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Influenza A Virus–Binding Activity of Glycoglycerolipids of Aquatic Bacteria

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As the aqueous sphere has been proposed to be an important source medium for the virus infection of land animals, the glycolipids of some aquatic organisms were examined for human influenza A virus–binding activity. Active compounds were not found among the eight echinoderm gangliosides, but two active non-sialylated glycoglycerolipids were isolated from an aquatic bacterium, *Corynebacterium aquaticum*. The structural formula of one of them, H632A, was elucidated to be 1-14-methyl-hexadecanoyl-3-α-D-galactopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol. The latter together with reported one elsewhere, S365A, 1-14-methyl-hexadecanoyl-3-[α-D-mannopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol, apparently bound to three human influenza viruses, A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2), exhibiting 7–12% (H632A) and 10–22% (S365A) of the activities of the control substances (Neu5Acα2-3-paragloboside and Neu5Acα2-6-paragloboside). Additionally, these glycolipids were assumed to have virus-neutralizing activities for the following two reasons: (i) The hemagglutination and hemolysis activities of the viruses were inhibited by the glycolipid. (ii) The leakage of a cytosolic enzyme (lactate dehydrogenase) from Madin-Darby canine kidney cells on virus infection was prevented by the glycolipids to nearly the same extent as by fetuin. This is the first evidence of the binding- and neutralizing-abilities of native glycoglycerolipids as to influenza viruses.

Key words: *Corynebacterium*, glycoglycerolipid, glycolipid, influenza, receptor.

The prevalence of influenza A viruses around the world sometimes leads to the loss of millions, or tens of millions of human lives per year. Recently, the virus infection mechanism has been intensively studied. The receptors are glycoproteins or glycolipids containing a terminal Neu5Ac or Neu5Gc linked to galactose through an α2-3 or α2-6 linkage (*1–4*).

Compounds mimicking the receptors have been searched for and studied as to their binding abilities to these viruses. The target compounds are mainly oligosaccharides or glycolipids. Recent studies have shown that a non-sialylated sulfatide also has apparent receptor activity (*5*). As non-sialyl glycolipids are not substrates for sialidase, a receptor-destroying enzyme which is integrated into the influenza virus envelope, they might be useful as anti-influenza drugs.

Here, we searched for virus-binding glycolipids among echinoderm gangliosides and glycoglycerolipids produced by aquatic microorganisms. The aqueous sphere was proposed to be an important source medium for the virus infection of land animals (*6*), but the host organisms in the aquatic sphere have not been determined. Furthermore, aquatic organisms have not been studied for the virus receptor, except whales and seals. Although fishes and echinoderms contain some amount of sialic acid, it has not been found in other lower aquatic animals or aquatic plants. Whether fishes and echinoderms have a virus receptor and whether non-sialylated receptors function in virus infection are unsolved problems.

Glycoglycerolipids are widely distributed in the cell membranes of Gram-positive bacteria, but their physiological roles have not been fully elucidated, compared to the compelling reports on the roles of glycosphingolipids in higher organisms (*7*). Recently, the roles of some glycoglycerolipids in cell adhesion were reported (*8*, *9*). In this study, two active non-sialylated glycoglycerolipids were examined. This is the first evidence of the binding ability of glycoglycerolipids as to influenza viruses.

MATERIALS AND METHODS

Viruses and Cells—Influenza A viruses, A/PR/8/34

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Abbreviations: FAB, fast atom bombardment mass spectrometry; HAU, hemaglutination units; HMBC, ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectrum; LDH, lactate dehydrogenase; MDCK, Madin-Darby canine kidney.

(H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2), and Madin-Darby canine kidney (MDCK) cells were used in this study. The details of their propagation were given elsewhere (*6*).

Gangliosides—Echinoderm gangliosides were prepared as described elsewhere (*10–12*). Neu5Acα2-6-paragloboside and Neu5Acα2-3-paragloboside were from human meconium and red blood cells, respectively (*13*, *14*).

Glycoglycerolipids—S365A is a glycoglycerolipid, 1-14-methyl-hexadecanoyl-3-[α-D-mannopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol. M874 is also a glycoglycerolipid, di-*O*-12-methyl-tetradecanoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol. The details of S365A and M874 have been submitted for publication. Although S365A was isolated from *Corynebacterium aquaticum* S365, the compound was already known as a metabolite in some other bacteria (*15–17*).

