Simple Method of Changing the Permeation Property of Surfactant Vesicle by Ion Pairing

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Received April 1, 1999

Various synthetic surfactants have been proposed as the alternative for lipid.[1] Ion pairing of oppositely charged surfactants through electrostatic attraction help forming vesicle and numerous ion-paired surfactants (IPS) have been reported, together with the polymerizable one.[2,3] One IPS vesicle can sensor specific ions such as Al^{3+} and Ce^{4+}, and collapse to micelle.[4] Ion pairing technique can be also applied to control the permeation of vesicular membrane. In this paper, the permeability of IPS vesicle is checked with encapsulated ions. Some IPS vesicle shows improvements in stability, and encapsulation of ion and fluorescence marker. Details of this finding and plausible explanation for the results are briefly discussed.

As shown in Figure 1, IPS (I), (II), and (III) are prepared. IPS (IV) is used as a control for IPS (II). IPS (V) and (VI) have a intervening spacer for ion-pairing. IPS was prepared by literature method.[2c] The solid product showed ion-pairing as confirmed by FT-IR in which disappearance of carboxyl peak at 1700 cm^{-1} was observed. The protons of ammonium group shifted from 3.5 to 3.3 ppm in NMR. Vesicle was made by bath-type sonicator, while probe sonicator was too powerful to form vesicle, resulting in clear solution. Extrusion method was not useful because of the clogging problem during extrusion.[5] IPS (I) surpassed other IPS in reliability and stability of vesicle formation. IPS (II), (III), (IV), (V), and (VI) did not form homogeneous solution, showing either precipitation or aggregation. Despite of the unstability, IPS (II) formed good vesicle when 20 mol% excess of dicetylphosphate was combined. But it was not stable either for more than a day. Accordingly, attention was paid to IPS (I) excluding the unstable ND14C6 vesicle. Vesicle formation of the remaining four IPS (I) was confirmed by transmission electron microscopy (TEM), staining with 2% uranyl acetate. Their sizes were about 400-700 Å. TEM picture of ND16C14 vesicle is shown in Figure 2.

Membrane properties of the four IPS (I) vesicle were checked by permeation and phase transition. Encapsulation and release of the fluorescence marker such as 4(5)-carboxyfluorescein (CF), riboflavin and fluorescein isothiocyanate-dextran (FITC-dextran, M_w = 4,000) was tried by dialysis method.[6] Riboflavin was avoided due to low capture efficiency, and CF showed aggregation and precipitation during vesicle preparation. FITC-dextran was encapsulated well and the vesicle was stable enough to measure permeation for more than 100 hrs. Thus it was the suitable marker for IPS vesicle, enabling the result shown in Table 1.

