Spontaneous Vesicles from Aqueous Solutions of Aerosol OT and Choline Chloride Compounds

Single-walled, nearly monodisperse, equilibrium vesicles form spontaneously when cholinergics such as choline chloride, acetylcholine chloride, calcium salt of phosphorylcholine chloride, or succinylcholine chloride, a muscle relaxant, are added to the solutions of sodium bis[2-ethylhexyl]sulfosuccinate (AOT). Partial phase diagrams mapping the stable vesicle region are presented for the systems AOT/water/choline chloride, AOT/water/acyethylcholine chloride, AOT/water/phosphorylcholine chloride calcium salt, and AOT/water/succinylcholine chloride. The measured diameters for the vesicles made with these choline chloride compounds are 2000 Å for acetylcholine chloride and 1000 Å for choline chloride, succinylcholine chloride, and the calcium salt of phosphorylcholine chloride. © 1991 Academic Press, Inc.

INTRODUCTION

Single-walled monodisperse equilibrium vesicles form spontaneously when cholinergics such as choline chloride, acetylcholine chloride, the calcium salt of phosphorylcholine chloride, or succinylcholine chloride, a muscle relaxant, are added to aqueous solutions of sodium bis[2-ethylhexyl]sulfosuccinate (AOT).

Previously, biological membranes have been mimicked with vesicles formed with double-tailed surfactants (1). Preparation of single-bilayer vesicles required input of considerable mechanical energy, as opposed to their spontaneous formation in vivo. Spontaneity was often achieved with the addition of a third component, such as a single-tail surfactant (2) or surfactants with hydroxide counterions (3–5). Recently, we reported spontaneous formation of single-bilayer vesicles from aqueous mixtures of single-tailed cationic and anionic surfactants (6). This self-organization is driven by electrostatic attractions between the two oppositely charged head groups (6, 7).

Vesicles, with their capacity to encapsulate water-soluble solutes, are attractive vehicles for the controlled release of drugs, enzymes, hormones, or chelating agents (8). Vesicles act as reservoirs by storing water-soluble solutes in their interior cores, as synaptic vesicles store the neurotransmitter acetylcholine (9). Here we show that the addition of succinylcholine chloride, acetylcholine chloride, choline chloride, and the calcium salt of phosphorylcholine chloride to aqueous solutions of the double-tailed anionic surfactant AOT yields vesicles spontaneously.

EXPERIMENTAL

AOT, acetylcholine chloride, choline chloride, succinylcholine chloride (Serva, pharmaceutical grade), and calcium salt of phosphorylcholine chloride (Aldrich) were used as received. Stock solutions of individual components were prepared in deionized water. AOT stock solutions (micellar solutions containing less than 1.4 wt% AOT) (10) were titrated with stock solutions of each cholinergic to determine the vesicle phase boundaries. Vesicle formation has been demonstrated by quasi-elastic light scattering (QLS), freeze-fracture transmission electron microscopy (TEM), and glucose entrapment experiments as described earlier (6). Glucose entrapment experiments in choline chloride mixtures were carried out as follows. Vesicles were prepared in 0.075 M isotonic salt solution (to enable the glucose–enzyme reaction) and two separate experiments were performed with glucose added either before or after vesicle formation. In either case, 3.0 ml of vesicle solution was dialyzed for 12 h against 500 ml of 0.075 M isotonic salt solution. The vesicles were then destroyed by adding Triton X-100, 1.75 ml of this micellar solution was further dialyzed against 10.0 ml of 0.075 M isotonic salt solution for 6 h, and the presence of glucose in each of the dialyzates was determined (11). The vesicles prepared in 0.075 M isotonic salt solutions remained very stable and showed no increase in the turbidity as compared with their preparations in water.

