Colloid Polym Sci (2005) 283: 1376–1381
DOI 10.1007/s00396-005-1347-9

SHORT COMMUNICATION

Jan Cocquyt
Ulf Olsson
Gerd Olofsson
Paul Van der Meeren

Thermal transitions of DODAB vesicular dispersions

Received: 1 April 2005
Accepted: 8 April 2005
Published online: 22 June 2005

J. Cocquyt (✉) · P. Van der Meeren
Particle and Interfacial Technology Group,
Ghent University, Coupure Links 653,
9000 Ghent, Belgium
E-mail: Jan.Cocquyt@Ugent.be
Tel.: +32-9-2646006
Fax: +32-9-2646242

U. Olsson · G. Olofsson
Physical Chemistry 1,
Center of Chemistry and Chemical
Engineering, Lund University,
Box 124 221 00 Lund, Sweden
E-mail: Ulf.Olsson@fkem1.lu.se
Tel.: +46-46-2228159
Fax: +46-46-2224413

Abstract Two endothermic transitions, at 36°C and 44°C, were observed with differential scanning calorimetry (DSC) upon heating dioctadecyldimethylammonium bromide vesicle dispersions that were equilibrated below 15°C while in samples kept at 25°C there was only the transition at 44°C, which was shown to be the gel to liquid–crystalline transition by ^{1}H-NMR measurements. The transition at 36°C was reversed in an exothermic transition around 13°C upon cooling. The slowness of this transition at ambient temperatures suggests that the presence of the transition at 36°C in a DSC upscan depends strongly on the sample history.

Keywords Bilayer · DODAB · DSC · Melting

Introduction

Dioctadecyldimethylammonium bromide (DODAB) is a synthetic double- and long-chained cationic amphiphile forming vesicle. Using differential scanning calorimetry (DSC) several authors observed for DODAB vesicles a single endothermic peak around 45°C, which they ascribe to liquid–Gúcrystalline phase transition [1–4]. However, based on a fluorescence investigation, Sarpal and Durocher [5] claimed that the phase transition temperature of DODAB vesicles is 36°C whereas several authors found with DSC two almost equally sized endothermic peaks at 36°C and 45°C [6–10]. The aim of this study was to find the reason for these conflicting observations.

Experimental

Chemicals

Dioctadecyldimethylammonium bromide with purity higher than 99% was used as received from Acros Organics (Belgium). Ultrapure water of Milli-Q-Plus quality was used.

Preparation of vesicles

Nonsonicated vesicle dispersions were prepared by gently stirring at 65°C during 2 h using a magnetic stirrer. Unless otherwise stated, the sample was then

cooled in a water bath at room temperature during 30 min. The sonicated vesicles were prepared as described previously [10].

Proton nuclear magnetic resonance (^{1}H-NMR)

The method was described previously [10].

Differential scanning calorimetry (DSC)

A high-sensitivity differential scanning calorimeter Microcal VP-DSC (Microcal Inc., Northampton, MA, USA) equipped with 0.5 ml twin total-fill cells was used.

Turbidity

The turbidity was measured at a wavelength of 633 nm using a Shimadzu UV-VIS 1205 spectrophotometer. A 1 mm path-length quartz cuvet was used. The sample cuvet was equilibrated at selected temperatures in an external water bath. It was then taken out and the turbidity was measured as quickly as possible. Water was used as a reference.

Dynamic light scattering (DLS)

A Photon Correlation Spectroscopy 4700 (Malvern, UK) equipment was used, provided with a vertically polarized 10 mW laser with a wavelength of 633 nm. The scattering angle was 90°C.

