Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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John M. Seddon [a], Oscar Ces [a], Richard H. Templer [a], David A. Mannock [b] & Ron N. McElhaney [b]

[a] Department of Chemistry, Imperial College, London SW7 2AY, UK

[b] Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7

Version of record first published: 15 Jul 2010

To cite this article: John M. Seddon, Oscar Ces, Richard H. Templer, David A. Mannock & Ron N. McElhaney (2003): STRUCTURE AND PHASE BEHAVIOUR OF SYNTHETIC GLYCOLIPIDS, Molecular Crystals and Liquid Crystals, 402:1, 77-84

To link to this article: http://dx.doi.org/10.1080/744816839

Mol. Cryst. Liq. Cryst., Vol. 402, pp. 77/[313]–84/[320], 2003

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400390243345

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STRUCTURE AND PHASE BEHAVIOUR OF SYNTHETIC GLYCOLIPIDS

John M. Seddon, Oscar Ces, and Richard H. Templer
Department of Chemistry, Imperial College, London SW7 2AY, UK

David A. Mannock and Ron N. McElhaney
Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7

We have used X-ray diffraction and differential scanning calorimetry (DSC) to study the structure and lyotropic phase behaviour of two synthetic dialkyl glycolipids having β-D-glucose and β-D-galactose headgroups and two saturated tetradecyl (C_{14}) chains. The diastereomeric compounds, 1,2-di-O-tetradecyl-3-O-(β-D-glucopyranosyl)-sn-glycerol (di-14:0–β-D-GlcDAG) and 1,2-di-O-tetradecyl-3-O-(β-D-galactopyranosyl)-sn-glycerol (di-14:0-β-D-Gal-DAG) show striking differences in their phase behaviour, particularly in the ordered lamellar phase region. Both compounds adopt the fluid lamellar L_α phase upon cooling from the H_{II} phase, but below the chain-melting transition, di-14:0-β-D-GlcDAG forms a metastable L_β gel phase, whereas di-14:0–β-D-GalDAG forms only crystalline lamellar phases, on the timescale of our measurements. We have determined the limiting hydrations of the various phases, and compare the findings from these glycolipids with those from our previous studies of the phospholipid didodecyl phosphatidylethanolamine (di-12:0-PE).

Keywords: synthetic glycolipids; gel phases; non-lamellar phases; model membranes; X-ray diffraction; calorimetry

INTRODUCTION

Glycolipids, biological amphiphiles whose polar headgroups contain one (monosaccharide) or more (oligosaccharide) cyclic sugar groups, are vitally important components of biological membranes, being involved in many regulatory and recognition processes. There is increasing interest

This work was supported by grant GR/L48065 from the EPSRC (UK) and by a grant from the Canadian Institute of Health Research.

in their structural roles in membranes, since they are the major membrane lipids of all plants (mainly galactose-based) and many micro-organisms (mainly glucose-based) [1]. The reason why such species utilise glycolipids rather than phospholipids may be to do with conserving phosphorus, which is usually a precious commodity in the environment. Frequently, monosaccharide-based lipids are the major glycolipid component of such membranes. The reasons why some organisms utilise galactose-based lipids, and some glucose-based lipids, as the main structural component of their membranes is poorly understood. It is therefore important to establish whether there are any differences in the lyotropic phase behaviour of these two classes of glycolipid.

A well-established and important finding is that fully-hydrated monoglycosyl diacylglycerols and dialkylglycerols, with either glucose-based or galactose-based headgroups, have a strong tendency to adopt inverse, nonlamellar phases such as the inverse hexagonal H_{II} and bicontinuous cubic phases. This is found both in naturally-occurring glycolipids extracted from biological membranes, and in purely synthetic glycolipid systems [2]. The phase behaviour of monosaccharide-based glycolipids is strikingly similar to that of the phosphatidylethanolamines (PE), one of the major classes of phospholipid in animal cell membranes. For synthetic dialkyl (saturated, ether-linked) PE's, it was found that the effect of reducing the chainlength was first to raise the fluid bilayer – inverse hexagonal (L_α–H_{II}) phase transiton temperature, and then when the chainlength was reduced below C_{14}, to induce inverse bicontinuous cubic phases to form, between the L_α and H_{II} phases [3–6].

