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Phase Behavior of Galactocerebrosides from Bovine Brain†

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ABSTRACT: Bovine brain cerebrosides (BOV-CER) were separated by high-performance liquid chromatography into cerebroside fractions with a single acyl chain type or with a relatively homogeneous acyl chain distribution. The thermal behavior of these isolated cerebroside fractions was studied by differential scanning calorimetry. Nonhydroxy (*n*-acyl) fatty acid cerebrosides (NFA-CER) possessing a saturated acyl chain (C16:0, C18:0, C24:0) exhibit their major order–disorder transition temperature T_M at 83 °C, independent of chain length. NFA-CER possessing primarily unsaturated acyl chains (C24:1) exhibits T_M at 70 °C. 2-Hydroxy fatty acid cerebrosides (HFA-CER), which possess a saturated hydroxyacyl chain (C18:0h, C24:0h), exhibit T_M at 70–72 °C. Thus, naturally occurring cerebrosides exhibit high T_M's that do not depend significantly on acyl chain length and that depend only to a small degree on unsaturation and the presence of a 2-hydroxy branch in the amide-linked chain. Isolated NFA-CER's each exhibit metastable polymorphism of the type previously described for unfractionated NFA-CER [Curatolo, W. (1982) *Biochemistry 21*, 1761]. Polymorphism in HFA-CER is complex, with a different type of thermal behavior observed for each isolated acyl chain fraction studied. On prolonged storage at low temperature, unfractionated HFA-CER and unfractionated BOV-CER reach a highly ordered gel state similar to that which is readily reached by NFA-CER's. These results indicate that all cerebrosides exhibit metastable polymorphism. However, the kinetic barriers to reaching the stable gel state are greater for HFA-CER and BOV-CER than for NFA-CER.

Cerebroside, the simplest mammalian glycosphingolipid, is found in large quantities in the myelin membrane and in the brush border membrane of the intestinal wall (Norton, 1975; Hauser et al., 1980; Hansson, 1983). The order–disorder transition temperature (T_M) of bovine brain cerebroside model membranes is extremely high (67 °C) relative to body temperature (Curatolo, 1982), and it is likely that this property provides the basis for a major function: imparting increased order to membranes. In the case of myelin, this would decrease permeability to ions, thus facilitating saltatory conduction. In the case of the brush border, decreased ionic permeability assures proper ion gradients for ion gradient driven pumps for active transport. In addition, some special stabilization of the brush border may be necessary to prevent dissolution by bile salts. The involvement of interlipid hydrogen bonding in this membrane stabilization by sphingolipids has been emphasized by Pascher (1976). While more complex glycolipids probably

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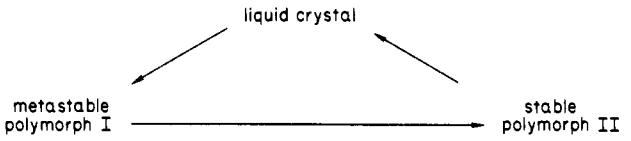
function as cell surface receptors, it is unlikely that simple cerebrosides serve such a function, since the monoglycosyl head group is located too close to the membrane surface to be recognized by carbohydrate-binding proteins (Curatolo et al., 1978).

Bovine brain cerebrosides (BOV-CER)[1] can be fractionated by thin-layer chromatography into two general classes: those that contain amide-linked 2-hydroxy fatty acyl groups (HFA-CER) and those that contain non-hydroxylated fatty acyl (or *n*-acyl) groups (NFA-CER). The NFA-CER and HFA-CER fractions exhibit quite different physical properties. Differential scanning calorimetry (DSC) studies have shown that NFA-CER liposomes exhibit a low-enthalpy exotherm on heating, followed by a high-enthalpy endothermic acyl chain order–disorder transition (Curatolo, 1982). Similar behavior has been observed for liposomes composed of synthetic palmitoylcerebroside or glucocerebroside from Gaucher's spleen (Freire et al., 1980; Ruocco et al., 1981). This behavior can be schematically summarized as



Ruocco et al. (1981) have demonstrated by X-ray diffraction that stable polymorph II[2] possesses a highly ordered acyl chain packing mode and is more ordered than the gel state of phosphatidylcholines (PC's). We have demonstrated that unfractionated HFA-CER and unfractionated BOV-CER do not exhibit this type of metastability when subjected to the same thermal protocol as unfractionated NFA-CER (Curatolo, 1982).

