A Mixture of Surfactants that Can Induce the Release of Encapsulated Markers in the Presence of Specific Ions

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Since the pioneering finding that vesicle can be prepared from ion-paired surfactants (IPS),[1,2] various forms of IPS have been studied[3-13] together with the polymerizable ones.[14,15] Because the composed surfactants are loosely attracted by electrostatic and hydrophobic interactions, some IPS vesicles are collapsible to micelles in the presence of specific ions which can strongly bind one of the head groups and interrupt the ionic interactions among them.[16] Those IPS vesicles starts working at above certain ion concentration, and if the dissembled surfactants can attack other vesicles containing encapsulated markers, release of them will be triggered. The strategy is in line with the switch-on of release of encapsulated markers by pH,[17] photo irradiation,[18] and polymeric surfactants.[19] The intriguing IPS in Figure 1 were prepared and their bilayer properties were checked in the points of selective response to specific ions, release rate of markers, stability upon temperature change, and vesicle formation.

Previously, IPS was prepared by replacing counter ions with hydroxide ions through ion exchange and combining two oppositely charged surfactants at equimolar ratio in methanol, rendering tight ion pair between head groups. In this case we preferred weakly paired IPS which could better sense ions or temperature change and be more easily collapsible. As for IPS (I) the positively charged myristyltrimethylammonium bromide (MTAB) and sodium dodecylsulfate (SDS) were dissolved in methanol without ion exchange at various molar ratios, and stirred overnight. After removal of solvent, the residual white solid was recrystallized from ethylacetate/hexane 3 times. Because SDS was already well known as a membrane disrupting surfactant,[20] it could attack and break other vesicles when liberated from IPS after ion contact. The chain length of ammonium surfactant used as the cationic counterpart in IPS was designed to be slightly longer than that of SDS to bring some unstability in vesicle formation (see reference 21). In comparison, IPS (II) was made by passing MTAB and SDS through AG 1-X8 anion exchange resin (OH^- form) and IRP-64 cation exchange resin (H^+ form), respectively, and each solutions were combined and stirred in methanol at equimolar ratio overnight. Melting point of IPS (I) was 157 °C which was much lower than 204 °C of SDS and 237 °C of MTAB, and that of IPS (II) was 170 °C. Chemical shift of the methyl protons around ammonium group in proton NMR sptectrum shifted upfield from 3.5 ppm of MTAB to 3.0 ppm of IPS (I) and 3.2 ppm of IPS (II). Strong C-N stretch at 1470 cm^{-1} of MTAB was significantly reduced after ion pairing in FT-IR spectrum. On TLC, R_fs of IPS (I) and IPS (II) were 0.11 and 0.23 which were in between 0.35 of MTAB and 0.05 of SDS ($CHCl_3$: MeOH=5 : 1). Based on the above results, the mixture of surfactants was bound together through ionic interac-

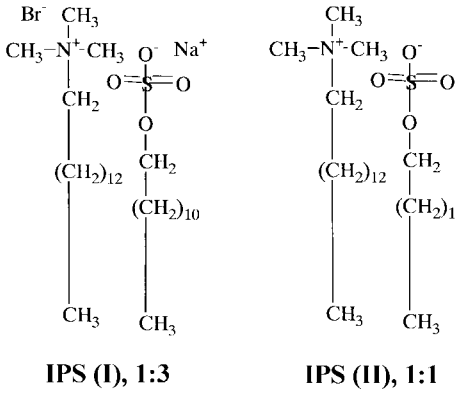

Figure 1. Structures of IPS (I) and IPS (II).