Screening of Bacterial Glycolipids—Two thousand bacteria were isolated from various types of seashore or riverside soil samples in Japan. About 10 mg of soil was added to a test tube containing 3 ml of YMPG medium [0.3% yeast extract (Difco), 0.3% malt extract (Difco), and 0.5% polypepton (Nihon Pharmaceutical), 1% glucose, pH 7.0], followed by cultivation for 40 h at 28°C on a reciprocal shaker (80 rpm with a 50-mm stroke). In the cases of seashore samples, 0.9% NaCl was added to the YMPG medium. The culture broth (0.1 ml) was spread on a YMPG (with or without 0.9% NaCl) agar plate, followed by incubation for 40 h. The isolated bacteria were incubated in 250-ml Erlenmeyer flasks containing 30 ml of YSG medium (0.5% yeast extract, 0.5% soy flour, 2% glucose, 0.1% K_2HPO_4, 0.1% $MgSO{\cdot}7H_2O$, 0.2% NaCl, and 0.2% $CaCO_3$) on a rotary shaker (220 rpm with a 50-mm stroke). In the cases of seashore samples, 0.9% NaCl was included in the YSG medium. The culture broth was extracted with 30 ml of a solvent system (chloroform:methanol = 1:1). The organic solution was evaporated to dryness at 40°C *in vacuo*. The residue was dissolved in 100 μl of the solvent system. Ten microliters of the solvent layer was spotted onto a silica gel plate (Merck Art. 5715) and developed with chloroform:methanol:water = 85:15:1. Glycolipid was detected by spraying with an orcinol reagent [(sulfuric acid:water = 11.4:88.6) containing 2% orcinol], followed by heating at 110°C for 10 min.

Influenza Virus–Binding Assay—One nanomole of a ganglioside sample or 50 μl of a bacterial glycolipid solution was applied to a silica gel plate and developed as described in the above section. The plate was incubated with 2^8 hemaglutination units (HAU) of the three viruses at 4°C for 12 h, followed by detection by immunostaining method previously described (*18*).

Fermentation Conditions for Strain H632—This strain was cultivated in a 250-ml Erlenmeyer flask containing 30 ml of YMPG medium for 20 h at 28°C. An aliquot (0.7 ml) was added to a 500-ml Erlenmeyer flask containing 70 ml of YSGG medium (0.4% yeast extract, 0.1% soyflour, 2% glycerol, 1% glucose, 0.1% K_2HPO_4, 0.1% $MgSO_4{\cdot}7H_2O$, 0.2% NaCl, and 0.2% $CaCO_3$) unless otherwise described. Cultivation was carried out for 4 days at 28°C. Glycolipid H632A was detected and quantified at 203 nm by a HPLC method. The column was an Asahipack GS-310 7E one (Showa Denko; 7.6 × 250 mm), and the mobile phase was 50% acetonitrile, at the flow rate of 0.5 ml/min.

Glycolipid Preparation—The culture broth (3.5 liters) was centrifuged at 6,000 rpm for 20 min and then the precipitated cells were extracted twice with 200 ml of a solvent system (chloroform:methanol = 1:1). The organic solution was evaporated to dryness at 40°C *in vacuo*. The residue was dissolved in 1 ml of the solvent system and then spotted all onto a silica gel plate (Merck Art. 5715; 20 × 20 cm) with chloroform:methanol:water = 85:15:1. The silica gel was scraped off from the part corresponding to an R_f value of 0.35 to 0.4 containing all the active glycolipid, and eluted with an excess of the solvent system. The extract was evaporated and rechromatographed on a reverse phase silica gel plate (Whatman LKC18F; 20 × 20 cm). The plate was developed with 90% methanol. The silica gel was scraped off from the part corresponding to an R_f value of 0.65 to 0.70 containing all the active glycolipid, and eluted with an excess of the solvent system, followed by evaporation to dryness. After purification by TLC, HPLC-separation was performed as described in the above section.

Structure Determination—The methanolysis of fatty acids and conversion of glycosides to alditolacetate were performed as reported previously (*19*). The experimental conditions for ^{13}C-NMR, GLC, and FABMS/MS were also given then.

Assaying of Lactate Dehydrogenase Activity Released from Virus-Infected Cells—The activity of cytosolic lactate dehydrogenase (LDH), which was extracellularly released from the MDCK monolayer on virus infection, was measured by the method described previously (*5*).

Preparation of Glycolipid-Coated Gauze—Some sheets of gauze (2 × 2 cm) were immersed in a methanol solution of a glycolipid (10 mg/ml) for 5 min. After removing the remaining solution with several other sheets of gauze, they were dried *in vacuo*.

Anti-Hemolysis Activity of Glycolipid—Four sheets of gauze coated with a glycolipid were immersed in 200 μl of a virus suspension [A/Aichi/2/68 (H3N2); 2^{13} HAU in 400 μl of 0.9% NaCl] for 3 min at room temperature and then washed with 1.1 ml of 0.9% NaCl. To 1 ml of the rinsing, 0.1 ml of 200 mM acetate buffer (pH 4.9) and 1 ml of 2.5% human erythrocytes were added. After keeping in ice for 10 min and incubation at 37°C for 30 min, 1 ml of PBS (131 mM NaCl, 14 mM Na_2HPO_4, 1.5 mM KH_2PO_4, and 2.7 mM KCl) was added to it, followed by centrifugation at 400 ×*g* for 5 min. Hemolyzed hemoglobin in the supernatant was measured at 540 nm. Experiments were carried out five times and the results were presented as averages.

RESULTS

Influenza Virus–Binding Activity of Echinoderm Gangliosides—Eight ganglioside compounds were prepared from whole bodies of several echinoderm species, and their binding activity as to three influenza A viruses was examined by a TLC overlay method. As shown in Table I, the compounds bound to none of the viruses, *i.e.* A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2).

