Biochimica et Biophysica Acta 859 (1986) 266–270
Elsevier

BBA 70333

BBA Report

An infrared spectroscopic study of metastable and stable forms of hydrated cerebroside bilayers

David C. Lee, Israel R. Miller * and Dennis Chapman **

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine (University of London), Rowland Hill Street, London, NW3 2PF (U.K.)

(Received February 7th, 1986)

Key words: Cerebroside; Bilayer; Hydrogen bonding; Phase transition; Fourier transform; Infrared spectroscopy

Fourier transform infrared spectroscopy has been used to study the stable and metastable forms of a range of cerebrosides in aqueous systems. The spectra provide evidence for different degrees of inter- and intra-molecular hydrogen bonding, involving principally the amide group, in these different states. A comparison has been made with the spectra of a cerebroside containing an α-hydroxyl group in the fatty acyl chain. This cerebroside does not show metastability and its hydrogen bonding characteristics are shown to be different.

In aqueous dispersion, cerebrosides form lamellar arrays which undergo complex phase transitions as determined by differential scanning calorimetry (DSC) upon heating or cooling. Cerebrosides containing saturated or predominantly saturated (as in glucocerebrosides from Gaucher's patient spleen) acyl chains which are void of α-hydroxy residues give metastable states upon fast cooling from the liquid-crystalline state [1–4]. During the reheating scan the metastable state transforms into the stable one, producing an exothermic peak at approx. 65°C, depending on the scan rate, for Gaucher's, palmitoyl or stearoyl cerebroside [2] and for the type II fraction of brain cerebrosides in which the acyl chains do not contain α-hydroxy groups [4]. This behaviour is primarily due to head group-head group interactions and is accompanied by hydration-dehydration processes which cause further changes in hydrocarbon chain packing. X-ray diffraction and DSC measurements have shown that this results in two forms in the rigid crystalline state: a stable tightly packed form and looser metastable form. The stable form has, as expected, a higher transition enthalpy into the liquid-crystalline state than the looser metastable form.

Pascher and co-workers carried out infrared spectroscopy and X-ray investigations on crystalline *N*-acyl ceramides [5] and cerebroside [6] in the absence of water. They concluded that, in the latter, the sugar groups are bent almost perpendicular to the hydrocarbon chains. This conformation involved extensive lateral hydrogen bonding between the amide groups and neighbouring sugar headgroups.

Skarjune and Oldfield [7,8] have obtained ^{2}H-NMR spectra of hydrated cerebrosides which were labelled in the carbohydrate or acyl chain moieties. They found that the carbohydrate residue is mobile with a small order parameter and projects directly into the aqueous phase. The results indicated that there was little interaction between neighbouring sugar residues thereby permitting maximum hydration of the sugar hydroxyls by bulk water molecules [8].

In the hydrated state, intermolecular hydrogen

* Permanent address: Membrane Research Department, The Weizmann Institute of Science, Rehovot, Israel.
** To whom correspondence should be addressed.

bonding is likely to involve the amide groups located between the fatty acyl chain and the sphingosine base together with the adjacent hydroxyls of the ceramide grouping which are screened from the aqueous environment. It has been suggested that the failure to form intermolecular hydrogen bonds between headgroups which remain out of register on rapid cooling from the liquid-crystalline state may be the origin of metastability [2]. Lateral, inter-molecular hydrogen bonding may be important in increasing membrane stability, promoting close packing of the hydrocarbon chains and creating a barrier to both unpolar and polar molecules in those biological membranes of which cerebrosides form a significant part [5].

Here, we report a Fourier transform infrared spectroscopic study of cerebrosides in aqueous systems which provides evidence for different degrees of hydrogen bonding in the formation of the different states first observed by calorimetric measurements. Infrared band frequencies, intensities and band widths are sensitive to the degree of hydrogen bonding of the vibrating groups [9,10]. We concentrate on effects on the amide I and II bands as a study of the cerebroside O-H and N-H stretching vibrations was precluded by overlap with sugar O-H stretching modes. We illustrate, however, differences in sugar C-O stretching frequencies between the stable and metastable states.

