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Polymorphism of Model Glycosphingolipids Evidenced by Calorimetric and Polarizing Microscopic Study

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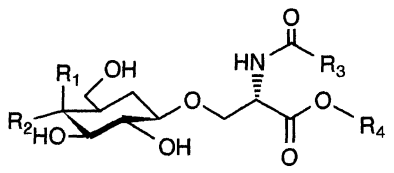
Novel glycolipids having a L-serine derivative as the hydrophobic region, have been synthesized as a model glycosphingolipid. Differential Scanning Calorimetry and polarizing microscopic study of the aqueous dispersion exhibited a polymorphism similar to that observed for certain glycosphingolipids. Significantly higher chain order-disorder transition temperature was also observed for a galactolipid than for a glucolipid.

Glycolipids, as well as phospholipids, are one of the important constituents of biomembranes. They serve a variety of functions such as stabilization, shape determination, recognition and ion-binding in biomembranes.[1] However, glycolipids have attracted much less attention compared to glycerophospholipids. Especially, glycosphingolipids are present in most animal cell membranes although the exact structural and/or functional role still remains obscure. Thus, for the last decade there has been a growing demand for the pure synthetic glycolipids having a well-defined stereochemistry and homogeneous hydrocarbon chains.

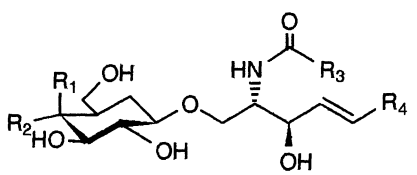
In this study, we have synthesized novel synthetic glycolipids **1** and **2** whose reducing end is ß-glycosidically linked to an acyl serine alkyl ester. These compounds can be regarded as a simplified model compound of the glycosphingolipids **3** and **4**, because only the 3-hydroxy-propenylene group of the ceramide moiety is replaced by an ester bond. These analogues are also of interest, because they possess hydrogen-bonding capacity through the amide and ester bond of the serine moiety and thus may afford different intermolecular interactions from those exhibited by a glycero-based hydrophobic backbone.[1] We wish to report here the polymorphism exhibited by the synthetic glycolipid-water dispersion. We also found the remarkable difference in the phase behavior between this synthetic gluco- and galactolipid.

The synthetic glycolipids **1** and **2** were prepared by glycosylation of reactive acetyl-protected O-(α-D-glucopyranosyl or α-D-galactopyranosyl) trichloroacetimidate[2] with N^{α}-tetradecanoyl-L-serine dodecyl ester[3] and subsequent deacetylation with 1%-sodium methoxide in dry methanol (pH= 8.0).[4] The synthetic intermediates and the final products were purified with medium pressure liquid chromatography (MPLC) with a Lichroprep Si 60 [chloroform/methanol (9/1,v/v) or toluene/acetone(15/1-4/1, v/v)]. Their chromatographic



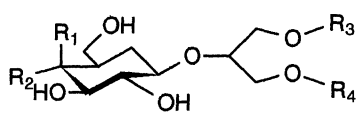
$R_3=(CH_2)_{12}CH_3$, $R_4=(CH_2)_{11}CH_3$