Screening of Bacterial Glycolipids Having Influenza Virus–Binding Activity—Two thousand aquatic soil samples were collected in Japan and 257 bacteria were isolated which produced more than detectable levels of glycolipids,

of which 27 strains were selected because of their high productivity. The TLC-separated glycolipids were assayed for binding activity as to influenza viruses A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2). Strains H632 and S365 gave one active spot, respectively, which had similar R_f-values (Fig. 1).

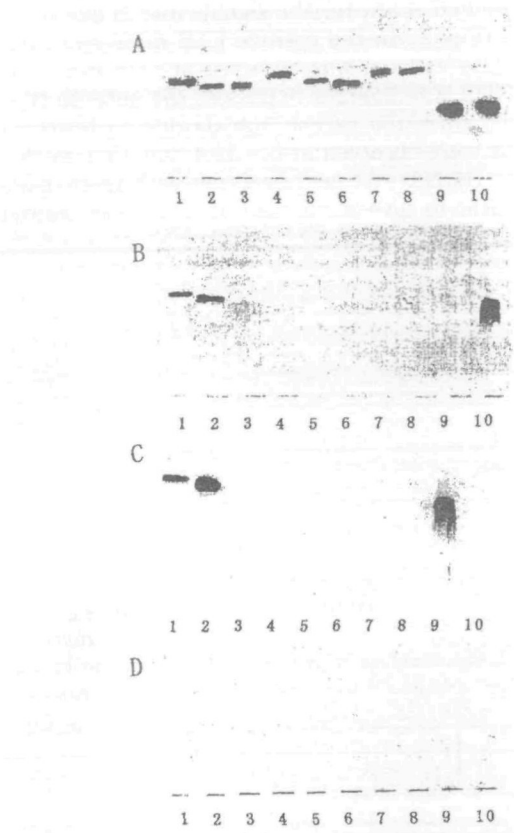

Fig. 1. **Screening of bacterial glycolipids for influenza virus-binding activity.** Glycolipids were purified from the culture broth of miscellaneous glycolipid-producing bacteria as described under "MATERIALS AND METHODS." After development on TLC, the glycolipids were detected by the orcinol-H_2SO_4 method (A) and then examined for binding activity as to two viruses (B, A/PR/8/34; C, A/Aichi/2/68; D, virus-free) by a TLC-immunostaining method (*18*). Lanes: 1, H632A; 2, S365A; 3–8, miscellaneous glycolipids prepared from bacterial cultures; 9, Neu5Acα2-6-paragloboside; 10, Neu5Acα2-3-paragloboside.

Taxonomical Identification of Producer Strains—Strains H632 and S365 were identified and characterized as *Corynebacterium aquaticum* according to Bergey's Manual of Systematic Bacteriology (*20*) and other literature (*21*). Their microbiological properties are presented in Table II.

TABLE II. **Microbiological properties of the active strains.**

	H632	S365
Colony		
Shape	Flat and round	Flat and round
Tone	Buttery, opaque, and yellow	Buttery, opaque, and yellow
Cell shape		
Shape	Long-rod	Long-rod
Width	0.6–0.8 μm	0.6–0.8 μm
Moving	–	–
Gram-staining	+	+
Spore formation	–	–
Enzymatic properties		
Pyrazinamidase	+	+
Pyrrolidonearylamidase	–	–
Alkaline phosphatase	+	+
Catalase	+	+
β-Glucuronidase	–	–
β-Galactosidase	+	+
β-Glucosidase	+	+
N-Acetyl-β-glucosaminidase	+	+
Urease	–	–
Esculin hydrolization	+	+
Gelatin hydrolization	–	+
Nitrate reduction	–	+
Fermentation ability		
Glucose	–	–
Ribose	–	–
Xylose	–	–
Mannitol	–	–
Maltose	–	–
Lactose	–	–
Sucrose	–	–
Glycogen	–	–

TABLE I. **Binding activity of echinoderm gangliosides as to influenza A viruses.**

Origin	Trivial name	Structure	Binding activity		
			A/PR/8/34	A/Aichi/2/68	A/Memphis/1/71
Control samples					
		NeuAc(2→3)Gal(1→4)Glc(1→1)Ceramide	+	+	+
		NeuAc(2→3)Gal(1→3)GlcNac(1→3)Gal(1→1)Ceramide	++	++	++
		NeuAc(2→6)Gal(1→ 4)Glc(1→1)Ceramide	+	+	+
		NeuAc(2→6)Gal(1→3)GlcNac(1→3)Gal(1→1)Ceramide	++	++	++
		NeuGc(2→3)Gal(1→4)Glc(1→1)Ceramide	–	–	–
		NeuGc(2→3)Gal(1→3)GlcNac(1→3)Gal(1→1)Ceramide	–	–	–
Echinoderm samples					
Sea cucumber					
Holothuria pervicax	HPG-8	4-O-SO_3^--NeuAc(2→6)Glc(1→1)Ceramide	–	–	–
Holothuria pervicax	HPG-3	NeuGc(2→4)NeuAc(2→6)Glc(1→1)Ceramide	–	–	–
Holothuria pervicax	HPG-1	Fuc(1→8)NeuGc(2→4)NeuAc(2→6)Glc(1→1)Ceramide	–	–	–
Holothuria pervicax	HPG-7*	Fuc(1→11)NeuGc(2→4)NeuAc(2→4)NeuAc(2→6)Glc(1→1)Ceramide	–	–	–
Starfish					
Luidia maculata	LMG-2bm*	8-O-Me-NeuGc(2→3)Gal(1→4)Glc(1→1)Ceramide	–	–	–
Acanthaster planci	AG-2	Galf(1→3)Gal(1→4)NeuAc(2→3)Gal(1→4)Glc(1→1)Ceramide	–	–	–
Linckia laevigata	LLG-3	8-O-Me-NeuAc(2→11)NeuGc(2→3)Gal(1→4)Glc(1→1)Ceramide	–	–	–
Linckia laevigata	LLG-5*	NeuGc→NeuGc→NeuGc(2→3)Gal(1→4)Glc(1→1)Ceramide	–	–	–