Glucocerebrosides extracted from Gaucher's spleen, *N*-palmitoyl cerebroside, type I and type II galactocerebrosides from bovine brain were each purchased from Sigma (Poole, U.K.). Type I bovine brain cerebroside contains approximately 98% α-hydroxy fatty acids whereas type II contains approximately 98% non-hydroxy fatty acids.

For infrared spectroscopy, cerebrosides were dispersed in distilled water to a concentration of 4–10 mg/ml. In the case of Gaucher's cerebroside, *N*-palmitoyl cerebroside and brain cerebroside type II, the suspensions were heated to 90°–95°C and then quenched in ice to obtain the metastable states. These samples were then incubated at 70°C for 5 min to obtain the stable states. Infrared spectra of bovine brain cerebroside type I were recorded before and after quenching from 70°C; this fraction does not show metastable behaviour [4].

Infrared spectra were recorded using a Perkin-Elmer 1750 Fourier transform spectrometer at 2 cm^{-1} resolution and using a medium Beer-Norton apodisation function. Samples were introduced into a Beckmann FH-01 CFT cell fitted with CaF_2 windows and a 7 μm tin spacer. 64 scans were signal-averaged for each sample while maintaining a temperature of 20°C using a cell jacket of circulating water. Difference spectra were generated by subtraction of the spectrum of water using an interactive difference routine to yield a level baseline. During data acquisition the spectrometer was continuously purged with dry air at a dew point of −40°C and a flow rate of 100 l/min. This was found to be necessary in order to eliminate the spectral contributions of atmospheric water vapour [11].

The calorimetric behaviour of fully hydrated samples of the cerebrosides studied by Fourier transform infrared spectroscopy was investigated using a Perkin-Elmer DSC-2 and was identical to previous studies [2,3].

Fig. 1 (a) presents infrared spectra of glucocerebrosides from Gaucher's spleen in the metastable and stable states after subtraction of the background water absorption. There are two main absorption bands in the spectral region shown, namely, the amide I band (1670 cm^{-1}–1600 cm^{-1}) and the amide II band (1580 cm^{-1}–1500 cm^{-1}). These amide bands are 'in-plane' modes but cannot be described by a single displacement coordinate. However, the amide I band is essentially a C = O stretching vibration weakly coupled with C-N stretching and N-H bending [9]. The amide II band is essentially an N-H bending vibration strongly coupled to C-N stretching [9].

On conversion from the metastable to the stable state there are some notable changes in the infrared spectrum. The shift of the amide I component at 1630 cm^{-1} to 1623 cm^{-1} in the stable state indicates increased hydrogen bonding of the amide C = O group [9,10]. The reduced half-width and increased intensity of both amide I components is also consistent with a more rigid, tightly packed structure. The composite amide I band suggests the existence of two different amide group conformations [1]. The amide II band shifts slightly from 1547 cm^{-1} to 1548 cm^{-1} in the stable state. The formation of a linear C-O---H-N hydrogen

