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Dynamics and orientation of glycolipid headgroups by ^{2}H-NMR: gentiobiose *

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Deuterium nuclear magnetic resonance has been used to investigate the dynamics and determine the orientation of the headgroup of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-*sn*-glycerol (β-DTDGL), in aqueous multilamellar dispersions. In addition, its anomeric analog, having an α glucose-glycerol linkage, was prepared and examined. The lipids were labelled with deuterium at specific positions in the disaccharide moiety. Analysis of the deuterium quadrupolar splittings for the first glucose ring (glycerol-linked) gave segmental order parameters of 0.43 and 0.35 for the β and α isomers, respectively. Both isomers had similar orientations of the sugar ring relative to the bilayer surface, as determined for lipid in the liquid-crystalline phase. ^{2}H-NMR results for the lipid labelled at C-6′ are consistent with a single conformation about the C-5′–C-6′ bond of the first glucose residue, with a dihedral angle (O-5′–C-5′–C-6′–O-6′) of −17°. The results obtained for the second sugar ring suggest that two conformers may be present, which are in slow exchange on the ^{2}H-NMR timescale. Measurements of longitudinal relaxation times, T_{1z}, gave similar values for both sugar moieties in the headgroup, suggesting that the disaccharide does not exhibit the flexibility expected about the 1 → 6 linkage. Since T_{1z} for ^{2}H in these compounds decreases with increasing temperature and increases with magnetic field strength, the motion(s) dominating relaxation is in the long-correlation-time regime ($(\omega_0\tau_c)^2 > 1$). Thus, the gentiobiosyl headgroup undergoes the slowest motion of the glycolipid headgroups studied to date.

Introduction

Cell surface carbohydrates, in particular the headgroups of glycolipids, are involved in important biological processes [1]. The elucidation of their role in membrane surface interactions first requires a good knowledge of their physicochemical properties. More specifically, the conformation(s) of the glycolipid headgroup and its orientation relative to the membrane surface are of prime interest. The spatial restrictions (ordering) imposed on the headgroup motions, as well as the types and rates of these motions, are also valuable parameters. While high resolution ^{1}H-NMR has proven to be a powerful approach to elucidating glycolipid conformation of isolated molecules in isotropic solutions [2], it is not clear whether these reflect the corresponding properties of the same carbohydrate moieties in a membrane environment. For some time, ^{2}H-NMR has been recognized as having the potential to provide insight into these properties of membrane surface carbohydrates [3]. Until now, attention has been focused on the orientational and motional properties of glycolipids having a monosaccharide headgroup [3–7]. These studies have been extended recently to a glycolipid bearing a disaccharide headgroup, the biologically relevant lactosyl residue [8]. The results have revealed a single headgroup conformation which differs from that of crystalline lactose and of methyl lactoside in aqueous solution. Since the lactosyl residue was expected to exhibit one conformation and little internal motion, we have sought a system which might give more than one headgroup conformation and possibly exchange between conformers. The 1 → 6 linkage between pyranosides is expected to be more flexible than the 1 → 4, 1 → 3 and 1 → 2 linkages. Gentiobiose, 6-*O*-β-D-gluco-

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Abbreviations: DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; DTDGL, 1,2-di-*O*-tetradecyl-3-*O*-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-*sn*-glycerol; DPPC, dipalmitoylphosphatidylcholine; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; ^{2}H-NMR, deuterium nuclear magnetic resonance; β-DTLL, 1,2-di-*O*-tetradecyl-3-*O*-(β-lactosyl)-*sn*-glycerol.

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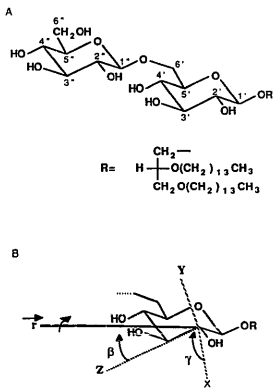

Fig. 1. (A) Structure of β-DTDGL; (B) Molecule-fixed coordinate system and definition of angles relating the motional axis of the ring, r, to this system. The Z axis lies along the C-2′–C-3′ bond and the X axis falls in the plane spanned by C-3′–C-2′–O-3′. β is the angle between the motional axis and the Z axis and γ is the angle between the X axis and the projection of r onto the XY plane.

pyranosyl-β-D-glucopyranose (Fig. 1A), occurs as one of the headgroups of the membrane lipids of methanogenic bactcria and is therefore of biological interest [9]. The theoretical calculations of Rees and Scott [10] have led to the conclusion that, for gentiobiose, the average conformation about the glycosidic bond is antiperiplanar. Interestingly, Melberg and Rasmussen [11] have found 24 conformational minima by empirical force-field calculations, and that, on average, β-gentiobiose is not fully extended but is slightly coiled. This is consistent with the crystallographic data of Rohrer et al. [12] who have observed a C-5′–C-6′–O-6′–C-1″ dihedral angle of −156.3°. Solution NMR studies [13–15] of the disaccharide or its derivatives have concluded that there is limited flexibility about the glycosidic linkage with specific rotameric preferences being manifest. It is therefore of considerable interest to elucidate the conformational properties of a gentiobiosyl residue constrained within the anisotropic environment of a membrane surface.

