CHEMISTRY LETTERS, pp. 33-36, 1994.

Thermal Stability and Specific Dye Binding of a Hydrogen-Bond-Mediated Bilayer Membrane[1)]

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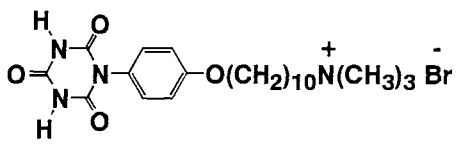
A novel aqueous bilayer membrane that is formed from amphiphilic complexes of complementary hydrogen bond pairs provided a specific dye binding site similar to that of the conventional ammonium bilayer. Partial dissociation of the hydrogen bond pairs and the consequent component separation occurred by heating the aqueous bilayer to 80 - 100 °C.

Molecular recognition via hydrogen bonding is a fundamental biological process and constitutes a rapidly growing branch of organic chemistry.[2)] Construction of hydrogen-bond-directed molecular assemblies in water is a chalenging target, since hydrogen bonding is most effective in solid states or in noncompetitive (aprotic) organic media. We have recently shown that suitably designed isocyanuric acid derivative **1** and substituted melamine **2** form in water a stable bilayer membrane that contains extended arrays of complementary hydrogen bonds.[3)] The conventional bilayer membranes often possess specific surface structures that are derived from regular molecular alignment. The hydrogen-bond array may lead to an additional unique feature. In this study, we conducted a careful DSC study and used anionic cyanine dye NK 2012 and 1,6-diphenyl-1,3,5-hexatriene (DPH) as spectral probes and examined their spectral changes in relation with the bilayer phase transition. The cyanine dye is bound specifically onto the bilayer surface of conventional double-chain ammonium amphiphiles and displays marked spectral changes.[4,5)] DPH has been used as a fluorescence probe of bilayer phase transitions.[6)]

A transparent aqueous dispersion of the **1**-**2** complex (ca. 30 mM) was



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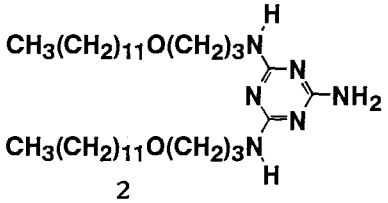

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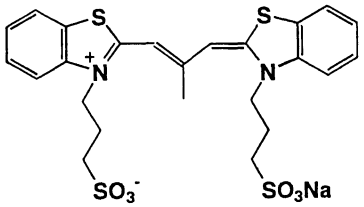

NK 2012

prepared by ultrasonication.[3] Differential scanning calorimetry (DSC ; instrument, Seiko Electronics DSC 120) of the aqueous **1-2** showed concentration-dependent endothermic peaks. At a concentration of 20 mM, a broad endothermic peak was observed between 70 °C to 100 °C (ΔH; ca. 50 kJ mol^{-1}) in the first heating process, whereas a 5 mM dispersion possessed a broad peak which was located at a lower temperature range of ca. 50 to 80 °C with ΔH of 80-100 kJ mol^{-1}. When the heating scan was repeated up to 100 °C, a new endothermic peak gradually grew at 53 °C in addition to the original broad peak. Reproducible isotherms were obtained after the 3rd scan, ΔH of the 53 °C-peak amounting to 18% (at 20 mM) or 6% (at 5 mM) of the total heat absorption. The new endothermic peak is ascribed to the gel-to-liquid crystal phase transition of the uncomplexed aggregate of **2**, since an aqueous dispersion of **2** (5 mM) gave an endothermic peak at the same temperature. On the other hand, when the heating scan was repeated up to 80 °C (5 mM dispersion), the original broad peak remained intact without a new peak. Thus, we conclude that the **1-2** composite undergoes partial dissociation at temperatures of 80 - 100 °C.

Figure 1 shows absorption and fluorescence spectra of NK 2012 (Nippon Kankoh-Shikiso Kenkyusho Co. Ltd) bound to aqueous **1-2** (solid line) and those observed in the presence of **1** alone (dotted line). Absorption maxima in aqueous **1** are located at 505 and 541 nm, and are similar to those in liquid crystalline bilayers.[5] The corresponding fluorescence emission appears at 585 nm. In contrast, a sharp absorption peak (at 565 nm) and remarkably enhanced fluorescence peak with a small Stokes shift (at 575 nm) are observed in the presence of aqueous **1-2**. The latter spectral characteristics are typical of the J-aggregated dye which is formed only in the presence of highly ordered bilayer surface.[4,5]

Figure 2 gives temperature dependence of the absorption spectrum in aqueous **1-2**. The absorption intensity at 565 nm was gradually weakened with increasing temperature, and a small peak appeared at 616 nm together with the broadened major peak at 560 nm (dotted line, at 57 °C). The 616-nm band has been observed as the transient species of NK 2012 during phase transition.[7] Further increase in temperature causes a λ_{max} shift to 542 nm with disappearance of the 616-nm absorption (Fig. 2b), indicating that the cyanine dye is mostly dispersed as the monomeric species. These spectral changes were reversible with respect to temperature change.