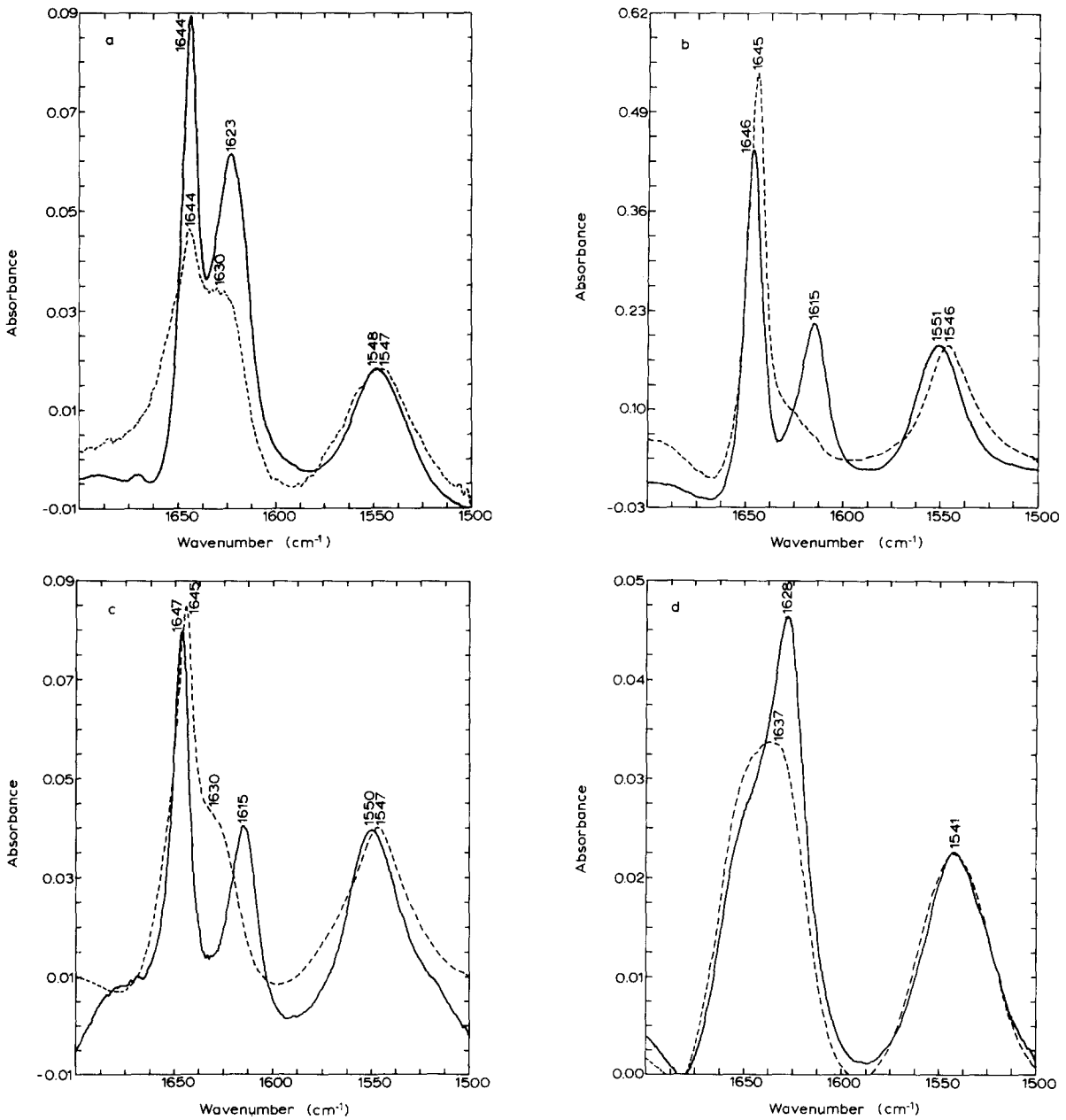

Fig. 1. Fourier transform infrared spectra after subtraction of the background water spectrum of suspensions of (a) glucocerebrosides from Gaucher's spleen, (b) type II galactocerebrosides from bovine brain, (c) *N*-palmitoyl cerebroside, and (d) type I galactocerebrosides from bovine brain in the amide I and II band region recorded at 20°C. Spectra (a) to (c) were recorded after quenching in ice from 90–95°C (broken lines) to produce the metastable states, and after reheating and incubation at 70°C for 5 min (solid lines) to produce the stable states. Spectra in (d) were recorded of a suspension of cerebroside powder as supplied (solid line) and after quenching from 85°C (broken line).

bond is expected to result in an increase in the amide II frequency which is comprised principally of N-H bending vibrations [10].

Infrared spectra in the amide I and II region of an aqueous suspension of the galactocerebrosides (type II extract and palmitoyl cerebroside) after

quenching from 90°C and again after reheating to 70°C and slow cooling are presented in Figs. 1b and 1c. The infrared spectrum of a suspension of type II bovine brain cerebroside which had not been heated (not shown) was almost identical with the spectrum of the metastable state. This is, however, incidental since the conformation in the dry state is dependent on the history of the sample, e.g. the solvent from which it has been dried. In the quenched, metastable samples the amide I band is comprised of two components, a strong band at 1645 cm^{-1} and a shoulder at 1630 cm^{-1}. On formation of the stable state the latter component shifts to an intense and sharp peak at 1615 cm^{-1} indicating that there is increased hydrogen bonding involving the C = O group. This shift is similar to that observed for Gaucher's glucocerebrosides (Fig. 1a) although the shift in the galactocerebrosides is more pronounced. Also the amide II band in the spectra of palmitoyl and type II galactocerebrosides shifts to higher frequency on formation of the stable state than in glucocerebrosides. This suggests the formation of linear C = O---H-N hydrogen bonds in the stable state.

