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EFFECTS OF ADDED UREA AND ALKYLUREAS ON GEL TO LIQUID-CRYSTAL TRANSITIONS IN DOAB VESICLES

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Abstract

The gel to liquid-crystal transition for vesicles in aqueous solution formed by dimethyldi-*n*-octadecylammonium bromide (DOAB) occurs at 44.7°C. Moreover, the shapes of the scans recorded by a sensitive DSC microcalorimeter are very similar when the vesicular solutions are prepared starting with solid DOAB and comparable amounts of either solid urea or solid alkylureas. Therefore, the DOAB vesicles in aqueous solution accommodate this class of solutes without marked changes in the melting temperature and the enthalpy of the transition. The contrast with effects of added surfactants and simple organic solutes such as THF and ethanol is particularly significant.

Keywords: alkylureas, DOAB vesicles, DSC, gel-liquid transitions, urea

Introduction

In aqueous solutions, the synthetic amphipathic solute dimethyl-*n*-octadecylammonium bromide (DOAB) forms closed bilayer vesicles [1, 2]. These systems are interesting in their own right and in terms of their link with natural lipid bilayer systems in, for example, cell membranes. The latter are often complex containing several phospholipids and proteins. In principle, therefore, vesicles formed by DOAB and similar dialkylphosphates [3, 4] offer simple, reasonably well-characterised systems for probing the physical and chemical properties of bilayers. An interesting feature is the gel to liquid-crystal transition [5] which occurs at a temperature characteristic of the vesicular system. The transition emerges as a strong feature in the scans recorded by ultra-sensitive differential scanning microcalorimeters [6, 7]. An important consideration in these experiments is the protocol for solution preparation. We have shown [6, 7] unequivocally

cally that protocols which involve injection into water of a solution of the monomer salt in an organic solvent (e.g. ethanol) produces vesicle solutions for which the scans recorded by DSC are complicated and not reproducible. Therefore, we have used a protocol based [6, 7] on dissolving amphipathic salts directly into hot water. The resulting scans are both simpler and reproducible. The transitions are reversible, the sample pattern being obtained through several heat-cool-heat-cool ... cycles. Moreover, this protocol allows the effects of added solutes on the gel to liquid-crystal transition to be examined in terms of, for example, the change in the melting temperature T_m characterising this transition. We have shown [8] in the case of vesicles formed by sodium di-*n*-dodecylphosphate that added ethanol shifts T_m to lower temperatures consistent with direct incorporation of ethanol molecules into the bilayer. In the case where THF was added, the presence of two extrema in the DSC scan pointed to the presence of domains in the bilayer containing different proportions of THF.

With reference to DOAB [aq; $2 \cdot 10^{-3}$ (monomer mol) dm^{-3}] a set of repeat scans for solutions containing *n*-hexadecyltrimethylammonium bromide (CTAB) showed [9] that T_m shifted to lower temperature, implying that CTAB is more readily incorporated by vesicles in the liquid-crystal (high temperature) state than in the gel (low temperature) state. However, when sodium dodecylsulphate (SDS) was added, T_m shifted [10] to higher temperature. The contrast between the effects of added CTAB and SDS is understood in terms of attractive charge-charge interactions involving SDS anionic head groups and trimethylammonium cationic head groups in CTAB. Otherwise, for both DDP and DOAB, neutral hydrophobic solutes are incorporated into the bilayer, destabilising the gel state. This possible generalisation prompted the experiments reported here where we examined the impact on the gel to liquid-crystal transition for DOAB of added urea and alkylureas. The changes in T_m and enthalpy of fusion $\Delta_m H$ are surprisingly small.

We recall the suggestion by Finney and Soper [11] that urea molecules in aqueous solutions are readily incorporated into the hydrogen-bonded water-asociates. In these terms, direct influence of the urea on the DOAB vesicular properties is significantly moderated. Alternatively, the DOAB bilayer structure is able to incorporate the urea molecules with little change in the structure of the bilayer. Unfortunately, thermodynamic information cannot discriminate between these two explanations.