It was subsequently found that the above-mentioned effects of chainlength also applied to glycolipids, which was to be expected since the underlying physical mechanisms are rather general in nature, and not specific to phospholipid systems. In fact the glycolipids have a stronger tendency to form inverse phases than the phospholipid PE system, the L_α–H_{II} phase transiton in excess water occurring at 55–56°C for di-14:0–β-D-GlcDAG [7,8], 62–63°C upon cooling for di-14:0–β-D-GalDAG [9], but at 96°C for the di-14:0-PE [3]. Changing the chain linkage from ether to ester is sufficient, for the di-14:0 glycolipids, to cause the appearance of inverse bicontinuous cubic phases above the L_α phase, but at higher temperatures of 72°C for the ester-linked Glc [10] and 81°C for the ester-linked Gal [11], compared to the L_α–H_{II} transition temperatures of the corresponding dialkyl compounds.

For glycolipids, chirality may be expected to play a greater role than in phospholipids, since the 1,2-***sn*** and 2,3-***sn***-glycolipid diastereomers are not mirror images of one another and so can have different physical properties. Indeed, studies in excess water of ether-linked di-12:0–β-D-GlcDAG found significant differences in phase behaviour between the 1,2-***sn*** and 2,3-***sn***

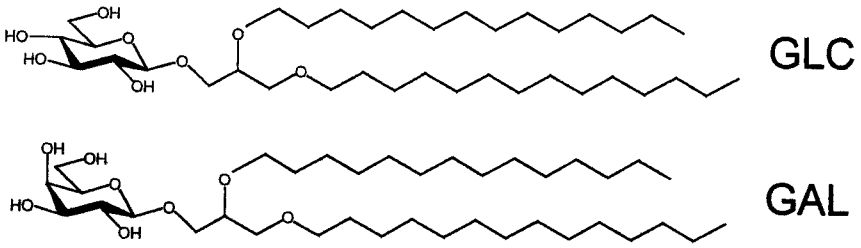

FIGURE 1 Chemical structures of the synthetic dialkyl glycolipids, di-14:0–*β*-D-GlcDAG and di-14:0–*β*-D-GalDAG.

isomers [12,13]. For the longer chainlength *β*-D-glucosyl compounds, however, the effects of chirality were very small. For galactose-based lipids, on the other hand, the effects of chirality are still quite marked for the 14 carbon chainlength compounds [9,14], and are very striking for the di-12:0 and di-13:0 compounds [15].

We have chosen to study the dialkyl compounds because the ether linkages confer enhanced chemical stability at higher temperatures compared to the ester-linked compounds. The particular homologues we chose for detailed study are the diastereomers 1,2-di-14:0–*β*-D-GalDAG and 1,2-di-14:0–*β*-D-GalDAG, which have the natural 1,2-***sn***-glycerol configuration.

This 14 carbon chainlength is just long enough to suppress the formation of any cubic phases. Our initial aim was to establish the binary temperature-composition phase diagrams for these two compounds at atmospheric pressure, and then to study the effects of hydrostatic pressure on the phase behaviour. We report some of our preliminary findings here.

