Determination of Critical Aggregation Concentrations of Self-Assembling Lipids in Nonpolar Organic Media Using Spiropyrans as Photochromic Probes

Hiroshi Hachisako,* Hiroki Nakayama, and Hirotaka Ihara†

Department of Applied Chemistry, Kumamoto Institute of Technology, 4-22-1 Ikeda, Kumamoto 860-0082

†*Graduate School of Science and Technology, Department of Applied Chemistry and Biochemistry, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555*

(Received July 21, 1999; CL-990639)

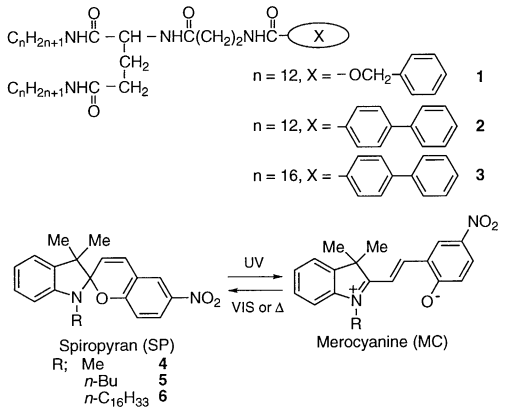
Critical aggregation concentrations of organic gel-forming lipids in nonpolar organic media such as benzene were found to be determined by measuring the first-order rate constants of merocyanine-to-spiropyran thermal isomerizations for spiropyrans added as probes.

There has been increasing interest in supramolecular assemblies from low molecular weight molecules in organic solvents. The aggregation occurs with an increase in the concentration of self-assembling molecules across a critical concentration referred to as critical aggregation concentration (CAC). The CAC is one of the most important properties for the self-assembling molecules, and therefore, its direct and precise evaluation is of great significance in supramolecular chemistry. Conventionally, surface tension, conductivity, fluorescence and visible absorption spectroscopies using chromophoric probes, etc. have been used for aqueous solution systems to determine the CAC. It is, however, difficult to apply these methods to organic solvent systems since hydrophobic and electrostatic interactions, which play major roles in determining the CAC in aqueous solution systems, are much less operative in nonpolar organic media. Although the CAC can be estimated to some extent from visually observed critical gelling concentration (CGC)[1] for organic gel-forming lipids, the establishment of a novel method applicable to a wide variety of self-assembling molecules in organic solution/gel systems is desired.

Our interests have been focused on spiropyran (SP) which is known as one of the representative photochromic compounds, because the SP changes not only in its structure from a bulky SP form to a planar merocyanine (MC) form but also in the color and polarity from a colorless and nonionic SP to a colored and zwitterionic MC form when exposed to UV light. This MC species is converted to the original SP form thermally even in the dark, and the rate of thermal MC-SP isomerization is known to be affected by the microenvironment in which the MC species are surrounded.[2-7] Therefore, the change in microenvironment around the MC species due to aggregation of lipids is considered to affect the rate of the MC-SP decoloration detected by visible absorption spectroscopy. In this paper, we demonstrate a novel method for the determination of the CAC by using three kinds of SPs as photochromic probes with different length of *N*-alkyl groups (Me-, **4**; *n*-Bu-, **5**; *n*-$C_{16}H_{33}$-, **6**).

It has been reported that L-glutamic acid-derived lipids, e.g., **1** with at least three amide groups per molecule can form organic gels through highly-oriented aggregation in benzene and other nonpolar organic media.[1,8] In this study, organic gel-forming lipids **1-3** were used as model compounds. Visual observation of the gelation is also convenient for the detection of their aggregation in benzene.

Lipids **1-3** were thermally dissolved (ca. 70 °C) in benzene containing 1.0×10^{-4} mol dm^{-3} of a SP as a probe,[9] and then gradually cooled to 20 °C to form gels.[10] UV-irradiation experiments for SP-MC photoisomerization and subsequent first-order kinetic analyses for MC-SP thermal recovery were conducted as reported previously.[1]



Prior to UV-irradiation experiments using SP as a probe, the CAC of biphenyl chromophore-containing lipid **3** alone in benzene was directly determined using fluorescence spectroscopy.[11] Figure 1a shows the relationships between the fluorescence maximum wavelength (λ_{em}), relative fluorescence intensity and the concentration of **3**. The λ_{em} red-shifted with an increase in its concentration across 8×10^{-4} mol dm^{-3}. The red-shift of λ_{em} is ascribed to the excimer formation between highly-organized biphenyl headgroups through highly-oriented aggregation (gelation) of the L-glutamic acid-derived lipid tailgroups due to complementary hydrogen bondings.[1,8,12-14] Quenching resulting from the excimer formation was also observed. The critical concentration at which excimer of **3** was formed well coincided with the critical concentration at which fragmentary gels were formed.[15] From these results, it is safely concluded that the CAC of **3** in benzene is 8×10^{-4} mol dm^{-3}.