Figure 3 displays effects of temperature on the fluorescence intensity of NK 2012 and fluorescence depolarization of DPH (Tokyo Kasei) bound to aqueous **1-2** (instrument, Hitachi 650-10S spectrofluorimeter).

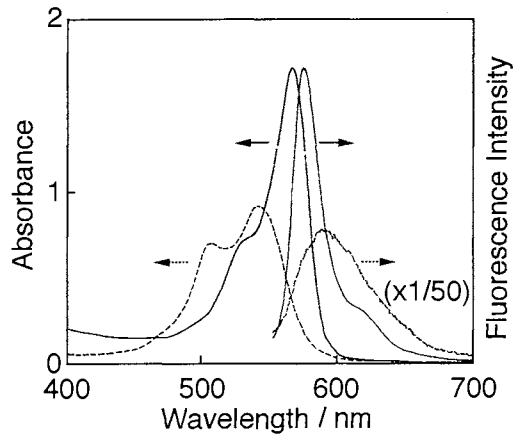

Fig. 1. Absorption and fluorescence spectra of NK 2012 in aqueous **1-2** (solid line) and in aqueous **1** (dotted line) at 20 °C. absorption spectra; [**1-2**] = [**1**] = 3 X 10^{-4} M, [NK 2012] = 5 X 10^{-5} M, fluorescence spectra; [**1-2**] = [**1**] = 2 X 10^{-4} M, [NK 2012] = 5 X 10^{-8} M.

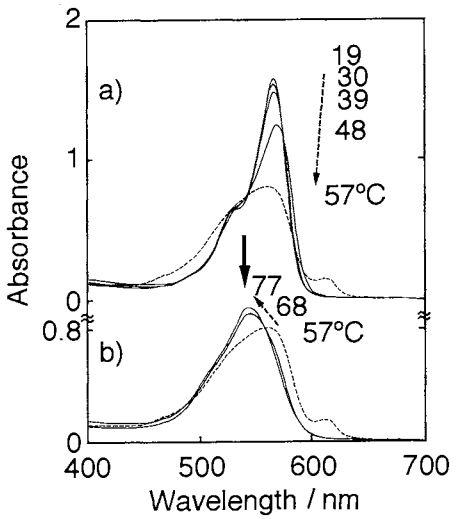

Fig. 2. Temperature dependence of absorption spectrum of NK-2012 in aqueous **1-2**. a) 19 - 57 °C, b) 57 - 77 °C. [**1-2**] = 3 X 10^{-4} M, [NK 2012] = 5 X 10^{-5} M.

The fluorescence intensity due to the J-aggregate formation rapidly decreases at 40 - 50 °C. This decrease is clearly related to the onset of the gel-to-liquid crystal phase transition of the bilayer. A good correspondence was found for the drop in fluorescence depolarization of DPH from 0.35 to 0.12. these values are comparable to those reported for the phase transition of conventional aqueous bilayer aggregates.[8] Apparently, NK 2012 is bound electrostatically to the bilayer surface, while DPH is solubilized in the hydrophobic interior of the bilayer. A schematic illustration of bilayer **1-2** and membrane-bound dyes is given in Fig. 4.

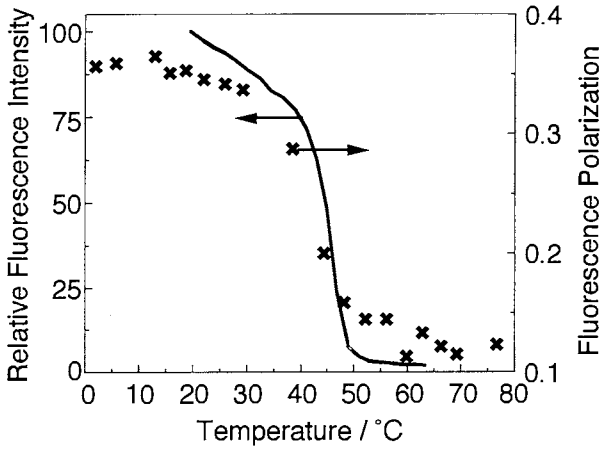

Fig. 3. Temperature dependence of fluorescence intensity of NK 2012 (solid line) and fluorescence depolarization of DPH (**X**). [**1-2**] = 2 X 10^{-4} M, [NK 2012] = 5 X 10^{-8} M, [DPH] = 2 X 10^{-7} M.