EXPERIMENTAL

The diastereomeric compounds, 1,2-di-***O***-tetradecyl-3-***O***-(*β*-D-glucopyranosyl)-***sn***-glycerol (di-14:0–*β*-D-GlcDAG) and 1,2-di-***O***-tetradecyl-3-***O***-(*β*-D-galactopyranosyl)-***sn***-glycerol (di–14:0–*β*-D-GalDAG), were synthesised according to procedures which have been previously described [16]. The compounds were pure as assessed by TLC, using a solvent system of $CHCl_3$ / CH_3OH (9:1 v/v). Samples for differential scanning calorimetry (DSC) were weighed into aluminium pans along with a weighed amount of HPLC grade water. The estimated accuracy of the water content was ±1 wt%. DSC scans were obtained with heating/cooling rates of 0.5 or 1°C per minute, using either a TA Instruments DSC 2910 or a Perkin-Elmer DSC7 calorimeter. Heating and cooling scans were repeated typically three times each. Low-angle and wide-angle X-ray diffraction patterns were

obtained using a Guinier camera (Huber Diffraktionstechnik) operating with Cu $K_{\alpha 1}$ radiation ($\lambda = 1.5405$ Å). The scanned films were analysed using software written within the IDL 5.4 (Research Systems) data processing package. The estimated accuracy of the X-ray spacings is ± 0.5 Å (low-angle) and ± 0.05 Å (wide-angle).

RESULTS AND DISCUSSION

DSC scans were measured for both compounds over a wide range of water contents. Figure 2 shows representative scans for the dry samples, and samples containing excess water.

For the di-14:0–β-D-GlcDAG, the single, highly energetic phase transition seen for the dry sample on heating at 54.5°C is replaced in the sample containing excess water by a highly energetic phase transition at 53.2°C, followed by a low-enthalpy transition at 59.5°C. Two corresponding phase transitions are observed in the cooling scans, indicating that these transitions are reversible.

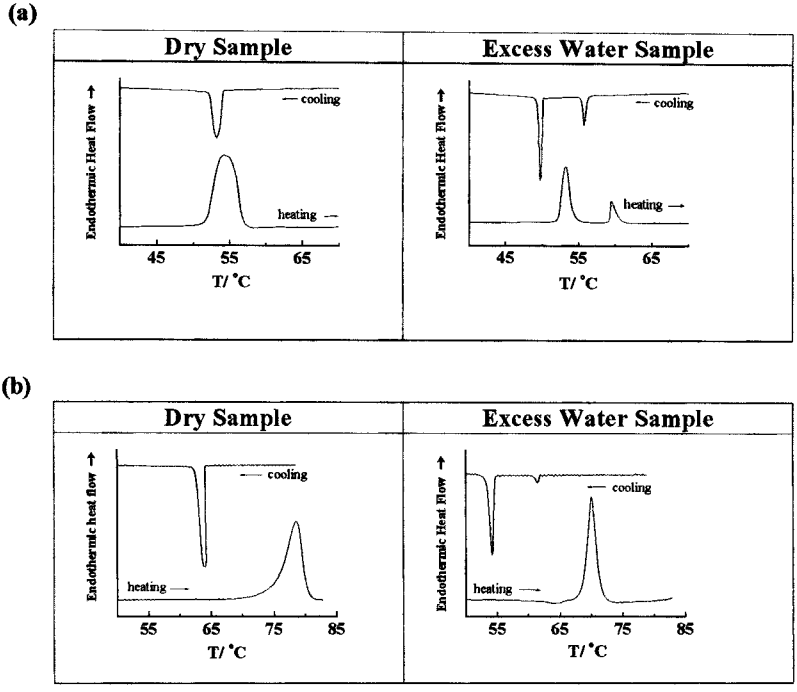

FIGURE 2 DSC scans of (a) di-14:0-β-D-GlcDAG and (b) di-14:0-β-D-GalDAG.