This paper describes a ^{2}H-NMR study of the orientation and dynamics of a β-gentiobiosyl glycerolipid, namely β-DTDGL (Fig. 1A), as aqueous multilamellar dispersions. This lipid is of interest both as a conceptual model upon which to develop further understanding of membrane surface carbohydrate and as an analog of a naturally occurring glyceroglycolipid [9].

Materials and Methods

Synthesis

Octa-O-acetyl-β-[6-$^2H_2$]gentiobiose. Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside was prepared by an established procedure [16] except that the hydrogenolysis was performed with lithium aluminum hydride-aluminum(III) chloride [17]. The benzyl derivative was oxidized with Jone's reagent in acetone to give the crude uronic acid. The crude acid was reduced with lithium aluminum deuteride (Aldrich Chemical, Milwaukee, WI) in dry diethyl ether and the product isolated by standard procedures. The crude product was crystallized from ethanol/water to give the C-6 labelled compound having m.p. 92–94° C (unlabelled material had m.p. 92–93° C) and elemental analysis for $C_{34}H_{34}{}^2H_2O_6$: calc. C, 75.25%; H, 7.06%; found C, 75.08%; H, 6.98%.

Tetra-*O*-acetyl-D-glucopyranosyl bromide (2 mmol) was added to a stirred mixture of the deuterated benzyl derivative (1.9 mmol), mercury(II) bromide (1 mmol) and mercury(II) cyanide (1 mmol) in dry acetonitrile. After 2 h, more glycosyl bromide (1 mmol) was added and the mixture stirred for 2 h. The crude product was isolated by standard procedures and purified by chromatography on silica gel with ethyl acetate/hexane (1 : 3 followed by 2 : 3 (v/v)).

The benzylated disaccharide was hydrogenated in glacial acetic acid (30 ml) with 30% palladium on charcoal. Acetylation of the product with acetic anhydride-sodium acetate afforded octa-*O*-acetyl-β-[6-$^2H_2$]gentiobiose (797 mg, 65% from the starting benzyl glycoside). The product had m.p. 185° C (lit. 191.5–192.5° C [18]) and elemental analysis for $C_{28}H_{36}{}^2H_2O_{19}$: calc. C, 49.41%; H, 5.92%; found C, 49.23%; H, 5.80%. Chemical ionization mass spectrometry (methane) gave an $M+1-$HOAc ion at 621.

Octa-O-acetyl-β-[2,3,4-$^2H_3$]gentiobiose. 1,6-Anhydro-β-D-glucose [19] was deuterated with Raney nickel-^2H_2O according to the procedure of Koch and Stuart [20]. ^{13}C-NMR of the product indicated deuterium incorporation of 20, 100 and 37% at C-2, C-3, and C-4, respectively. The labelled anhydroglucose (10 mmol) was converted into 2,3,4-tri-*O*-benzyl-1,6-di-*O*-acetyl-D-glucopyranose according to established procedures [21]. The acetyl derivative was treated overnight with benzyl alcohol (50 mmol) and boron trifluoride-etherate (5 ml) in dry dichloromethane (40 ml). The reaction mixture was diluted with dichloromethane, washed with water, and concentrated. The residue was coevaporated with toluene and the product acetylated with acetic anhydride (10 ml) in pyridine (30 ml). Methanol was added and the resulting mixture concentrated. The crude product was chromatographed on silica gel with a step-

gradient of 0–10% ethyl acetate in hexane to give benzyl 2,3,4-tri-*O*-benzyl-6-*O*-acetyl-α,β-D-[2,3,4-$^2H_3$]glucopyranoside (6.5 mmol). Deacetylation with sodium methoxide in methanol afforded the deacetylated derivative which co-migrated on TLC with the authentic unlabelled compound (Giziewicz, J.B., unpublished results).

The benzyl derivative was converted to octa-*O*-acetyl-β-D-[2,3,4-$^2H_3$]gentiobiose as described for the C-6-labelled derivative. The compound had a ^{13}C-NMR spectrum consistent with that expected for the titled compound.