Difference infrared spectra in the C-O stretching region of type II galactocerebroside and glucocerebrosides are presented in Figs. 2a and 2b, respectively. In the case of the type II fraction there are some notable band shifts. C-O stretching modes at 1143 cm^{-1} and 1128 cm^{-1} do not vary but those at 1072 cm^{-1}, 1053 cm^{-1} and 1023 cm^{-1} in the metastable state occur at 1064 cm^{-1}, 1045 cm^{-1} and 1018 cm^{-1}, respectively, in the stable state. This suggests that a number of sugar oxygen atoms and/or the hydroxyl group at C3 of the sphingosine base are involved in formation of the stable state as hydrogen bond donors. Assignment of these absorption bands to particular C-O groups is difficult and would require a series of isotopic substitutions. The band at 1023–1018 cm^{-1} is absent from the spectrum of the glucocerebrosides and may be related to the change of -OH orientation between the gluco- and galacto-moieties. In addition there are much smaller band shifts between metastable and stable states of the glucocerebrosides which suggests a comparatively smaller involvement of hydrogen bonding in stable state formation in glucocerebrosides.

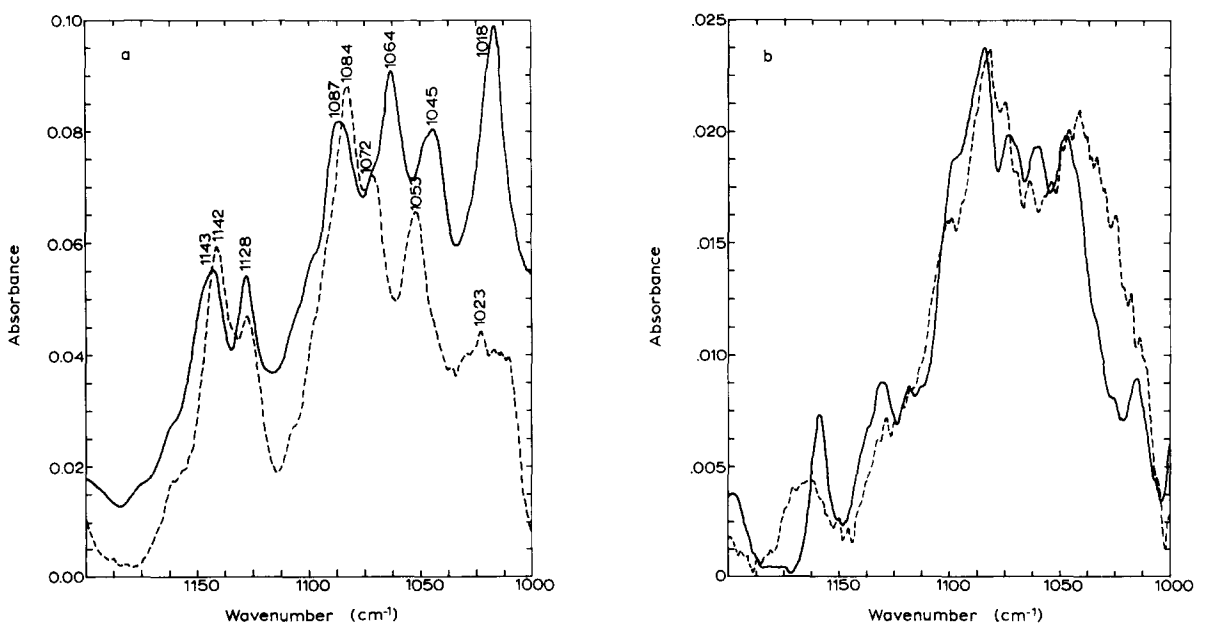

Fig. 2. Fourier transform infrared spectra after subtraction of background absorption of suspensions of (a) type II galactocerebrosides from bovine brain and (b) glucocerebrosides from Gaucher's spleen in the C-O stretching region recorded at 20°C. Metastable (broken lines) and stable states were produced as in Fig. 1.