In the present work, we have used high-performance liquid chromatography (HPLC) to fractionate bovine NFA-CER and HFA-CER into cerebroside fractions that possess a single acyl chain type or that possess an acyl chain composition simpler than that found in the natural extract. The phase behavior of aqueous dispersions of these various HFA- and NFA-CER's has been studied by differential scanning calorimetry. We have observed that, unlike the whole HFA-CER fraction, certain isolated homogeneous HFA-CER species exhibit polymorphism similar to that observed for NFA-CER's. Furthermore, the whole BOV-CER and HFA-CER fractions have also been observed to form an additional stable low-temperature state after incubation at low temperatures for extended time periods. The kinetic barriers to achieving this ordered state in BOV-CER and HFA-CER are considerably larger than those for NFA-CER.

Materials and Methods

Cerebrosides were extracted from fresh bovine brains by using the procedure of Radin (1976). Alkenyl ether and ester linkages were cleaved by iodinolysis and alkaline methanolysis, respectively (Radin, 1976). Cerebrosides were then isolated by chromatography on diethylaminoethylcellulose according to Rouser et al. (1976) to remove sulfatides and subsequently on silicic acid. Cerebrosides were eluted from silicic acid with ~10% methanol in chloroform. NFA-CER and HFA-CER were separated by preparative thin-layer chromatography on silica plates with $CHCl_3/CH_3OH/H_2O$ (65:25:4) as solvent (Kishimoto, 1978). NFA-CER and HFA-CER were further fractionated by HPLC, according to Koul & Jungalwala (1981). Fatty acid analyses of the purified fractions are presented in Table I. The C18:0-NFA-CER and C18:0-HFA-CER are chromatographically pure. It was not possible to isolate other fractions with a single acyl chain type. However, repeated HPLC purification resulted in fractions that are significantly enriched in certain acyl chain species. The C24:0/C26:1-NFA-CER fraction consists primarily of 76% C24:0 and 21% C26:1; the C24:1/C22:0-NFA-CER fraction consists primarily of 73% C24:1 and 23% C22:0. The C24:0-HFA-CER fraction is almost pure (86% C24:0), while the C22:0/C24:1-HFA-CER fraction consists of 63% C22:0 and 37% C24:1. The sphingosine composition of bovine brain cerebroside is as follows: 88.5% C18-sphingenine, 8.5% C18-sphinganine (i.e., dihydrosphingosine), and 3% C20-sphingenine (Jungalwala et al., 1983). The sphingosine composition of the HPLC-purified cerebroside fractions was not determined. Because of the high overall C18-sphingenine content, it is unlikely that any of the purified cerebroside fractions has an unusual sphingosine composition.

Table I: Fatty Acid Composition of HPLC-Purified NFA-CER's and HFA-CER's

(A) NFA-CER

fraction	% of total fatty acids 18:0	22:0	24:0	25:0	24:1	26:1
C18:O-NFA-CER	>99	0	0	0	0	0
C24:O/C26:1-NFA-CER	0	0	75.6	3.4	0	21
C24:1/C22:0-NFA-CER	0	23	0	3.4	73	0

(B) HFA-CER

fraction	% of total fatty acids 18:0h	22:0h	24:0h	24:1h
C18:O-HFA-CER	>99	0	0	0
C22:O/C24:1-HFA-CER	0	63.3	0	36.7
C24:O-HFA-CER	0	4.3	86.2	9.5

BOV-CER were cleaved to form psychosine according to Radin (1972). *N*-palmitoylgalactocerebroside (C16:0-NFA-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 (1972).

Cerebroside samples in 2:1 CH_2Cl_2/CH_3OH were dried under N_2 in Perkin-Elmer DSC samples pans (50-μL capacity), desiccated under a vacuum overnight, hydrated with 30 μL of distilled deionized H_2O, and sealed. The cerebroside concentrations were in the range 2–8 wt %. Scanning calorimetry was carried out on a Perkin-Elmer DSC-2 scanning calorimeter.