Results

Differential scanning calorimetry

Examples of DSC results on a nonsonicated DODAB vesicle dispersion are shown in Fig. 1. The freshly prepared 5.47 mM DODAB dispersion was filled into the sample cell at 25°C. The temperature was lowered and kept at 2°C for 5 min before starting an upscan to 65°C. The thermogram showed two large, closely similar, endothermic peaks with maxima at 36.0°C and 44.2°C and enthalpy changes of 43.6 kJ/mol and 45.9 kJ/mol, respectively (Fig. 1a, curve a). After 5 min at 65°C, a downscan to 2°C was made (Fig. 1a, curve b). Besides an exothermic peak at 38.8°C with an enthalpy change of −43.6 kJ/mol, there was another exothermic peak starting at 13.7°C. A second upscan looked the same as the first scan. These experiments were made at a scan rate of 1°C/min. When the downscan rate was decreased to 0.3°C/min, the low temperature exothermic transition

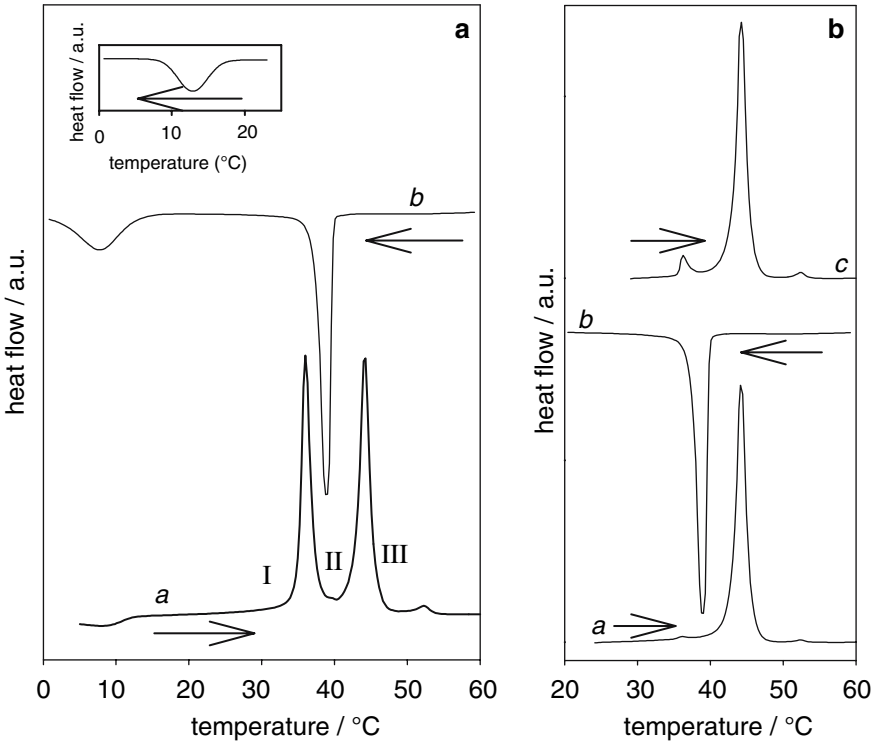

Fig. 1 Differential scanning calorimetry (DSC) thermograms of a 5.47 mM nonsonicated Dioctadecyldimethylammonium bromide (DODAB) dispersion (**a**) *a* first upscan, *b* subsequent downscan, *insert* downscan from 25°C. (**b**) *a* First upscan, *b* subsequent downscan, *c* upscan after being kept at room temperature during seven days after preparation. Scan rate was 1°C/min except in insert were it was at 0.33°C/min. For clarity, curves have been displaced on the heat capacity axis. The absolute scaling in the insert is the same as in the rest of the figure

started at 19.6°C, reached a maximum at 13°C and was characterized by an enthalpy change of −33.0 kJ/mol. The return to the baseline showed that the transition was completed (Fig. 1a, insert). Within limits of experimental errors, the same results were found for a 25-mM DODAB dispersion. Thus, samples that had equilibrated at temperatures well below 25°C showed two, about equally sized endothermic peaks as was seen by several authors [6–10]. Both peaks in the heating mode had a symmetric shape characteristic for a two-state transition [11]. The exothermic peak at −38.8°C in the cooling mode was asymmetric with a sharp descent indicating undercooling.