Experimental

Materials

Aqueous solutions containing DOAB vesicles were prepared using the hot-water method described previously [6] using the DOAB monomer. Aqueous systems containing DOAB vesicles and either urea or an alkylurea were prepared using one of two protocols. Weighed amounts of solid DOAB monomer salt and

either solid urea or solid alkylurea were dissolved in hot water and held at 55°C for approximately one half-hour.

Electron microscopy

An electron micrograph of DOAB [aq; 10^{-4} (monomer mol) dm^{-3}] was recorded at magnification $4.1 \cdot 10^4$. The vesicles were stained with uranylacetate and viewed using a Siemens transmission electron microscope.

Calorimetry

A MicroCal DSC calorimeter was used to measure the differential isobaric heat capacities of DOAB solutions containing urea over the range 15 to 90°C at a scan rate of approx. 60°C h^{-1} . The sample cell held 1.2112 cm^3 of solution; the reference cell contained water. The solutions were degassed before loading into the calorimeter and allowed to equilibrate at 15°C before a run was initiated. The operation of the calorimeter was computer controlled. The data were analysed using ORIGIN software supplied by MicroCal Ltd.

Results and discussion

Confirmation that the protocol used in these studies produces closed spherical DOAB vesicles is shown by the electron micrograph in Fig. 1. The fact that the protocol for preparing vesicles by us produces samples that are well-behaved thermodynamically (DSC) and show uniform structure in the electron micrographs is particularly pleasing.

Fig. 1 Electron micrograph of vesicles produced by DOAB [aq; $1 \cdot 10^{-4}$ (monomer mol) dm^{-3}]

The DSC scans for DOAB [aq; $2 \cdot 10^{-3}$ (monomer mol) dm^{-3}] vesicles showed evidence for a gel to liquid-crystal transition [10] at $44.7 \pm 0.1^\circ\text{C}$ where $\Delta_m H^\circ \text{ kcal mol}^{-1} = 8.72 \pm 0.51$; the co-operative melting involving a patch of 214 ± 13 monomers.

Fig. 2 Scans recorded by a differential scanning microcalorimeter for DOAB [aq; $2 \cdot 10^{-3}$ (monomer mol) dm^{-3}] and urea [aq; $1 \cdot 10^{-3}$ mol dm^{-3}]. The scans have been displaced for clarity on the heat capacity axis

A typical set of scans for DOAB [aq; $2 \cdot 10^{-3}$ (monomer mol) dm^{-3}] vesicular system containing urea [aq; $1 \cdot 10^{-3}$ mol dm^{-3}] is shown in Fig. 2. The set of scans in this figure were recorded after the solution in the sample cell had been heated to 90°C in the sample cell, allowed to cool for 3 h and then cycled to 90°C once more. The three scans were recorded at a scan rate of 60°C h^{-1} , the solutions being allowed to stand for 3 h at 5°C between scans. The reproducibility of the scans over a period of at least 12 h confirmed that a series of thermodynamic equilibrium states were scanned and that no slow reorganisation took place in these systems.

The original aim of this investigation was to compare the effects of added salts and added ureas on the gel to liquid-crystal transitions in DOAB vesicles. The intention was to contrast the effect of added solutes on this transition which, on the one hand, are adsorbed into the bilayer and, on the other hand, might perturb water-water interactions in the solution surrounding the vesicles.

In the first series of experiments we planned to contrast the effects of adding to DOAB(aq) approximately equimolar amounts of either sodium bromide or tetramethylammonium bromide. In these two cases, the recorded scans were complicated and not reproducible. Inspection of the solutions removed from the sample cell of the DSC calorimeter showed that during the heating of the solutions extensive precipitation had occurred. We conclude that both salts are probably fusogenic agents [2, 12] leading to extensive flocculation. If this phenomenon represents one end of the range of effects of added solutes on DOAB vesicles and if the impact of added CTAB (see above) represents the other extreme, we speculated that a useful comparison could be drawn with the effects of added urea and alkyl-ureas. The interest in these effects was prompted by two considerations.

