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Evaluation of the hydrophobicity of perfluoroalkyl chains in amphiphilic compounds that are incorporated into cell membrane

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ABSTRACT

Incubation of animal cells in the presence of amphiphilic glycosides having a lactoside residue (hydrophilic moiety) and various kinds of perfluoroalkyl chain (hydrophobic moiety) resulted to glycosylation of the saccharide chain by cellular enzymes and afforded glycolipid-like compounds. The amounts of amphiphilic glycoside and glycolipid-like compounds found in the cell and culture medium fractions, respectively, varied depending on the fluorine content of the perfluoroalkyl chain. To investigate further, the hydrophobicity of the perfluoroalkyl chain was estimated from the critical micelle concentration values and was found to be 1.5 times larger in hydrophobicity than the hydrocarbon chain [$CF_2 = 1.5CH_2$]. Liposomes resembling cells in size were also prepared and the amphiphilic glycosides were introduced. Results showed a positive correlation between hydrophobicity and localization of amphiphilic glycoside into liposomes. The amount of amphiphilic glycosides localized in liposomes increased with increasing hydrophobicity that is attributed mainly to the fluorine content of the aglycon. In spite of the low affinity of fluorous chain for hydrocarbon chain, the amphiphilic fluorous compounds showed high affinity for cell membrane that is composed of amphiphilic phospholipids.

1. Introduction

Fluorous compounds have generated a lot of interest because of their unique properties. Among the useful physicochemical properties [1] of fluorinated compounds include exceptional biological inertness, thermal and chemical stability, low dielectric constant, high gas capacity and lipophilicity [2]. There is also reported evidence of moderate to low toxicity as compared to hydrocarbon analogues [3]. Recently, possible application in biomedical and pharmaceutical fields due to haemolytic activity [4] and potential in drug delivery [3] has provided the motivation for the synthesis of various fluorous compounds and the assessment of biocompatibility.

A key feature of fluorous compounds is the perfluoroalkyl chain, commonly referred to as fluorous tags, that is linked to non-fluorinated domains. Depending on the fluorine content, the "heavy" fluorous tags (60% or more fluorine by molecular weight) have been distinguished from "light" fluorous tags (40% or less fluorine by molecular weight) [5]. The versatility and wide application of these perfluoroalkyl chains have been demonstrated in fluorous technology [6]. Since the seminal publication of Horváth and Rábai [7], substantial progress has been achieved in the aspects of synthesis, catalysis and separation [8]. Fluorous technique has gained wide application in solid phase synthesis and combinatorial chemistry. The perfluoroalkyl chains are inert to chemical reactions and have rarely affected the reactivity of the attached molecules.

We have embarked on a novel concept in oligosaccharide synthesis that incorporates glycosides with perfluoroalkyl chains into animal cells to serve as substrate for cellular enzyme mediated glycosylation [9]. Careful consideration of the hydrophilic (glycoside head) and hydrophobic (perfluoroalkyl tail) balance of the amphiphilic glycosides is a prerequisite for efficient cellular uptake, saccharide elongation by glycosyl transferases residing in the Golgi and efficient release of products from the cells to the culture medium. Recently, we reported the chemical synthesis of a series of lactose and N-acetyl glucosamine derivatives with different contents of fluorine atoms in the aglycon moeity and the incorporation into mouse melanoma B16 cells [10]. The fluorous-tagged lactosides were sialylated to afford GM3-type oligosaccharide. On the other hand, the fluorous-tagged N-acetyl glucosamine were galactosylated and gave a lactosamine derivative that was further sialylated by cellular enzymes and afforded a sialylated lactosamine. Differences in the amount of products obtained have been observed.

In this study, the effect of fluorine content on cellular uptake and glycolipid production was delved into. Several lactosides with different perfluoroalkyl chain [LacH6F6 (each number indicates

