Stabilization of Small Unilamellar Liposomes: Polymerization of Surfactants in Phospholipid Vesicles

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Cosonication of a styrene-containing surfactant $H_2C{=}CHC_6H_4NHCO[CH_2]_{10}N^+(C_{16}H_{33})Me_2Br^-$ (**1**) with dipalmitoylphosphatidylcholine (DPPC) leads to mixed DPPC–(**1**) 300 Å diameter unilamellar vesicles which subsequent to photopolymerization maintain their morphologies for months; conversely, nonpolymerized vesicles undergo spontaneous growth to 700 Å diameter vesicles.

Phospholipid vesicles, liposomes, are increasingly utilized as drug carriers, membrane models, and unique reaction media.[1,2] Many potential applications require stable small unilamellar vesicles. Below their phase transition temperatures small unilamellar vesicles undergo, however, spontaneous fusion.[3–6] The diameter of vesicles prepared from dipalmitoylphosphatidylcholine (DPPC) vesicles increases from *ca.* 300 Å to *ca.* 700 Å in the course of a few days.[4,7] We report here that cosonication of the styrene-containing surfactant (**1**)[8] with DPPC and subsequent photopolymerization completely obviates the growth of 300 Å diameter unilamellar vesicles.

$$H_2C{=}CHC_6H_4NHCO[CH_2]_{10}\overset{+}{N}(C_{16}H_{33})Me_2Br^-$$
(**1**)

Small unilamellar vesicles were prepared by the ultrasonic dispersal and subsequent ultracentrifugation[9] of DPPC, (**1**), and 2:1 w/w mixtures of DPPC and (**1**).† Phosphorus determinations[10] and absorption spectrophotometry‡ were utilized to verify vesicle concentrations.‡ The ratios of DPPC to (**1**) in the DPPC–(**1**) vesicles used† were found to be 1.6:1.0 (±10%). Vesicles prepared from (**1**) and from DPPC–(**1**) were polymerized by irradiation by a 450 W Xenon lamp for 2—6 h at 60 °C.[8] Absorption spectroscopy[8] and t.l.c.§ indicated the complete disappearance of styrene in polymerized (**1**) and DPPC–(**1**). Excitation of polymerized vesicles at 275 nm resulted in emission peaks at 330 nm (monomer) and 400 nm (excimer).[11] Conversely, the fluorescence spectrum of nonpolymerized DPPC–(**1**) vesicles contained predominantly monomers (emitting at 330 nm). Taken together, these results provide evidence for the formation of polystyrene surfactants [from (**1**)] in irradiated DPPC–(**1**) vesicles.

Hydrodynamic diameters of vesicles, monitored by dynamic laser light scattering,¶ as functions of incubation times at 23 °C are shown in Figure 1. DPPC and unpolymerized DPPC–(**1**) vesicles are seen to undergo spontaneous time-dependent growth. Further, increasing the concentration of a given vesicle or decreasing the temperature of incubation resulted in increased growth rate. Entirely analogous be-

† Typically, surfactants were dispersed in 0.01 M tris–HCl, pH = 7.5, buffer (3—6 mg/ml) at 60 °C for 15—20 min under N_2 by means of a Braunsonic 1510 ultrasonifier set at 150 W. Sonicated samples were promptly ultracentrifugated at 150 000 g, 45 °C for 3.5 h. A portion (*ca.* 1/4 of the total sample) of the supernatant liquid was filtered through 0.2 μm polycarbonate nucleopore membrane filter and used for subsequent measurements. The purity of DPPC (Sigma) was established by t.l.c.

‡ Extinction coefficient of (**1**) in MeOH at 270 nm = 1.68×10^4 mol dm^{-3} cm^{-1}. Extinction coefficient of (**1**) in DPPC–(**1**) vesicles at 262 nm determined for methanolic solutions of freeze-dried DPPC–(**1**) vesicles = 1.08×10^4 mol dm^{-3} cm^{-1}.

§ On Eastman 13179 silica gel sheets, using $CHCl_3$–MeOH–H_2O (65:35:4 v/v) as eluant. R_f values: unpolymerized (**1**), 0.88; unpolymerized DPPC, 0.31; polymerized DPPC–(**1**) 1:0.31, 0.41—0.81 broad.

¶ A Malvern 2000 light scattering system and a Spectra Physics 171 Ar^+ ion laser were used. Data were collected at $\theta = 90$ and 23 °C. Typical sampling times were 3—5 μs. Each measurement was carried out at least in triplicate. Q values (polydispersities) for the nonpolymerized DPPC–(**1**) vesicles increased from *ca.* 0.2 to 0.7. Conversely, Q values for polymerized DPPC–(**1**) and nonpolymerized DPPC remained *ca.* 0.2 for 7 and 1 week, respectively.

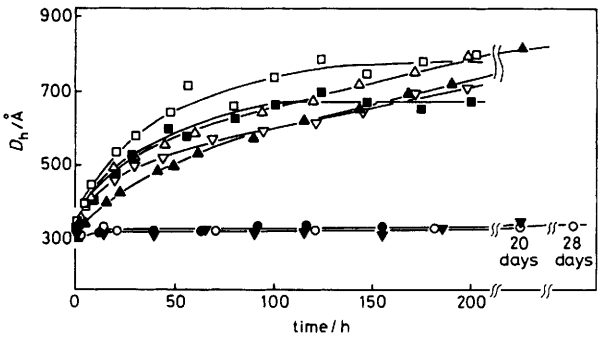

Figure 1. Spontaneous growth of DPPC (□, 1.02 mM; ■, 0.5 mM) and non-polymerized DPPC–(**1**) {△, [DPPC], 0.74 mM + [(**1**)], 0.44 mM; ▲, [DPPC], 0.47 mM + [(**1**)], 0.28 mM; ▽, [DPPC], 0.34 mM + [(**1**)], 0.23 mM} vesicles as a function of incubation time. Polymerized DPPC–(**1**) vesicles are seen to retain their sizes for extended periods {▼, [DPPC], 0.53 mM + [(**1**)], 0.35 mM; ●, [DPPC], 0.36 mM + [(**1**)], 0.25 mM; ○, [DPPC], 0.26 mM + [(**1**)], 0.16 mM} (M = mol dm^{-3}). Plotted are the hydrodynamic diameters (D_n) of the vesicles, determined by dynamic light scattering,¶ against incubation time at 23 °C.

haviour was reported previously for the spontaneous growth of DPPC vesicles.[4,7] The identical behaviour of DPPC and mixed DPPC–(**1**) vesicles further substantiates the similar morphologies of these aggregates. The different behaviour of polymerized DPPC–(**1**) vesicles is striking. Hydrodynamic radii and polydispersity indices (Q values)[7] of polymerized DPPC–(**1**) vesicles, unlike their nonpolymerized counterparts, remained essentially constant for months (Figure 1).¶

To the best of our knowledge, the polymerization of DPPC–(**1**) vesicles is the only method available at present which leads to the formation of such small *stable* unilamellar bilayer vesicles. In addition to potential utilizations, our approach allows the investigations of fusion mechanisms.

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