Octa-O-acetyl-β-D-[1′-$^2H_1$]gentiobiose. Benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (2.38 mmol) was treated with tetra-*O*-acetyl-D-[1-$^2H_1$]glucopyranosyl bromide (2.6 mmol) (prepared from D-[1-$^2H_1$]glucose (MSD Isotopes, Montreal, Canada)) as described for the synthesis of the C-6-labelled gentiobiose. The product (1.2 mmol) had the same mobility on TLC (hexane/ethyl acetate, 2.2 : 1 (v/v)) as authentic octa-*O*-acetyl-β-gentiobiose. ^{13}C-NMR spectra were identical to the unlabelled compound, except for the expected spectral differences associated with deuterium at C-1′. Mass spectrometry (CI methane) gave an ion corresponding to $M + 1 -$ HOAc at 620; that of the unlabelled material appeared at 619.

Gentiobiosyl lipid. 1,2-Di-*O*-tetradecyl-*sn*-glycerol was synthesized by the procedure of Ogawa and Beppu [22] and had m.p. 41–42°C, in good agreement with the reported value of 42–43°C. The coupling with specifically deuterated gentiobiosyl halides was performed according to the procedures reported in the synthesis of the corresponding lactosyl glycolipids [8]. The coupling reaction proved not to be stereospecific yielding both anomeric glycosides. ^{13}C-NMR spectra indicated an α : β ratio of 1 : 2 for the [2′,3′,4′-$^2H_3$]glycolipid and of 1 : 1 for the [6′-$^2H_2$] isomer. The [1′′-$^2H_1$]gentiobiosyl-lipid spectrum showed only the β anomer. The C-6′-labelled lipid had m.p. 110–114°C and elemental analysis for $C_{42}H_{82}{}^2H_2O_{13}$: calc. C, 63.67%; H, 10.69%; found C, 63.50%; H, 10.51%. The lipid labelled at C-2′, C-3′ and C-4′ had m.p. 112–114°C; elemental analysis for $C_{43}H_{82}{}^2H_2O_{13} + H_2O$: calc. C, 62.29%; H, 10.70%; found C, 62.40%; H, 10.67%.

Calorimetry

Calorimetry was performed on a Microcal MC-1 differential-scanning calorimeter, at 60 C°/h, using 1 mg of lipid dispersed in 1.5 ml of distilled water.

Spectroscopy

^{2}H-NMR spectra were obtained at 30.7 MHz on a 'home-built' spectrometer, or at 46.1 MHz on a Bruker MSL-300 spectrometer, as described elsewhere [4]. Spectra were acquired on resonance in quadrature using the quadrupolar echo sequence [23] with full-phase cycling of the radiofrequency pulses, 90° pulses (2.3–2.5 μs, 5 mm solenoid coil) separated by a 60 μs delay and a recycle time of 30–100 ms. Longitudinal relaxation times, T_{1z}, were measured by the inversion-recovery procedure in combination with the quadrupolar echo sequence as described elsewhere [24]. The sample temperature was electronically regulated to within ±0.5°C. Spectral dePaking was performed according to the procedure of Bloom et al. [25] to give the 90°-oriented sample spectrum. The samples were prepared from 30–50 mg of dry lipid hydrated with 0.2 ml of deuterium-depleted water (Aldrich Chemical, Milwaukee, WI) in a 5 mm (o.d.) sample tube sealed with epoxy resin. For [1′′-$^2H_1$]gentiobiosyl lipid, only 5 mg was available for ^{2}H-NMR. The suspensions were heated to 40–50°C, vortex mixed and cooled down to 15°C, with the cycle repeated at least three times.

^{13}C-NMR spectra of lipids in a C^2HCl_3-$C^2H_3O^2H$ (2 : 1, v/v) solution were acquired on a Bruker MSL-300, at a frequency of 75.47 MHz with Waltz ^{1}H-decoupling [26], and with the DEPT pulse sequence [27] with Waltz ^{1}H-decoupling.

Computational methods

Segmental ordering and the orientation of the first glucopyranose ring relative to the bilayer normal were determined from the deuterium quadrupolar splittings as described previously [4]. The relative atomic positions for the β-gentiobiosyl residue which were required for data analysis were obtained from X-ray diffraction data of β-gentiobiose [12,28]. Atomic coordinates of methyl α-D-glucopyranoside from neutron-diffraction studies [29] were used for the α-anomer because no crystallographic data were available in the literature for α-gentiobiose. All calculations were performed on an IBM 3090 computer.

Results and Discussion

Differential-scanning calorimetry of β-DTDGL revealed a single endothermic transition at 27.5°C on a heating scan from 10 to 90°C which is attributed to the gel to liquid-crystalline transition. The gentiobiosyl lipid is presumed to be organized in a lamellar structure since a number of other pure glyceroglycolipids having a disaccharide headgroup have been shown to exhibit only lamellar structures [30,31]. The presence of a second glucopyranosyl ring has a dramatic effect on the transition temperature which is 24°C lower than for the corresponding β-DTGL [4]. A similar decrease has been noted in the transition temperature of a 1 → 6 digalactosyl diacylglycerolipid relative to its monogalactosyl precursor [32]. This result illustrates further the general conclusion that the thermotropic properties of glycolipids depend strongly on the headgroup structure [31].