FITC-dextran was not released even by the temperature

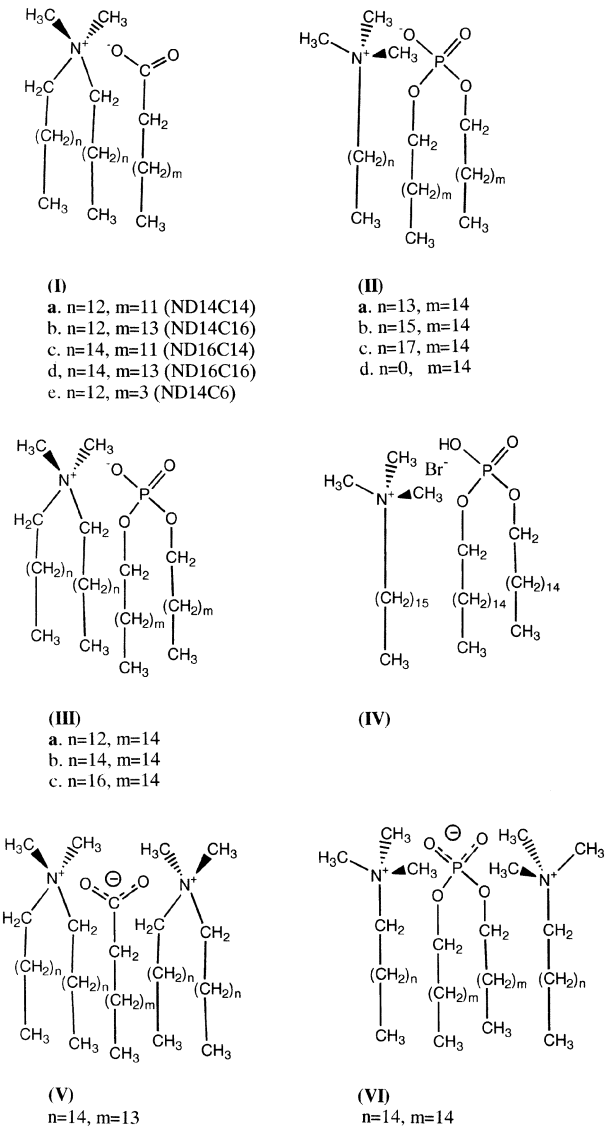

Figure 1. Structures of the Ion Paired Surfactant.

increase from 25 °C to 45 °C, 0.03% (w/v) SDS or Triton X-100,[7] and 0.2 mM Al^{3+} which induced the collapse of some IPS vesicle.[4] Ions could be also encapsulated and their release was followed with the ion sensitive indicators. For instance, release of encapsulated Al^{3+} could be monitored with CF outside of vesicle by following the decrease of fluorescence. Fluorescence of CF was drastically reduced at $[Al^{3+}] > 1\times10^{-4}$ M. As a control experiment, Al^{3+} solution was added to the hollow vesicle, followed by gel-filtration to

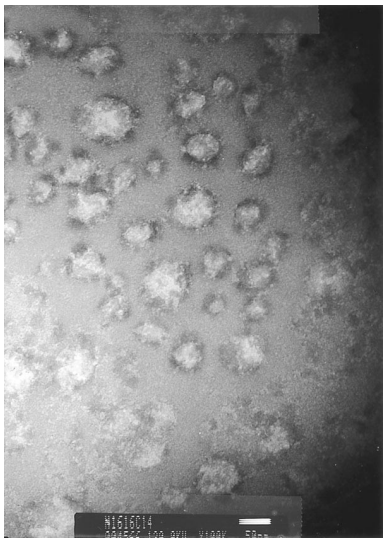

Figure 2. Transmission electon micrograph of the vesicle of ND16C14.

Table 1. Permeation rate (*P*) and phase transition temperature of IPS (I) vesicle

IPS	$P^a \times 10^9$, cm/hr			Transition Temp. (°C)	
	Dextran[b]	Al^{3+}	Mg^{2+}	DSC	Polarization
ND14C14	NR[c]	NR	NR	39	38-44
ND14C16	NR	13	5.4	38, 51	50-55
ND16C14	NR	3.5	3.6	51, 72	55-60
ND16C16	NR	NR	1.8	60	65-70

[a]Permeation rate is an average of two independent experiments and the temperature was kept at 25 °C. [b]FITC-Dextran, M_w=4,000. [c]NR=no release. Temperature was raised to 45 °C for NR, but the release was not observed.

remove unadsorbed ion, and the release was monitored by the same method. After initial fast but slight decrease by adsorbed ion, fluorescence intensity was almost constant, indicating that the slow decrease was originating from the permeation of ion within vesicle (Figure 3). Release of Mg^{2+} was followed with eriochrome black T (EBT) and the characteristic absorption peak at 705 nm decreased upon binding Mg^{2+}. As in table 1, release of Mg^{2+} is very slow for the four IPS (I) vesicle. A vesicle solution was heated to attain complete liberation of the ion and the decreased absorbance was used as 100% release for the calculation of permeation rate. Similar to the control experiment of Al^{3+}, the adsorbed Mg^{2+} showed very little absorbance drop.