*Not yet published. ++, strong binding; +, binding; –, no binding.

The two strains differed only in two characters, nitrate reduction and gelatin-hydrolyzing ability.

Glycolipid Production and Purification—Two adjacent peaks of glycolipids (named H632A and H632B) were detected in the HPLC profile of a H632, the active one, H632A, being major in quantity and lower in polarity, although only one spot was detected in the TLC profile. The H632A productivity in YSGG medium was 15 mg/liter of the cell culture. The glycolipid was only detected in the cell debris, *i.e.* not in the supernatant fraction. From 3.5 liter culture broth of strain H632, the active glycolipid was prepared by TLC- and HPLC-procedures. A white active powder (H632A) was obtained (18 mg).

Determination of the Structure—The ^{13}C-NMR spectrum of H632A exhibited characteristic signals due to diacylglycoglycerolipid (Fig. 2). In the positive FAB mass spectrum of H632A, a $[M+Na]^+$ ion was observed at m/z 915. Furthermore, H632A was thought to possess the ante-iso type of side chains, since the carbon signals due to terminal methyl groups were observed at δ = 11.4 and 19.3 on ^{13}C-NMR (Fig. 2). H632A was methanolyzed with methanolic hydrochloric acid to give a mixture of fatty acid methyl

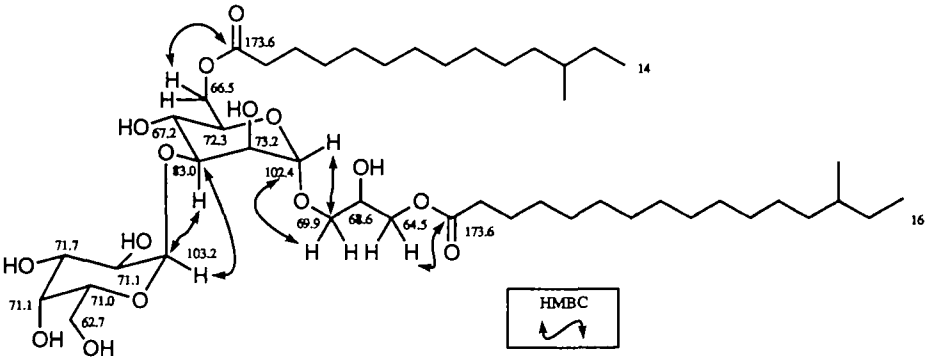

Fig. 2. **^{13}C-NMR spectral data and HMBC correlations of H632A.**

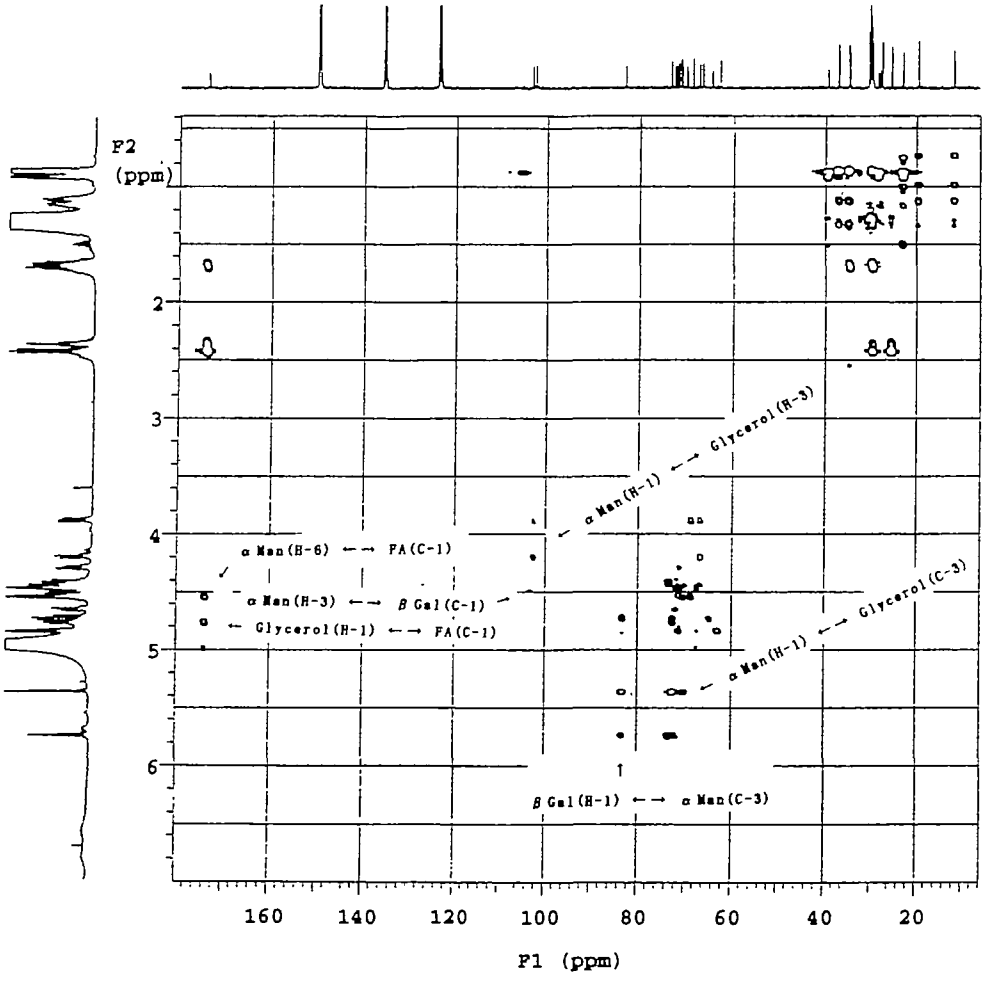

Fig. 3. **HMBC spectrum of H632A.**

esters (FAMs). Analysis of the FAM mixture by GC-MS showed the existence of two components which were characterized as C15:0 and C17:0. Taking the ante-iso type side chains into account, the FAMs of H632A are methyl-12-