If the freshly prepared 5.47 mM nonsonicated DODAB vesicle dispersion was first kept in the DSC cell at 20°C for 5 min, there was only one peak at 44.2°C with an enthalpy change of 46.4 ± 0.3 kJ/mol in the subsequent upscan (Fig. 1b, curve a). In the downscan from 65°C after 5 min waiting, a single exothermic peak at 38.9°C with an enthalpy change of −43.9 ± 0.1 kJ/mol was observed (Fig. 1b, curve b). Three additional upscans from 20°C to 65°C followed by downscans to 20°C gave identical results. Five consecutive runs under the same conditions on a 5.77 mM DODAB sample were equally reproducible and gave within experimental error the same transition temperatures and molar enthalpies. The difference between the enthalpy changes for the upscan and downscan transitions is to a large extent due to premelting in the upscans seen as a deviation from the baseline well below the main peak. The scan rate in these experiments was 1°C/min. These results fully agree with results reported by several authors [1–4]. The uppermost curve in Fig. 1b shows the DSC upscan on the 5.47 mM DODAB sample after standing for 7 days at room temperature. The appearance of the small additional peak at 36°C indicates that the low-temperature exothermic transition proceeds slowly at room temperature.

In DODAB vesicle dispersions conditioned below 15°C, we denote the state below 36.0°C state I, between 36.0°C and 44.2°C state II, and above 44.2°C state III (Fig. 1a). The transition from state II to state III at 44.2°C was not affected by the presence of the transition I → II at 36.0°C. This indicates that the transition in downscans at 38.9°C is III → II but undercooled by couple of degrees. The transition II → I at 36.0°C was inhibited upon cooling. Only around 15°C did the process continue to completion reasonably fast. Because of this slowness at ambient temperatures, some researchers have seen only one peak while others [6–10] have seen two peaks in DSC upscans, depending on the sample storage conditions.

Figure 2 shows the DSC result on a sonicated 5.62 mM DODAB sample. No exothermic peak is seen in the first scan down from 25°C to 2°C (Fig. 2, curve a). The subsequent upscan (Fig. 2, curve b) showed a broad peak starting at 33°C and ending at 46°C with maxima at 36 and 43.7°C. The downscan, after waiting 5 min at 65°C, showed a sharp peak at 40°C that continued as a broad exothermic bulge down to 25°C (Fig. 2, curve c). Hardly any deviation from the baseline was seen on further decrease in temperature to 5°C. A second scan up to 60°C showed the same features as the first upscan. Sonication broadened both transitions and reduced the sum of the enthalpies by roughly 50% [10]. After sonication, the transition at I → II is fast as hardly any hysteresis was observed.

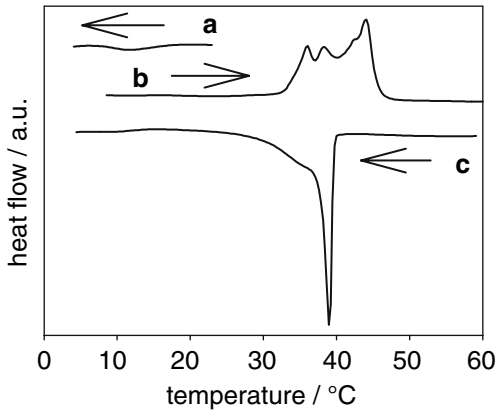

Fig. 2 Differential scanning calorimetry thermograms of a 5.62-mM sonicated DODAB dispersion. *a* first downscan, *b* subsequent upscan, *c* second downscan. The first downscan was at 0.33°C/min, subsequent scans were at 1°C/min. For clarity, curves have been displaced on the heat capacity axis. The absolute scaling is equal as in Fig. 1