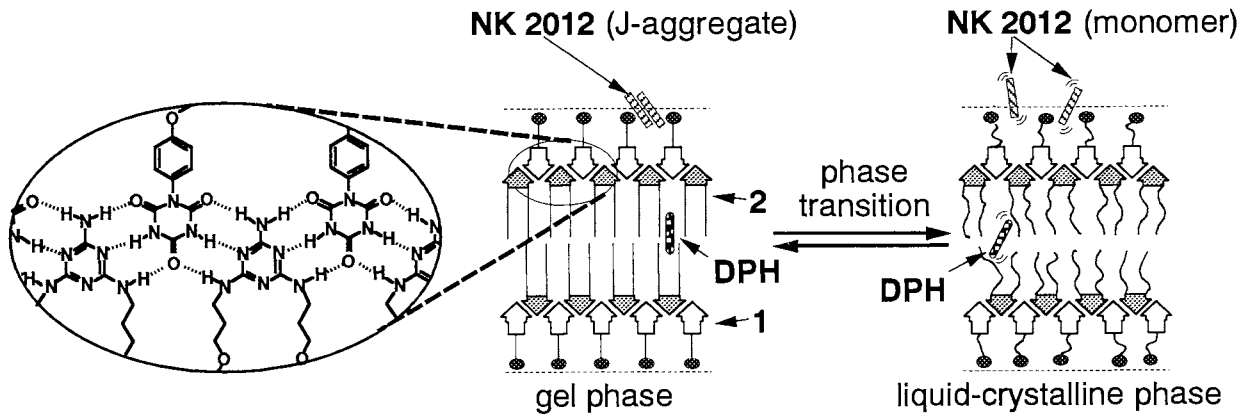

Fig. 4. Schematic illustration of bilayer **1**-**2** and membrane-bound dyes.

In conclusion, the aqueous bilayer formed via complementary hydrogen bonding possesses highly ordered molecular alignments. Multiple non-covalent interactions such as complementary hydrogen bonds, van der Waals force, aromatic stacking, and hydrophobic association contribute to the bilayer stability in the gel state as well as in the liquid crystalline state. At higher temperatures of 80 - 100 °C, partial dissociation of the complementary units proceeds. Thermal structural changes are one of the common features of biological self-assembly systems such as proteins and nucleic acids, and are determined by delicate balances of secondary valence forces. Similar balances in the present system will offer finely-controlled functions.

References

1) Contribution No. 990 from Department of Chemical Science and Technology.
2) For example, G. M. Whitesides, J. P. Mathias, and C. Seto, Science., **254**, 1312(1991); J-M. Lehn, M. Mascal, A. DeCian, and J. Fischer, J. Chem. Soc., Perkin Trans. 2., **1992**, 461; J. Rebek, Jr., Angew. Chem., Int. Ed. Engl., **29**, 245(1990); S. K. Chang, D. V. Engen, E. Fan, and A. D. Hamilton, J. Am. Chem. Soc., **113**, 7640(1991).
3) N. Kimizuka, T. Kawasaki, and T. Kunitake, J. Am. Chem. Soc., **115**, 4387(1993).
4) N. Nakashima, R. Ando, H. Fukushima, and T. Kunitake, J. Chem. Soc., Chem. Commun., **1982**, 707; N. Nakashima, A. Tsuge, and T. Kunitake, ibid., **1985**, 41.
5) N. Nakashima and T. Kunitake, J. Am. Chem. Soc., **104**, 4261(1982).
6) M. Shinitzky and Y. Barenholz, Biochim. Biophys. Acta., **515**, 367(1978).
7) M. Tsukamoto, Master Thesis, Faculty of Engineering, Kyushu University (1988).
8) For example, N. Kimizuka, H. Ohira, M. Tanaka, and T. Kunitake, Chem.Lett., **1990**, 29.

(Received September 21, 1993)