The MC-SP thermal isomerization processes of SP probes **4-6** (at the constant concentrations of 1×10^{-4} mol dm^{-3}, respectively) in benzene in the presence of lipids **1-3** under various concentrations were evaluated by first-order kinetic analyses. The first-order plots gave good linearity regardless of whether the gels were formed or not, suggesting that no appreciable interaction between lipid and probe is present.[17] Figure 1b shows the concentration dependence of lipid **3** on the rate constant (k_1) of MC-SP thermal isomerization for SP **4** added as a probe. The critical concentration (8×10^{-4} mol dm^{-3}) at which the k_1 values abruptly increased from 5.0×10^{-2} to 6.3×10^{-2} s^{-1} (the change of k_1 at the CAC; $\Delta k_1 = 1.2 \times 10^{-2}$ s^{-1}) coincides with the CAC estimated by fluorescence spectroscopy (Figure 1a). This clearly indicates that the acceleration of k_1 is caused by the aggregation of lipids. Similar results were obtained for lipids **1** and **2** using probe **4** in benzene and in toluene, indicating that this method is useful for the determination of CAC of the self-assembling lipids **1-3** in nonpolar organic media.

Effect of alkyl chain length of SP probe was investigated. It

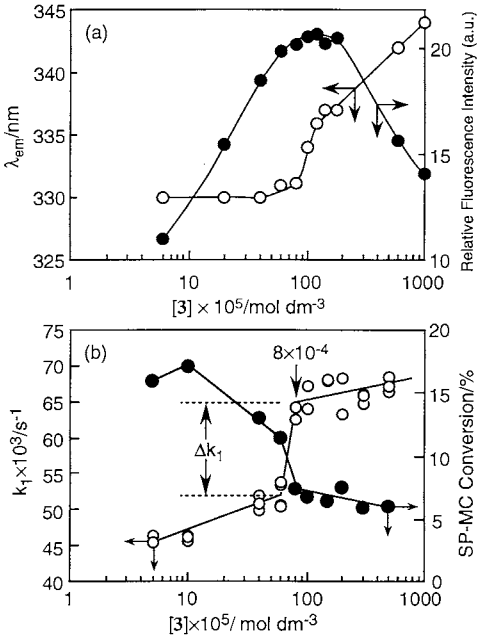


Figure 1. (a) Relationships between relative fluorescence intensity, wavelength at emission maximum (λ_{em}) and concentration of **3**. (b) Relationships between concentration of **3**, percentage of SP-MC conversion, and first-order rate constant (k_1) for MC-SP thermal isomerization of **4** in benzene at 20 °C. [**4**] = 1.0×10^{-4} mol dm^{-3} = const.

is noted that the shorter the *N*-alkyl chain is, the greater the Δk_1 becomes. The Δk_1 values in lipid **1** system are 10×10^{-3} s^{-1} for SP **4**, 5×10^{-3} s^{-1} for SP **5**, and 1×10^{-3} s^{-1} for SP **6**. On the other hand, the Δk_1 was not detected for SPs **5** and **6** with longer alkyl chains in the systems of lipids **2** and **3**. These results indicate that the SP **4** with the shortest *N*-alkyl substituent is the most suitable probe for this method. This is probably because of the least interaction between *N*-methyl group of SP **4** and lipid assemblies. Table 1 summarizes the CAC values for lipids **1-3** determined using probes **4-6**. It is noted that the same CAC values (3×10^{-4} mol dm^{-3}) were obtained for lipid **1** by using SP probes **4-6**. This strongly supports the validity of this method.

The acceleration of k_1 with the suppression of the percent of SP-MC conversion[18] can be ascribed to the local high concentration of the SP probe in bulk benzene by being excluded from the lipid aggregates tightened by complementary hydrogen bondings.[12-14] The following results are consistent with the mechanism: (i) Increasing the concentration of SP **4** alone in benzene accelerated k_1 with the suppression of SP-MC conversion.[19] As shown in Figure 1b, a percentage of the SP-MC conversion was decreased with increase in the lipid concentrations across the CAC, and the k_1 values were accelerated in spite of constant SP concentration; (ii) No appreciable difference in morphology of physically cross-linked fibrillar aggregates[1,8,12,14] was observed by addition of **4**; (iii) Endothermic peaks of the gel-to-sol phase transitions of **1-3** were not affected by addition of **4** as detected by differential scanning calorimetry; (iv) The λ_{max} of solvatochromic MC species of **4** did not change across the CAC of lipids **1-3**, indicating that **4** was not incorporated into the lipid aggregates. (v) The gelation behavior of lipid **1** was not affected by addition of **4**; (vi) First-order plots for the MC-SP thermal isomerization processes of **4** did not deviate from simple first-order kinetics, giving good linearities regardless of the