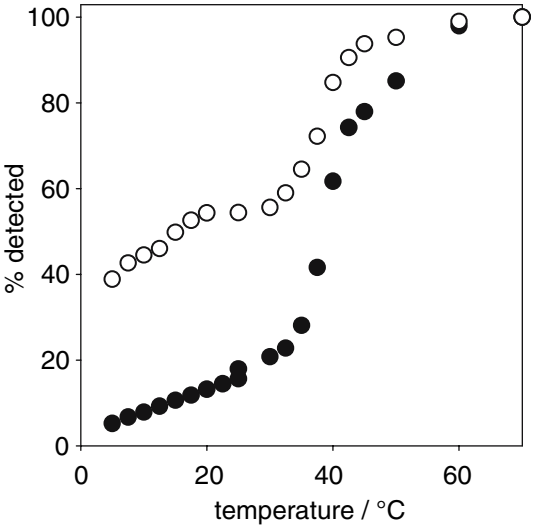

Fig. 3 Dioctadecyldimethylammonium bromide alkyl chain fraction detected with ^{1}H-NMR at various temperatures in a 6-mM DODAB in D_2O dispersion. It was assumed that all DODAB was detected at 70°C. (*filled circle*) before sonication; (*open circle*) after sonication. Data after sonication between 25°C and 65°C were taken from [10]

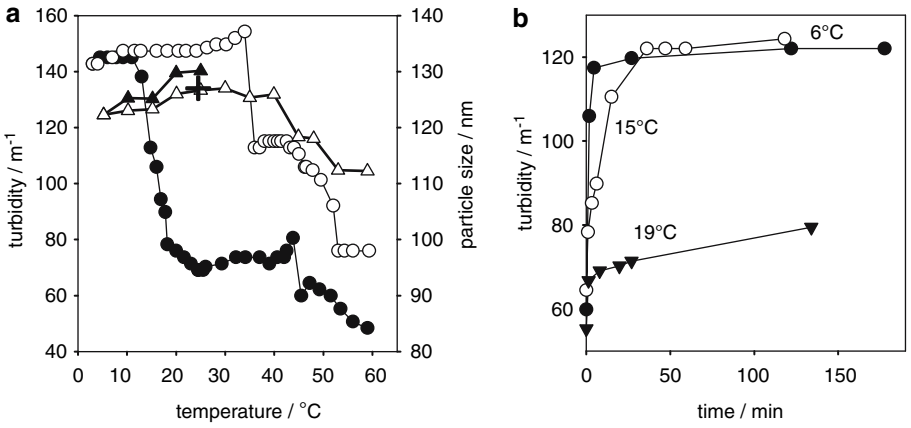

Fig. 4 **a** Turbidity of 5.7 mM nonsonicated DODAB where temperature was first decreased from 60°C to 3°C (*filled circle*) and then increased from 3°C to 60°C (*open circle*) and particle size of a 1 mM extruded DODAB sample where the temperature was first decreased from 25°C to 5°C (*filled triangle*), then increased from 5°C to 60°C (*open triangle*) and then decreased to 25°C (*plus*). **b** Temperature-quenched turbidity of a 5.9 mM nonsonicated DODAB sample from 60°C to 6°C (*filled circle*), 15°C (*open circle*) and 19°C (*filled inverted triangle*)

^{1}H-NMR

Due to line broadening, essentially no signals from the alkyl chains were seen in ^{1}H-NMR spectra of a 6 mM nonsonicated DODAB dispersion in D_2O at 25°C, i.e. where the DODAB alkyl chains are in the gel state. Heating the sample above the gel to fluid state transition temperature T_m makes the signal appear because alkyl chains in the fluid state give narrower NMR bandwidths due to faster local anisotropic motions [12]. Thus, the integrated ^{1} H-NMR signal intensities can be used to estimate the fraction of fluid alkyl chains [10]. Figure 3 shows the fraction of alkyl chains detected with ^{1} H-NMR at various temperatures assuming that at 70°C all alkyl chains are fluid and thus detected. It is clear from Fig. 3 that the DSC peak at 44.2°C corresponds to the melting of alkyl chains. Accordingly this temperature was denoted as the main transition temperature T_m. The drop in the pyrene fluorescence anisotropy at this temperature is consistent with this assignment [9]. Figure 3 confirms that about 50 % of the alkyl chains remained fluid upon cooling below T_m after sonication above T_m [10].