TABLE 1 Thermodynamic Data (Transition Temperatures, Enthalpies and Entropies) Relating to the DSC Scans Shown in Figure 2

Sample	Heating Sequence	Phase Transition Temperature(°C)	Enthalpy ΔH (kJ mol^{-1})	Entropy ΔS (J K^{-1}mol^{-1})
Glc (Dry)	Heating	54.5	43.0	131.3
	Cooling	53.2	42.6	130.6
Glc (Excess Water)	Heating	53.2	27.6	84.6
		59.5	6.9	20.8
	Cooling	55.7	7.3	22.2
		49.7	27.8	86.2
Gal (Dry)	Heating	78.9	80.9	229.9
	Cooling	64.0	63.3	187.8
Gal (Excess Water)	Heating	70.2	86.6	252.3
	Cooling	61.5	6.2	18.5
		54.2	81.2	248.2

For the di-14:0–β-D-GalDAG on the other hand, the single, highly energetic phase transition seen for the dry sample on heating at 78.9°C persists at higher hydrations, with the transition temperature progressively becoming lowered to a limiting value of 70.2°C. In this case, quite different behaviour is observed on the cooling scans. The single, exothermic phase transition oberved in the cooling scans of the dry sample at 64.0°C is replaced in samples containing excess water by a weakly exothermic phase transition at 61.5°C, and a highly exothermic phase transition at 54.2°C. It is clear that the phase transition behaviour of the Gal compound is not reversible, indicating the formation of metastable phases.

The thermodynamic data are summarised in Table 1.

In order to understand the DSC results, the two glycolipids were studied by X-ray diffraction. In addition to establishing the structures and symmetries of the phases, these measurements also yielded the lattice parameters of each phase. Figure 3 shows representative low-angle X-ray diffraction powder patterns from the various lyotropic phases observed for these two glycolipids.

The stable low-temperature phase of the di-14:0-β-D-GlcDAG compound is a lamellar crystalline phase (L_{C1}), with a layer spacing d of 52.5 Å, and strong wide-angle reflections at 4.60 and 3.90 Å. After heating above the chain-melting transition, the low-temperature phase becomes an untilted L_β lamellar gel phase, with a layer spacing in excess water of 55.7 Å, and a single, quite sharp symmetrical wide-angle peak at 4.15 Å. This gel phase is stable on the timescale of our experiments, but is known to be metastable upon longer incubation [11]. At low hydrations, the chain-melting transition

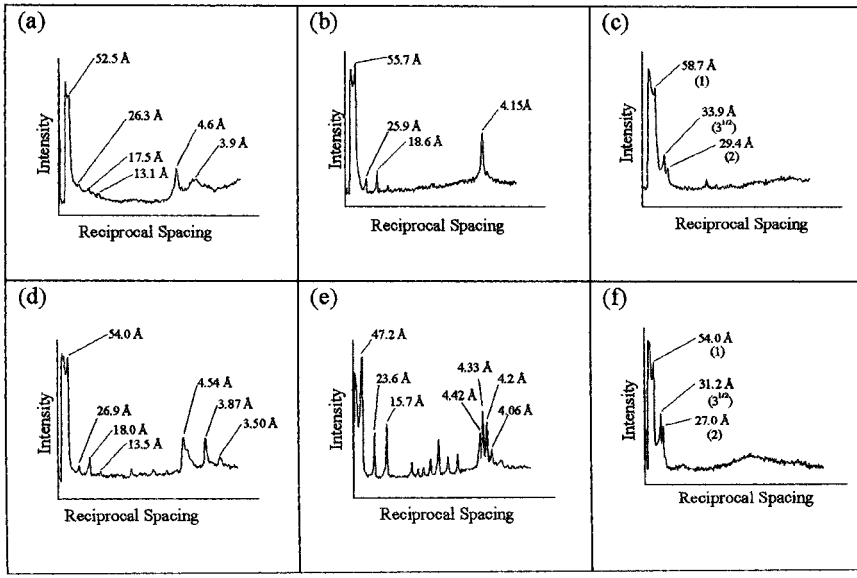

FIGURE 3 X-ray powder diffraction patterns from the various phases of di-14:0-β-D-GlcDAG (a: L_{C1}; b: L_β; c: H_{II}), and di-14:0-β-D-GalDAG (d: L_{C1}; e: L_{C2}; f: H_{II}) in excess water.

occurs directly to the inverse hexagonal H_{II} phase, whereas in excess water the transition at 53.2°C is to the fluid lamellar L_α phase ($d = 51.8$ Å), followed by a further transition at 59.5°C to the H_{II} phase (lattice parameter $a = 67.8$ Å).