Results

NFA-CER. Three isolated NFA-CER fractions were extensively studied by DSC: C18:0-NFA-CER, C24:0/C26:1-NFA-CER, and C24:1/C22:0-NFA-CER, in addition to synthetic C16:0-NFA-CER. In Figure 1, DSC heating runs (5 °C/min) are shown that follow a quench cool (40 °C/min). A number of interesting characteristics are immediately obvious. Each of the NFA-CER's studied exhibits one or more heating exotherms. This behavior is similar to that previously observed for unfractionated NFA-CER (Curatolo, 1982) and is indicative of the existence of two ordered low-temperature states, one metastable (polymorph I) and the other highly

[1] Abbreviations: BOV-CER, bovine brain cerebrosides; HFA-CER, 2-hydroxy fatty acid containing cerebroside; NFA-CER, nonhydroxy (*n*-acyl) fatty acid containing cerebroside; C24:0/C26:1-NFA-CER, a mixture of C24:0-NFA-CER and C26:1-NFA-CER in the ratios presented in Table I; DSC, differential scanning calorimetry; PC, phosphatidylcholine.

[2] Polymorphs I and II in the present study correspond to the metastable A form and the stable E form, respectively, of Ruocco et al. (1981).

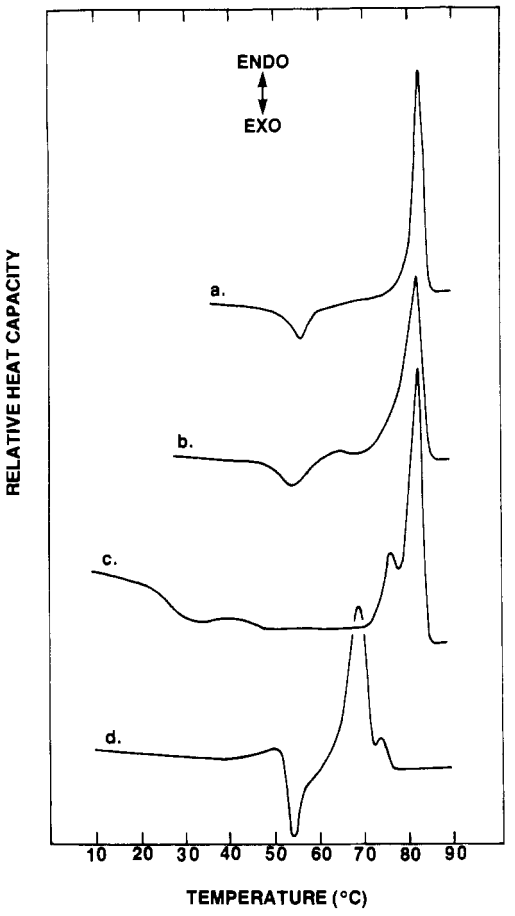


FIGURE 1: Differential scanning calorimetry traces for aqueous dispersions of (a) synthetic C16:0-NFA-CER, (b) isolated C18:0-NFA-CER, (c) isolated C24:0/C26:1-NFA-CER, and (d) isolated C24:1/C22:0-NFA-CER. These are heating runs at 5 °C/min, which immediately followed a quench cool at 40 °C/min.

ordered and stable (polymorph II), as described in the introduction. Observation of the cooling behavior of these NFA-CER's provides further support for this scheme. For example, C18:0-NFA-CER exhibits a single exotherm C at 61 °C (ΔH = −4.9 kcal/mol) on cooling from the liquid-crystalline state at 5 °C/min (Figure 2). A subsequent heating run exhibits a broad exotherm A at ~55 °C (ΔH = −3.6 kcal/mol), followed by a large endotherm B at 83 °C (ΔH = 9.7 kcal/mol). Thus, cooling from the liquid-crystalline state results in transition C to metastable low-temperature polymorph I. On immediate heating, metastable polymorph I undergoes exothermic transition A to a more stable polymorph II, which, on continued heating, undergoes endothermic transition B to the liquid-crystalline state. After prolonged storage at low temperature, a first heating run exhibits no exotherm, and only a single endotherm at 83 °C (ΔH = 11.0 kcal/mol) is observed (not shown). Thus, prolonged low-temperature storage results in conversion of metastable polymorph I to stable polymorph II.

