Communications

Ion-Sensing Property of an Ion Pair Amphiphile

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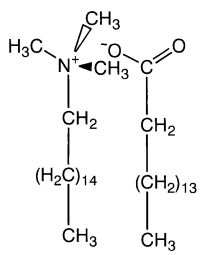
Continuous effort has been made in the field of controlled release, triggering the unloading of encapsulated content at a desired point by pH change,[1~3] photo-irradiation,[4] pore formation,[5] and the addition of surfactant sensitive to osmotic pressure difference in and out of the membrane.[6] The ion-sensing method that we are going to present in this paper is very unique in this area, contrasting with using the conventional liposome which is not instantly transformed by the presence of ions, so that a totally different type of surfactant is needed for the ion-sensing purpose.

Meanwhile, ion pair amphiphile (IPA) was introduced not long ago, showing that the micelle-forming single chain surfactant could also form bilayer structure through ionic interaction between positive and negative charge on their head groups, together with the hydrophobic interaction along hydrocarbon chains.[7~14] Although several solid evidences for the formation of bilayer structure have been reported, low stability of the IPA vesicle hinders widespread application in the area where liposome is deeply involved. One exception is the polymerized one in which the head group is covalently cross-linked to resolve the stability problem.[15,16] Adversely, the relatively unstable characteristic of IPA vesicle may be conducive to the quick response to the presence of some ions. Here, we introduce the new and original ion-sensing method of the IPA with 16 carbon chain (IPA-16) which shows more reliable vesicle formation than 14, and 18 carbon chain IPA.

To prepare IPA-16 cetyltrimethylammonium bromide (CTAB) was ion-exchanged to hydroxide form by passing through AG 1-X8 ion-exchange resin (Bio-Rad), and stirred with one equivalent of palmitic acid (PA) in methanol for a day, producing a white solid after 3 recrystallization from ethyl acetate. Ion pairing was checked by monitoring the shift of the carboxyl peak from 1700 cm^{-1} of free form to 1560 cm^{-1} of ion pair in FT-IR spectrum. The formation of vesicle from IPA-16 was confirmed by transmission electron microscopy (TEM), and gel-filtration profile after encapsulation of 4(5)-carboxyfluorescein (CF) showed good entrapment. Because one of the main forces holding two single chains in IPA-16 was ionic interaction, buffer solution was avoided and only deionized water was used during the experiment. Actually, clogging problem occurred during gel-filtration and membrane filtration in case of using buffer solution.



IPA-16

Structure 1

Because IPA-16 has two 16-carbon chains, the bilayer property can be compared to that of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC). Differential scanning calorimetry (DSC) showed the main transition at 41.6 °C which happened to be the same as DPPC (T_c= 41.6 °C), contrasting with the 1:1 mixture of CTAB and PA which did not show any transition over the same temperature range. Polarization data by 1,6-diphenyl-1,3,5-hexatriene (DPH) embedded in the IPA-16 bilayer also presented a sharp decrease at around the 40 °C. Both data supports similar phase transition behavior of IPA-16 as DPPC.

With the IPA-16 in hand, various ions were tested against sonicated vesicle solution to find out the ions capable of rupturing bilayer structure by following their turbidity change at 300 nm. Ions are diversified depending on the type of charge, the number of charge, and the hydrophobicity. The turbidity decrease by $AlCl_3$, and NaCl is shown in Figure 1 over a range of ion concentration.

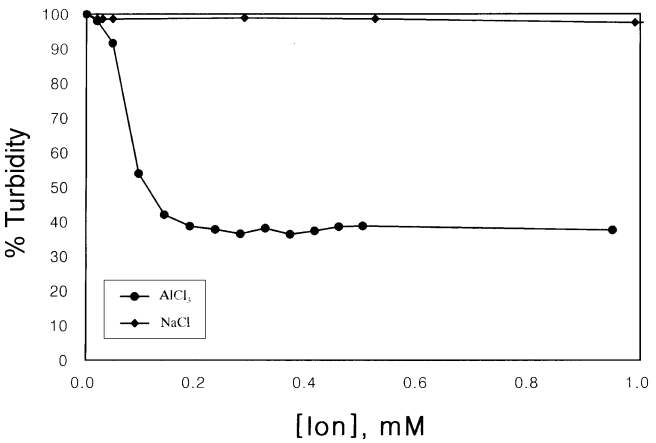


Figure 1. Turbidity decrease of IPA-16 vesicle at various ion concentration.

Table 1. Classification of Effective Ions Toward the Rupture of IPA-16 Vesicle

Effective[a]	Less Effective[b]	Aggregating[c]
$AlCl_3$, $Al(NO_3)_3$	NaCl, NH_4Cl, $CaCl_2$,	PbO_2, EDTA
$FeCl_3$, $Fe(NO_3)_3$	[d]TAB, [e]DTAB	NaH_2PO_4
$CrCl_3$, $Cr(NO_3)_3$	[f]HTAB, [g]OTAB	
$SnCl_2$, $Ce(SO_4)_2$	[h]SA, [i]SH, [j]SO, [k]SD	

[a]At 0.2 mM of added ion, the turbidity has decreased by more than 50%, [b]At 0.2 mM of added ion, the turbidity has decreased by less than 20%. [c]Addition of ion has increased the turbidity. [d]TAB: Tetramethylammonium Bromide, [e]DTAB: Decyltrimethylammonium Bromide, [f]HTAB: Hexadecyltrimethylammonium Bromide, [g]OTAB: Octadecyltrimethylammonium Bromide, [h]SA: Sodium Acetate, [i]SH: Sodium Hexanoate, [j]SO: Sodium Octanoate, [k]SD: Sodium Decanoate.