Turbidity and dynamic light scattering

Samples for turbidity experiments were kept unstirred at 60°C after preparation at 65°C. In a first series of experiments, the turbidity of a 5.7-mM nonsonicated DODAB sample, which was kept at 60°C for 2 h, was measured at selected temperatures upon cooling at a rate of about 0.2°C/min in an external water bath from 60°C to 3°C. Turbidity is plotted against the water bath temperature in Fig. 4a. The temperature of the sample during the measurements may differ somewhat from this temperature. Upon cooling, turbidity increases steeply around 15°C. Upon heating, the high turbidity remains constant up to about 35°C when there is a sudden drop. After staying constant for about 10°C the turbidity further decreases to a constant level at about 55°C. When this sample was slowly cooled from 60°C down to 20°C, the turbidity increased slightly down to 40°C and then stayed constant. In the subsequent upscan there were no drastic changes.

Figure 4a also shows the particle size in a 1-mM DODAB sample measured with dynamic light scattering (DLS) at several temperatures. As DLS has difficulties measuring broad size distributions [13], the nonsonicated sample was extruded ten times through polycarbonate membranes with 100 nm pore size (Whatman, UK) at 60°C and at 7 bar in order to obtain a narrow size distribution. This treatment did not alter significantly the melting behaviour observed with DSC (result not shown). After extrusion the sample was cooled to 25°C, then the particle size was measured at selected temperatures while first cooling and then heating the sample in the equipment. It is clear from Fig. 4a that the vesicle size hardly varied with temperature. After the complete temperature cycle, the size measured at 25°C was within experimental error equal to the size measured at the start of the experiment.

In a second series of experiments, the turbidity of samples which were quenched from 60°C by placing the sample cuvet in a water bath at 6°C, 15°C and 19°C, respectively, was measured versus time. The turbidity increased fast at the lower temperatures but rather slowly at 19°C (Fig. 4b).

Discussion

The main alkyl chain melting transition of DODAB was at 44°C (Fig. 3). This transition has similar features as

the main transition of the phopholipid dioctadecyl phosphatidylcholine (DOPC) that occurs with an enthalpy change of 43.4 kJ/mol lipid in dilute aqueous suspension [11]. The large enthalpy change in DODAB vesicles at 36°C shows that this transition differs from the pretransition observed for the corresponding phosphatidylcholine where the enthalpy change is only 5.9 kJ/mol [11]. The negatively charged phospholipid dimyristoyl phosphatidylglycerol (DMPG) presents a large gel-fluid transition region ranging from 18°C to 35°C at low salt concentration [14]. The thermal behaviour of DODAB may be related. Despite extended experimental work [15–18], the structural characteristics of this DMPG transition remain not fully understood.

Turbidity measurements confirm that the transition seen in the DSC downscan below room temperature is the undercooled transition seen at 36°C in the upscan curves (Fig. 4a). The larger turbidity observed in state I is not due to flocculation as the particle size did not increase (Fig. 4a). Besides,no sedimentation or flocculation was observed in a 3-mM sample stored at 3°C during 3 months. The process occurring upon cooling gets completed (Fig. 4b) whereas aggregation is expected to continue and to result in sedimentation. It is concluded that the phenomenon occurring between state I and state II is local leaving the vesicle aggregation number unaffected. Possible reasons for the turbidity increase upon cooling below 15°C are manifold. Higher turbidities upon cooling can be due to a decrease in the DODAB projected surface area and a concomitant increase in bilayer thickness since the vesicle form factor decreases less with scattering angle [19, 20]. The changes in turbidity with temperature may also be caused by changes in DODAB refractive index as has been observed before for phospholipids [20]. Sonicated vesicles were found to be prolate with cryo-TEM [10]. However, it is unlikely that observation of two peaks is due to differences in vesicle shapes or bilayer curvature since nonsonicated DODAB vesicles were found to be spherical and several hundreds of nanometers with cryo-TEM [1]. Nevertheless, the ratio of the two peaks is about equal before and after sonication. We must conclude that the exact nature of this transition is unknown but we will continue to study this phenomenon.