The three primarily saturated NFA-CER fractions (C16:0-NFA-CER, C18:0-NFA-CER, and C24:0/C26:1-NFA-CER) each undergo the acyl chain order–disorder transition at the same temperature, 83 °C (Figure 1). T_M for the primarily unsaturated C24:1/C22:0-NFA-CER is at 70 °C. Table II compares the T_M's of the order–disorder transitions of these NFA-CER's with other appropriate lipids. Mixed acyl phosphatidylcholines exhibit T_M's that increase with increasing length of the *sn*-2 acyl chain. The saturated cerebrosides show no such temperature dependence. The

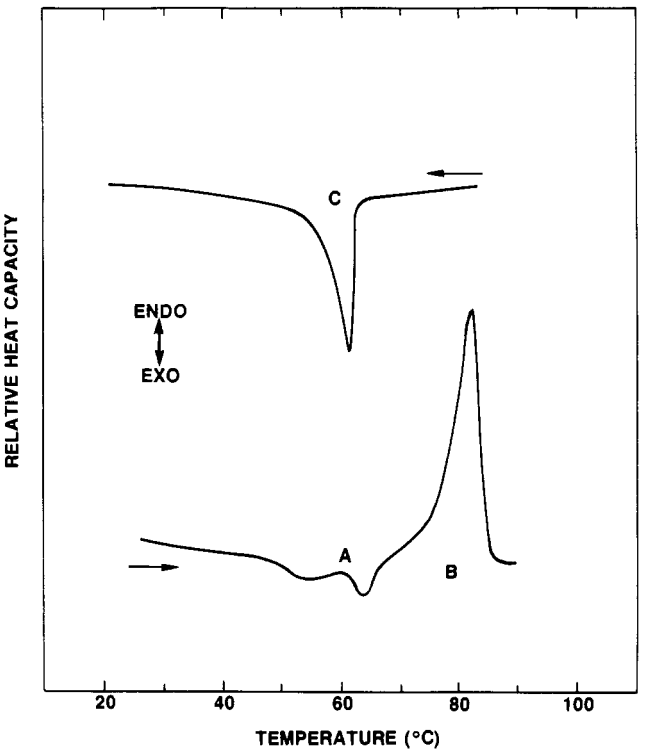


FIGURE 2: Thermal behavior of C18:0-NFA-CER: (upper trace) cooling run at 5 °C/min; (lower trace) subsequent heating run at 5 °C/min.

Table II: Order–Disorder Transition Temperature (T_M) and Enthalpy (ΔH) for NFA-CER's, HFA-CER's, and Selected Mixed Acylphosphatidylcholines

lipid	T_M (°C)	ΔH (kcal/mol)[a]
C16:O-NFA-CER	83	16.6
C18:O-NFA-CER	83	9.9
C24:O/C26:1-NFA-CER	83	13.9
C24:1/C22:O-NFA-CER	70	12.6
C18:O-HFA-CER	70	5.7[a]
C22:O/C24:1-HFA-CER	63	4.8[a]
C24:O-HFA-CER	72	12.4
1-C14:O-2-C14:O-PC[b]	23.9	6.1
1-C14:O-2-C16:O-PC[b]	37	7.3
1-C14:O-2-C18:O-PC[b]	42	8.2
1-C16:O-2-C14:O-PC[c]	27	5.2
1-C16:O-2-C16:O-PC[c]	41	6.4
1-C16:O-2-C18:O-PC[c]	49	8.3
1-C16:O-2-C18:1-PC[d]	−3	4.9

[a] Cerebroside ΔH's are from 5 °C/min heating runs, after a quench (40 °C/min) cool. With this thermal protocol, the reported enthalpies for C18:O-HFA-CER and C22:O/C24:1-HFA-CER are for the transition between the metastable gel state and the liquid-crystalline state. For the other cerebroside fractions, the reported enthalpies are for the transition between the stable gel state and the liquid-crystalline state. For further clarification, see the text and Table III. [b] From Stumpel et al. (1981). [c] From Chen & Sturtevant (1981). [d] Curatolo et al. (1985b).

primarily unsaturated C24:1/C22:0-NFA-CER has a T_M that is only 13 °C lower than that of the saturated species. This small decrease again indicates an insensitivity of T_M to acyl chain composition. By comparison, introduction of a single double bond into the *sn*-2 position of PC can reduce T_M by 52 °C (compare 1-C16:0-2-C18:0-PC and 1-C16:0-2-C18:1-PC in Table II).