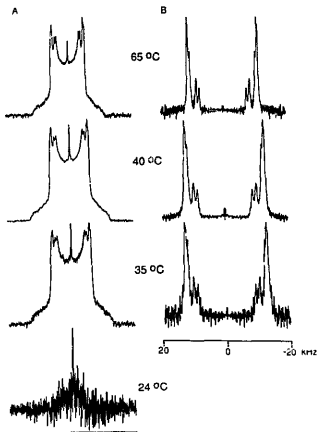

Fig. 2. (A) Temperature-dependence of the ^{2}H-NMR spectra (30.7 MHz) of DTDGL labelled at positions C-2′, C-3′ and C-4′ of the gentiobiosyl moiety. 99000 accumulations at 24, 35 and 65 °C, and 891000 accumulations at 40 °C. (B) Corresponding dePaked spectra (90° orientation) calculated from the powder spectra in (A).

Fig. 2A shows the ^{2}H-NMR spectra of β-DTDGL deuterated at positions 2′,3′ and 4′ (first ring). The spectrum at 24 °C is broad and of low intensity, reflecting a short transverse relaxation time. Above 30 °C, the spectra have an axially symmetric lineshape which is attributed to the lamellar to liquid-crystalline phase, in agreement with the differential-scanning calorimetry data. The spectra are composed of a superposition of four powder patterns. This is more easily seen in the dePaked spectra (Fig. 2B) where the 90°-oriented sample spectra have been calculated according to Bloom et al. [25]. The spectral assignment is not straightforward since in addition to there being three labelled positions on the glucose ring, and therefore three possible overlapping powder patterns, approx. 33 mol% of the α-gentiobiosyl lipid is present. This lipid mixture resisted all attempts to separate the two anomers by chromatographic techniques. While it is clearly preferable to deal with anomerically pure lipid systems, in the context of the present study, the presence of the two isomers is not viewed as detrimental to the validity of the conclusions. The aim of the present study is focused on molecular properties and not on those of the ensemble such as T_c, bilayer stability and membrane permeability. In addition, for the C-6′-labelled lipid a change in the α to β ratio from 1 : 1 to 1 : 2 had no effect on the quadrupolar splittings, $\Delta\nu_Q$ (vide infra). To aid in spectral assignments, the ratio of the α-gentiobiosyl to β-gentiobiosyl lipid as well as the relative extent of ^{2}H-labelling at each ring position was determined by high-resolution ^{13}C-NMR. The dePaked spectrum (40 °C) was simulated with overlapping lines having a Lorenzian lineshape, and resonance assignments based upon the known ^{2}H-labelling pattern and α/β glycolipid ratios (Table I).

Since the lineshape associated with the lipid at 40 °C indicates that the headgroup motion has effective axial symmetry, it is assumed that this reflects axially symmetric ordering. As a result, the average orientation of the first glucose ring relative to its axis of motional averaging may be determined using the method described previously [4,33,34]. The motion of the first ring of the gentiobiosyl residue consists of a rotation and a fluctuation about an axis which, for lipids forming a bilayer, is usually coincident with the bilayer normal as has been established for other glycolipids [6,8]. The quadrupolar splitting of the ith deuteron is given by

$$\Delta\nu_Q = \frac{3}{4} \cdot \frac{e^2qQ}{h} \cdot \frac{(3\cos^2\alpha_i - 1)}{2} \cdot S$$

where e^2qQ/h is the quadrupolar coupling constant (158 kHz for ^{2}HCOH and 164 kHz for $^2H_2$COH groups) [5], S is the segmental order parameter and α_i is the angle between the C–^{2}H bond and the motional axis. The product of the last two terms corresponds to the geometrical order parameter, S_{CD}. The orientation of the motional axis relative to the glucopyranosyl ring is determined using the ratios R_i of the quadrupolar splittings:

$$R_i = \frac{3\cos^2\alpha_i - 1}{3\cos^2\alpha_i - 1}$$

Using a molecule-fixed axis system (Fig. 1B), the cos α_i can be written as

$$\cos\alpha_i = a_{i1} \cdot \cos\gamma \cdot \sin\beta + a_{i1} \cdot \sin\gamma \cdot \sin\beta + a_{i2} \cdot \cos\beta$$

where (a_{i1}, a_{i1}, a_{i2}) and (cos γ · sin β, sin γ · sin β, cos β) are the direction cosines of the ith C–^{2}H bond and of the molecular axis, respectively. The first set of direction cosines is calculated from the crystallographic data [12,28]. γ and β are varied stepwise and the pairs of angles which give the calculated R_i ratios closest to the experimental values are retained as the possible solutions. Corresponding $\Delta\nu_Q$ values and S are then calculated, as shown in Table I.