Phase transition of the IPS (I) vesicle was detected by both differential scanning calorimetry (DSC) and polarization change of the probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) from 15 °C to 75 °C.[8] Polarization method provided somewhat intermediate value compared to DSC. Notably, the IPS vesicle showed higher transition temperature than dialkylammonium surfactants by more than 20 °C. Based on DSC results, 39 °C of ND14C14 and 60 °C of ND16C16 can be compared to the 16 °C of ditetradecyldimethylammonium bromide and 28 °C of dihexadecyldimethylammonium bromide respectively.[9] This supports denser and less permeable membrane of the IPS (I) vesicle.

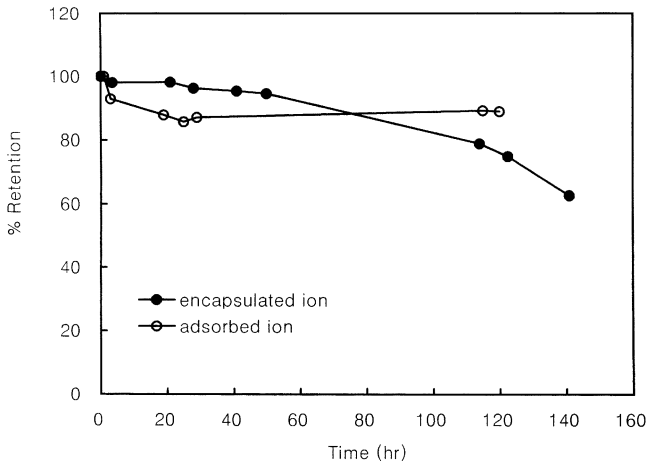

Figure 3. Comparison of the release of Al^{3+} from the vesicle of ND14C16 and the adsorbed one.

In conclusion, the stability of IPS vesicle is highly dependent on the component. The fact that IPS (I) vesicle encapsulates FITC-dextran and ions like Mg^{2+} and Al^{3+} well supports the enhanced membrane tightness together with the DSC data. As for IPS (I), intervening carboxylate group reduces repulsion between ammonium head groups and attracts nearby ammonium surfactant. Presence of four oxygen atoms and bulkiness of dicetylphosphate do not help forming tight ion pair in IPS (II), (III), and (IV) vesicle. In IPS (V) and (VI), the inserted monovalent anionic chain does not strongly attract two ammonium surfactants, unlike the dicarboxylate chain.[10] Enhanced membrane tightness established simply by ion-pairing an oppositely charged surfactant will be very useful in permeation control. Considering the antibacterial activity of the ammonium surfactant,[11] it is quite feasible to design a new carrier with controlled permeability and ion-sensing capability.

Experimental Section

***N,N*-dimethyl-*N,N*-dihexadecylammonium hexadecanoate.** 287 mg (0.500 mmol) of *N,N*-dimethyl-*N,N*-dihexadecylammonium bromide in 10 mL of methanol was converted to hydroxide form by passing through ion-exchange resin (Bio-Rad AG-1X8), and mixed with 128 mg (0.500 mmol) of *n*-hexadecanoic acid. After stirring overnight, solvent was evaporated and residue was recrystallized from ethylacetate/hexane to get 350 mg of colorless solid (93%): mp = 94 °C; R_f = 0.9 ($CHCl_3$/MeOH, 1/1); ^{1}H NMR ($CDCl_3$, 300 MHz) 0.90 (t, 15H, $C\underline{H}_3$), 1.0-1.5 (s, 72H, $C\underline{H}_2$), 1.65 (m, 4H, $C\underline{H}_2CH_2CH_2N^+$), 2.0 (m, 4H, $C\underline{H}_2CH_2N^+$), 2.15 (t, 2H, $C\underline{H}_2CO_2$), 2.35 (t, 2H, $C\underline{H}_2CH_2CO_2$), (3.1-3.3, 10H, $C\underline{H}_3N^+C\underline{H}_2$); IR (NaCl) 1573 cm^{-1} (C = O), 1468 cm^{-1} (C-H), 719 cm^{-1} (N-CH_3). Anal. Calcd. for $C_{50}H_{103}NO_2$. $1H_2O$: C, 78.16; N, 1.82. Found: C, 78.37; N, 1.56.

