Modulation of vesicular properties by variation of shapes of bolaform counter ions in hybrid-ion paired surfactants

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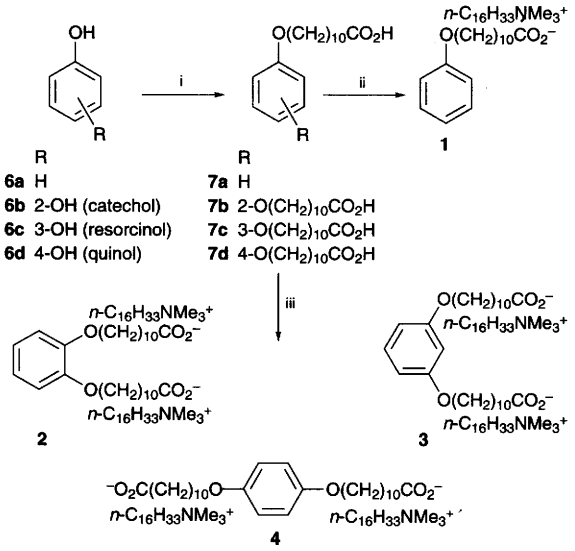
Four new vesicle-forming bolaphile/amphiphile ion pairs are synthesized; the bolaphile shapes in such hybrid systems strongly control their vesicular properties.

Spontaneous formation of specific folding motifs or supramolecular patterns can often be rationalized on the basis of stabilizing interactions. Ion-pairing typically brings about *ca.* 5 kcal mol^{-1} stabilization (cal = 4.184 J). Thus, ion pairs are encountered inside different proteins frequently.[1] In membrane proteins, *e.g.* rhodopsin,[2] the existence of such salt-bridges has been demonstrated. Ion-pairing is important in other biological events; thus when amphiphiles of identical charges are mixed, micelles are formed,[3] but spontaneous vesicle formation is observed when amphiphiles with oppositely charged headgroups are mixed.[4] These examples illustrate how specific ion-pairing influences unrelated biological phenomena *e.g.* enzymic catalysis, membranous aggregate formation.

Bolaform amphiphiles[5] in contrast to their monopolar counterparts (single chain/single polar headgroup) contain two polar headgroups connected through a hydrophobic segment. Synthetic bolaphiles are mimics of archaebacterial membranes and form ultra-thin 'monolayer' membranes.[5] These are being investigated for diverse purposes such as entrapment and release,[6] or for the design of metallo-aggregate catalysts.[7] However, nothing is known about the properties of hybrid systems comprising a bolaform and a monopolar amphiphile. Here we describe the syntheses of such a new family of novel ion-paired systems and the formation and characterization of vesicles from aqueous dispersions of such systems. We also examined the vesicular properties of the corresponding amphiphile (monopolar) anion/cation pair, cetyltrimethylammonium (CTA) 11-phenoxyundecanoate **1** and CTA palmitate **5**.

Four new ion-paired systems (**1–4**) were synthesized (Scheme 1) by passage of a methanolic solution of CTABr through an ion-exchange column (Amberlite IRA-900, OH^- form), then (*i*) treatment with 1 equiv. of freshly recrystallized **7a** or 0.5 equiv. of **7b**, **c** or **d** in dry MeOH, (*ii*) removal of solvent under reduced-pressure rotary evaporation and (*iii*) repeated recrystallization from MeOH–EtOAc.

Upon dispersal in water (conc. 2.5 mmol dm^{-3}) either by sonication or vortexing **1–4** gave translucent suspensions. Examination of aqueous dispersions (reverse-phase evaporation)[8] of **1–4** by TEM (JEOL 200 CX; 0.5% uranyl acetate) revealed the existence of closed vesicular structures. **1** gave mostly unilamellar vesicles of 750–800 Å diameter, whereas **2**



Scheme 1 *Reagents and conditions*: i, (*a*) $Br(CH_2)_{10}CO_2Et$, anhydrous K_2CO_3, dry acetone, reflux, 30 h, yield 90%; (*b*) aq. KOH (5%), reflux, cool, H_3O^+, yield 95%; ii, *n*-$C_{16}H_{33}N^+Me_3$, OH^- (1 equiv.), MeOH, room temp., stir, 24 h, yield 90–95%; iii, *n*-$C_{16}H_{33}N^+Me_3$, OH^- (2 equiv.), MeOH, room temp., stir, 24 h, yield 80–90%

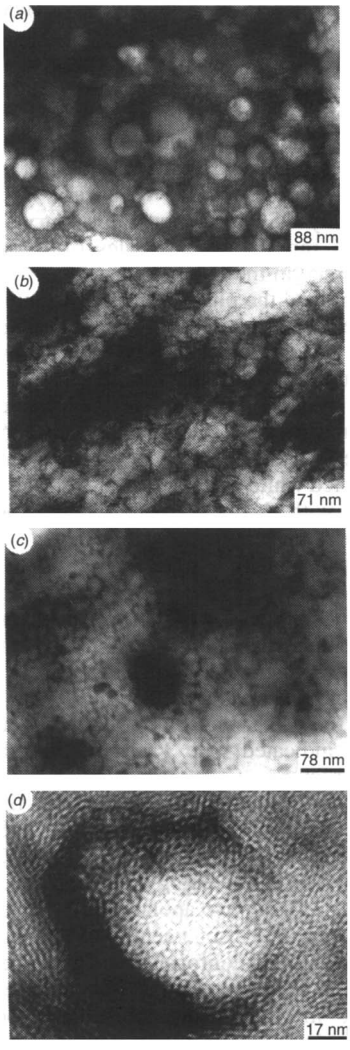


Fig. 1 Negative stain transmission electron micrographs of vesicles: (*a*) **1**, (*b*) **2**, (*c*) **3** and (*d*) **4**