The calculations indicate a very similar orientation of the first ring of the α- and β-DTDGL (Table I), suggesting that there is some compensation in the glyco-

TABLE I

Quadrupolar splittings [a] and the orientation of the first ring of DTDGL

Lipid	$\Delta\nu_Q$ [b] (kHz)			Orientation		S
	C-2	C-3	C-4	β	γ	
β-DTDGL	24.0 (24.0)	25.4 (25.4)	24.0 (24.1)	78	45	0.43
α-DTDGL	17.6 (17.6)	20.2 (20.2)	17.6 (17.5)	78	52	0.35

[a] At 40 °C.

[b] Values in parenthesis calculated for the reported orientation.

sidic torsional angles for the difference in the glycosidic bond orientation relative to the glucose ring. The segmental order parameter, S, of β-DTDGL is very close to what was found in the liquid-crystalline phase for the analogous monosaccharide lipid β-DTGL (0.45) [4], which may be considered as the precursor of β-DTDGL. It is of interest to note that an S value of 0.52 was determined for the linear disaccharide lipid 1,2-di-*O*-tetradecyl-3-*O*-(β-lactosyl)-*sn*-glycerol (β-DTLL). However, while an increased value of S (0.56) was observed on going from β-DTGL to its epimer, α-DTGL [5], the reverse is observed for DTDGL. One possible explanation for a decrease in S is an increase in the torsional oscillations about the glucose-glycerol bond for α-DTDGL relative to those which occur for the corresponding β-anomer. The latter argument assumes that for both lipid systems the glycerol backbone exhibits similar orientational averaging. Fig. 3 is a representation of the average orientation of the first ring with respect to the bilayer normal n which is depicted as in the plane of the figure going from bottom to top. Inspection of Fig. 3 reveals that the orientation which gives the best fit to the ^{2}H-NMR data is somewhat surprising in that it brings hydroxyl groups close to the lipid/water interface. This contrasts with previous ^{2}H-

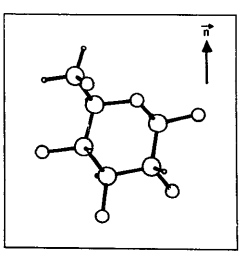

Fig. 3. Orientation of the first glucose ring of β-DTDGL relative to the director, n, which is vertical and in the plane of the figure.

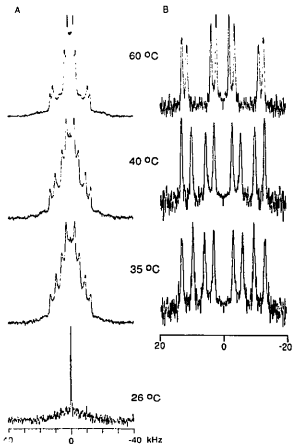

Fig. 4. (A) Temperature-dependence of ^{2}H-NMR spectra (30.7 MHz) of [6′-$^2H_2$]DTDGL, 90000 accumulations for spectra taken above 35 °C and 99000 accumulations for the one at 26 °C; (B) DePaked spectra calculated from the corresponding powder spectra in (A).

NMR studies of other glycolipids which concluded that the carbohydrate moieties extend away from the bilayer surface into the aqueous phase. However, the orientation given in Fig. 3 does allow the second sugar ring to be further from the bilayer surface. Interestingly, Ram and Prestegard [35] have deduced a similar orientation for a β-galactopyranosyl residue of a glycolipid analog which was anchored to a micelle surface. In their study, the headgroup orientation was established to be consistent with both NMR data and conformational energies, as calculated by molecular mechanics procedures using intramolecular potential energy terms. In the case of the gentiobiosyl lipid, it would be useful to confirm the deduced orientation by further experiments with ^{2}H-labelling on the glycerol backbone and on the second sugar ring.

Of particular importance in defining the conformation(s) of the 1 → 6 glycosidic bond of the gentiobiosyl residue is the orientation of the C-6′–O-6′ bond relative to the glucose ring. Also of interest is the question of the degree, if any, to which rotation about this bond occurs. To gain insight into these aspects, gentiobiosyl lipid, ^{2}H-labelled at C-6′ of the first ring was prepared and examined as aqueous dispersions. ^{2}H-NMR spectra