***N,N*-dimethyl-*N,N*-dihexadecylammonium tetradeca-**

noate. 574 mg (1.00 mmol) of *N,N*-dimethyl-*N,N*-dihexadecylammonium bromide and 228 mg (1.00 mmol) of *n*-tetradecanoic acid were used and 480 mg of colorless solid was obtained (66%): mp = 87 °C, R_f = 0.89 ($CHCl_3$/MeOH, 1/1); ^{1}H NMR ($CDCl_3$, 300 MHz) 0.80 (t, 9 H, C**H**$_3$), 1.1-1.4 (s, 68H, C**H**$_2$), 1.60 (m, 4H, C**H**$_2$$CH_2CH_2N^+$), 2.00 (m, 4H, C**H**$_2$$CH_2N^+$), 2.10 (m, 2H, C**H**$_2$$CH_2CO_2$), 2.40 (t, 2H, C**H**$_2$$CO_2$), 3.1-3.3 10H, C**H**$_2$$N^+$C**H**$_3$); IR (NaCl) 1570 cm^{-1} (C = O), 1467 cm^{-1} (C-H), 759 cm^{-1} (N-CH_3). Anal. Calcd. for $C_{48}H_{99}NO_2$. 1H_2O: C, 77.87; N, 1.89. Found: C, 77.94; N, 2.00.

***N,N*-dimethyl-*N,N*-ditetradecylammonium tetradecanoate and *N,N*-dimethyl-*N,N*-ditetradecylammonium hexadecanoate** were prepared according to the above procedure, and NMR, IR, and elemental analysis data agree with the structures.

Permeation Measurement. Encapsulation of FITC-dextran and permeation measurement was done following the previous method.[10]

Into a 3 mg of surfactant thin film, 1 mL of 1×10^{-3} M $Al(NO_3)_3$ was added, and the mixture was vortex-mixed and sonicated for 3 minute. After removing the unencapsulated Al^{3+} by gel-filtration, fractions containing the encapsulated ion were collected. 1 mL of the vesicle was mixed with 2 mL of 1×10^{-3} mM 4(5)-carboxyfluorescein. Decrease of fluorescence was followed at 25 °C or 45 °C (E_x: 480 and E_m: 518). As a control, 3 mg of surfactant and 900 μL of water were used to prepare the empty vesicle, into which 100 μL of 1×10^{-2} M $Al(NO_3)_3$ was added, followed by gel-filtration. 1 mL of the solution was mixed with 1 mL of 1×10^{-3} mM CF, and the decrease of fluorescence was followed.

As for the permeation of Mg^{2+}, 3 mg of surfactant and 1 mL of 1×10^{-3} M $MgCl_2$ was used and encapsulated vesicle was prepared by the same method as above. 500 μL of the solution was mixed with 500 μL of 1×10^{-3} M NaOH and 500 μL of 1×10^{-4} M eriochrome black T (EBT). The absorbance decrease at 705 nm was followed for more than 100 hrs. As a control experiment, 3 mg of surfactant in 900 μL of deionized water was used to obtain the hollow vesicle and 100 μL of 1×10^{-3} M $MgCl_2$ was added, followed by gel-filtration. Into 500 μL of the vesicle was added 500 μL of 1×10^{-3} M NaOH, 500 μL of 1×10^{-4} M of EBT, and 1.5 mL of water. Absorbance decrease at 705 nm was monitored.

Polarization and DSC was measured following previous method.[10]

Acknowledgment. Authors would like to thank Myung-Hoon Chung for technical assistance and Prof. Hoseup Yoon at Ajou University for DSC measurement.

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