T_M for the NFA-CER's is extremely high compared to the phosphatidylcholines, probably due to the highly ordered acyl chain packing of polymorph II and inter-cerebroside hydrogen bonding (see Discussion). The enthalpy (ΔH) of the order–disorder transition is presented in Table II for the various

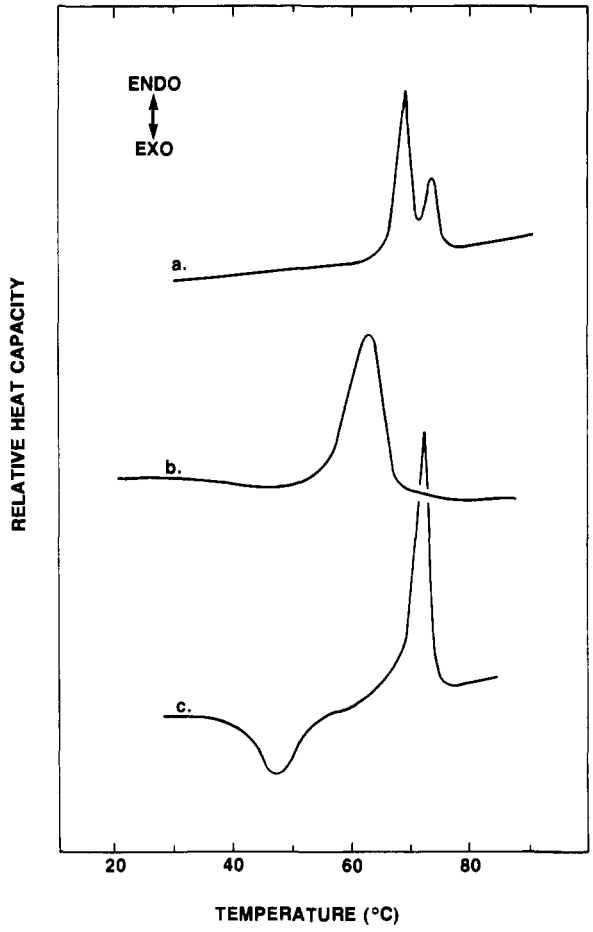


FIGURE 3: Differential scanning calorimetry traces for aqueous dispersions of (a) C18:0-HFA-CER, (b) C22:0/C24:1-HFA-CER, and (c) C24:0-HFA-CER. These are heating runs at 5 °C/min, which immediately followed a quench cool at 40 °C/min.

isolated NFA-CER's. C16:0-NFA-CER exhibits an unusually high enthalpy compared to the other NFA-CER fractions and phosphatidylcholines.

HFA-CER. Three HFA-CER fractions were extensively studied by DSC: C18:0-HFA-CER, C22:0/C24:1-HFA-CER, and C24:0-HFA-CER. In Figure 3, DSC heating runs (5 °C/min) are shown that follow a quench cool (40 °C/min). The major order–disorder transition temperature is similar for these three HFA-CER fractions and is ~10–20 °C lower than that observed for the saturated NFA-CER's (Table II). The C18:0-HFA-CER and C22:0/C24:1-HFA-CER do not exhibit heating exotherms of the type observed for the NFA-CER's, although C18:0-HFA-CER exhibits a double-peaked transition. C24:0-HFA-CER, on the other hand, behaves like the NFA-CER fractions; i.e., it exhibits an irreversible heating exotherm and thus undergoes metastable polymorphism. Unfractionated HFA-CER from bovine brain exhibit a single broad endotherm at 68 °C (Curatolo, 1982). Table II presents enthalpies for the order–disorder transition of the HFA-CER fractions. The C24:0-HFA-CER fraction, which behaves like an NFA-CER, has a relatively high ΔH (12.4 kcal/mol). C18:0-HFA-CER and C22:0/C24:1-HFA-CER exhibit much lower enthalpies.

