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HYDRATION OF *N*-PALMITOYLGALACTOSYLSPHINGOSINE COMPARED TO MONOSACCHARIDE HYDRATION

MARTIN J. RUOCCO * and G. GRAHAM SHIPLEY

Biophysics Institute, Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, MA 02118 (U.S.A.)

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Differential scanning calorimetry (DSC) studies of the ice-water transition of *N*-palmitoylgalactosylsphingosine (NPGS) (cerebroside)/water mixtures indicate 4 ± 1 non-freezable water molecules per molecule NPGS. This hydration level, representing strongly bound water, is identical to that observed previously for human glucocerebroside (Bach, D., Sela, B. and Miller, I.R. (1982) Chem. Phys. Lipids 31, 381). Comparison of gluco- and galacto-cerebroside hydration with hydration measurements on simple monosaccharides suggests a favored orientation of the glycosyl polar group at the cerebroside-water interface.

Studies to quantitate lipid hydration have been primarily concerned with phospholipids. Elworthy [1] originally used the vapor adsorption isotherm technique to quantitate phosphatidylcholine hydration. Chapman et al. [2] and Lundberg et al. [3] used differential scanning calorimetry (DSC) to quantitate the non-freezable bound water. This approach indicated hydration numbers at 0°C of 10, approx. 9, approx. 9 for gel state dipalmitoylphosphatidylcholine (DPPC), liquid-crystal state egg- and dioleoylphosphatidylcholine bilayers, respectively. Recently, Ter-Minassian-Saraga and Madelmont [4] used a modified calorimetric approach to quantitate the non-freezable, bound water to DPPC and to demonstrate the relevance of the ice-water transitions to the surface forces emerging from the lipid bilayers and affecting the interbilayer water.

Using a calorimetric approach, Bach et al. [5] have recently determined the hydration for a number of glycosphingolipids, including human Gaucher's glucocerebroside and bovine brain galactocerebroside. Our own studies characterizing the structure and thermotropic behavior of synthetic cerebroside, *N*-palmitoylgalactosylsphingosine (NPGS) [6,7] have included a hydration study of NPGS. In this communication we compare these hydration data of NPGS with those obtained by Bach et al. for natural cerebrosides. The hydration of cerebrosides is then discussed in terms of simple monosaccharide hydration.

N-Palmitoylgalactosylsphingosine was synthesized from pig brain cerebroside according to methods described by Skarjune and Oldfield [8]. It is estimated that NPGS contains approximately 5% of the dihydrosphingosine derivative. The purity was checked by thin-layer chromatography (TLC). The NPGS gave a single spot in the solvent system, chloroform/methanol/water/acetic acid (65 : 25 : 4 : 1, v/v) and was used without further purification. Certain batches of NPGS containing fatty acid, sphingosine and other impurities were purified by silicic acid column chromatography and the final lipid product recrystallized from chloroform/methanol (2 : 1, v/v). The purified

* Present address: Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

Abbreviation: NPGS, *N*-palmitoylgalactosylsphingosine.

lipid gave a single spot by TLC.

Hydrated samples were prepared by weighing NPGS and doubly distilled water into stainless steel pans and hermetically sealing the pans. Samples were initially equilibrated at $t > 82°C$ (t_c (NPGS)) in the calorimeter. Subsequent to this equilibration the NPGS samples were annealed into the stable hydrated bilayer phase (crystal E form) as described in Ref. 6. Heating and cooling scans over the temperature range $-20 \rightarrow +90°C$ were performed on a Perkin-Elmer (Norwalk, Connecticut) DSC-2 calorimeter calibrated with gallium. In all cases, multiple scans were performed. The thermograms presented in Fig. 1 are representative of the reproducibly observed calorimetric behavior. The scanning rate was 5 (deg/min). Enthalpy measurements were determined from the area under the transition peak by comparison with those for a known standard (gallium). Baselines in the region of the ice-water transition were determined by extrapolating the pre-transition baseline to the post-transition baseline.

