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The effects of ethylene glycol and dimethyl sulfoxide on cerebroside metastability

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Aqueous dispersions of *n*-acyl cerebrosides are known to exhibit metastable polymorphism of the type:

Liquid crystal
↙ ↖
metastable polymorph I → stable polymorph II

The involvement of hydration in this metastable polymorphism has been investigated by differential scanning calorimetric studies of aqueous palmitoylgalactocerebroside (C16:0-CER) dispersions in the presence of agents which disrupt water structure. In the presence of 50 vol% ethylene glycol or 50 vol% dimethyl sulfoxide, only a single reversible ordered → liquid-crystalline transition is observed. This single ordered → liquid-crystalline transition exhibits a smaller enthalpy and occurs at a lower temperature than the major Polymorph II → liquid-crystal transition observed for dispersions in water alone. These results indicate that metastable polymorphism in C16:0-CER is related to hydration.

Introduction

Cerebrosides are polar membrane lipids which are composed of a galactosyl or glucosyl headgroup linked to an apolar ceramide. These compounds form bilayers in aqueous suspension, and exhibit phase behavior which differs significantly from that of phospholipids. *n*-Acyl cerebrosides, for instance, exhibit two ordered low-temperature states, one metastable and the other stable [1–5]. Diacylglycerol-based glycolipids of plant or bacterial origin exhibit similar polymorphism [6]. Finally, synthetic sphingomyelins and carbamyloxyphosphatidylcholines, which contain a hydrogen bond donating NH group, also exhibit polymorphic low-temperature states [7,8]. These studies, taken together, indicate that hydrogen-bonding capability is a sufficient condition for the kind of metastable phase behavior observed in sphingolipids.

It has been proposed that metastability in *n*-acyl cerebrosides involves a change in hydration of the glycosyl headgroup [3,4]. In the present communication, evidence is presented which supports this hypothesis.

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; C16:0-CER, palmitoylcerebroside; DSC, differential scanning calorimetry; DMSO, dimethyl sulfoxide; T_M, peak temperature of a thermal transition.

Materials and Methods

DMPC was synthesized by acylation of glycerylphosphorylcholine with an imidazole adduct of myristic acid [9]. DPPC was similarly synthesized, and was converted to POPC according to published procedures [10]. Cerebrosides were extracted from fresh bovine brains by using the procedure of Radin [11]. Alkenyl ether and ester linkages were cleaved by iodinolysis and alkaline methanolysis, respectively [11]. Cerebrosides were isolated by chromatography on diethylaminoethylcellulose according to Rouser et al. [12] (to remove sulfatides) and subsequently on silicic acid. Cerebrosides were eluted from silicic acid with 10% methanol in chloroform. Cerebrosides were cleaved to form psychosine according to Radin [13]. *N*-Palmitoylgalactocerebroside (C16 : 0-CER) was synthesized by reaction of psychosine with palmitoyl chloride in a two-phase system composed of equal volumes of aqueous sodium acetate and methylene chloride, a variation of the method of Radin [13]. After purification, all lipids exhibited only one spot on thin-layer chromatography in $CH_2Cl_2/CH_3OH/H_2O$ (65 : 25 : 4, v/v), with the exception of brain cerebrosides which exhibit two spots corresponding to hydroxy fatty acid-containing and *n*-acyl fatty acid-containing cerebrosides.

DSC was carried out using a Perkin-Elmer DSC-2 calorimeter calibrated with indium. Lipid samples in (2 : 1, v/v) CH_2Cl_2/CH_3OH solution were transferred to Perkin-Elmer DSC pans (50 μl capacity), dried under N_2, and desiccated overnight under vacuum. Samples were hydrated with 30 μl H_2O, 50 vol% ethylene glycol, or 50 vol% DMSO, and the pans were sealed. The lipid concentration was 5–10 wt%.

Results

The calorimetric behavior of C16 : 0-CER in water is presented in Fig. 1a,b. On cooling from the liquid-crystalline state at 5 deg. C/min, a sharp transition A is observed, followed by a smaller broad transition B. A subsequent heating run at 5 deg. C/min exhibits a small exotherm C followed by a large endotherm D. Transition temperatures and enthalpies for these DSC runs are