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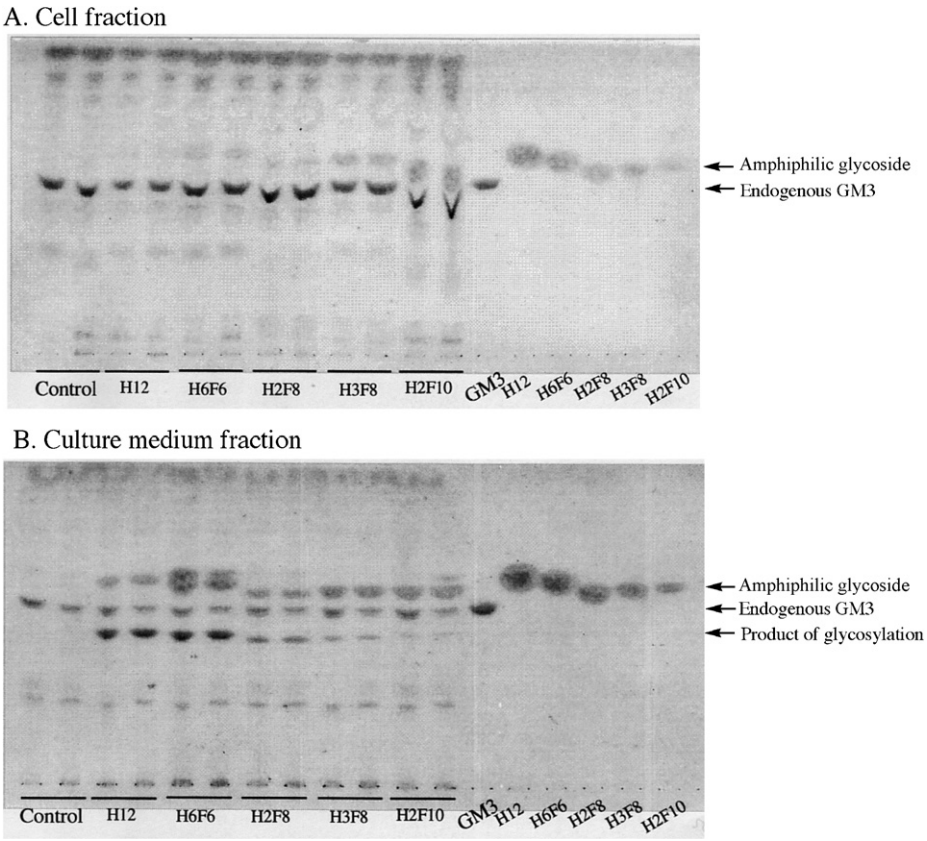


Fig. 1. HPTLC profile of lipids from the cell and culture medium fraction obtained after incubation of B16 cells in the presence of fluorous-tagged glycosides. 2×10^6 B16 cells were seeded into 10 cm dishes containing 7 ml of 10% FBS DMEM/F12 and incubated for 48 h. 50 μM fluorous-tagged glycosides in 7 ml ITS-X DMEM/F12 were administered into cells. After incubation for 48 h, culture medium was collected and lipids were extracted and separated.

the number of CH_2 and CF_2, respectively), LacH2F8, LacH3F8, LacH2F10] were chemically synthesized and introduced to mouse melanoma B16 cells. Factors such as critical micelle concentration that may account for the differences in cellular response on uptake and glycosylation was investigated. Moreover, the localization of amphiphilic glycosides into liposomes resembling cells was also established.

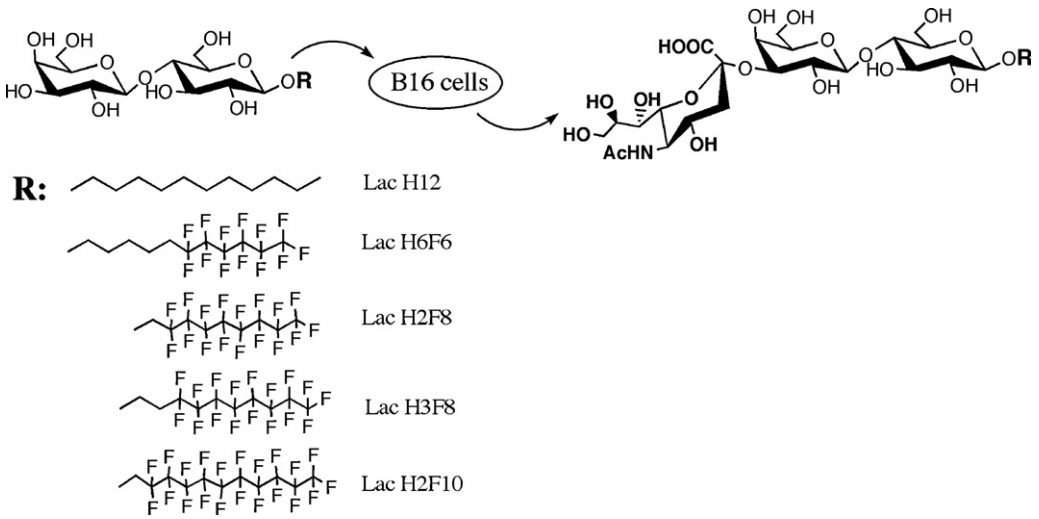
2. Results and discussion

The fluorous-tagged lactosides (LacH6F6, LacH2F8, LacH3F8, LacH2F10) were synthesized by glycosylation followed by deacylation as reported in literature [10,11]. Glycosylation of the corresponding fluoroalcohol was carried out at room temperature using peracetylated lactose as donor and Lewis acid (boron trifluoride etherate) as catalyst. Dodecyl β-lactoside (control) was also prepared by glycosylation of dodecyl alcohol under similar condition. Purification was achieved by silica gel column chromatography with hexane:ethyl acetate as eluent. Deacylation under Zemplen conditions was accomplished using sodium methoxide and methanol.

