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FUSION AND PHASE SEPARATION OF AMMONIUM BILAYER MEMBRANES[1)]

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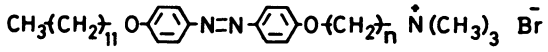
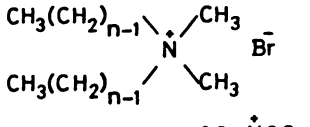
The fusion process of separate aggregates and the phase separation in mixed membranes were demonstrated by using the blue shift of stacked azobenzene chromophores in the aqueous ammonium bilayer system.

Synthetic bilayer membranes have been shown to possess physicochemical characteristics common to those of the biolipid bilayer membrane. For instance, the bilayer of dialkyl amphiphiles undergoes the gel-to-liquid crystal phase transition in a manner similar to that of lecithin bilayers,[2)] and the phase transition was shown to affect the catalytic property of ammonium bilayer membranes in nucleophilic displacement[3,4)] proton abstraction[5)] and decarboxylation.[6)]

Fusion and phase separation are other important bilayer characteristics related to the membrane function. It was shown previously in the ammonium bilayer system that an intra-vesicle reaction is much faster than the inter-vesicle counterpart,[7)] although the process of vesicle fusion is yet to be elucidated. We describe in this article that aggregate fusion and phase separation can be studied spectroscopically by using membrane-forming amphiphiles with azobenzene chromophore.



1 n = 2, 4, 10.



2 2CnN+2C1

Azobenzene amphiphiles 1 were shown to form bilayer aggregates when dispersed in water.[8)] Their absorption maxima are located at 355 - 360 nm in organic media (ethanol, chloroform and benzene), but shift to shorter wavelengths in water: λ_{max} = 340 nm for 1 (n = 2 and 4), and 330 nm for 1 (n = 10). It has been known that Methyl Orange (an azobenzene derivative) shows extensive blue shifts due to aggregation in the domain of aqueous cationic polymers[9)] and aqueous CTAB micelles.[10)] The blue shifts observed for the azobenzene amphiphiles in water are similarly attributable to stacking of the chromophore. In fact, the blue shift disappears when 1 is diluted by a large excess of aqueous CTAB micelles or $2C_nN^+2C_1$ membranes. The spectral shift due to chromophore stacking is a useful tool for elucidation of membrane fusion and phase separation phenomena. Amphiphile 1 (n = 10) was used for

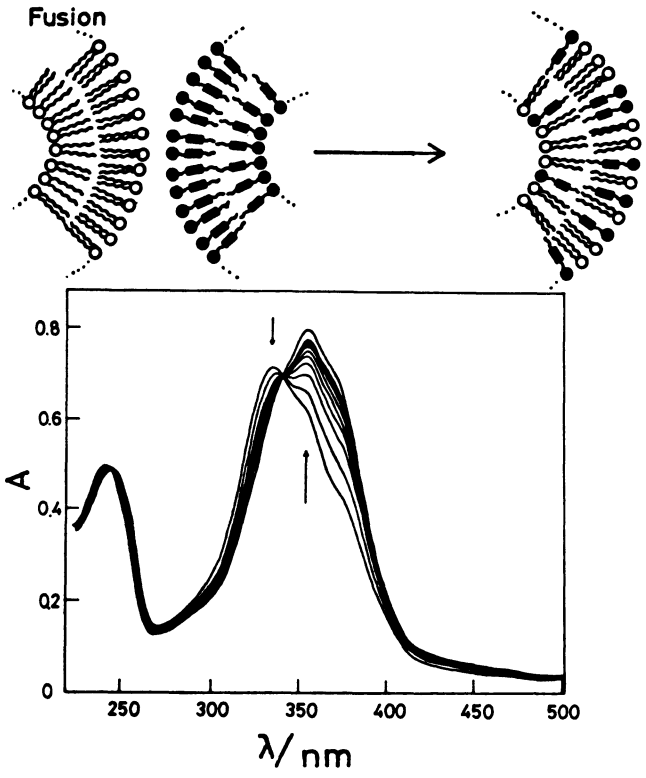


Fig. 1 Spectral change due to membrane fusion. $[2C_{18}N^{+}2C_1] = 5 \times 10^{-4}$ M. $[\underset{\sim}{1}(n = 10)] = 5 \times 10^{-5}$ M. 50°C. The arrow indicates the direction of the spectral change.