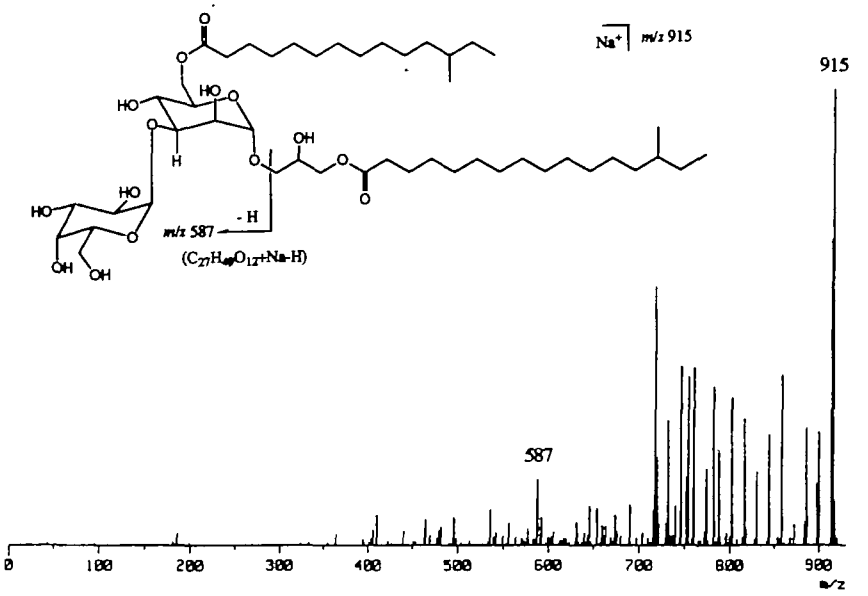

Fig. 4. **FABMS/MS of the [M+Na]⁺ ion obtained on positive FABMS of H632A.**

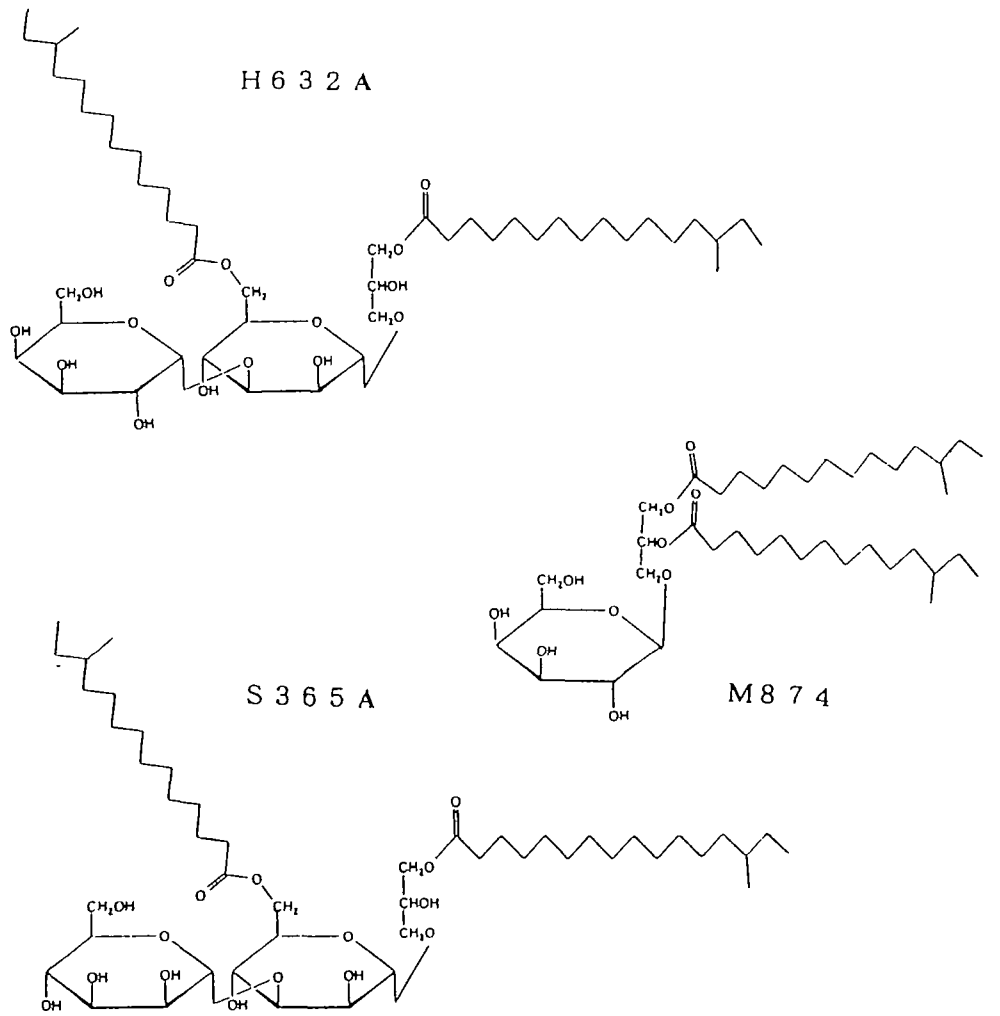

Fig. 5. **Structural formulae of the glycoglycerolipids, H632A, S365A, and M874.**

methyl-tetradecanoate and methyl-14-methyl-hexadecanoate. The GLC analysis of hexitol acetate derivatives of the neutral sugars, which were obtained by hydrolysis, reduction, and acetylation of H632A, showed the existence of 1 mol each of galactose (Gal) and mannose (Man). The configuration of each monosaccharide was considered to be α on the basis of the chemical shift of the anomeric proton signal (Man, δ = 5.36), and on the basis of the coupling constant of the anomeric proton signal (Gal, J = 3.9). The sites of the linkage of the glycerol and the sugar moiety were determined by means of two-dimensional NMR spectroscopy. In the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectrum (HMBC spectrum) of H632A, $^3J_{CH}$ correlations were observed from C-3 of glycerol to 1-H of Man, from C-1 of Man to 3-H of glycerol, from C-3 of Man to 1-H of Gal, and from C-1 of Gal to 3-H of Man (Figs. 2 and 3). This indicates the existence of a glycerol-(3→1)-Man-(3→1)-Gal moiety. Furthermore, $^3J_{CH}$ correlations were also observed from C1 of fatty acid to 6-H of Man and 1-H of glycerol, which indicates the sites of linkage of fatty acid moiety (Figs. 2 and 3). On positive ion FABMS/MS of the $[M+Na]^+$ ion, a characteristic fragment ion was observed at m/z 587, which indicates the site of linkage of each fatty acid (Fig. 4). Consequently, if Gal and Man are assumed to belong to the most commonly found D series, then H632A is 1-14-methyl-hexadecanoyl-3-[α-D-galactopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol. Through similar procedures, another minor glycolipid, H632B, was found to be 1-13-methyl-pentadecanoyl-3-[α-D-galactopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol (Fig. 5).

Binding Activity of Glycolipids as to Influenza Viruses— By means of a TLC-overlay method, the binding activity of glycoglycerolipids as to influenza viruses, such as the A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2) strains, was compared with that of Neu5Acα2-3-paragloboside and Neu5Acα2-6-paragloboside (Fig. 6). Neu5Acα2-3-paragloboside preferentially recognized A/PR/8/34 (H1N1), and Neu5Acα2-6-paragloboside preferred to bind to A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2). H632A and S365A bound to all the three viruses more evenly, although the activity of S365A was about twice higher than that of H632A. The activity of H632A was 7–