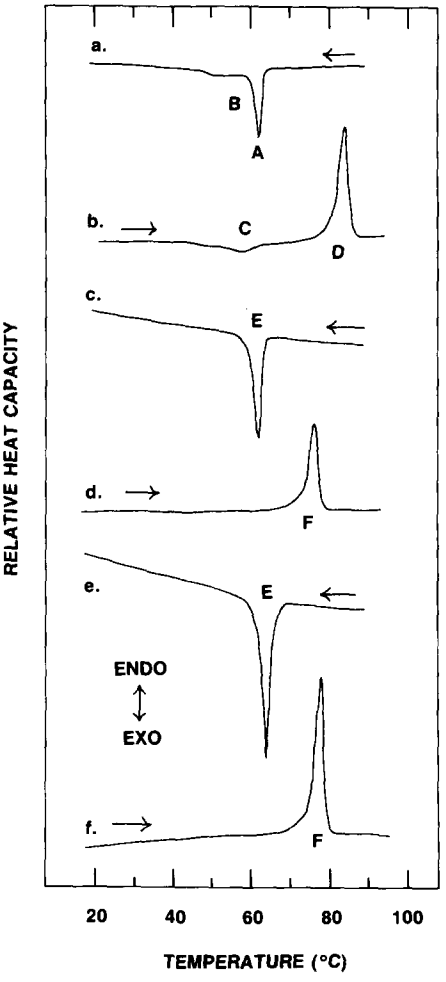

Fig. 1. Differential scanning calorimetry of dispersions of C16 : 0-CER in (a,b) water, (c,d) 50 vol% ethylene glycol, and (e,f) 50 vol% DMSO. a, c, and e are cooling runs; b, d, and f are their respective subsequent heating runs. The cooling/heating rate is 5 deg. C/min.

tabulated in Table I. The multiple transitions on cooling and heating indicate the existence of two low-temperature states, one metastable and one stable. This behavior has been extensively characterized previously [4], and is schematically summarized in Fig. 2a. On cooling from the liquid-crystalline state at 5 deg. C/min (Fig. 1a), transition A to the metastable polymorph I occurs, followed by transition B of the metastable polymorph to stable polymorph II. On subsequent heating (Fig. 1b), an exothermic transition C occurs which consists of a transformation of the remaining metastable material into the stable form.

TABLE I

THERMAL BEHAVIOR OF C16:0-CER IN H_2O, 50% ETHYLENE GLYCOL, AND 50% DMSO

Heating and cooling runs at 5 deg. C/min.

Solvent	T_M (°C)						ΔH (kcal/mol)				
	cool			subsequent heat			cool		subsequent heat		
	A	B	E	C	D	F	A+B	E	C	D	F
H_2O	63	55	–	58	83	–	−10.3	–	−2.7	16.5	–
50% Ethylene glycol	–	–	62	–	–	76	–	−12.6	–	–	13.7
50% DMSO	–	–	64	–	–	78	–	−12.6	–	–	13.2

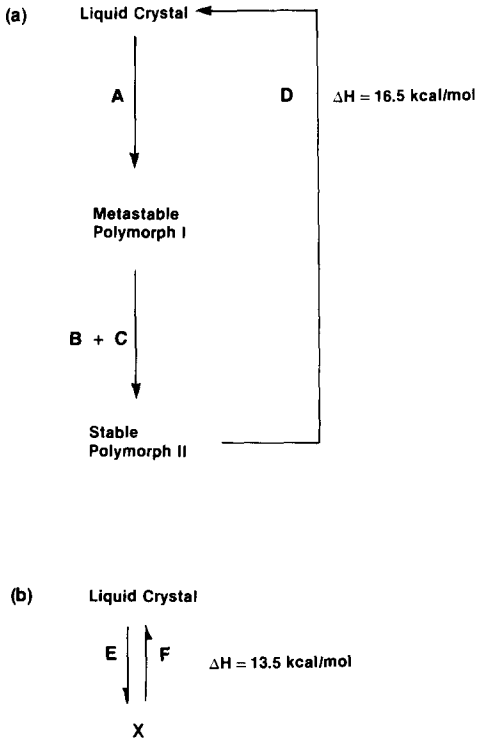

Fig. 2. Schematic representation of the phase behavior of C16:0-CER in (a) water, and (b) 50 vol% ethylene glycol or DMSO. Polymorphs I and II are the same as the A and E forms (respectively) of Ruocco et al. [4].

Continued heating results in transition D from the stable low-temperature polymorph II into the liquid-crystalline state.

C16:0-CER samples hydrated with 50% ethylene glycol or 50% DMSO exhibit thermal behavior which is quite different from that observed in water. In 50% ethylene glycol, C16:0-CER exhibits a single transition on cooling and heating, as shown in Fig. 1c,d. Significant super-cooling occurs, with the cooling exotherm E at 62°C and the heating endotherm F at 76°C. The cooling and heating enthalpies are approximately equal, and are smaller than that observed for the order-disorder transition D when C16:0-CER is heated in water alone (Table I). C16:0-CER samples in 50% DMSO exhibited thermal behavior which is essentially identical to that observed in 50% ethylene glycol (Fig. 1e, f; Table I).