For the production of oligosaccharides using animal cells, a dodecyl (C12) aglycon teethered to a lactoside has been reported as the appropriate chain length for the efficient cellular uptake and release of products to the culture medium for collection [12]. Hence, the glycosides used in this research were designed with (perfluoroalkyl)alkyl chains that subscribe to the recommended chain length but with varied number of fluorine atoms.

The glycosides were non-cytotoxic to mouse melanoma B16 cells at 50 μM. After 48 h incubation of the cells in the presence of the fluorous-tagged glycosides, the cell and culture medium fractions were collected and the lipids were extracted and analyzed. The HPTLC profile of lipids obtained from the cell and culture medium fractions in Fig. 1 showed putative bands corresponding to products of glycosylation. Mass spectral analysis of products confirmed sialylation of the fluorous-tagged glycosides. These results agree with previous reports [9]. We could deduce that the fluorous-tagged glycosides were taken into the cell, transported to the site of glycosylation (Golgi), and served as substrate for glycosylation by endogenous glycosyl donors and glycosyl transferases and afforded glycolipid-like compounds that were mostly found in the culture medium. Incubation of mouse melanoma B16 cells in the presence of fluorous-tagged lactosides afforded GM3-type ganglioside as shown in Scheme 1. The yield (%) is as follows: SA (sialylated) LacH12: 19.4; SALacH6F6: 14.4; SALacH2F8: 3.8; SALacH3F8: 3.2; and SALacH2F10: 1.2. The amount of amphiphilic glycoside (%) found in the cell fraction is LacH12: 1.2; LacH6F6: 2.1; LacH2F8: 3.6; LacH3F8: 14.0; and LacH2F10: 22.5. The amounts of product and amphiphilic glycoside found in the culture medium and in the cell fractions, respectively, varied relative to the number of fluorine atoms in the perfluoroalkyl chain. Among the amphiphilic glycosides used in this study, LacH6F6 having six units of CF_2 gave the most amount of product.

Lac H2F10 with the highest fluorine content gave the least amount of product. On the other hand, among the fluorinated compounds, Lac H6F6 having the least number of fluorine atoms gave the highest amount of product although the amount is relatively smaller compared to control that does not have any fluorine atom at all. Significantly, a relatively large amount of Lac H2F10 was found in the cell fraction. Results suggested that as the fluorine content of the perfluoroalkyl tail increases, the amount of



Scheme 1. Production of glycolipid-like compounds using amphiphilic glycosides with different kinds of perfluoroalkyl chain and B16 cells.

lactoside found in the cells also increases. On the other hand, an increase in the fluorine content results to a decrease in the amount of product. To investigate further, the hydrophobicity of the amphiphilic glycosides was estimated from the critical micelle concentration (CMC) values. Liposomes resembling cells in size were also prepared and the amphiphilic glycosides were introduced.

Surface tension measurement was carried out to determine the critical micelle concentration of the amphiphilic glycosides. As shown in Fig. 2, the CMC values obtained are as follows: LacH12: 57.9 μM; LacH2F8: 12.1 μM; LacH3F8: 6.9 μM; LacH6F6: 6.9 μM; and LacH2F10: 6.0 μM. CMC decreased with increasing fluorine content of the perfluoroalkyl chain. Moreover, the CMC of glycosides with perfluoroalkyl chains was very low as compared with the hydrocarbon counterpart suggesting that the fluorous-tagged glycosides are relatively more hydrophobic. It is well known that fluorinated amphiphiles are more hydrophobic than their corresponding hydrogenated analogues in terms of lowering the interfacial tension and critical micelle concentration [2]. We could surmise that among the glycosides used, H2F10 having the highest fluorine content is the most hydrophobic.

Significantly, Lac H3F8 and Lac H6F6 having a perfluoroalkyl chain of $(CH_2)_3(CF_2)_8$ and $(CH_2)_6(CF_2)_6$, respectively, gave the same CMC value of 6.9 μM. Since these glycosides have the same saccharide unit, the hydrophobicity of the perfluoroalkyl chain could be estimated and was found to be 1.5 times larger in hydrophobicity than the hydrocarbon chain [$CF_2 = 1.5CH_2$]. This is quite reasonable with reference to the reported value of 1.44 as cited in literature [2].