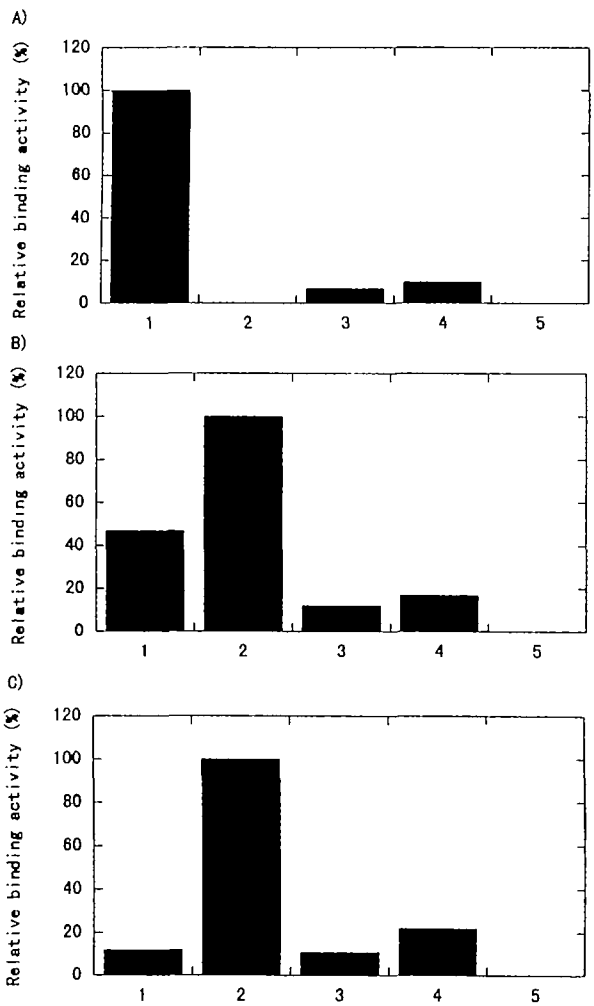

Fig. 6. **Binding activity of glycoglycerolipids as to influenza viruses.** One nanogram of a test sample was applied on a thin layer plate, and then binding activity as to three virus strains was assayed by a TLC-overlay method. The highest activity of the five glycolipids was taken as 100 and the relative activities are indicated. A, A/PR/8/34 (H1N1); B, A/Aichi/2/68 (H3N2); C, A/Memphis/1/71 (H3N2); 1, Neu5Acα2-3-paragloboside; 2, Neu5Acα2-6-paragloboside; 3, H632A; 4, S365A; 5, M874.

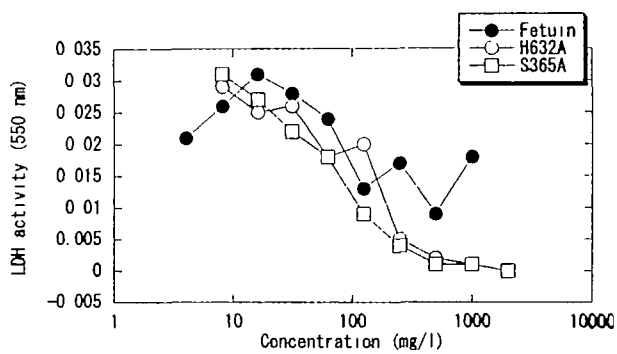

Fig. 7. **Influenza virus-neutralizing activity of glycoglycerolipids.** The virus-induced release of cytosolic LDH activity was measured with various concentrations of the glycoglycerolipids and compared with that in the case of an anti-viral agent, fetuin.

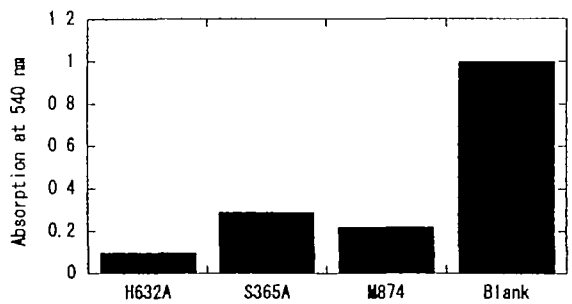

Fig. 8. **Anti-hemolysis activity of coated gauze.** Glycolipid-coated gauze was immersed in a virus suspension [A/Aichi/2/68 (H3N2)] and then rinsed in water. The virus-trapping and -neutralizing activities of the glycolipid in the gauze was evaluated by measuring the hemolysis activity of the gauze rinsings.

12% of the highest control level and that of S365A was 10–22%. Other glycoglycerolipids including the monoglucosyldiacyl-glyceride, M874, were not active at all.

Influenza Virus-Neutralizing Activity of Glycoglycerolipids—Influenza virus-neutralizing activity can be assayed in different manners. Here, the extracellular release of a cytosolic enzyme, LDH, from the host MDCK monolayer cells on viral infection was first examined. The preventive activity of H632A and S365A was the same as that of a well known antiviral agent, fetuin (Fig. 7). Virus-induced hemaglutination and hemolysis were also examined. H632A and S365A had apparent anti-hemaglutination and anti-hemolysis activities (data not shown).

Glycolipid-Coating on Gauze—The water solubility of the glycoglycerolipids, H632A and S365A, was about 800 mg/liter at room temperature. Gauze was dipped in and picked out of a methanol solution of a glycolipid. After removing the remaining solution with excess sheets of gauze, a coated-sheet was estimated to contain 0.5 mg of a glycolipid. The dried coated-gauze was dipped in water and then the amount of effluent glycoglycerolipid was measured. The effluent level was less than 0.2% of the amount of glycoglycerolipid on the gauze.