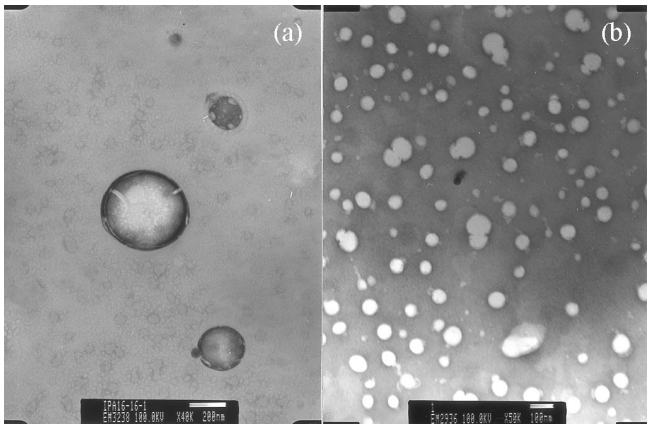


Figure 2. TEM micrograph of the IPA-16 vesicle (a) without ions and (b) with $AlCl_3$.

As in Table 1, monovalent ions are less effective than trivalent or highly charged ions in clearing the vesicle solution, and ammonium and carboxylate type ions are generally less effective. Hydrophobicity change among the ions with different chain length do not contribute much to the disruption of vesicle, while some additives like EDTA, PbO_2, NaH_2PO_4 have increased turbidity by inducing aggregation of each vesicle. Highly charged ion can more strongly associate with the oppositely charged chain of IPA than monovalent one and thus hinder the ion pairing, which eventually dissociates the double chain structure into a single chain one, preferring the micellar structure. In Figure 2, TEM picture shows that the diameter of the IPA-16 vesicle has been greatly reduced by the addition of $AlCl_3$, the effective ion.

After turbid solution was cleared with the addition of the effective ion, it was then dialyzed against deionized water for a few days. 80 percent and 150 percent increase in turbidity was observed after 3 days and 5 days of dialysis, respectively, suggesting that dismantling and association of vesicular structure could be reversibly controlled. The idea of ion-sensing by IPA was also supported by gel-filtration with and without $AlCl_3$ or $Al(NO_3)_3$, Vesicle fractions without ions showed much higher turbidity than those with ions, indicating that most of vesicle had reassembled into smaller micellar structure in the presence of ions.

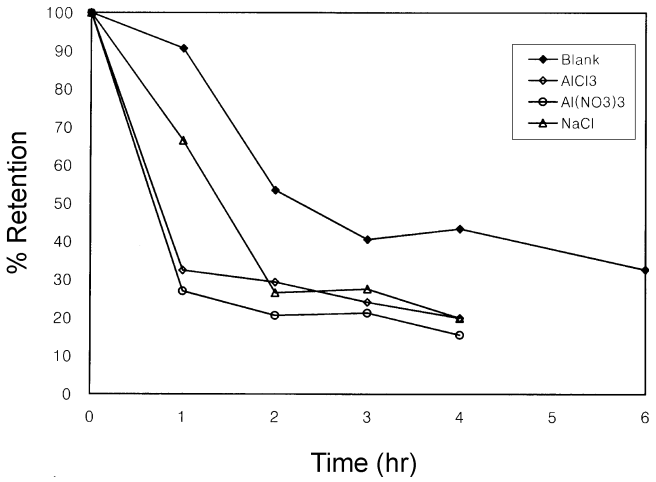


Figure 3. Release of FITC-Dextran from the IPA-16 vesicle at 0.2 mM of ion.

To take advantage of the above ion-sensing characteristic, fluorescein isothiocyante-dextran (FITC-Dextran, Mw= 4,000) was encapsulated with IPA-16 vesicle and the unencapsulated FITC-Dextran was removed by gel-filtration. Into the vesicle solution with encapsulated FITC-Dextran was added one of the tested ions to get a final concentration of 0.2 mM, and the release of the marker was monitored at room temperature by dialysis method.[17] As in Figure 3, $AlCl_3$, and $Al(NO_3)_3$, the effective ions, affected the release profile most significantly, and NaCl, the less effective one, did also increase the permeation but not as much as $AlCl_3$. Permeation property was also checked by encapsulating CF, but the quenching of fluorescence intensity by $AlCl_3$ and $Al(NO_3)_3$ prohibited further investigation.

The ion-sensing of IPA can be explained through the following equilibrium, in which C, and A represent positively and negatively charged component, respectively, and XY is the salt added. Depending on relative affinity between IPA component and added ion, equilibrium position is determined, and concomitant upkeep of vesicular structure and breakdown to micellar structure will be distributed.

$$C^+A^- + X^+Y^- \rightleftharpoons C^+Y^- + X^+A^-$$

These findings open a new horizon in the application of IPA surfactants, and some modification such as varying the number of chains to improve stability is required for practical application. Additionally, ion-sensing ability of IPA can be utilized as an actuator for the release of content encapsulated within the vesicle of phospholipid, in which dissociated components of IPA work as disrupting surfactant toward phospholipid vesicle.[18] A compromise between stability and ion-sensing needs to be precisely controlled.

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