Conclusions

In DODAB vesicle dispersions, the alkyl chains were observed with ^{1}H-NMR to be in the fluid state above 44°C, which is therefore termed the gel to fluid transition. The enthalpy change of this fast and reproducible transition was 45.9 kJ/mol. Upon cooling, the transition occured reproducibly at 38.9°C with an enthalpy change of −43.6 kJ/mol. Upon cooling below 15°C, a second much slower −33.0 kJ/mol exothermic transition was observed. Although turbidity doubled during this transition, no flocculation or fusion occured as the particle size remained constant. Besides, sedimentation was not even observed after 3 months at 3°C. Although the exact nature of this transition is not fully understood, this phenomenon occurred within the vesicle leaving the vesicle aggregation number unchanged. Moreover, this phenomenon was only reversed at 36°C in a 43.6 kJ/mol endothermic transition. This brings clarity in the conflicting observations in literature: only samples that had been cooled below 15°C showed the peak at 36°C in a DSC-upscan beside the main transition at 44°C.

Acknowledgments Jan Cocquyt thanks the Marie Curie Training Site (contract no. HPMT-CT-2000-00150) and Ulf Olsson thanks the Swedish Research Council (V.R.), both for financial support.

References

1. Barreleiro PCA, Olofsson G, Brown W, Edwards K, Bonassi N, Feitosa E (2002) Langmuir 18:1024
2. Feitosa E, Barreleiro PCA, Olofsson G (2000) Chem Phys Lipids 105:201
3. Jung M, Hubert DHW, van Veldhoven E, Frederik PM, Blandamer MJ, Briggs B, Visser PM, van Herk AM, German AL (2000) Langmuir 16:968
4. Linseisen FM, Bayerl S, Bayerl TM (1996) Chem Phys Lipids 83:9
5. Sarpal RJ, Durocher, GJ (1994) Photochem Photobiol A Chem 80:307
6. Blandamer MJ, Briggs B, Cullis PM, Green JA, Waters M, Soldi G, Engberts JBFN, Hoekstra DJ (1992) J Chem Soc Faraday Trans 88:3431
7. Blandamer MJ, Briggs B, Cullis PM, Engberts JBFN, Kacperska A (1995) J Chem Soc Faraday Trans 91:4275
8. Blandamer MJ, Briggs B, Cullis PM, Irlam KD, Kirby SD, Engberts JBFN (1998) J Mol Liq 75:181
9. Benatti CR, Tiera MJ, Feitosa E, Olofsson G (1999) Thermochim Acta 328:137
10. Cocquyt J, Olsson U, Olofsson G, Van der Meeren P (2004) Langmuir 20:3906
11. Hinz HJ, Sturtevant JM (1972) J Biol Chem 247:6071
12. Wennerström H, Ulmius J (1976) J Magn Reson 23:431
13. Dyuzheva MS, Klyubin VV (2003) Colloid J 65:567
14. Lamy-Freund MT, Riske KA (2003) Chem Phys Lipids 122:19

15. Riske, KA, Politi MJ, Reed WF, Lamy-Freund MT (1997) Chem Phys Lipids 89:31
16. Riske KA, Nascimento OR, Peric M, Bales BL, Lamy-Freund MT (1999) Biochim Biophys Acta 1418:133
17. Riske KA, Amaral LQ, Lamy-Freund MT (2001) Biochim Biophys Acta 1511:297
18. Riske KA, Fernandez RM,Nascimento OR, Bales BL, Lamy-Freund MT (2003) Chem Phys Lipids 124:69
19. Matsuzaki K, Murase O, Sugishita K, Yoneyama S, Akada K, Ueha M, Nakamura A, Kobayashi S (2000) Biochim Biophys Acta 1467:219
20. Yi PN, MacDonald RC (1973) Chem Phys Lipids 11:114