Determination of the Absolute Configuration of Sialic Acids in Gangliosides from the Sea Cucumber Cucumaria echinata

Fumiaki KISA, Koji YAMADA, Tomofumi MIYAMOTO, Masanori INAGAKI, and Ryuichi HIGUCHI*

Faculty of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.
Received March 15, 2007; accepted April 6, 2007

Enantiomeric pairs of sialic acid, D- and L-NeuAc (N-acetylneuraminic acid), were converted to D- and L-arabinoses, respectively, by chemical degradation. Using this method, the absolute configuration of the sialic acid residues, NeuAc and NeuGc (N-glycolylneuraminic acid), in the gangliosides from the sea cucumber Cucumaria echinata was determined to be the D-form. Although naturally occurring sialic acids have been believed to be the D-form on the basis of biosynthetic evidence, this is the first report of the determination of the absolute configuration of the sialic acid residues in gangliosides using chemical methods.

Key words  sialic acid; absolute configuration; ganglioside; sea cucumber; Cucumaria echinata

Gangliosides, an important group of glycosphingolipids due to their biological functions,1) are characterized by the presence of one or more moles of sialic acid, N-acetylneuraminic acid (NeuAc) or N-glycolylneuraminic acid (NeuGc), in their carbohydrate parts. In general, naturally occurring sialic acids have been believed to be the D-form on the basis of biosynthetic evidence, i.e., NeuAc is synthesized from pyruvic acid and N-acetyl-D-mannosamine enzymatically.1) However, there has been no report on the determination of the absolute configuration of the sialic acid residues in gangliosides. We attempted to determine the absolute configuration (D or L) of the sialic acid residues in the gangliosides2,3) from the sea cucumber Cucumaria echinata.

For the determination of the absolute configuration of aldoses in gangliosides, we have used the Hara method.3,4) However, this method cannot be applied to sialic acids directly. Therefore we prepared aldose from sialic acid, preserving the absolute configuration of the parent sialic acid, and then applied the Hara method to the aldose. As shown in Fig. 1, D(L)-arabinose should be obtained from D(L)-sialic acid in the process. First, this assumption was proved.

D-NeuAc (1) was acetylated to give pentaacetate (2), which was methanolyzed with HCl–MeOH to yield methyl 2-O-methylneuraminate (3). Protection of the diol in the side chain of 3 with acetone afforded an isopropyridene derivative (4). Oxidation of 4 with NaIO4 gave a dial derivative (5). Finally, compound 5 was hydrolyzed to separate arabinose (6). The absolute configuration of 6 was verified as being the D-form using the Hara method. On the other hand, L-NeuAc (7), which was synthesized from L-glucose using the authentic method,5) gave L-arabinose in the same manner as 1. Thus the fact that D(L)-sialic acid could be converted to D(L)-arabinose was confirmed, as shown in Fig. 1.

Next, this method was applied to the gangliosides SJG-1, CG-1, CEG-3, CEG-4, CEG-5, CEG-6, HLG-3, CEG-8, and CEG-93,4) from the sea cucumber C. echinata. The nine gangliosides were each methanolyzed and the sialic acid derivatives (3) produced were converted to ketals (4). Periodic acid oxidation of 4 followed by acidic hydrolysis of the oxidation products 5 gave arabinoses (6). Compound 6, obtained from each ganglioside, was determined to be the D-form using the Hara method, as shown in Fig. 2. Accordingly, the sialic acid residues (NeuAc and NeuGc) in the gangliosides from C. echinata must be the D-form.

To the best of our knowledge, this is the first report of the determination of the absolute configuration of the sialic acid residues in gangliosides from the sea cucumber C. echinata.

Fig. 1. Preparation of D(L)-Arabinose from D(L)-NeuAc
Reagents and conditions: (a) Ac2O, pyridine; (b) 10% HCl-MeOH, 80 °C, 18 h; (c) DMF, acetone, 10% HCl-MeOH; (d) NaIO4, H2O; (e) 90% HCOOH–10% CF3COOH (1/1), 100 °C, 12 h.