Long-Term Metastability in HFA-CER's and Unfractionated BOV-CER. Our initial study of the whole NFA-CER and HFA-CER fractions from bovine brain indicated that NFA-CER exhibited metastable polymorphism, while HFA-CER did not (Curatolo, 1982). We concluded that the 2-hydroxyl group in HFA-CER sterically prevented the formation of the highly ordered stable polymorph II observed in

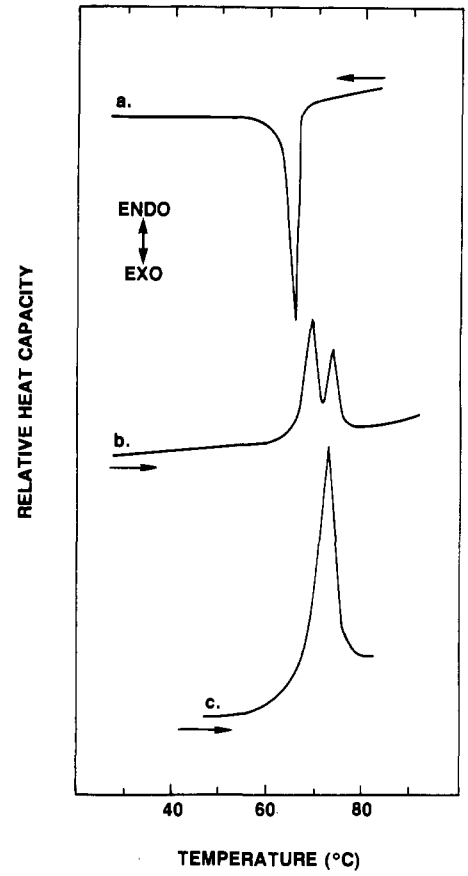


FIGURE 4: Metastability in C18:0-HFA-CER: (a) cooling run at 5 °C/min; (b) subsequent heating run at 5 °C/min; (c) heating run at 5 °C/min after holding for 2 h at 27 °C.

NFA-CER. This conclusion is also consistent with Raman spectroscopic studies that found the acyl chains of HFA-CER in the low-temperature state to be less ordered than those of NFA-CER (Bunow, 1979; Bunow & Levin, 1980). Our more recent observation that C24:0-HFA-CER behaves like NFA-CER's was unexpected and indicates that the presence of a 2-hydroxyacyl group in HFA-CER's does not per se guarantee the absence of metastability. Furthermore, even though C18:0-HFA-CER exhibited no heating exotherm, the observed double-peaked heating endotherm (Figure 3) was suggestive of some metastability. These observations prompted us to look more closely into the behavior of HFA-CER's and unfractionated BOV-CER.

When C18:0-HFA-CER was cooled at 5 °C/min, a single exotherm was observed at 66 °C with an enthalpy of −5.3 kcal/mol (Figure 4a). A subsequent heating run at 5 °C/min exhibited a double-peaked endotherm with peaks at 70 and 74 °C, with a total enthalpy of 6.1 kcal/mol (Figure 4b). These enthalpies indicate that this transition is essentially reversible on this time scale. When this sample was cooled to 27 °C, held at this temperature for 2 h, and then heated at 5 °C/min, a single heating endotherm was observed at 73 °C with an enthalpy of 11.6 kcal/mol (Figure 4c). This result indicates that the gel state that is reached on cooling at 5 °C/min is in fact metastable and transforms to a more stable state upon prolonged incubation at low temperature.

C18:0-HFA-CER, C22:0/C-24:1-HFA-CER, unfractionated HFA-CER, and unfractionated BOV-CER were further investigated for the presence of metastability as follows. Samples were equilibrated in the calorimeter at 3 °C directly after 3-months uninterrupted storage at −20 °C. On the first heating run (5 °C/min) after prolonged storage, these samples

Table III: Calorimetric Behavior of HFA-CER's and Unfractionated BOV-CER after Long-Term Storage at −20 °C[a]

cerebroside	ΔH (cal/g) first heating run	ΔH (cal/g) second heating run
C18:O-HFA-CER	16.1 (12.0)[b]	8.1 (6.0)
C22:O/C24:1-HFA-CER	13.5 (10.9)	6.6 (5.4)
unfractionated HFA-CER	15.9	7.3
unfractionated BOV-CER	15.8	8.0

[a] See text for details. [b] Values in parentheses are in kcal/mol.

each exhibited one endothermic transition with a surprisingly large ΔH (Table III). Following a 40 °C/min cooling run, the second heating run exhibited typical endotherms for each sample, with enthalpies that were reduced in magnitude by 50% compared with the first run (Table III). These results indicate that each of these fractions exhibits long-term low-temperature metastability.

