Vesicle Formation by Double Long-chain Amidines

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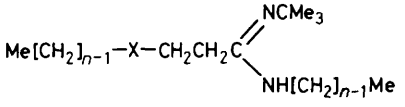
The formation and characterization of synthetic surfactant vesicles prepared from aqueous suspensions of a new class of amphiphiles with an amidine function as the polar head group are reported.

Phospholipids are known to assemble in several kinds of aggregates: micelles, inverted micelles, and bilayers.[1] The phospholipid bilayer vesicles or liposomes have received intense interest as models for biological membranes[2] and drug carriers.[3] Since a first report in 1973[4] vesicles have been obtained from a large variety of synthetic single chain, double chain,[5,6] and recently triple chain amphiphiles.[7] Vesicles derived from synthetic surfactants are now being extensively investigated for drug entrapment and release or for use in photochemical solar energy conversion.[6,8]

We report here on the formation and characterization of vesicles prepared from aqueous solutions of novel synthetic double chain surfactants with an amidine function as the polar head, compounds (**1**). The amidines (**1a—d**) were prepared by aminolysis of ethyl *N*-t-butylacrylimidate with the corresponding fatty amine. This previously unreported imidate[9] was obtained by ethanolysis of *N*-t-butylacrylonitrilium tetrachloroferrate.[10] Amidine (**1e**) was prepared from 4-oxaoctadecanitrile *via* aminolysis of its *N*-t-butylnitrilium tetrachloroferrate with tetradecylamine following the general experimental conditions reported earlier.[11] The structure of these new amidine molecules (**1a—e**) is in agreement with their elementary analyses, i.r., ^{1}H n.m.r., and mass spectral data.

Vesicle formation has been substantiated by substrate entrapment and gel filtration for compounds (**1b**) and (**1e**); thermotropic phase transitions have been observed for the compounds (**1b—d**) (see Table 1). Mild agitation (vortex) of a solid suspension or of a thin film of product (**1b**) or (**1e**) in hot water (40—70 °C) produced stable opalescent dispersions similar to liposome suspensions. Upon sonication, the turbidity (monitored at 400 nm) of an amidine (**1b**) dispersion in water decreased with time of exposure to the ultrasonic radiation down to a plateau value. Increasing the sonication time resulted in a decrease in hydrodynamic diameter from 138 nm (vortexed vesicles) to 87 nm (vesicles sonicated 8 min) as determined by dynamic laser light scattering experiments at 22 °C.[12] The formation under these conditions of closed vesicles was further demonstrated by the successful entrap-



(**1**) **a**; n = 12, X = NH
b; n = 14, X = NH
c; n = 16, X = NH
d; n = 18, X = NH
e; n = 14, X = O

Table 1. Comparison between melting point of the anhydrous compound and phase transition temperature (T_c) of amidines (**1a—e**) and saturated phosphatidylcholines (PC's).[a]

Compound	M.p./°C	T_c/°C		M.p./°C[b]	T_c/°C[b]
(**1a**)	26—30	<5	Dilauroyl PC	230	−1.8
(**1b**)	32—36	23.4	Dimyristoyl PC		23.9
(**1c**)	34—40	41.8	Dipalmitoyl PC		41.4
(**1d**)	39—46	51.2	Distearoyl PC		55
(**1e**)	Liquid				

[a] For d.s.c. measurements, the amidine dispersions (25 mM in 0.2 M Tris/HCl buffer initial pH 7.2) were prepared by vortex mixing. Measurements were carried out on 100 µl aliquots sealed in stainless steel sample pans on a Setaram DSC 111 apparatus operating at a scan rate of 2 K min^{-1}. [b] From refs. 14, 15.

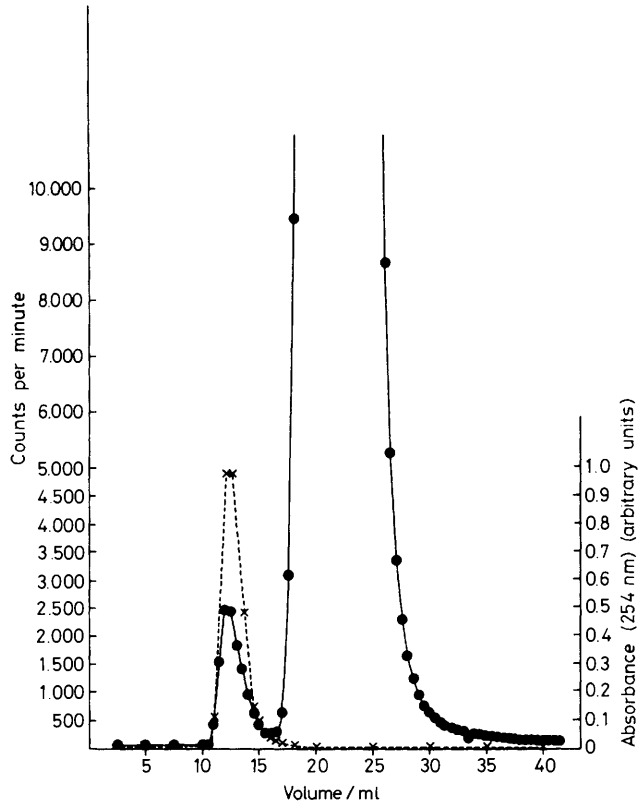


Figure 1. Gel filtration separation of free ^{14}C-labelled sucrose and ^{14}C-labelled sucrose entrapped in vesicles of (**1b**). Elution profile (·· × ···· × ··) and (^{14}C) radioactivity (—●—●—). Typically, 5.36 mg of (**1b**) were suspended in 2 ml of distilled water containing 2 μCi of ^{14}C-labelled sucrose, 1 ml of the suspension was added to a Sephadex G 25 column (1.6 × 16 cm) and eluted with distilled water.