Liposomes having almost the same size as cells were also prepared [13] and each amphiphilic glycoside was administered to the liposome solution. The amount of glycoside localized in the liposome was quantified to be: 6.4% (LacH12); 24.0% (LacH2F8); 37.8% (LacH3F8); 42.6% (LacH6F6); 59.9% (LacH2F10). In Fig. 3, the plot of the amount of amphiphilic glycoside incorporated into the liposome against the reduced chain length [$(CH_2)_n(CF_2)_m$: $n + 1.5m$] revealed a positive correlation between hydrophobicity estimated from the CMC and localization of the amphiphilic glycosides in liposomes. The amount of amphiphilic glycosides localized in liposomes increased with increasing hydrophobicity that is attributed mainly to the fluorine content of the aglycon.

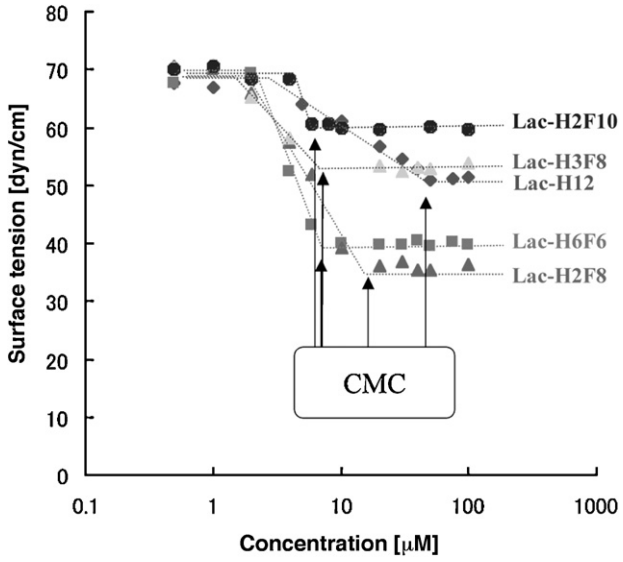


Fig. 2. Critical micelle concentration (CMC) of fluorous-tagged lactosides.

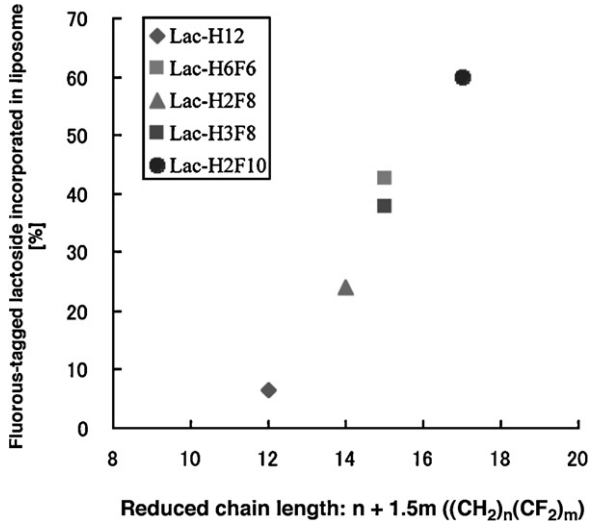
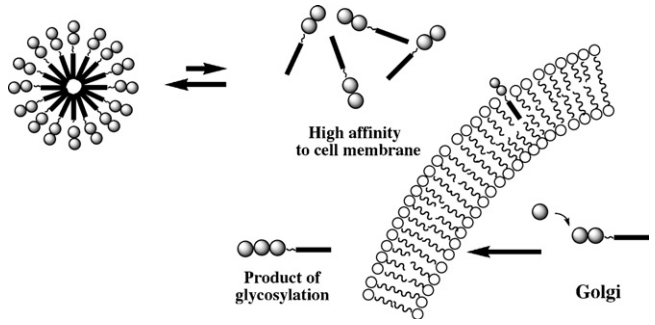


Fig. 3. Amount of fluorous-tagged lactoside incorporated into liposome against reduced chain length.



Scheme 2.

The affinity of amphiphilic glycosides to the cell membrane brought about by the number of fluorine atoms in perfluoroalkyl tail possibly accounts for the differences in the amount of amphiphilic glycoside found in the cell fraction and the amount of products found in the culture medium fraction. Membrane affinity apparently increased with increasing fluorine content, and the interaction between the lipid bilayer and the amphiphilic glycosides affected diffusion of the later (Scheme 2). The glycosides with high fluorine content possibly remained embedded in the cell membrane. For glycosylation to occur, the amphiphilic glycosides should reach the glycosylation site. The numerous fluorine atoms may have obstructed the transport of the amphiphilic glycoside to the Golgi or the glycosylation by the enzyme. These considerations could justify why only a small amount of product was obtained from H2F10 although a large amount was found in the cell fraction.