Anti-Hemolysis Activity of Coated Gauze—Glycoglycerolipid-coated gauze was immersed in a virus suspension [A/Aichi/2/68 (H3N2)] and then rinsed in water. The virus-trapping and -neutralizing activities of the coated glycolipid were assessed as the hemolysis activity in the rinsings. In the cases of H632A and S365A, the activity was reduced to 10 and 30% of the blank (methanol-treated gauze) level, respectively (Fig. 8), indicating the low number of active virus particles in the rinsings. As the applied amount of glycolipid was 2 mg and the initial number of influenza particles was 2^{13} HAU, and a 1 mg aliquot was assumed to be enough to trap and neutralize a virus level of 2^{11} HAU. In the case of glycoglycerolipid M874, the activity was also reduced, to 23% of the blank level, suggesting its anti-viral effect, which might be caused by its prominent anti-oxygen radical activity (submitted for publication).

DISCUSSION

Recently, the influenza virus infection mechanism was intensively studied. The receptors are glycoproteins or glycolipids containing a terminal Neu5Ac or Neu5Gc linked to galactose through an α2-3 or α2-6 linkage (*1–3*). We used human influenza A viruses, A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2), and A/Memphis/1/71 (H3N2), in this study. These viruses differ in the recognition types of their receptors. A/PR/8/34 (H1N1) preferentially recognizes the NeuAc-(2→3)Gal linkage in sialo sugar chains over the NeuAc-(2→6)Gal linkage, whereas A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2) preferentially recognize the NeuAc-(2→6)Gal linkage (*4*, *18*). In this study, although none of the tested echinoderm gangliosides exhibited binding activity, the data in Table I conformed with the so far proposed rules. Sugar addition to the terminal sialic acid resulted in a loss of binding activity.

The route of infection of humans by influenza viruses has been studied to some extent. Waterbirds are candidate primary carriers. Viruses in their feces supposedly diffuse into the aqueous sphere, and then shore animals (secondary carriers) might resultantly be infected by the viruses. Then, infection of humans might be caused by close contact with the second carriers, but, in this hypothetical infection route, the host organisms in the aqueous sphere have not been considered. In this study, we isolated glycolipids of aquatic organisms (echinoderms and aquatic bacteria) and examined their binding activity as to influenza viruses. Although all ganglioside compounds might not yet have been isolated from echinoderms, it is possible that the phylum defects of receptors to human influenza A viruses. We obtained two glycolipid producing bacteria, of which glycolipids exhibited distinct virus-binding activity. As the producer, *C. aquaticum*, is a ubiquitous bacterium in natural fresh water (*21*), the discovery of virus-binding glycolipids in the aqueous sphere might facilitate the total elucidation of the virus infection route.

Strains H632 and S365 were isolated from aquatic soil samples from Hokkaido and Hiroshima, Japan, respectively. Although both were identified as *C. aquaticum*, the chemical structures of their glycolipid's were a little different. H632A had the structure, 1-14-methyl-hexadecanoyl-3-[α-D-galactopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol. In the case of S365A, the structure, 1-14-methyl-hexadecanoyl-3-[α-D-mannopyranosyl-(1→3)-6-(12-methyl-tetradecanoyl)-1-α-D-mannopyranosyl]-*sn*-glycerol, was proposed. Although the present data are too few to elucidate the mechanism of binding of these glycoglycerolipids to influenza viruses, their structural similarity to sialo-glycolipid chains is tentatively proposed here. Some ligands have been reported to fairly change the conformation of Neu5Ac, and to shorten the distance between OH at C8 and COOH at C1 (*22*). Although the distance might also be determined by some other functional groups, when the distance was provisionally minimized, a mimic aldohexose (-C2-C1-C8-C7-C6-O-) could be assumed. In that particular case, the (α2→3)-bonded two hexose moiety (the mimic hexose and galactose) somewhat resembles the diglycosides of S365A (mannose and mannose). The important role of the mimic hexose was supported by the report by Suzuki, who has already shown the importance of the C7, C8, and C9 conformation of Neu5Ac for the virus-binding activity by using synthetic glycolipids (*6*). H632A and S365A evenly bound to viruses of both the (2→3) and (2→6) types, which was a different characteristic from Neu5Acα2-6-paragloboside and Neu5Acα2-3-paragloboside.

Glycoglycerolipids exhibit much variety and are widely distributed among animals, plants, and microorganisms, but their physiological roles have been scarcely elucidated. As some glycoglycerolipids were recently found which participate in cell adhesion (*8*, *9*), glycoglycerolipids might supposedly participate in the recognition of the complicated structures of cell components. The discovery of virus-binding activity of the present glycoglycerolipids strongly supports this idea.

Representative physiological characteristics of the glycoglycerolipids, H632A and S365A, are as follows. (i) They apparently bound to both an α2-3 linkage-type virus [A/PR/8/34 (H1N1)] and α2-6 linkage-type viruses [A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2)]. (ii) They had prominent virus-neutralizing activity, so they significantly inhibited the virus-induced hemagglutination and hemolysis, and prevented the virus-induced leakage of cytosolic components, *e.g.* LDH. (iii) In contrast with a sialylglycolipid type of adsorbent, the glycolipids are resistant to virus-

derived sialidase (data not shown). (iv) Their moderate hydrophobicity is a beneficial character for a virus-adsorbent.

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