Discussion

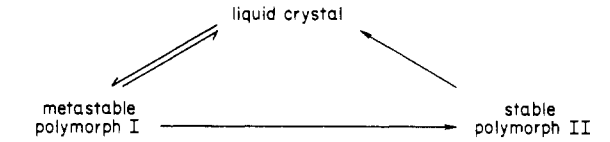
Comparison of the thermal behavior of various cerebroside fractions demonstrates that cerebroside order–disorder transition temperatures are not dominated by the length of the amide-linked acyl chain. The NFA-CER's exhibit no variation in T_M on going from C16:0 to C24:0. The presence of a single double bond in C24:1/C22:0-NFA-CER does not have a large effect on T_M and results in a decrease of only 13 °C compared with the saturated NFA-CER's. With the exception of C16:0-NFA-CER, the NFA-CER's exhibit ΔH's for the order–disorder transition that are similar to those of PC's. For instance, ΔH for C18:0-NFA-CER (9.9 kcal/mol) is similar to that of 1-C18:0-2-C18:0-PC (10.6 kcal/mol) (Maybrey & Sturtevant, 1976). The enthalpies of the two long-chain NFA-CER's (C24:0/C26:1-NFA-CER and C24:1/C22:0-NFA-CER) are comparable to that of 1-C22:0-2-C22:0-PC (14.9 kcal/mol) (Ladbrooke & Chapman, 1969). C16:0-NFA-CER, however, exhibits an order–disorder transition with an exceptionally large ΔH (16.6 kcal/mol). This difference between C16:0-NFA-CER and the longer chain NFA-CER's is not understood at present but may be related to the relative length of the sphingosine and the amide-linked chain in each cerebroside.

The independence of T_M with variation of the acyl chain length is very different from the behavior of phosphatidylcholines, as demonstrated in Table II. Sphingomyelins have recently been shown to possess a dependence of T_M on acyl chain length that is weaker than that of the phosphatidylcholines (Cohen et al., 1984). This may be a property of sphingolipids in general, related to their capacity for interlipid hydrogen bonding. Recent studies of a series of (carbamyloxy)phosphatidylcholines (CMPC) of varying chain length indicate that hydrogen-bonding capability per se does not result in invariance of T_M with acyl chain length. CMPC's, which possess interlipid hydrogen-bonding capability via the carbamyl group, exhibit T_M's that are similar to those of phosphatidylcholines, although the enthalpies are significantly higher (Curatolo et al., 1985a). In the case of cerebrosides, the invariance of T_M with acyl chain length must be a result of lipid–lipid interactions involving the glycosyl head groups.

The most obvious characteristic of the thermal behavior of the NFA-CER's is the occurrence of metastable low-temperature polymorphism. This has been observed previously in unfractionated NFA-CER (Bunow, 1979; Curatolo, 1982), in *n*-acylglucocerebroside from Gaucher's spleen (Freire et al., 1980), and in synthetic C16:0-NFA-CER (Ruocco et al., 1981; Curatolo, 1985). The present work suggests that this metastable polymorphism must result from features of the glycosyl head group and sphingosine backbone, since all the studied NFA-CER's are metastable, regardless of acyl chain length. It is likely that interlipid hydrogen bonding via the glycosyl hydroxyls and backbone amide is responsible for this behavior. This proposition is supported by (1) the observed metastability in certain sphingomyelins, which possess hydrogen-bonding capability via the backbone amide (Barenholz et al., 1976; Estep et al., 1980), and (2) the observed metastability in (carbamyloxy)phosphatidylcholines, which possess hydrogen-bonding capability via the carbamyloxy group (R–NH–COO–) (Curatolo et al., 1982, 1985a).