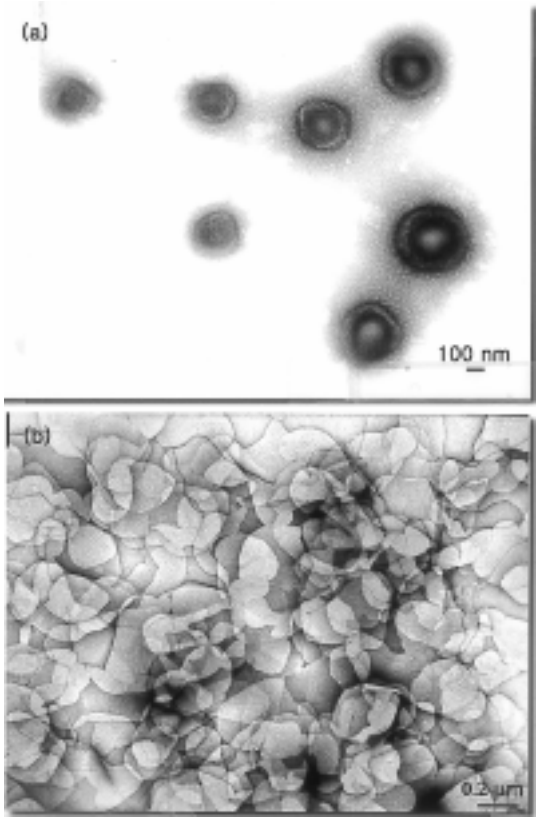

Figure 2. Transmission electron microscopy of (a) IPS (I) vesicles and (b) IPS (II) vesicles stained with uranylacetate. Bars represent the relative sizes.

tion instead of separate presence.

Vesicles of the IPS were prepared by extrusion through 0.4 μm Nuclepore polycarbonate filter under argon pressure at room temperature and the slightly milky dispersion was stable for a few days without showing any precipitates. In Figure 2, TEM (transmission electron microscopy) picture of the IPS (I) vesicles stained with uranylacetate confirmed vesicle formation with an average diameter of *ca*. 300 nm, coupled with that of IPS (II) vesicles. In order to get the gel to liquid crystalline phase transition temperature of the IPS vesicles, multilamellar vesicle (MLV) solutions of each IPS were scanned by differential scanning calorimeter (MAC science DSC 3100) from -20 ^{o}C to 50 ^{o}C to find the phase transition temp. of 9.8 ^{o}C for IPS (I) and 10.1 ^{o}C for IPS (II).

Critical aggregation concentrations of IPS vesicles were found by tensiometry in which surface tension was measured from 0.01 mM to 10 mM by Fisher Tensiomat 21. Compared to the critical micellar concentration (CMC) of MTAB (0.9 mM) and SDS (1 mM), the low critical concentrations of IPS (I) (0.03 mM) and IPS (II) (0.1 mM) were coming from ion pairing. Turbidity of the IPS solution (MLV) was measured at 400 nm by Hewlett Packard 8453A diode array spectrophotometer, scanning the spectrum with the increase of ion concentration. Turbidity of IPS (II) vesicles was not affected by the ions (K^+, Li^+, Mg^{2+}, Ca^{2+}, Al^{3+} and Ce^{4+}), but IPS (I) vesicles showed abrupt decrease in turbidity by the ions such as Al^{3+} and Ce^{4+} while K^+, Li^+, Mg^{2+}, and Ca^{2+} were not effective. Although we have tried various IPS with different combination of MTAB and SDS, the best sensitivity to the ions was observed at the combination of 1 mole of MTAB to 3 mole of SDS. The turbidity profile was shown in Figure 3.

Using the ion-sensing ability of IPS (I) vesicle, possibility of rupturing phospholipid vesicles carrying encapasulated markers was checked. We have used 1,2-dimyristyl-*sn*-glycerophosphatidylcholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero phosphatidylcholine (DPPC), and 1,2-distearyl-*sn*-glycerophosphatidylcholine (DSPC) to encapsulate 5,6-carboxyfluorescein (CF) as fluorescence marker. 2 mL of 0.1 mM CF in deionized water was used to disperse 10 mg of phospholipids and extruded vesicle solution through 0.4 μm filter was prepared by following the literature method.[22] Unencapsulated CFs were removed by gel-filtration through Sephadex G-50 column (40 cm $\times$ 1 cm), and the fractions containing encapsulated CFs were collected and divided into 3 parts. In the first one, 2 mL of the vesicle solution was mixed with 0.5 mL of deionized water. 0.5 mL of 5 mM IPS (I) solution was added for water in the second one. Into the third one, 100 μL of 0.1 M $Al(NO_3)_3$ was additionally added into the content of the second one. The three mixtures are loaded into three dialysis bags (spectrapore No. 2, 2.5 cm $\times$ 5 cm) in 300 mL of deionized water at 25 ^{o}C. 100 μL of the solution inside the bag was taken every 30 minutes and mixed with 2 mL of deionized water. Fluorescence intensity was measured at 490 nm (ex) and 518 nm (em), and % retention of the markers with time was compared for the three mixtures. To see the ion-sensing and actuating effect against more stiff membrane, 50 mol % of cholesterol was incorporated into the all three phospholipid membranes and CF release was compared by the same method. Addition of both IPS (I) and Al^{3+} induced leakage from all three phospholipid vesicles, showing instant release of most of CFs irrespective of cholesterol incorporation, while IPS (I) by itself was not so effective. In Figure 4 the release profiles of DMPC with cholesterol system were compared and other phospholipid systems, although not shown, also followed similar trends.