TABLE II

EFFECTS OF ETHYLENE GLYCOL AND DMSO ON THE THERMAL BEHAVIOR OF DMPC AND POPC

Lipid	Solvent	T_M (°C)		ΔH (kcal/mol)	
		pre	main	pre	main
DMPC	H_2O	14.5	24	0.6	5.9
	50% Ethylene glycol	–	24	0	7.4
	50% DMSO	–	31	0	6.8
POPC	H_2O	a	−2	a	4.7
	50% DMSO	a	8	a	5.9

[a] No pretransition is observed for POPC, but this may be due to interference by the ice-water transition.

For comparison, the effects of ethylene glycol and DMSO on the phospholipid DMPC were determined. Hydration of DMPC with 50% ethylene glycol results in elimination of the thermal pretransition, no change in the T_M of the order-disorder transition, and a 25% increase in the enthalpy of the order-disorder transition (Table II). Hydration of DMPC with 50% DMSO resulted in elimination of the pretransition, a 7°C increase in the T_M of the order-disorder transition, and a 15% increase in the enthalpy of the order-disorder transition. The effect of DMSO on the T_M of the DMPC order-disorder transition was unexpected, so the effect of DMSO on POPC was investigated for comparison. In 50% DMSO, theT_M of the order-disorder transition of POPC increases by 10°C, and the enthalpy is 26% larger than in water alone.

Discussion

The observation that ethylene glycol eliminates the thermal pretransition of DMPC verifies a previous report [14]. X-ray and calorimetric studies of the effect of hydration on the DMPC pretransition indicate that this transition from a lamellar gel structure ($L_{\beta'}$) to a rippled lamellar gel structure ($P_{\beta'}$) requires a complete hydration shell (11 H_2O molecules) for the DMPC molecule [14]. The absence of the $L_{\beta'} \rightarrow P_{\beta'}$ transition in the presence of ethylene glycol or DMSO results from disruption of this hydration shell. In the presence of ethylene glycol, the enthalpy of the DMPC order-disorder transition is increased, while T_M is unchanged. These observations are similar to the reported effects of glycerol on DPPC [15]. DMSO increases the transition enthalpy for both DMPC and POPC, but also increases T_M. These observations are consistent with a partial dehydration of the phospholipid, since previous studies have indicated that the order-disorder transition of DMPC increases in temperature and enthalpy at reduced water contents [16]. The observed increases in T_M and ΔH in the presence of ethylene glycol and DMSO indicate stabilization of the phosphatidylcholine gel state. The dehydration induced by these agents may allow stronger ion-pairing between the zwitterionic headgroups of adjacent phosphatidylcholine molecules, with concomitant tighter acyl chain packing.

The major point of the present study is that metastability in C16 : 0-CER appears to be related to cerebroside hydration. Addition of ethylene glycol or DMSO to C16 : 0-CER results in loss of metastable phase behavior. Only one transition is observed on cooling and heating, and the enthalpy on cooling is within experimental error of the enthalpy of the subsequent heating transition. Furthermore, the data are consistent with the proposals that polymorphs I and II are not formed in the presence of ethylene glycol or DMSO, and that the order-disorder transition F is a transition to the liquid-crystalline state from a low-temperature state X which is intermediate in order between polymorphs I and II (Fig. 2b). Four observations support these proposals. In the presence of ethylene glycol or DMSO, (1) the heating exotherm is not observed, (2) the cooling exotherm is a single peak rather than a double peak, (3) the order-disorder transition temperature is lower, and (4) the order-disorder transition enthalpy of transition F (approx. 13.5 kcal/mol) is intermediate between the enthalpies of transition A and transition D. X-ray diffraction studies by Ruocco et al. [4] indirectly suggest that the stable polymorph II is a hydrated structure with a highly ordered hydrocarbon chain packing arrangement, while metastable polymorph I is a less hydrated species *. The present work with ethylene glycol and DMSO does not indicate which of the two low-temperature polymorphs is less hydrated.

Thus, low-temperature metastable polymorphism in 16 : 0-CER is a hydration-related phenomenon. Metastable polymorphism in natural *n*-acyl cerebrosides must have the same origin. We have proposed that 2-hydroxy fatty acids in myelin cerebrosides have the function of altering this metastable polymorphism, thus avoiding the possibility of a disruptive hydration-dehydration cycle in the cerebroside-rich multilamellar myelin membrane [5].

* Polymorphs I and II in the present study correspond to the metastable A form and the stable E form, respectively, of Ruocco et al. [4].

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