RESULTS AND DISCUSSION

The vesicle phase boundaries (Fig. 1) depend on the cholinergic structure. The vesicle phase extends to higher concentrations when choline chloride is replaced by its esters, and the esters of monocarboxylic acid are more effective than esters of dicarboxylic acid. The smallest vesicular region (the unshaded region in Fig. 1) is observed with the calcium salt of phosphorylcholine chloride, which may be due to the fusogenic character of calcium ions. Vesicle diameters range from 600 to 2500 Å as shown by TEM (Fig. 2) and QLS. The vesicles appear to be unilamellar as can be seen from the cross-fractured vesicle in the center of Fig. 2. Vesicle size decreases with increasing choline chloride and phosphorylcholine chloride concentration, and multieponential fits of QLS data show the vesicles to be monodisperse. To determine the stability of these vesicles, AOT/water/choline chloride vesicles were subjected to a 22–60–22°C heating–cooling cycle. The
vesicles grew slightly during heating, and within the first 2 h of cooling to 22°C, the vesicle population became bimodal, with the smaller size vesicles becoming more numerous. After 24 h, the larger component of the population had disappeared and the vesicles recovered their original size. The largest vesicles are those made with acetylcholine chloride, and their size increased with increasing concentration of acetylcholine chloride. Multieponential analysis of the correlation function showed the population to be bimodal. The measured diameters for the vesicles made with these choline chloride compounds are 2000 Å for acetylcholine chloride and 1000 Å for choline chloride, succinylcholine chloride, and the calcium salt of phosphorylcholine chloride.

The glucose trapping experiments showed the amount of glucose present in each dialyzed was similar regardless of whether the glucose was added before or after vesicle formation. This result strongly suggests that glucose is either bound to the vesicle surface or entrapped in the vesicles during exchange of the vesicle contents with the solution exterior to the vesicle. Accordingly, it is difficult to estimate the entrapped volume quantitatively with this glucose experiment.

Aggregate behavior of ionic surfactants in aqueous solutions is known to depend strongly on the counterions and co-ions present in the medium (12, 13). A cationic

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**Fig. 1.** Partial phase diagram mapping the equilibrium vesicle region in AOT/water/cholinergic systems. Different shaded regions for succinylcholine chloride and acetylcholine chloride indicate their respective extensions of the vesicle regions beyond that of choline chloride. The molecular sizes of these three cholinergics are in the order acetylcholine chloride > succinylcholine chloride > choline chloride.

**Fig. 2.** TEM micrograph confirming vesicle formation in an AOT/water/choline chloride mixture (1.4/97.6/1.0% by weight). The arrow indicates a rare event of cross-fracturing of a vesicle, revealing a single bilayer wall.
double-tailed surfactant, didodecyldimethylammonium bromide (DDAB), shows greatly enhanced (10^4 times) solubility in water when the bromide counterion is replaced by the hydroxide counterion. Vesicles form spontaneously in mixtures of water and didodecyldimethylammonium hydroxide and bromide (5, 14). The hydroxide counterion with its high hydration number increases the area per head group and thus the curvature of the surfactant film at the surfactant-water interface. Evidently, the curvature of the surfactant film can be varied from highly curved to flat by using various mixtures of hydroxide and bromide counterions. Similarly, the choline chloride compounds examined here likely control the curvature of the AOT film by their increased hydrophilicity. That larger vesicles are obtained with acetylcholine chloride suggests that acetylcholine binds more strongly to AOT than do any of its cousins. This results in more screening of electrostatic repulsions between the AOT head groups that decreases the curvature of the surfactant film and so increases the vesicle size.

This is a novel method for the preparation of single-walled, equilibrium vesicles from aqueous solutions of AOT in the presence of cholingerics. These vesicles form spontaneously on mixing the surfactant solutions with the cholingeric compounds. Safran et al. (7) have presented a theoretical model to explain the spontaneous vesicle formation in aqueous mixtures of cationic and anionic single-tailed surfactants (6). According to this model the vesicular phase is energetically favorable over the lamellar phase when the two monolayers have different surfactant concentrations (nonideal mixing) and complexing interactions (such as cationic–anionic head group attractions) are present. In a similar way, we expect that nonideal mixing and complexing interactions (7) between the cholingerics and AOT are responsible for the observed spontaneous vesicle formation.

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