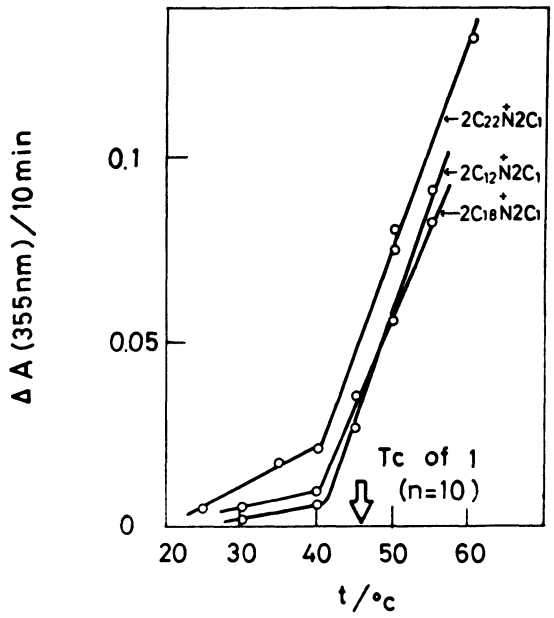


Fig. 2 Temperature dependence of rate of fusion. $[2C_nN^{+}2C_1] = 5 \times 10^{-4}$ M. $[\underset{\sim}{1}(n = 10)] = 5 \times 10^{-5}$ M. The rate of fusion(ordinate) is estimated by the absorbance increase at 355 nm in 10 min after mixing of aggregate solutions.

this purpose in most cases, because it gave the largest blue shift.

In the fusion experiment, aqueous bilayer dispersions of $2C_{18}N^{+}2C_1$ and $\underset{\sim}{1}$(n = 10) were prepared separately by sonication(Bransonic cell disruptor, 185) and mixed in a thermostated 1-cm quartz cell. Figure 1 shows the spectral change with time at 50°C. The initial λ_{max} at 335 nm(stacked species) disappears slowly and a new maximum appears at 355 nm(isolated species). This spectral change is apparently produced by dilution of $\underset{\sim}{1}$ by the dialkylammonium matrix due to aggregate fusion(see illustration). The fusion process could be confirmed by differential scanning calorimetry(DSC). The phase transition temperatures(T_c) of aqueous dispersion of $2C_{16}N^{+}2C_1$ and $\underset{\sim}{1}$(n = 10) are 28° and 46°C, respectively.[2,8] When an equimolar mixture of these dispersions was subjected to repeated DSC scan, the original peaks gradually disappeared along with appearance of new peaks at 24 and 30°C.[11]

Figure 2 illustrates the temperature dependence of the initial rate of fusion. The dependence is expressed by two lines with breaks at 40 - 42°C with the three dialkylammonium membrane matrices. The T_c values of these membranes are quite different: 66 - 70°C for $2C_{22}N^{+}2C_1$; 45°C for $2C_{18}N^{+}2C_1$; 10°C for $2C_{12}N^{+}2C_1$. Therefore, the common inflection temperature observed may be associated with