1: R_1=H, R_2=OH **2**: R_1=OH, R_2=H



$R_3=(CH_2)_{14}CH_3$, $R_4=(CH_2)_{12}CH_3$

3: R_1=H, R_2=OH **4**: R_1=OH, R_2=H



$R_3=R_4=(CH_2)_{13}CH_3$

5: R_1=H, R_2=OH **6**: R_1=OH, R_2=H

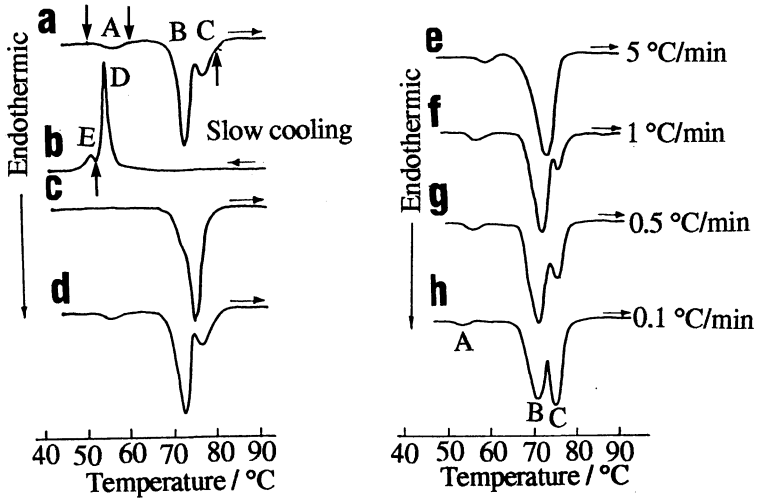


Fig.1. DSC thermograms for aqueous dispersions of synthetic glucolipid **1** (Sample concentration; 44 g/dm^3). (a-d) Heating and cooling scan (Heating rate in a,c, and d; 1 °C/min, cooling rate in b; 0.5 °C/min) (e-h) Effect of heating rate on transition endotherms.

purity was confirmed by thin-layer chromatography on silica gel plates (Merck F60 254). As a reference glycolipid, 1,3-di-O-tetradecyl-2-O-(β-D-glycosyl)-glyceols **5** and **6** were synthesized in the same manner as reported before.[5] All new compounds reported here gave satisfactory analytical data. The stereochemical purity of these synthetic glycolipids was checked by 270 MHz ^{1}H-NMR. It revealed clearly the presence of doublet with J=7.25-7.58 Hz for an anomeric proton, indicating the β-glycosidic linkage. We prepared hydrated samples by adding pure water to a dry glycolipid and subsequent sonication for 30 min at 90 °C. At least three heating and cooling scans over the temperature range 15-90 °C gave complete hydrated samples which displayed reproducible differential scanning calorimetry (DSC) thermograms.

Figure 1a shows the DSC thermogram of the hydrated lipid **1**. The glucolipid **1** displayed a small low temperature endotherm **A** and a large high temperature endotherm **B** as well as an additional higher temperature endotherm **C**. The main transition **B** can be safely ascribed to the hydrocarbon chains order-disorder transition (Tm=72 °C). This transition is about 15 °C lower than that of the synthetic glucocerebroside **3**.[6] The chain melting temperature of cerebroside do not depend significantly on acyl chain length.[7] Therefore, the result can be attributed to the difference between the ester group of **1** and 3-hydroxy-propenylene group of **3**. The cooling run at slow rate (<0.5 °C/min) displayed two exothermic transitions (Fig. 1b); an exotherm **D** at 53 °C followed by a smaller exotherm **E** at 50 °C. Surprisingly, the low temperature endotherm **A** was absent in the reheating scan (Fig. 1c). This phenomenon indicates that two types of gel phases exist. Upon rapid cooling (>5 °C/min), the subsequent reheating gave the same thermorgrams as that observed in the first scan (Fig. 1d). The effect of heating rate on the thermal behavior is shown in Fig. 1e-h. The relative area of the endotherm **C** increased with decreasing in the heating rate. This means that the transition **C** is a relatively slow process. The total transition enthalpy of both endotherms **B** and **C** remained almost constant (ΔH_{B+C}=44.9 kJ/mol).

To correlate the energetic changes with phase changes, we performed polarizing optical microscopy. We employed the same heating/cooling scan as that used in the calorimetric studies. Heating of the aqueous dispersion at 1 °C/min caused an evident phase change in a gel-phase at 56 °C (G-I → G-II) (Fig. 2a and Fig. 2b). In addition, heating to 65 °C (intermediate between endotherms **A** and **B**) followed by cooling and reheating from 30 °C afforded no significant phase change which corresponds to the first endotherm **A**. This result indicates that the low endothermic transition is irreversible. Heating to 80 °C resulted in the transition from a hydrated crystal (G-II) to a liquid crystal whose optical texture is characterized as a mixture of minor oily streaks (LC-I) and dominant unidentified droplets (LC-II) (Fig. 2c)[8] However, we obtained a homogeneous lamellar liquid crystalline phase at 52 °C by slow cooling from 90 °C (oily streaks texture in Fig. 2d).[8] After-