(Fig. 4) of [6′-^{2}H$_2$]DTDGL exhibit a superposition of four powder patterns and are representative of axially symmetric motion. ^{13}C-NMR of the glycolipid revealed that the lipid was an equimolar mixture of α- and β-DTDGL. After extensive chromatography, the mixture was enriched in β-DTDGL ($\alpha:\beta$, 1:2) facilitating resonance assignments. Thus, the smallest (5.5 kHz) and largest (26.5 kHz) splittings were assigned to the β-anomer. The integrated intensities, as measured from the dePaked spectrum, of the two spectral components attributed to the β-anomer, were equal. The latter result may be explained in three possible ways. The first is that there is only one orientation or rotamer about the C-5′–C-6′ bond and that the two splittings arise from the inequivalent orientations of the two C-6′–^{2}H bond vectors in the molecular frame. The second explanation requires the presence of two equally populated conformers having either both deuterons in each rotamer giving rise to the same quadrupolar splitting, that is, one $\Delta\nu_Q$ value for each rotamer, or the same two quadrupolar splittings occur for each conformer. These possibilities assume that the interconversion between conformers is slow on the ^{2}H-NMR timescale ($\ll 10^{-5}$ s). A third possibility is that there are at least two rotamers which are exchanging rapidly on the timescale of 10^{-5} s. The first explanation appears the most consistent with the experimental results for the following reasons. If two or more rotamers were interconverting with a jump rate slower than the rate of axial diffusion of the lipid molecule as a whole, an axially symmetric lineshape would be difficult to explain. Conversely, if the jump rate were as fast or faster than the rate of molecular axial diffusion, an axially symmetric lineshape might be expected. However, a further consequence of the latter type of segmental motion is that an S^2_{CD} dependence of the spin-lattice relaxation rate, T_{1z}^{-1}, would be expected [36,37]. Inspection of Table II reveals that both deuterons at C-6′ exhibit similar relaxation times. In addition, the relaxation rates associated with the ring positions are very similar to those of the exocyclic hydroxymethyl residue. There is no obvious dependence of T_{1z}^{-1} on S^2_{CD}, suggesting further that there is no rotameric interconversion about the C-5′–C-6′ bond on the timescale of the Larmor frequency. The second possibility may be discounted in the following manner. Given the ring order parameter, S, and the orientation of the director relative to the sugar ring, a search over the various rotamers about the C-5′–C-6′ bond for the predicted quadrupolar splittings for each of the two deuterons did not give two conformers with identical pairs of $\Delta\nu_Q$ values. However, two conformers were found which gave one $\Delta\nu_Q$ value for both deuterons in each conformer. One of these corresponds to a rotamer with O-6′ and C-4′ fully eclipsed. The second rotamer giving rise to a $\Delta\nu_Q$ value of 25.4 ± 0.2 kHz may be one of three possibilities in which O-6′ is *gauche*(+), *gauche*(−) or nearly *trans* to O-5′. The terms *gauche*(−), *gauche*(+) and *trans* refer to dihedral angles, as defined by the O-5′–C-5′–C-6′–O-6′ segment, of −60, +60 and 180°, respectively. It seems reasonable to anticipate that a fully eclipsed conformer would be energetically less favoured than any of the latter three rotamers. However, inspection of the ^{2}H-spectra for the C-6′-labelled position as a function of temperature (30–60 °C) reveals that the relative intensities of the spectral components of interest (5.5 and 26.5 kHz) are equal and invariant. The latter observation suggests that the presence of two rotamers about the C-5′–C-6′ bond is unlikely. Consequently, the explanation most consistent with the present results is that there is one conformer about the C-5′–C-6′ bond. Interestingly, high-resolution ^{1}H-NMR studies of the disaccharide gentiobiose in solution [13] have concluded that three rotamers were in fast exchange on the timescale of approx. 10^{-2} s with populations given as: 56% *gauche*(−), 41% *gauche*(+) and 3% *trans*. Assuming a fast exchange between the three corresponding rotamers for β-DTDGL weighted by their respective populations, as found for gentiobiose in solution, leads to calculated quadrupolar splittings that do not agree with the present data. This further supports the conclusion that only one rotamer is present for β-DTDGL. The best fit with experimental values corresponds to a dihedral angle of −17° as shown in Fig. 5B. This result clearly does not correspond to any of the classical minimum energy conformations for this type of molecular fragment where *gauche*(+), *gauche*(−) and *trans* are expected. A similar, partially eclipsed conformation at C-6′ of the corresponding β-glucolipid has been calculated from ^{2}H-NMR data [4]. The dihedral angle of −17° represents the best fit to the experimental data based upon the assumption that there is one fixed conformation about the C-5′–C-6′ bond. However, if small-amplitude librational motion about the C-5′–C-6′ bond were occurring, a different dihedral angle would result. At present, the latter possibility can not be completely discounted. It should be emphasized that if the orientation of the

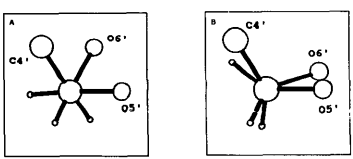

Fig. 5. Orientation of the hydroxymethyl group in β-gentiobiose moiety as viewed along the C-5′–C-6′ bond. (A) Atomic positions as calculated from the X-ray crystallographic data [12]; (B) Relative atom positions as determined from ^{2}H-NMR data for the β-DTDGL headgroup.

