Inhibition of 2,7-Dideoxy-7-fluoro-2,3-didehydrosialic Acid for Binding of Influenza Virus H1 Hemagglutinin to GM3

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A sialidase inhibitor, 2,7-dideoxy-7-fluoro-2,3-didehydrosialic acid was found to effectively inhibit the binding of influenza virus H1 hemagglutinin to ganglioside GM3.

Influenza virus has hemagglutinin and sialidase as membrane proteins, which play an important role for the infection process.[1] Those membrane proteins are known to recognize sialylglycolipids and sialylglycoproteins.[1] To prevent the infection of influenza virus, inhibitors for sialidase and hemagglutinin binding have been developed. For example, sialidase activity of influenza virus was inhibited by 2-deoxy-2,3-didehydrosialic acid (**1**),[2] its 4-guanidino derivative (4G-**1**),[2] and its 7-fluoro derivative (7F-**1**).[3] IC_{50} values of these sialic acid derivatives were 12, 0.04, and 4 μM, respectively. On the other hand, macromolecules such as sialo-dendrimer[4] and sialic acid-bearing polymer[5] are known as an inhibitor for the binding of influenza hemagglutinin. Those macromolecules, however, did not inhibit silalidase. A compound that can simultaneously inhibit sialidase and hemagglutinin binding has never been reported. In this paper, we report the unexpected findings that the silalidase inhibitor 7F-**1** also inhibited the binding of hemagglutinin to GM3.

GM3, GlcCer, and sialyllactose were obtained from Snow Brand Milk Products Co., Ltd., Japan. Sialic acid derivatives, **1**,[6] 4G-**1**,[7] 7-F-**1**,[8] and 9F-**1**[9] were synthesized according to the literature. The chemical structures of these compounds were

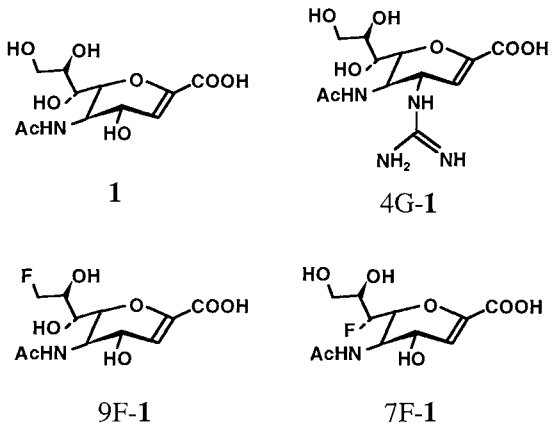

Figure 1. Sialic acid derivatives employed in this study.

shown in Figure 1. Hemagglutinins, which were extracted from influenza virus (A/PR/8/34(H1) or A/bukan/359/95(H3)) with ether, were kindly gifted by Mr. Yujirou Suzuki (The Kitasato Institute). Hemagglutinin contents were determined to be 50% by SDS-polyacrylamide gel electrophoresis. Neuraminidase activities per total protein were decreased by function of 500 compared with intact virus. Preparation of GM3 containing monolayers and quantitative analyses for the binding of influenza virus to the monolayer were done according to the previous paper.[10-13] A mixed solvent of chloroform and methanol (4: 1, v/v) containing GM3, was spread on an aqueous solution (10 mM phosphate buffer, pH 7.2) in a Teflon-coated trough with a microcomputer-controlled Teflon barrier (USI, Fukuoka, Japan). A 9 MHz AT-cut QCM plate was attached horizontally on the mixed monolayer at the surface pressure of 30 mN m^{-1}. The frequency decrease of the QCM (mass increase) responding to the addition of hemagglutinin with or without inhibitors was followed with time. A notional illustration for a experimental

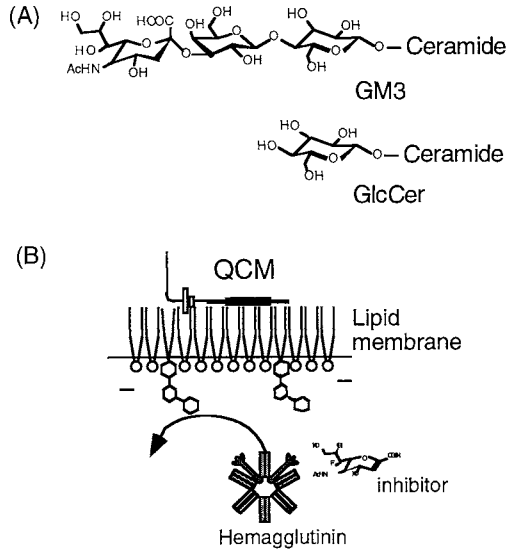

Figure 2. (A) Chemical structures of ganglioside (GM3) and glucosylceramide (GlcCer) and (B) an experimental apparatus of a QCM attached horizontally on a monolayer.