Table 1 Main phase-transition temperatures (T/°C) of vesicular **1–5** and the unit-layer thicknesses of their self-supporting cast films

	Main transition[a]/°C				Unit-layer thickness/Å	
Compound	DSC	Microcal.	Fluor. Pol.	UV method	obs.[b]	calc.[c]
1	31.3	35.3	30.5	*d*	33.4	40.5
2	*e*	39.1	37.0	38.0	46.8	44.0, 33.0
3	*e*	*e*	*e*	*e*	39.1	39.0, 33.7
4	85.0	84.9	*e*	*e*	36.1	34.5
5	41.6	41.8	41.0	42.0	37.9	41.0

[a] Average deviation is ±1 °C for DSC; ±0.5 °C for microcalorimetry; ±1 °C for fluorescence polarization method and ±2 °C for UV method. [b] As obtained from reflection XRD of cast films. [c] Molecular lengths of unit layers of ion-pairs as estimated from energy-minimized CPK models (INSIGHT). [d] Not determined. [e] No detectable transition.

and **3** formed multilamellar vesicles having diameters of 510 ± 10 and 975 ± 25 Å respectively (Fig. 1). In contrast, dispersions of **4** contained both open and closed multilamellar structures (1250 ± 50 Å).

DSC (Perkin Elmer DSC-2C) of aqueous gels (60 mmol dm^{-3}) of **1**, **4** and **5** displayed sharp endothermic peaks at 31.3, 85.0 and 41.6 °C respectively. In contrast, **2** and **3** did not give any peak within the range 15–90 °C. Examination of the corresponding vesicles (2.5 mmol dm^{-3}) using microcalorimetry (MC-2) generally reproduced the peaks related to T_m although with **4**, the cooperativity was drastically reduced upon dilution. Remarkably, the vesicles of **2** (2.5 mmol dm^{-3}) now gave a pronounced peak at 39.1 °C under microcalorimetry, while **3** showed no detectable peak as was observed with aqueous gels under DSC at higher concentration (60 mmol dm^{-3}).

To probe this apparent unusual concentration dependence of the thermotropic transition process, the relationship between steady-state fluorescence anisotropy (r) due to the vesicle doped 1,6-diphenyl-1,3,5-hexatriene and temperature was examined.[9] The temperatures related to the break in an r *vs.* T plot (not shown) for vesicular **1**, **2** and **5** were very similar to the T_m values obtained by DSC and microcalorimetry (Table 1). But, importantly, 2.5 mmol dm^{-3} vesicles of **3** and **4** gave no evidence of a break in the r *vs.* T plot.

We then examined the phase transition behaviour of the 2.5 mmol dm^{-3} vesicular matrices **2–5** by studying the temperature-dependent absorption changes of benzoyl acetanilide (BAA) doped in individual vesicular suspensions.[10] The breaks related to the plots of the ratios of the vesicle-doped BAA absorbencies due to enol (315 nm) and keto (250 nm), A_{enol}/A_{keto} *vs.* T gave T_m values (Table 1). No apparent T_m for **3** and **4** could be detected while vesicles of **2** and **5** gave T_m values that coincided well with those obtained by the above unrelated measurements (Table 1).

The aqueous dispersions were converted to regular self-supporting multilayered films by casting on glass slides.[11] Reflection XRD (Scintag XDS-2000) of these cast films gave single layer thicknesses of 33.4, 46.8, 39.1, 36.1 and 37.9 Å for **1–5** respectively. As estimated from CPK models, the molecular lengths of the ion-pair units are 40.5, 44.0, 39.0, 34.5 and 41.0 Å respectively for **1–5**, which show that while a tilted bilayer arrangement is indicated with **1** and **5**, **2–4** should support interdigitated structures.

To explore the origin of such exceptional findings on the basis of molecular modelling studies, ion pairs **1–5** were created using DISCOVER package of INSIGHT II (ver. 2.3.5, Biosym Tech. software) and their energy minimized conformations were calculated. In **2**, the two apolar undecanoate chains in the bolaphilic core originate from a central catechol unit and are at *ca.* 60° in their extended conformation. Even after ion-pairing, chain propagation in an angular fashion could lead to packing impairment. As indicated from molecular modelling, other conformational plans (tilted) are also possible to minimize apolar chain/water contacts. A number of such arrangements could coexist leading to concentration dependent phase-transition behaviour. In **3**, the undecanoate chains stem from a resorcinol and remain at *ca.* ≈ 120°. In the conformational plan with unit layer thickness *ca.* 39 Å (from reflection XRD experiment), the intramonomer van der Waals interactions between the chains become severely 'loose'. Variation of its concentration may lead to the formation of alternative structures, but the structural 'flaw' with the bolaphilic core persists leading to a lower phase-transition temperature (< 15 °C). The presence of an aromatic quinol moiety at the bolaphilic core of **4** allows the formation of tightly organized assemblies as the undecanoate chains from the central quinol unit span along opposite directions (*ca.* 180°). This conformational plan helps optimal van der Waals contacts upon ion-pairing with CTA^+ and also interaromatic π-stacking association. It is difficult to explain the observed effect on dilution of **4** on the basis of molecular-mechanics calculations alone. Dilution may lead to formation of other plans that may not support bilayer type organization. Whatever the exact reason, these observations show for the first time that vesicle formation may be controlled by variation of concentration.

These findings illustrate a novel approach to fine-tune vesicular properties and suggest new recipes for stable vesicular structures that may be of practical value. In particular, mixed bilayers composed of bacterial lipid (bolaamphiphile) and fatty acids of mammalian lipids (monopolar amphiphiles) could be tailored to have useful biological properties. These aspects are currently under examination.

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