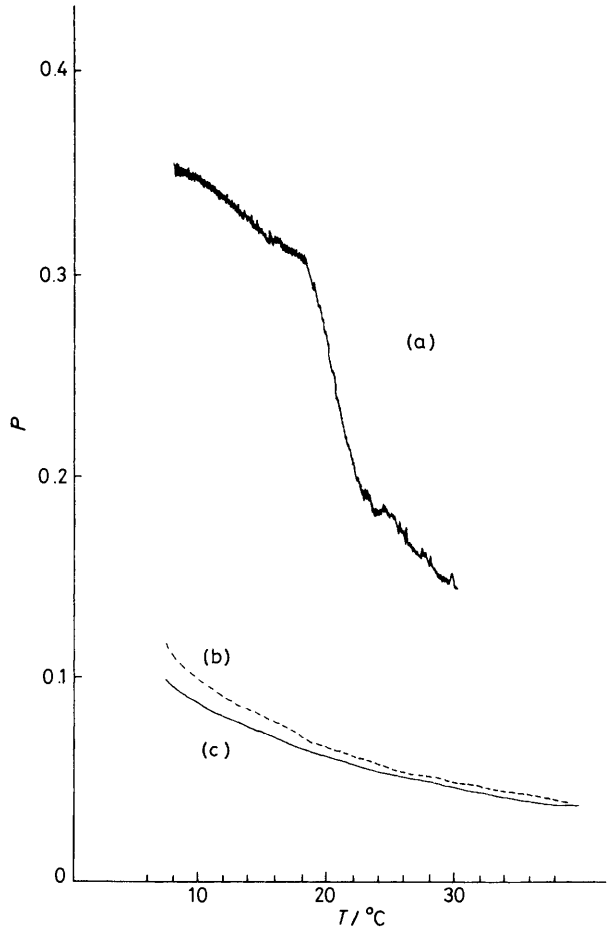


Figure 2. Temperature dependence of fluorescence polarization P of DPH solubilized in amidine suspensions (0.5 mM in H_2O). (a) Compound (**1b**) after vortex mixing; (b) single chain amidine $Me[CH_2]_{13}NHC(Me)=NCMe_3$ after sonication and (c) after vortex mixing.

ment of substrates like 2-aminopyridine[13] and ^{14}C-labelled sucrose (Figure 1) which were co-sonicated with the compound (**1b**) or (**1e**). Gel filtration on Sephadex G 25 M (Figure 1) separated the free molecules from those entrapped in the vesicles. Elution of these probes in the void volume (Figure 1) can only be explained in terms of their association with the surfactant vesicles. In the case of 2-aminopyridine at acid pH in particular, electrostatic repulsions mean that the solute is located in the vesicle-entrapped water compartments.† 0.5% of the ^{14}C-labelled sucrose was found associated with the vesicles of (**1b**), which corresponds to encapsulation of 0.9 l mol^{-1} amidine. Encapsulation values of 0.2 to 1.5 l mol^{-1} lipid (depending on lipid composition of the vesicles) have been reported for small unilamellar liposomes.[14]

Phospholipid vesicles undergo distinct structural changes at a characteristic temperature, the phase transition temperature (T_c, Table 1).[15] Below the T_c, the lipid molecules in the bilayer are in a highly ordered gel state, with their alkyl chains in an all-*trans* conformation. Above T_c, the lipid molecules become 'fluid' as the consequence of *gauche* rotational isomers and kink formation. Buffered dispersions of product (**1b**), (**1c**), and (**1d**) show temperature dependent phase transitions as determined by differential scanning calorimetry (d.s.c.). The T_c values obtained for these dispersions are nearly identical to those of the saturated phosphatidylcholines of corresponding chain length (Table 1). These results are highly suggestive of a bilayer organization in the amidine suspensions. Amidines having only one long alkyl chain (Figure 2) cannot form vesicles on vortexing or sonication. Indeed, the 'turbid' suspension obtained cannot be separated by chromatography and upon incorporation of the hydrophobic probe diphenylhexatriene‡ only the monotonic fluorescent depolarization profile characteristic of micelles was observed (Figure 2). The highly co-operative depolarization profile of compound (**1b**) is characteristic of a bilayer mode of organization.

A semi empirical conformational analysis which makes it

† D.s.c. and fluorescence polarization measurements have demonstrated that the phase transition characteristic of the bilayer organization was only obtained when the amidine was positively charged; the amidines thus form cationic surfactant vesicles which should be highly impermeable to cationic solutes such as 2-aminopyridine.

‡ The fluorescent label 1,6-diphenylhexa-1,3,5-triene (DPH) was introduced into the amidine film before hydration (DPH/lipid molar ratio 1:500); measurements were carried out on dilute (0.5 mM) vortexes or sonicated labelled dispersions with an Elscint microviscosimeter at a heating rate of 2.5 K min^{-1}.

possible to assemble amphiphilic molecules[16] indicates that (**1a**, **b**) adopt a cylindrical shape, highly favourable towards organization in bilayers.

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