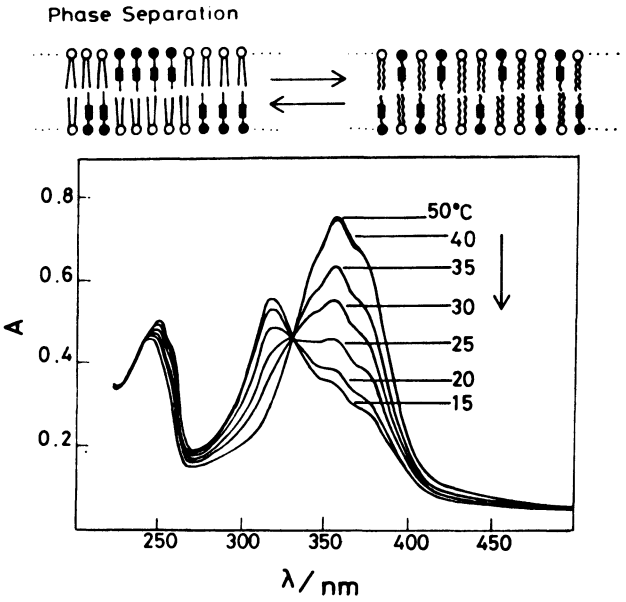


Fig. 3 Spectral change due to phase separation in mixed bilayer membranes. $[2C_{18}N^{+}2C_1] = 5 \times 10^{-3}$ M. [1(n = 10)] = 5×10^{-4} M.

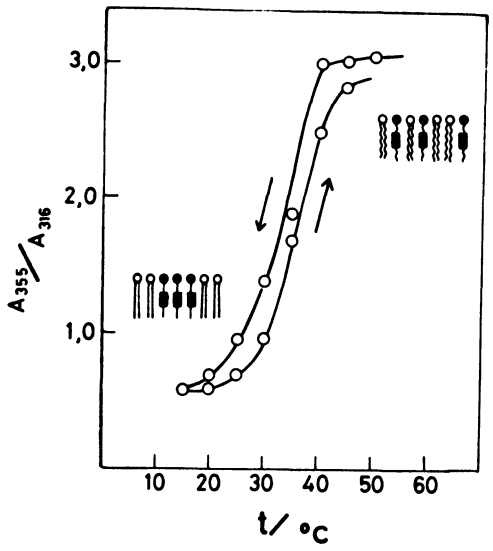


Fig. 4 Temperature dependence of relative absorbance(A_{355} / A_{316}).

T_c(46°C) of 1(n = 10) rather than those of $2C_nN^{+}2C_1$ membranes.

The absorption spectrum of the fusion product changes with temperature. Figure 3 shows the absorption spectrum of a well-sonicated 1:10 mixture of 1(n = 10) and $2C_{18}N^{+}2C_1$ in water. When the mixture was incubated for 1 h at 50°C, λ_{max} appeared at 355 nm, indicating the presence of monomeric azobenzene species. The monomer peak started to decrease upon cooling to temperature below 40°C and a new peak due to the azobenzene cluster appeared at 316 nm. Each plot was measured after incubation for 1 h at the respective temperature. The trend continued until 15°C at which temperature the monomer peak disappeared almost completely. Analogous spectral changes were noted for 1:10 mixtures of 1(n = 10) with $2C_{16}N^{+}2C_1$ and $2C_{14}N^{+}2C_1$ membranes.

The ratio of monomeric and clustered azobenzene chromophores is estimated by the relative absorbance at 355 and 316 nm. Figure 4 describes the dependence of relative absorbance(A_{355} / A_{316}) on temperature, as estimated from the data of Fig. 3. The absorbance ratio decreases with temperature lowering from 50°C and a fairly sharp inflection is present at around 40°C which corresponds approximately to T_c of the $2C_{18}N^{+}2C_1$ matrix membrane. The heating curve does not coincide with the cooling curve in spite of 1-h aging.

These data are explained by the schematic illustration of phase separation shown in Fig. 3. The azobenzene-amphiphile is isolated in the matrix membrane at

temperatures above 40°C where the bilayer matrix is in the liquid crystalline state. When the bilayer solidifies at low temperatures, the azobenzene chromophore separates as impurity, leading to cluster formation.

In conclusion, fusion and phase separation are explicitely discussed for the first time for the synthetic bilayer assembly. The functional regulation of the synthetic bilayer membrane would be realized by appropriate uses of these phenomena.

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11) The experimental procedure for DSC is described in Ref. 2.

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