Difference infrared spectra were also obtained of the type I fraction of bovine brain galactocerebrosides which contains an α-OH group in the fatty acyl chain. This fraction does not form a metastable state on fast cooling from the liquid-crystalline state [4]. The broad amide I band has a maximum at 1637 cm^{-1} with a shoulder at 1650 cm^{-1} (Fig. 1d) in contrast to the stable forms of the other galactocerebrosides which suggests that the C = O group is less involved in inter-molecular hydrogen bonding. However the amide II band appears at 1541 cm^{-1}, much lower in frequency than in the spectra of the other cerebrosides studied in this work. This may reflect the formation of bent intramolecular hydrogen bonds between N-H and the adjacent α-OH of the fatty acyl chain. To interpret this phenomenon we have to bear in mind that the α-hydroxy group in the acyl residue draws electrons from the adjacent carbonyl of the amide group. Thus it weakens the tendency of the carbonyl to bind hydrogen and it becomes itself a strong competitor for internal hydrogen bonding with the amine. Consequently the amine is strongly hydrogen bonded and the NH bending frequency of amide II decreases while the amide I band remains characteristic of the weakly hydrogen bonded carbonyl. The broad amide I band in the quenched sample supports the suggestion that the hydrogen bonding of the type I galactocerebroside is different from the non-α-hydroxy galactocerebroside in the stable state. The presence of the α-hydroxy group, to some extent, reduces intermolecular interactions in favour of intramolecular hydrogen bonding to give a less tightly packed structure. This is consistent with the reduced enthalpy for the gel to liquid-crystalline phase transition of α-hydroxy fatty acid-containing cerebroside compared to that of the non-α-hydroxy type observed by Curatolo [4].

In conclusion, we have found a series of band shifts in the amide I, amide II and C-O stretching band regions of the Fourier transform infrared spectra of aqueous dispersions of a number of cerebrosides. These shifts indicate the presence of increased hydrogen bonding in the stable compared to the metastable states. Conversion from the metastable to the stable state is known to involve rehydration of the lipid headgroups [3] and this may be responsible for at least some of the band shifts observed in the C-O region. The ordering of the hydrocarbon chains in the stable state [3] is likely to involve inter-molecular hydrogen bonding at the amide group which is consistent with the band shifts observed in the amide I and II region.

We would like to acknowledge the financial support of the Wellcome Trust and the Humane Research Trust.

References

1 Bunow, M.R. and Levin, I.W. (1980) Biophys. J. 32, 1007–1021
2 Freire, E., Back, D., Correa-Freire, M., Miller, I.R. and Barenholz, Y. (1980) Biochemistry 19, 3662–3664
3 Ruocco, M.J.,Atkinson, D., Small, D.M., Skarjune, R., Oldfield, E. and Shipley, G.G: (1981) Biochemistry 20, 5957–5966
4 Curatolo, W. (1982) Biochemistry 21, 1761–1764
5 Pascher, I. (1976) Biochim. Biophys. Acta 455, 433–451
6 Pascher, I. and Sundell, S. (1977) Chem. Phys. Lipids 20, 175–191
7 Skarjune, R. and Oldfield, E. (1979) Biochim. Biphys. Acta 556, 208–218
8 Skarjune, R. and Oldfield, E. (1982) Biochemistry 21, 3154–3160
9 Pimentel, G.C. and McClellan, A.L. (1960) The Hydrogen Bond, pp. 67–141, Freeman, San Francisco
10 Susi, H. (1969) in Structure and Stability of Biological Macromolecules, (Timasheff, S.N. and Fasman, G.D., eds.), pp. 573–663, Marcell Dekker, New York
11 Lee, D.C., Hayward, J.A., Restall, C.J. and Chapman, D. (1985) Biochemistry 24, 4364–4373