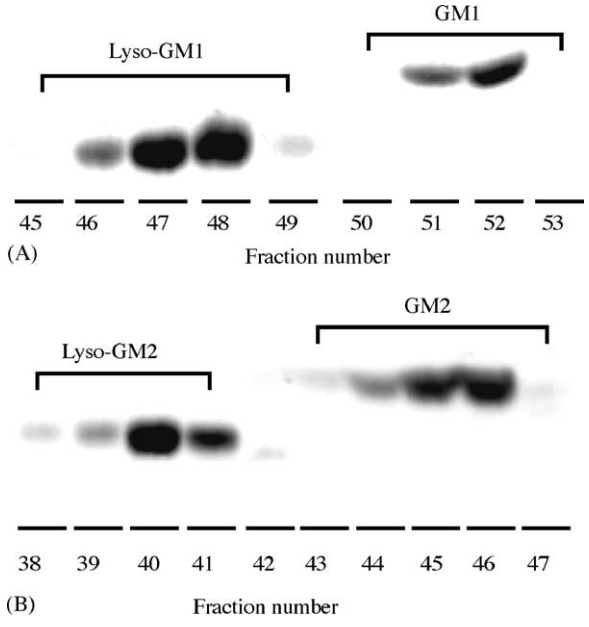

Fig. 2. TLC-analysis showing the separation of lyso-GM1 generated from GM1 (A) and lyso-GM2 generated from GM2 (B) by the recombinant SCDase using HPAEC with a Mono Q 5/5 column (10 cm × 1 cm) attached to the Amersham Biosciences FPLC system. For the HPAEC separation, the elution was performed at a flow rate of 1 ml/min and 1 ml fractions were collected. The column was initially eluted with methanol for 20 min followed by 60 min with a linear gradient formed by methanol and 0.25 M ammonium acetate in methanol. The conditions for TLC analysis are identical to that described in Fig. 1.

containing pure lyso-GM1 or lyso-GM2, as revealed by TLC analysis (Fig. 2), were pooled, evaporated to dryness and followed by desalting to obtain pure lyso-GM1 or lyso-GM2. By this HPAEC procedure, we obtained 10.3 mg of lyso-GM1 and 8.4 mg of lyso-GM2 in pure form. Thus, the recovery of enzymatically generated lyso-GM1 or lyso-GM2 is almost quantitative.

The removal of the fatty acid from GM1 or GM2 generates a free amino group in the lyso-derivatives. This makes these two lyso-derivatives less negatively charged than their parent gangliosides. Consequently, lyso-GM1 and lyso-GM2 should be, respectively, retained less strongly than GM1 and GM2 by the Mono Q column. By Mono Q/FPLC (shown in Fig. 2A), lyso-GM1 produced by the recombinant SCDase was eluted prior to and well separated from GM1. The same was also true for lyso-GM2 (Fig. 2B). The excellent separation of lyso-GM1 from GM1 and lyso-GM2 from GM2 using the Mono Q/FPLC system affords an effective and convenient way of preparing lyso-GM1 and lyso-GM2 enzymatically generated from GM1 and GM2, respectively. As this separation procedure can be accomplished in 3 h, it is much more convenient than using silicic acid chromatography for purification of lyso-GM1 and lyso-GM2.

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