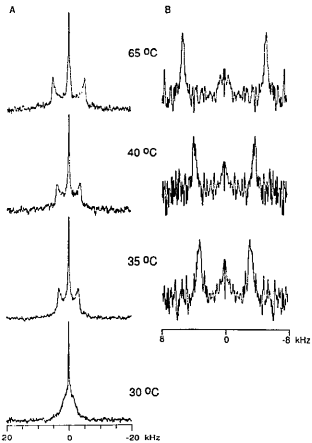

Fig. 6. (A) Temperature-dependence of ^{2}H-NMR spectra (30.7 MHz) of β-[1''-^{2}H$_1$]DTDGL, 90000 accumulations; (B) 90° oriented sample spectrum calculated from the corresponding powder spectra in (A).

first sugar ring relative to the bilayer surface were different from that proposed in Fig. 3, the data would still be consistent with a single conformer about the C-5′–C-6′ bond but the calculated dihedral angle would be different.

While data from the C-6′ position reflect conformation and motion about the C-5′–C-6′ bond, data associated with deuterons attached to the second glucose ring are sensitive to the conformation(s) about the intersaccharide bonds and the combined motions about the C-1''–O-6′, O-6′–C-6′ and C-6′–C-5′ bonds. ^{13}C-NMR of [1''-^{2}H$_1$]DTDGL (that is, labelled on C-1 of the second glucose ring) revealed the absence of the α-anomer thus simplifying spectral analysis. Besides a central isotropic component, the ^{2}H-NMR spectra of the lipid labelled at C-1'' (Fig. 6) have a lineshape characteristic of axially symmetric motion. From the latter spectral feature one may therefore conclude directly that if motion about the intersaccharide bonds (conformational exchange) exists, it must be on a timescale which is less than that for axial diffusion of the headgroup. If conformational interconversion were slower than axial diffusion, an axially asymmetric lineshape would be expected. Inspection of Fig. 6 reveals that as the temperature is raised from 35 to 65°C, the quadrupolar splitting increases from 8.6 to 10.8 kHz. The latter results indicate that either there is a change in the average orientation of the C-1''–^{2}H bond relative to the director (change in a single conformation) or that there are changes in the populations of two or more conformers that are in rapid equilibrium (on a timescale faster than axial diffusion of the lipid molecule). Relaxation times (Table II) for this position (for the anisotropic spectral component) are the same as for positions in the first glucose ring to within experimental error and show the same temperature-dependence. This is more consistent with a change in conformation about the disaccharide bond than a change in conformational equilibria. The central component is not residual HO^{2}H, since several exchanges with ^{2}H-depleted water did not alter the spectra. Therefore, it is tempting to speculate that two or more conformers are present and in slow exchange. According to spectral simulations (data not shown), the central component decreases slightly with increasing temperature, going from 21 to 17% of the total intensity over the temperature range 40–65°C. The present results are consistent with, but do not prove, the existence of two conformers about the disaccharide linkage. Further experiments involving additional ^{2}H-labelling of the second sugar ring are in progress and should provide more definitive information on these putative conformers.

Headgroup motion

The longitudinal relaxation times (T_{1z}) of ^{2}H in the labelled carbohydrates were measured to probe the headgroup dynamics. Deuterons on both rings exhibit, to within experimental error, identical relaxation times at 40°C (Table II), indicating similar mobilities. Therefore, it appears that for motions having frequencies near the Larmor frequency (ω_0), the gentiobiose moiety behaves as a rigid unit and that it does not manifest the anticipated flexibility about the $1 \rightarrow 6$ linkage. The DTDGL labelled on the first ring also shows a substantial increase in T_{1z} at higher magnetic field (Table II). The ratio of T_{1z}(46.1 MHz): T_{1z}(30.7 MHz) is 1.55, suggesting that the motion(s) dominating relaxation is approaching the long-correlation-time regime. The present results are reminiscent of the corresponding field-dependence (1.6–2.0) exhibited by cholesterol where the dominant motion was concluded to be axial diffusion [38]. This correlates well with the very short value of T_{1z} and indicates that the dominant headgroup motion is close to ω_0. Table II also reveals a small decrease in T_{1z} with increasing temperature, which is consistent with motion in the long-correlation-time regime ($\omega_0^2\tau_c^2 > 1$). Therefore, motions with τ_c shorter than 10^{-9} s are excluded. Inspection of Table III allows a comparison of T_{1z} of the gentiobiosyl moiety with various lipid headgroups. It is important to note that, except for DTDGL, the motions of the lipids reported in Table III

TABLE II

Longitudinal relaxation times of β-DTDGL

Sites of labelling	Temperature (°C)	T_{1z} (ms)
C-2′, C-3′, C-4′	40	3.3 ± 0.2
		5.1 ± 0.2 [a]
	50	3.1 ± 0.2
	60	3.0 ± 0.2
C-6′	30	3.6 ± 0.2
	40	2.9 ± 0.2
	50	2.8 ± 0.2
C-1″	40	3.3 ± 0.2 and 8 ± 2
	60	2.8 ± 0.3 and 7 ± 2
	65	2.8 ± 0.3 and 6 ± 2

[a] At B_0 = 46.1 MHz. All other values for B_0 = 30.7 MHz.