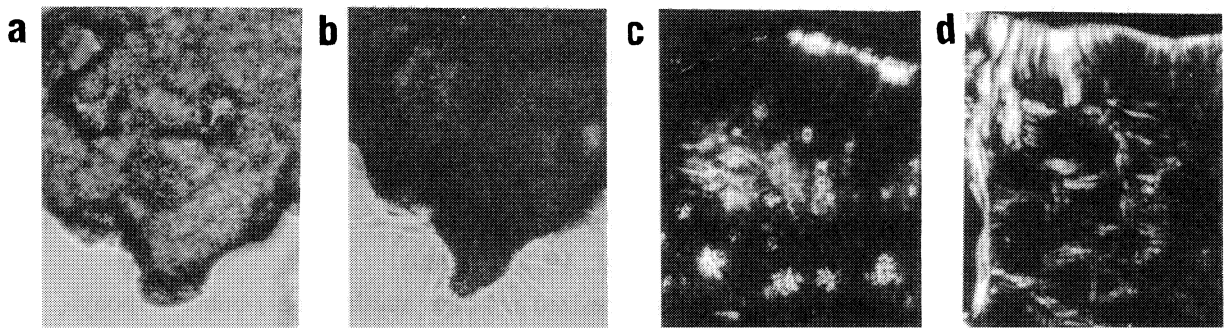


Fig. 2. Polarizing optical micrographs which were recorded at temperatures pointed by vertical arrows in Fig.1a and Fig. 1b (Sample concentration; 50 g/dm^3, Magnification; 100 times).

wards two types of crystallization were detected, depending strongly on the cooling rate. Based on the above calorimetric and optical microscopic evidence, we proposed a scheme of the phase change in Fig. 3. At temperatures below the chain melting temperature (Tm), the aqueous dispersion of **1** has potential to form two irreversible gel phase. One is a metastable gel-phase (G-I) which becomes predominant by rapid cooling (>5 °C/min). The other is a stable gel phase (G-II) induced by slow cooling (<0.5 °C/min). The small low temperature endotherm **A** coincides with (G-I) → (G-II) transition. At temperatures above Tm, two liquid crystalline phases exist. From the findings mentioned above, the endotherm **B** should correspond to (G-II) → (LC-I), and endotherm **C** (G-II) → (LC-II).

A similar metastable state in a gel phase has been observed for gluco- and galactocerebroside,[6,9] sphingomyelin,[10] and carbamyloxyphosphatidylcholine[11] as an exothermic transition on heating. These lipids are characterized as possessing ceramide moieties or carbamyloxy-linked fatty acids. Therefore, the present results afford a clear example of polymorphism exhibited by a non-natural glycolipid of short alkyl chain length without ceramide moiety. It is unknown, on the basis of the presently available data, which structural change is responsible for the endotherms. However, interlipid hydrogen bondings via amide and ester bonds would largely contribute to the metastable behavior of **1**. Actually, we observed that the synthetic glycolipids **5** and **6** having no hydrogen-bond donor groups displayed no metastability.[13] On the contrary, Kuttenreich et al has observed the polymorphism of 1,2-O-ditetradecyl-O-β-D-galactosyl-sn-glycerole.[12] Therefore, the above results show that the metastable behavior of glycolipids is not mediated only by the ceramide backbone.