* To whom correspondence should be addressed. e-mail: rhiguchi@phar.kyushu-u.ac.jp

© 2007 Pharmaceutical Society of Japan
Gangliosides from Cucumaria echinata (SJG-1, CG-1, CEG-3, CEG-6, HLG-3, CEG-8, CEG-9)

10% HCl in MeOH (20 ml) to give solids on silica gel (solvent CHCl₃–MeOH–H₂O, 6:4:1) to give 4 (10.5 mg, 90%). [α]ₐ₉ = -17.1° (c=1.0, H₂O). Positive-ion FAB-MS m/z: 336 [M+H]+. [1H-NMR (D₂O) δ: 4.44 (1H, m, 4-H), 4.34 (1H, m, 7-H), 4.14 (1H, m, 8-H), 4.12 (1H, m, 9-H), 3.90 (3H, s, COOCH₃), 3.77 (1H, m, 9-H), 3.56 (1H, m, 6-H), 3.30 (3H, s, OCH₃), 2.80 (1H, m, 5-H), 2.47 (1H, dd, J=12.9, 11.5 Hz, 3-H), 1.85 (1H, dd, J=12.9, 5.1 Hz, 3-H), 1.45, 1.40 [each 3H, s, C(CH₃)₃].

Preparation of O’-Arabinose (8) from 4 To the solution of 4 (10.5 mg) in H₂O (0.5 ml), NaOAc (1.0 mg) was added and stirred at room temperature for 1 h. The reaction mixture was diluted with H₂O (5 ml) and a small amount of ethylene glycol, and extracted with n-BuOH (3 ml×3). The organic layer was washed with H₂O and concentrated in vacuo to give 5 as a crude product. Compound 5 was heated with 90% HCOOH–10% CF₃COOH (1:1) (0.5 ml) at 100°C for 12 h, and the reaction mixture was concentrated in vacuo. The residue was purified on silica gel TLC (solvent CHCl₃–MeOH–H₂O, 7:3:0.5) to give 6 (2.3 mg, 50% from 4).

Preparation of α-Arabinose (8) from 1-NeuAc (7) In the same manner as for 1, compound 7 (8.4 mg) afforded 8 (1.8 mg, 44%).

Determination of the Absolute Configuration of 6 and (Hara Method) Compounds 6 (1 mg) and 8 (1 mg) were each heated with t-cys-methyl ester hydrochloride (1.5 mg) and pyridine (0.2 ml) at 70°C for 1 h. Then, 0.1 ml of 1-(trimethylsilyl) imidazole was added and the mixture was heated at 70°C for a further 15 min. The reaction mixture was diluted with 50% MeOH (0.8 ml), extracted with n-hexane (0.5 ml×3), and the n-hexane layer was concentrated in vacuo to yield trimethylsilyl ether of the methyl (4R)-thiazolidine-4-carboxylate derivative. Each derivative was analyzed using gas chromatography [column temperature: 180–300°C (rate of temperature increase 2.5°C/min); tₖ [min] = 23.5 (derivative of 6), 22.1 (derivative of 8) (derivative of α-arabinose, 23.5, α-arabinose, 22.1)].

Determination of the Absolute Configuration of Ganglioside Residues from C. echinata Each ganglioside (2 mg) shown in Fig. 2 was heated with 10% HCl in MeOH (0.5 ml) at 80°C for 18 h and concentrated in vacuo. The residue was dissolved in DMF (0.3 ml), and acetone (0.3 ml) and 10% HCl in MeOH (10 ml) were added. The mixture was heated at 60°C for 4 h, neutralized with Ag₂CO₃, centrifuged, and the clear supernatant solution was concentrated in vacuo. The reaction product was heated with 90% HCOOH–10% CF₃COOH (1:1) (0.5 ml) at 100°C for 12 h, and the reaction mixture was concentrated in vacuo. The arabinose from sialic acid, contained in the reaction product, was induced to the thiazolidine derivative, and the derivative was analyzed using gas chromatography in the same manner as in 6, tₖ [min] (original ganglioside): 23.5 (SGJ-1), 23.5 (CG-1), 23.5 (CEG-3), 23.4 (CEG-4), 23.4 (CEG-5), 23.5 (CEG-6), 23.5 (HLG-3), 23.5 (CEG-8), 23.4 (CEG-9) (derivative of α-arabinose, 23.5, α-arabinose, 22.1).

Acknowledgments We thank Mr. Y. Tanaka and Ms. Y. Soeda of the Faculty of Pharmaceutical Sciences, Kyushu University, for the NMR measurements. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 13024260, Priority Area A) from the Ministry of Education, Culture, Science, Sports and Technology, Japan, and a grant (No. 16510163, 18510187) from the Japan Society for the Promotion of Science, which are gratefully acknowledged.