The isolated HFA-CER fractions exhibit order–disorder transition T_M's at temperatures that are 10–20 °C lower than those of the NFA-CER fractions, presumably due to some disruption of acyl chain packing by the 2-hydroxy group in the low-temperature state. The three isolated HFA-CER fractions studied exhibit different thermal profiles when studied under the thermal protocol of Figure 3. C24:0-HFA-CER behaves similarly to the NFA-CER's, while C18:0-HFA-CER and C22:0/C24:1-HFA-CER exhibit thermal behavior similar to that of unfractionated HFA-CER (Curatolo, 1982). The enthalpies for the order–disorder transition of C18:0-HFA-CER and C22:0/C24:1-HFA-CER are quite low (Table II), as was also observed for unfractionated HFA-CER (Curatolo, 1982). C24:0-HFA-CER, on the other hand, exhibits an order–disorder transition enthalpy that is similar to the enthalpies exhibited by the NFA-CER's (Table II).

A consistent explanation for the behavior of all the studied cerebroside fractions becomes apparent upon consideration of the behavior of HFA-CER's and unfractionated brain cerebrosides[3] after prolonged storage at low temperature (Table III). It is clear that these fractions are also metastable and exhibit a stable low-temperature polymorph that is less kinetically accessible than that of NFA-CER's. The thermal behavior of HFA-CER's (except C24:0-HFA-CER) and unfractionated BOV-CER (HFA plus NFA) can be schematically summarized as



The atypical behavior of C24:0-HFA-CER can be explained by considering the 2-hydroxy group of HFA-CER's to cause disruption of the tight acyl chain packing of stable polymorph II, as has been demonstrated by Raman spectroscopy for unfractionated HFA-CER (Bunow, 1979; Bunow & Levin, 1980). In the case of C24:0-HFA-CER, the exceptionally long saturated chain may provide enough chain–chain contacts to overcome the disruptive effect of the 2-hydroxy group, thus decreasing the kinetic barrier to reaching stable polymorph II.

In summary, all pure cerebrosides studied exhibited metastable polymorphism in the gel state. The X-ray diffraction studies of Ruocco et al. (1981) on C16:0-NFA-CER have suggested that this metastability is associated with changes in hydration of the glycosyl head group. The present work demonstrates that the presence of a 2-hydroxy group in the amide-linked acyl chain can result in a significant kinetic

[3] HFA-CER is the predominant fraction (~60%) of whole brain cerebroside (O'Brien & Rouser, 1964) and apparently dominates the behavior of the natural mixture.

barrier to transformation into the stable polymorph II. As we have previously suggested, a function of 2-hydroxy fatty acids in cerebrosides may be to prevent a membrane-destructive hydration–dehydration cycle associated with metastability, particularly in the multilamellar myelin membrane, which possesses a high cerebroside content (Curatolo, 1982).

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Covalent Complexes Formed between Plasma Gelsolin and Actin with a Zero-Length Cross-Linking Compound†

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ABSTRACT: Actin and plasma gelsolin were covalently cross-linked with the zero-length cross-linker 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide. Two major intermolecularly linked products were identified on polyacrylamide gels. By use of ^{14}C-labeled actin and ^{125}I-labeled gelsolin, these were shown to be the 1:1 and 2:1 complexes of actin with gelsolin, respectively. The higher molecular weight complex predominated under all conditions tested including the presence and absence of Ca^{2+}. In titration experiments in which actin at different concentrations was reacted with a fixed concentration of gelsolin, end points were obtained for the formation of both cross-linked species at about two actins per gelsolin, implying that a 2:1 noncovalent complex is cross-linked. In 0.1 mM Ca^{2+}, the extent of cross-linking was independent of protein concentration down to 50 nM gelsolin. At low Ca^{2+} concentrations ($<10^{-8}$ M), the extent of cross-linking was very much reduced at micromolar gelsolin and fell to zero at about 100 nM gelsolin. The binding of actin to gelsolin to give a cross-linkable complex is therefore very strong at 0.1 mM Ca^{2+} but much weaker at low Ca^{2+} concentrations.

Control of the motile behavior of cells is dependent on the regulation of actin filament assembly and organization. A protein which is likely to be an important modulator of actin behavior in vivo is gelsolin, which solvates actin gels in the presence of Ca^{2+} (Yin & Stossel, 1979). It has been identified in a wide variety of cells and tissues (Yin et al., 1981a; Snabes et al., 1983) and is also found extracellularly, in plasma. The plasma and cytoplasmic forms of gelsolin are distinct but closely related polypeptides (Yin et al., 1984). Studies on the

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