Table 1. CAC[a] of lipids **1-3** in benzene determined by using SP probes **4-6**[b]

	4	**5**	**6**
1	3×10^{-4}	3×10^{-4}	3×10^{-4}
2	6×10^{-4}	not determined	not determined
3	8×10^{-4}	not determined	not determined

[a] mol dm^{-3}, temperature 20 °C. [b] [SP] = 1.0×10^{-4} mol dm^{-3}

concentrations of lipids **1-3**.[17] (vii) Among SPs **4-6**, the SP **4** with the shortest *N*-alkyl chain is the most effective as a probe.

In conclusion, we have established a novel method for the determination of CAC of **1-3** in nonpolar organic media by using first-order kinetic analyses for the thermal MC-SP isomerization of SP probe. This method is based on the non-incorporation of the SP probe into the lipid aggregates in nonpolar organic media. Applicability of this method to some polar organic media systems is under investigation although, in general, these lipids tend to precipitate in polar media.[20]

References and Notes

1 H. Hachisako, H. Ihara, T. Kamiya, C. Hirayama, and K. Yamada, *J. Chem. Soc., Chem. Commun.*, **1997**, 19.
2 G. Smets, *Adv. Polym. Sci.*, **50**, 17 (1983).
3 T. Tsutsui, A. Hatakeyama, and S. Saito, *Chem. Phys. Lett.*, **132**, 563 (1986).
4 E. Goldburt and V. Krongauz, *Macromolecules*, **19**, 247 (1986).
5 H. Tomioka and T. Itoh, *Nippon Kagaku Kaishi.*, **1988**, 1031.
6 T. Seki and K. Ichimura, *Macromolecules*, **23**, 31 (1990).
7 K. Takagi, T. Kurematsu, and Y. Sawaki, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 1667.
8 H. Ihara, H. Hachisako, C. Hirayama, and K. Yamada, *J. Chem. Soc., Chem. Commun.*, **1992**, 1244.
9 It is suggested that the concentration of SP probe should be below the visually determined CGC of the lipid alone. Also, absorbance of MC species should be below 1.5 or so.
10 In the gel systems in this study, the diameters of fibrillar aggregates were ca.100-2000Å as revealed by TEM observation.[1] Consequently, the gels were transparent enough to enable the measurement of visible absorption spectra of MC species added as probes.
11 Fluorescence spectrum of **1** cannot be measured in benzene because absorption bands of headgroup of **1** and benzene are almost identical.
12 H. Ihara, K. Shudo, M. Takafuji, C. Hirayama, H. Hachisako, and K. Yamada, *Jpn. J. Polym. Sci. & Tech.*, **52**, 606 (1995).
13 H. Hachisako, H. Ihara, and K. Yamada, *Recent Res. Devel. in Pure & Applied Chem.*, **2**, 59 (1998).
14 H. Ihara, M. Yoshitake, M. Takafuji, T. Yamada, T. Sagawa, and H. Hachisako, *Liq. Cryst.*, **26**, 1021 (1999).
15 The critical concentration (CAC) at which fragmentary gels are formed is slightly lower than the CGC evaluated by the inversion method,[16] because the solution containing fragmentary gels at the CAC flows by the inversion method.
16 Y.-C. Lin, B. Kacher, and R. G. Weiss, *J. Am. Chem. Soc.*, **111**, 5542 (1989).
17 Deviations from linearity are, in general, observed in many systems[2-7] such as in polymer matrices and in molecular assemblies when MC-SP thermal isomerization is affected by these matrices.
18 Percentage of SP-MC conversion was measured by UV-visible absorption spectroscopy immediately after completing UV-irradiation for 5 min, assuming that the molar extinction coefficient of MC species is 30000.[7]
19 Percentage of SP-MC conversion of SP **4** alone in benzene changed from ca.10 % to several % with an increase in the concentration from 1×10^{-4} mol dm^{-3} to 2×10^{-2} mol dm^{-3}.
20 It is also unknown whether this method is applicable to the perfluorocarbon amphiphile systems due to different driving forces of self-assembling: Y. Ishikawa, H. Kuwahara, and T. Kunitake, *J. Am. Chem. Soc.*, **116**, 5579 (1994).