The behaviour of the di-14:0–β-D-GalDAG compound is rather different. At low temperatures, depending on the sample history, it can form either the L_{C1} phase, with a layer spacing of 54.0 Å and strong wide-angle peaks at 4.54, 3.87 and 3.50 Å, or a second tilted lamellar crystalline phase L_{C2}, with a smaller layer spacing of 47.2 Å and a very different wide-angle pattern, containing numerous sharp peaks (Figure 3(e)). It has not yet been possible to identify the packing modes from these wide-angle powder patterns, but it has been suggested that monoclinic chain subcells may be involved [9]. On the other hand, Hinz and co-workers suggest that the wide-angle patterns indicate hybrid orthorhombic/triclinic subcell packing modes [17]. What does seem clear from both our data and these earlier results is that the packing within the L_{C1} phase is essentially the same for both the Glc and the Gal compounds. The wide-angle diffraction pattern we observe from the L_{C2} phase appears to be different from any previously reported, possibly indicating the formation of a new lamellar crystalline phase, with a

TABLE 2 The Limiting Hydrations (waters per lipid molecule) of the L_β gel, fluid Lamellar L_α, and Inverse Hexagonal H_{II} Phases

System	L_β	L_α	H_{II}
di-14:0-β-D-GlcDAG	7	13	21
di-14:0-β-D-GalDAG	–	11	19
di-12:0-PE[$]	6	12	16

[$]Reference [4].

different subcell packing, not previously seen. At all hydrations, the lamellar crystal phase melts directly to the H_{II} phase upon heating, generally from the L_{C2} phase at low hydration (at 78.9°C), but from the L_{C1} phase in excess water (at 70.2°C). The lattice parameter of the H_{II} phase in excess water is 62.3 Å.

On the timescale of our experiments, we see no evidence for gel phase formation in the di-14:0–β-D-GalDAG compound. However, previous X-ray experiments did observe a transient L_β lamellar gel phase, which reverted rapidly (less than one hour) to a lamellar crystalline phase [9,17]. The rate of reversion from the gel phase to the more ordered crystalline lamellar phases is known to be faster for β-D-Gal than for the β-D-Glc lipids, and also to depend on the chirality of the glycerol backbone [18]. Generally speaking, the rate is fastest for the 1,2-***sn*** compounds, intermediate for the racemate, and slowest for the 2,3-***sn*** compounds. The explanation for these differences is still unclear, but must lie in the different patterns of headgroup-headgroup and headgroup-water hydrogen bonding.

For hydration levels in excess of 20 wt% water, we observe a fluid lamellar L_α phase, for di-14:0-β-D-GalDAG upon cooling, at 61.5°C, followed by a transition to the lamellar crystal L_{C1} phase at 54.2°C. We infer that not only the L_β gel phase, but also the fluid lamellar L_α phase is metastable for this lipid, although this has not as yet been directly observed.

By plotting the lattice parameters as a function of water content (data not shown), we have determined the limiting hydration of each phase. We find the values given in Table 2 for the L_β gel, L_α fluid bilayer, and H_{II} phases (these are nearly independent of temperature within each phase).

The limiting hydration in the gel phase is similar to the hydration numbers of 8.4 and 8.7 for free glucose and free galactose in solution [19]. Our results are in quite good agreement with the hydration values for dialkyl glycolipids inferred indirectly by Hinz and co-workers [17], and demonstrate that the limiting hydrations of the three phases for the glycolipid systems are rather similar to those established earlier for the same phases of the phospholipid system didodecyl phosphatidylethanolamine (di-12:0-PE) [4]. It has been established that, as in the case of PE,

the lamellar crystalline phases of the glycolipids are essentially anhydrous (less than one water per lipid [17]).

The binary temperature-composition phase diagrams of these two synthetic glycolipid systems will be published at a later date.

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