Calorimetric heating curves of the ice-water transition for a range of NPGS/water compositions are presented in Fig. 1. Samples containing excess water, for example, 64.2 wt.% (Fig. 1A) and 49.1 wt.% water (Fig. 1B), exhibit a slightly asymmetric ice-water transition at approx. 0°C with a shoulder on the low temperature side of the transition. At lower water contents, for example 38.1 wt.% water (Fig. 1C), this low temperature shoulder is more pronounced and considerable tailing on the low temperature side of the ice-water transition is also observed. The tailing phenomenon increases at lower hydrations (Figs. 1D and E) and the low temperature shoulder in Fig. 1C develops into a defined peak which progressively decreases in temperature to approx. −2°C at 11.2% water (Fig. 1E). Note that the higher temperature peak of the original ice-water transition has diminished and the melting profile is now dominated by the broad peak with a maximum at approx. −2°C.

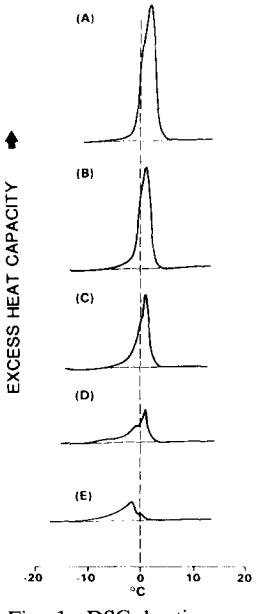

Fig. 1. DSC heating curves of the ice-water transition of NPGS/water mixtures. (A) 64.2 wt.% water; (B) 49.1 wt.% water; (C) 38.1 wt.% water; (D) 22.9 wt.% water; (E) 11.2 wt.% water. Heating rate 5 C deg/min.

Assuming that water tightly associated with NPGS does not contribute to the enthalpy of the ice-water transition at approx. 0°C, the 'bound' water can be quantitated. The enthalpy data for the ice-water transition derived from these heating curves are plotted in two ways. In Fig. 2A the transition enthalpy for the ice-water transition was calculated on a calorie per gram total material basis (see Ref. 2). The heavy dashed line gives the expected enthalpy assuming two non-interacting lipid and water components. The experimental data show a deviation from this theoretical line. Linear regression analyses of all the experimental data points (thin dashed line) or all data points excluding the 100 wt.% water (0 wt.% lipid) experimental point (solid line) give intercepts corresponding to 11.2 and 9.3 wt.% water, respectively. These data translate into five and four bound water molecules per molecule of NPGS. These water molecules are apparently not undergoing the bulk ice-water transition and presumably represent water 'tightly' bound to cerebroside at 0°C.

Alternatively the enthalpy data can be calculated on a kcal/mol NPGS basis as in Refs. 3 and 5. Plotting the enthalpy data as a function of mol H_2O/mol NPGS ratio indicates approx. three molecules of 'bound' water per NPGS molecule (Fig. 2B). The slope of this plot should give the enthalpy of the ice-water transition. However, the experimental slope is approx. 12% lower than the actual enthalpy of the ice-water transition (1.44