First, in aqueous DOAB systems vesicle formation, an example of supra-molecular self-assembly is driven, in part, by hydrophobic cohesion [13] between

the alkyl chains. If T_m is a measure of cohesion between the alkyl chains then, as in the case of dialkylphosphates, [14] T_m should increase with increase in alkyl chain length. The results show that this pattern is, in general, followed. Second and following on from the above, the effect of added urea on the properties of aqueous solutions is often described in terms of its effect on water-water interactions [11, 15]. In terms of the stability of macromolecules in aqueous solutions, added urea is often claimed to weaken hydrophobic interactions although in the case of proteins, the importance of direct urea-protein interaction cannot be ruled out [16]. Granted that urea does, in fact, perturb hydrophobic interactions, we speculated that useful comparisons could be drawn with the effect of added urea and alkyl-ureas where the concentrations of these ureas are comparable to the concentration of DOAB monomers. Indeed, it turned out that the scans were reproducible and the observed transitions are reversible. Nevertheless, a striking feature of the scans was their relative insensitivity to the nature and concentration of added urea. In the case of, for example, added 1,3-diethylurea, the characteristic melting temperature for DOAB (see above) is unaffected (Fig. 3). A similar pattern emerged for the other ureas (Table 1).

Fig. 3 Scans recorded by a differential scanning calorimeter for solutions prepared using DOAB [aq; $2 \cdot 10^{-3}$ (monomer mol) dm^{-3}] and 1,3-diethylurea; (a) 0.0, (b) $1 \cdot 10^{-3}$, (c) $1.5 \cdot 10^{-3}$, (d) $2.0 \cdot 10^{-3}$ and (e) $2.5 \cdot 10^{-3}$ mol dm^{-3} . The curves have been displaced on the heat capacity axis for clarity

The insensitivity of transitions was apparent not only in T_m but in two other parameters characterising the transition. Thus the patch number refers to the number of DOAB monomers involved in co-operative melting and the enthalpy term $\Delta_m H$ (kcal) refers to the calorimetric enthalpy change expressed in terms of a mole of DOAB monomers. The insensitivity of the three important parameters (Table 1) implies that none of these ureas are taken into the bilayer. This conclu-

Table 1 Effect of added ureas on the gel to liquid-crystal transition for DOAB vesicles in aqueous solutions^(a)

Solute	$T_m/^\circ\text{C}$	$\Delta_m H/\text{kcal mol}^{-1}$	Patch number n
(none)	44.8	8.9	133
Urea	43.8 $\pm$ 0.8	9.10 $\pm$ 0.42	137 $\pm$ 30
Ethylurea	44.2 $\pm$ 0.1	8.40 $\pm$ 1.0	188 $\pm$ 34
1,1-Diethylurea	44.2 $\pm$ 0.1	9.13 $\pm$ 0.40	137 $\pm$ 27
1,3-Diethylurea	44.1 $\pm$ 0.1	10.01 $\pm$ 0.44	124 $\pm$ 11
1,1-Dimethylurea	43.7 $\pm$ 0.5	8.69 $\pm$ 0.55	160 $\pm$ 32
1,3-Dimethylurea	44.3 $\pm$ 0.1	9.65 $\pm$ 0.42	143 $\pm$ 10

sion was confirmed by the titration calorimetric experiments [2] in which aliquots of 1,3-diethylurea (aq; 0.018 mol dm⁻³) were injected (5 · 10⁻⁶ mol dm⁻³) into DOAB solutions. For systems both below (25°C) and above (50°C) the T_m , the enthalpies of injection over 50 injections were zero after correction for the simple dilution into water of the urea solution.

The relative insensitivity of the gel to liquid-crystal transitions in DOAB(aq) to added ureas is particularly interesting when viewed in terms of the selectivity of the bilayers to added solutes. If the added solute cannot be incorporated into the bilayer, as in the case of CTAB, and if the added solute cannot interact with the polar double layer, there is no affinity between the added solute and DOAB vesicles. These observations prompt speculation concerning similar characteristics of lipid bilayer systems.

* * *

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