The synthetic galactolipid **2** which differs only in the configuration of the OH group at C4 position from the synthetic glucolipid **1**, gave a single endothermic transition at 102 °C (ΔH=63.5 kJ/mol). It is of great interest that the configurational change of the OH group produced no metastable behavior and 30 °C higher chain order-disorder temperature for the galactolipid **2**. To the best of our knowledge, such a difference in the chain melting temperature is the most remarkable among those so far observed.[10,11,14,15] Glycosphingolipids[6,9]

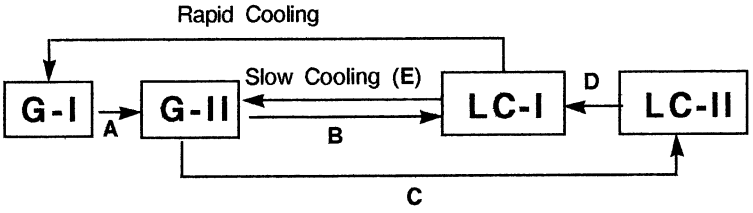


Fig. 3. Schematic representation of the generalized phase behavior of synthetic glucolipid **1**.

and the synthetic glycolipids **5** and **6**[13] display no such a difference. This finding would show the marked difference in the hydration properties of the two sugar head groups in the individual molecule. Galactose head group of **2** may have a lower degree of hydration because of strong interlipid sugar-sugar interactions.[9]

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4) **1**; Mp 116-117 °C, TLC[chloroform/methanol (9:1)]; Rf 0.70, $[\alpha]_D^{23}$ -9.9° (c 1.0, $CHCl_3/CH_3OH$, 3/1,v/v), ^{1}H-NMR ($CDCl_3/CD_3OD$, 2/1, v/v) δ 4.74 (t, 1H, Ser-CHα), 4.38 (dd, 1H, $J_{CH\beta,CH\beta}$ =10.2 Hz, $J_{CH\alpha,CH\beta}$=3.6 Hz, Ser-CHβ), 4.26 (d, 1H, $J_{1,2}$=7.6 Hz, 1-H), 4.14 (t, 2H, -$COOCH_2$-), 3.87 (dd, 1H, $J_{5,6a}$=2.6 Hz, 6a-H), 3.73 (dd, 1H, $J_{5,6b}$=5.1 Hz, 6b-H), 3.71 (dd, 1H, $J_{CH\alpha,CH\beta}$=3.3 Hz, Ser-CHβ), 3.42-3.25 (m, 4H, 3-H, 4-H, 5-H, 2-H), 2.26 (t, 2H, -$NDCOCH_2$-), 1.64(t, 4H, -$COOCH_2\underline{CH_2}$- and -$NDCOCH_2\underline{CH_2}$-), 1.25 (br. s, 38H, $19CH_2$), 0.88 (2t, 6H, $2CH_3$).
2; Mp 129.5-130 °C, TLC[chloroform/methanol(5:1)]; Rf 0.70, $[\alpha]_D^{23}$ -3.5° (c 0.5, $CHCl_3/CH_3OH$, 3/1, v/v), ^{1}H-NMR ($CDCl_3/CD_3OD$, 2/1, v/v) δ 4.72 (t, 1H, Ser-CHα), 4.40 (dd, 1H, $J_{CH\beta,CH\beta}$ =10.1 Hz, $J_{CH\alpha,CH\beta}$=3.8 Hz, Ser-CHβ), 4.21 (d, 1H, $J_{1,2}$=7.3 Hz, 1-H), 4.13 (m, 2H, -$COOCH_2$-), 3.88 (dd, 1H, 4-H), 3.81 (dd, 1H, $J_{5,6a}$=6.3 Hz, 6a-H), 3.77 (dd, 1H, $J_{5,6b}$=5.27 Hz, 6b-H), 3.72(dd, 1H, $J_{CH\alpha,CH\beta}$=3.6 Hz, Ser-CHβ), 3.55 (t, 1H, $J_{2,3}$=9.9 Hz, 2-H), 3.52-3.47 (m, 2H, 5-H, 3-H), 2.26 (t, -$NDCOCH_2$-), 1.64 (t, 4H, -$COOCH_2\underline{CH_2}$-, -$NDCOCH_2\underline{CH_2}$-), 1.25 (br. s, 38H, $19CH_2$), 0.88 (2t, 6H, $2CH_3$).

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