apparatus is shown in Figure 2. Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 3.1 ng cm^{-2} on the QCM electrode at the air-water interface. Initial binding rates and binding amounts were determined by the methods previously described.[10-13]

Figure 3 shows typical time courses of frequency changes of the QCM for 5 mol% of GM3 reconstituted in GlcCer matrix responding to the addition of H1 hemagglutinin (4.9 μg ml^{-1}) into the subphase. The frequency decreased with time by the addition of hemagglutinin and saturated within 5 h. Binding of H1 hemagglutinin to GM3 in the presence of 100 μM 7F-**1** was completely inhibited. Other derivatives (**1**, 4G-**1**, and 9F-**1**)

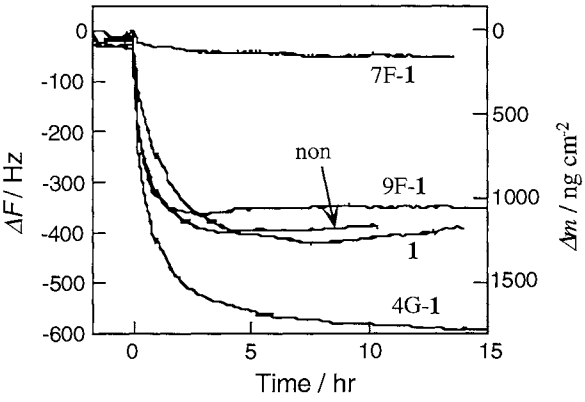

Figure 3. Time-courses of frequency decrease (-ΔF) of the QCM attached to GM3/GlcCer (5 : 95 by mol%) mixed monolayer in the absence and presence of 100 μM sialic acid derivatives when H1 hemagglutinin (4.9 μg ml^{-1}) was injected in the subphase.

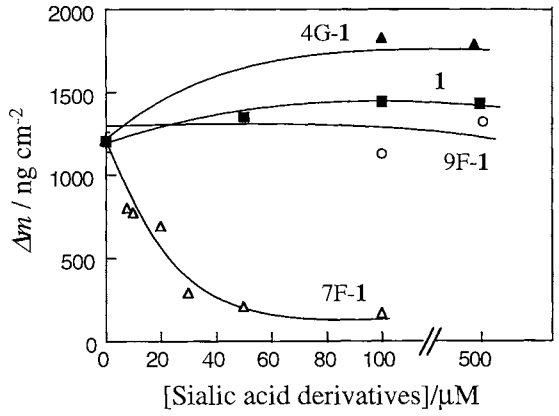

Figure 4. Dependence of the concentration of sialic acid derivatives on binding amounts (Δm) of H1 hemagglutinin.

showed no inhibition. Binding amounts (*Δm*) were shown in Figure 4 as a function of the concentration of sialic acid derivatives. The *Δm* values in the presence of 7F-**1** decreased depending on its concentration. IC_{50} value of 7F-**1** for hemagglutinin inhibition was 12 μM. Other compounds did not show any inhibition below 500 μM. Sialylactose also did not show any inhibition at 2 mM (data not shown).

Inhibition experiments using H3 hemagglutinin were carried out by the same method described above. When the concentrations of the inhibitors were 500 μM, bindings of H3 hemagglutinin were slightly inhibited by 7F-**1**, 9F-**1**, and **1**, but not by 4G-**1** at all. IC_{50} values of those compounds for hemagglutinin inhibition, however, were above 500 μM. The inhibition effects for H3 hemagglutinin, however, were a little compared with those for H1 hemagglutinin. Thus inhibition effect of 7F-**1** was specific for H1 hemagglutinin.

Though 4G-**1** is a very excellent sialidase inhibitor, it had no effect on the inhibition of hemagglutinin binding to GM3. On the other hand, 7F-**1** was inferior to 4G-**1** as sialidase inhibitor. However, it inhibited the binding of H1 hemagglutinin to ganglioside GM3 in high efficiency. If hemagglutinin binding and sialidase activity were simultaneously inhibited, transfection of influenza virus will be effectively prevented. Thus, it is expected that the inhibition effect of 7F-**1** on hemagglutinin and sialidase will open new way to the development of antimicrobial reagents.

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