TABLE III

Longitudinal relaxation times of lipid headgroups

Lipid	Temperature [a] (°C)	T_{1z} (ms)	
		30.7 (MHz)	46.1 (MHz)
β-DTDGL	40 (27.5)	3.3	5.1
β-DTGL [b]	52 (52)	5.3–5.6	
α-DTGL [b]	52 (52)	4.8	
DPPC [c]	50 (41)		29.7–38.4
POPG [d]	5 (−5)		5.6–5.8

[a] Value in parentheses is the transition temperature, T_c.
[b] Ref. 5.
[c] Ref. 39.
[d] Ref. 40.

fall within the short-correlation-time regime ($\omega_0^2\tau_c^2 < 1$) so that T_{1z} increases with temperature. Interestingly, the DTDGL headgroup, which is the most complex in Table III, has the shortest relaxation time and the lowest mobility. Indeed, one might speculate that compared with the relatively linear 1 → 4 linkage in the lactosyl moiety of the corresponding lipid (whose fast motion(s) fall within the short-correlation-time regime [8]), the 1 → 6 linkage of DTDGL leads to a bulkier surface residue with reduced motional rates.

Conclusions

The disaccharide-containing glycolipid β-DTDGL exhibits a gel to liquid-crystalline phase transition at 27.5 °C, which is 24 °C lower than that of the corresponding monoglucopyranosyl lipid, β-DTGL, and nearly 40 °C lower than that of a lipid having a lactosyl headgroup [8]. Since in the present study the lipid system was actually composed of an anomeric mixture, an attempt to explain the lowering of T_c by replacing the lactosyl residue with the gentiobiosyl moiety would be speculative. Nonetheless, two possible explanations are that the bulky headgroup is significantly affecting packing in the gel state, and/or that headgroup hydration effects may contribute. Both aspects need to be explored to elucidate their importance.

^{2}H-NMR spectra obtained for lipid in the liquid-crystalline phase reflect axially symmetric motion. Assuming that ordering is axially symmetric, the data were analyzed for each of the two lipid isomers to give segmental order parameters of 0.43 and 0.35 for the β- and α-epimers, respectively. These values are similar to those obtained with other glyceroglycolipids [3–8] and perhaps reflect similarities in the constraints imposed on orientational averaging of lipids in the bilayer. In addition to the segmental ordering of the headgroup, the average orientation of the first ring of the disaccharide was calculated from the quadrupolar splittings. For the α- and β-isomers of the first ring, the data were consistent with similar orientations relative to the bilayer surface, suggesting a difference in the conformation(s) about the glucose-glycerol linkage for the two anomeric isomers. This result differs with that observed with monosaccharide lipids, where significant differences were noted in the headgroup orientations for anomeric pairs. This may reflect a need to balance intramolecular conformational effects while satisfying the requirements of the bilayer structure. This may be the reason that the monogalactosyl- and digalactosyl(1 → 6 linkage) glyceroglycolipids have similar surface areas for the headgroups in lamellar structures, 57 and 61 Å^2, respectively [41]. Clearly, studies involving lipids labelled in the glycerol backbone would help to elucidate aspects of the glucose-glycerol linkage in the two anomers. Results obtained with the second glucose ring labelled at C-1″ suggest that two or more conformers about the 1 → 6 linkage are present and in slow exchange on the ^{2}H-NMR timescale (10^{-5} s). This tentative conclusion requires corroboration using additional labels in the second ring. However, if two conformers do exist, the present ^{2}H-NMR results are not consistent with either having the conformation of crystalline gentiobiose [12].

Since spin-lattice relaxation times associated with ^{2}H-labels in both sugar rings of the gentiobiosyl residue are the same at 40 °C, the disaccharide moiety behaves as a rigid unit with respect to motions with rates near the Larmor frequency, and the expected flexibility about the 1 → 6 linkage is not observed. Indeed, the temperature- and magnetic field-dependences of the relaxation rates indicate that the gentiobiosyl headgroup has the lowest mobility of the glyceroglycolipids studied to date.

The present study provides new insight into the behaviour of glycosidic headgroups at membrane surfaces, a field of growing interest. These important and unexpected results demonstrate the need for further investigation to confirm and complete the interpretations.

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