3. Conclusion

The interplay between the hydrophilic and hydrophobic balance is an important factor in glycolipid production using amphiphilic glycosides and animal cells. The fluorine content of the perfluoroalkyl chain affects the production of glycolipid-like compounds. Higher fluorine content renders the amphiphilic glycosides to be more hydrophobic. Moreover, the presence of many fluorine atoms may increase affinity of amphiphilic glycosides to the cell membrane and consequently prevent the saccharide elongation. Based on results, an amphiphilic glycoside with a perfluoroalkyl chain having six units or less of CF_2 is most suitable for glycolipid production in animal cells.

Generally, fluorous layer is immiscible with neither the aqueous layer nor the organic layer. The result in this study is particularly surprising in the view of the fact that amphiphilic compounds have a strong affinity for the cell membrane.

4. Materials and methods

4.1. Chemical synthesis of amphiphilic glycosides

The fluorous-tagged glycosides were prepared in two steps, glycosylation and deacylation, according to the literature cited in the text [10,11]. The structures were confirmed from the NMR and mass spectral results.

4.2. Cellular uptake of amphiphilic glycosides

General methods. B16 cells were obtained from Riken Cell Bank (Tsukuba, Japan). Dulbecco Modified Eagle's Medium: Nutrient Mixture F-12 (Ham) 1:1 (DMEM/Ham F12) and Insulin-Transferrin-Selenium-X solution were from GIBCO and fetal bovine serum (FBS) was from JRH Biosciences. A 50 mM stock solution of fluorous-tagged lactoside was prepared by dissolving in sterile DMSO. SepPak C18 was from Waters. HPTLC (Silica Gel 60) plates were from E. Merck, Darmstadt, Germany. Lipids from the cell homogenate and culture medium fractions were analyzed by HPTLC with $CHCl_3$:MeOH:0.25%KCl(aq) = 5:4:1 (v/v/v) as developing solvent. HPTLC plates were sprayed with resorcinol and heated to detect the separated glycolipids that were quantified using Scion Image (Scion Corporation). The bands corresponding to the products were scraped from the HPTLC plate, and the glycolipids were extracted with methanol. The mass spectrum of the products was recorded on a Bruker Esquire HCT Ultra ESI LC MS using MeOH:acetonitrile (1:1,v/v).

Cell culture. Mouse B16 melanoma cells were cultured in 1:1 DMEM-F12 supplemented with 10% fetal bovine serum (FBS) and were detached through application of 0.25% trypsin-EDTA, passaged every 3 days and maintained in humidified atmosphere of 5% CO_2 air at 37 °C.

Incubation of cells with glycosides. Inocula of 2×10^6 cells were seeded into 100-mm culture dishes containing 7 ml of medium and incubated for 24 h. Then, the cells were washed with TI-DF without phenol red (1:1 DMEM Hams-F12 supplemented with Insulin-Transferrin-Selenium-X solution), and cells were incubated with 50 μM of the lactoside for 48 h at 37 °C. After incubation, culture media were collected and cells were washed with PBS (−), harvested with 0.25% EDTA in PBS (−), and centrifuged at 1000 rpm for 5 min. Lipid extraction and identification of product were carried out according to literature [8,9].

4.3. Surface tension measurement

The critical micelle concentration was determined by measuring the surface tension of the glycosides using the Dynamic Contact Angle Weighing Device DCA-100W from ORIENTEC, Inc.

4.4. Incorporation of glycosides into liposomes

Liposome (GUV) was prepared from Phosphatidylcholine that was dissolved in chloroform:methanol (2:1) and evaporated under vacuum, followed by swelling in aqueous medium and heating at 70 °C [14].

To 5 ml of 2 mM liposome solution was added 5 ml of 50 μM of the glycosides and incubated under 5% CO_2 at 37 °C. After 48 h, the mixture was centrifuged (4000 rpm) for 30 min at 4 °C, the supernatant liquid discarded and the liposome washed again with water, centrifuged (15,000 rpm) for 30 min at 4 °C and the liposome fraction collected. Incorporated glycosides were extracted from the liposomes by using Triton-X100, then separated using fluorous solid phase extraction columns and analyzed by HPTLC.

Each glycoside was administered to the liposome solution, and the amount of the glycoside taken into the liposome was quantified from the HPTLC results by using a densitometer.

Acknowledgements

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