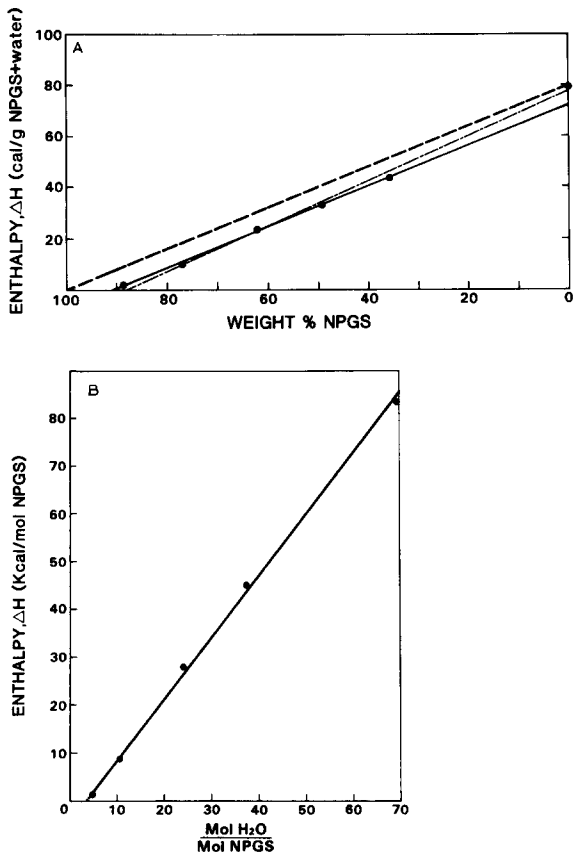

Fig. 2. Enthalpy (ΔH) of the ice-water transition of NPGS/water mixtures expressed as: (A) ΔH (cal/g total material) versus weight% NPGS. (- - - - - -) expected line for two non-interacting NPGS and water components; (·—·—·) linear regression line using all data points; (———) linear regression line using all data points except that at 0 wt.% lipid. (B) ΔH (kcal/mol NPGS) versus mol H_2O/mol NPGS. In both (A) and (B) the plotted data points represent the mean value of duplicate or triplicate experiments and the errors are contained within the plotted points.

kcal/mol). Bach et al. [5], originally reported a lower (approx. 10%) value for the ice-water transition enthalpy using the corresponding slopes of their hydration data for cerebrosides and gangliosides. They also point out a similar decrease in the slope of the hydration data of Lundberg et al. [3], for phosphatidylcholine. Interestingly if the data plotted in Fig. 2A are extrapolated by linear regression to 100 wt.% water using data points from all lipid/water compositions except pure water, an enthalpy value for the ice-water transition of 72 cal/g water is obtained, again, 10% less than the true value (see solid line in Fig. 2A).

The calorimetric results indicate that there are three to five unfreezable water molecules at the ice-water transition of NPGS/water dispersions. These water molecules are presumed to be associated with the polar region of *N*-palmitoylgalactosylsphingosine. Using a similar calorimetric approach, Bach et al. [5], have determined approximately four molecules of unfreezable water for glucocerebroside from Gaucher's spleen and eight to nine unfreezable water molecules for bovine brain galactocerebroside. Thus, NPGS, which has a single saturated fatty acid species, apparently hydrates less than bovine brain galactocerebroside which contains a significant proportion (approx. 40 mol%) of the unsaturated nervonic acid, $C_{24:1}$ [7]. Glucocerebroside, which contains approx. 90% saturated fatty acid species [9,10] gives a lower hydration value which is similar to that of NPGS. The structural characteristics of the lipid phase are strongly influenced by the nature of the fatty acid species linked to the molecule and, as proposed by Bach et al. [5], it seems reasonable that cerebroside hydration would be dependent upon the degree of hydrocarbon chain unsaturation and the molecular surface area. It should also be noted that isolated bovine brain cerebroside fractions contain significant amounts of α-hydroxy fatty acids. Their presence alters the thermotropic behavior of cerebrosides (see Ref. 11) perhaps as a result of different cerebroside hydration properties.

Although there is an apparent hydration dependence upon fatty acid unsaturation for cerebrosides, in contrast, no dependence was reported for phospholipids by Lundberg et al. [3]. This difference may possibly be explained in molecular terms if the structure and dynamics of cerebroside bilayers are considered. NPGS-water dispersions which have been shown to undergo complex polymorphic phase transitions can be annealed into a stable, hydrated bilayer form, crystal E form, (see Ref. 6). For the stable hydrated crystal E form of NPGS containing approx. four molecules of tightly bound water (see above), calorimetric [6], X-ray diffraction [6], and ^{2}H-NMR [8] data indicate that this phase exhibits a high temperature ($t_c = 82°C$), high enthalpy (17.5 kcal/mol NPGS) transition to a bilayer liquid crystal L_α phase.

Based on crystallographic [12], X-ray powder

diffraction [7] and ^{2}H-NMR [8] the unusually ordered hydrated 'crystal' bilayer is thought to be stabilized by both a highly ordered chain packing mode and a lateral intermolecular hydrogen bonding network involving the sphingosine backbone, galactosyl group and interbilayer water molecules. Cerebrosides have a large number of potential hydrogen bond donor groups (4 hydroxyls/sugar residue; 1 hydroxyl/sphingosine base and one amide group). These hydroxyl and amide groups can readily act as hydrogen bond vectors which allow specific intra- and intermolecular contacts. These specific and directional hydrogen bonds, would probably be sensitive to surface area changes. In cerebrosides containing saturated *N*-acyl groups, regular chain packing may allow an appropriate surface area for effective intermolecular hydrogen bonding. In contrast, cerebrosides containing unsaturated fatty acids pack less regularly yielding a larger surface area in which fewer direct intermolecular (H-bonding) contacts can occur. In such cases hydrogen bonded water ligands may replace lipid-lipid contacts yielding a cerebroside bilayer with a greater number of tightly bound water molecules.