Based on NMR, IR, DSC, surface tension, melting point, and TLC data, anionic and cationic surfactants were paired even when mixed at unequal ratio. IPS was known to form vesicle due to the cylindrical shape of each surfactants coming from the reduction of headgroup spaces by ion pairing, but single chain surfactants formed micelles because of the wedge shape due to the big headgroup.[2] The weak electrostatic interaction for IPS (I) formed by three SDS molecules surrounding a MTAB molecule could be easily interrupted by addition of Al^{3+} so that the free SDS molecules reassembled to micelles that can attack phospholipid vesicles. In contrast, IPS (II) having more tightly bound surfactants was

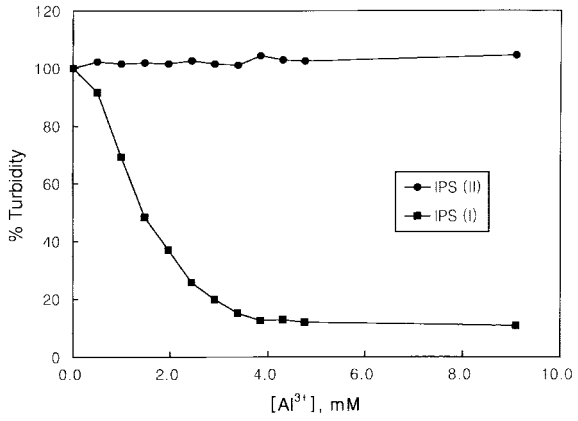

Figure 3. Turbidity change of IPS (I) and IPS (II) vesicles upon addition of Al^{3+}.

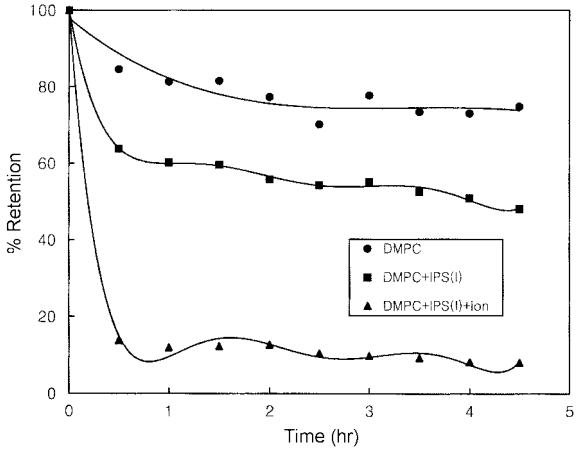

Figure 4. CF release profile of DMPC vesicles incorporated with cholesterol in the presence of IPS (I) only or both IPS (I) and Al^{3+}.

hard to be disintegrated by the ions. Having reported on the ion-sensing property of *n*-hexadecyltrimethylammonium palmitate IPS vesicle by Al^{3+} and Ce^{4+}, we presume that highly charged ions are necessary to break up head group interaction and reorganize surfactants assemblies. In addition to the ion-sensing effect, turbidity of IPS (I) vesicles was significantly reduced at 40 ^{o}C and was not recovered by lowering temperature, showing the possibility of temperature-sensing.

We have shown the possibility of IPS vesicles as inducer for the release of markers encapsulated by phospholipid vesicles. There are the critical combinations of cationic surfactants and anionic ones, and further structural optimization of the IPS vesicles can lead to the induction by monovalent or divalent ions, suggesting the possible application in drug delivery system targeting the infected cells with higher ion concentration than normal ones. There are still a lot of rooms to be developed in the application of IPS vesicle as a bridge between micelle and vesicle, and further work is undergoing in our laboratory.

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