Quantitation of the water of hydration for dilute monosaccharide solutions has been performed using compressibility [13], dielectric [14], NMR [15,17] and viscometric [16] methods and, in general, variation in the hydration numbers has resulted depending upon the method used. Early studies by Shiio [13] indicated approximately 0.5 to 0.9 molecules H_2O per hydroxyl group at 25°C for various pentoses and hexoses. For example, 3.5 water molecules were calculated to bind to glucose. Later studies by Franks et al. [14] showed that six water molecules hydrated glucose at 5°C. Extrapolation of viscometric and hydrodynamic data obtained by Ihnat et al. [16] to 0°C gives hydration numbers of approx. 4 and 5, respectively. In contrast, others have determined hydration numbers as large as 10 at 0°C for glucose (i.e., 2 H_2O per hydroxyl group) [15]. Although no hydration number for galactose has been reported, ^{17}O-NMR linewidth data for both glucose and galactose are indistinguishable suggesting that these sugars will have similar hydration numbers [17].

The number of 'bound', unfreezable water molecules for NPGS (4 ± 1), glucocerebrosides (4) and bovine brain cerebroside (8–9) at 0°C fall in the range of hydration numbers for monosaccharides (4–10 H_2O/hexose). Modeling studies applied to ^{2}H-NMR data of *N*-palmitoylglucosylsphingosine indicate that the sugar residue projects vertically from the bilayer surface [18]. The similar hydration numbers of saturated NPGS, glucocerebroside and hexoses suggest that the galactosyl and glucosyl moieties at the interface of the cerebroside bilayers are in a conformation such that both sides of the sugar ring are accessible to water molecules. Although this orientation would certainly favor maximum hydration of the hydroxyl groups, other intra- and intermolecular interactions will also contribute to the precise orientation of the sugar ring at the bilayer interface.

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References

1 Elworthy, P.H. (1961) J. Chem. Soc., 5385
2 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445
3 Lundberg, B., Svens, E. and Ekman, S. (1978) Chem. Phys. Lipids 22, 285
4 Ter-Minassian-Saraga, L. and Madelmont, G. (1982) J. Coll. Interface Sci. 85, 375
5 Bach, D., Sela, B. and Miller, I.R. (1982) Chem. Phys. Lipids 31, 381
6 Ruocco, M.J., Atkinson, D., Small, D.M., Skarjune, R.P., Oldfield, E. and Shipley, G.G. (1981) Biochemistry 20, 5957
7 Ruocco, M.J. (1983) Doctoral Dissertation, Boston University
8 Skarjune, R.P. and Oldfield, E. (1979) Biochim. Biophys. Acta 556, 208
9 Correa-Freire, M.C., Freire, E., Barenholz, Y., Biltonen, R. and Thompson, T.E. (1979) Biochemistry 18, 442
10 Freire, E., Bach, D., Correa-Freire, M., Miller, I. and Barenholz, Y. (1980) Biochemistry 19, 3662
11 Curatolo, W. (1982) Biochemistry 21, 1761
12 Pascher, I. and Sundell, S. (1977) Chem. Phys. Lipids 20, 175
13 Shiio, H. (1958) J. Am. Chem. Soc. 80, 70
14 Franks, F., Reid, D.S. and Suggett, A. (1973) J. Solut. Chem. 2, 99
15 Harvey, J.M., Symons, M.C.R. and Naftalin, R.J. (1976) Nature 261, 435
16 Ihnat, M., Szabo, A. and Goring, D.A.I. (1968) J. Chem. Soc. A, 1500
17 Tait, M.J., Suggett, A., Franks, F., Ablett, S. and Quickenden, P.A. (1972) J. Solut. Chem. 1, 131
18 Skarjune, R. and Oldfield, E. (1982) Biochemistry 21, 3154