Differences between Spontaneous Assemblage and Concentration-Induced Assemblage of Peptide-Containing Molecules in Organic Media

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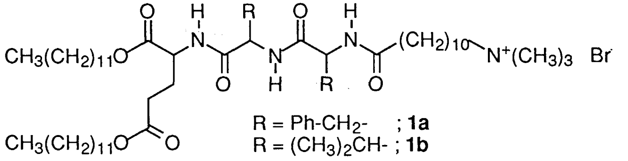
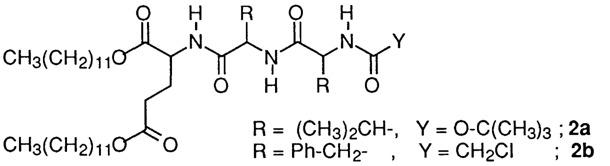
Assemblages of tripeptide-containing molecules in organic media were divided into the stable self-assemblage which was formed at a low concentration and the concentration-induced assemblage which was unstable upon heating.

Self-assembling molecules in organic media are attractive for designing a new molecular architecture system. According to recent research, the morphology and properties of the resultant aggregates have been predominantly discussed. [1-7] However, no report refers to the aggregation processes, and hence some important fundamentals, such as definitive evidence of aggregation, the mechanisms of aggregation and disintegration, and the critical concentration of aggregation, remain unclear. Using FT-IR spectroscopy, we first demonstrated herein the aggregation and disintegration processes in organic solvents and emphasized that the aggregate in organic solvents is divided into two classes, namely spontaneous assemblage and concentration-induced assemblage. These aggregates are entirely different in aggregation mechanism and the critical concentration of aggregation.

We use two kinds of molecules that contain a tripeptide moiety. One is amphiphilic (**1**) and the other is hydrophobic (**2**).[8]



R = Ph-CH$_2$- ; **1a**
R = (CH$_3$)$_2$CH- ; **1b**



R = (CH$_3$)$_2$CH-, Y = O-C(CH$_3$)$_3$; **2a**
R = Ph-CH$_2$- , Y = CH$_2$Cl ; **2b**

According to our previous report, the amphiphile **1** formed an aggregate in CCl_4, whereas no aggregate was formed in $CHCl_3$.[5,7] The aggregate formation can be detected from the FT-IR spectra, because this amphiphile produced a characteristic spectra attributed to a parallel-chain β-sheet structure when the aggregate formation was completed.[7] If a $CHCl_3$ and CCl_4 mixture was used as the solvent, we can observe the disintegration process of the aggregate of **1a** due to increasing temperature (Figure 1).[9,10] The bottom spectrum in Figure 1 is attributed to the parallel-chain β-sheet structure of the tripeptide. The bonding amide I mode at 1637 cm^{-1} quickly disappeared at temperatures above 55 °C and showed the same spectrum as that observed in $CHCl_3$. The disintegration was insensitive to the concentration. For example, the bonding amide I mode was unchanged in CCl_4 solution at 25 °C even if the concentration decreased to 0.01 mM which is the lower limit of detection. Therefore, the critical concentration of the aggregate formation of **1a** is lower than 0.01 mM in CCl_4 at 25 °C. In addition, the solution was not a gel in the range of 0.01 to 0.1 mM despite the orderly structure of the peptide observed in the FTIR spectra. This result means that the aggregate formation does not always accompany gel formation, and hence gelation is not definitive evidence for the aggregate formation. As another example, the hydrophobic molecule (**2a**) dissolves in almost all organic solvents. Although the molecule dissolved in CCl_4, gelation was not observed if the concentration was increased to 30 mM. Nevertheless, the amide bands started to shift from 1678 cm^{-1} to 1649 cm^{-1} when the concentration was increased from 1 mM to 5 mM at 25 °C (Figure 2). Although the molecule **2b** produced a gelatinous solution if the concentration was increased to 10 mM, the same spectral change can be observed for the CCl_4 solutions of **2b**. This band shift to a lower wavenumber and the decrease in the half-band width mean the formation of an ordered H-bond structure between amide groups, which is apparent evidence for the aggregate formation. Therefore, the molecule **2a** aggregated at the concentration of 5 mM, which was at least 100-fold higher than the critical concentration for **1a**.

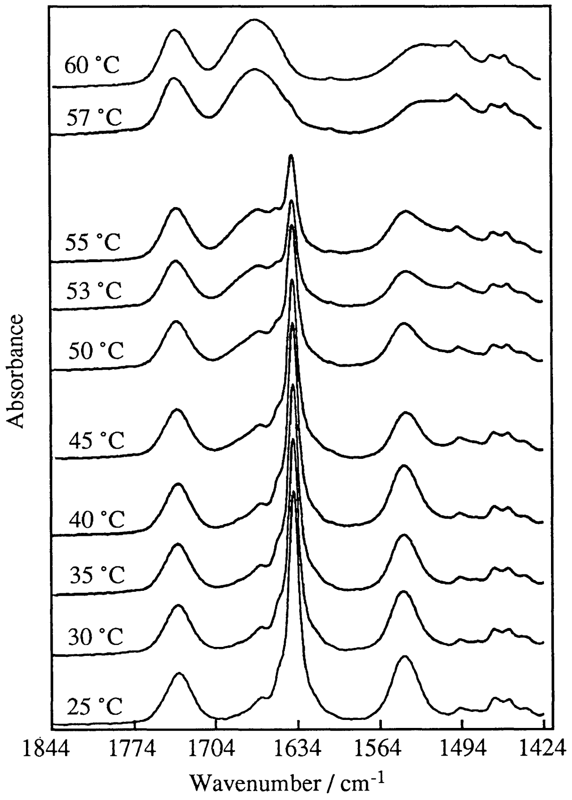


Figure 1. Temperature dependence of FT-IR spectra of **1a** in a 1:4 mixture of $CHCl_3$-CCl_4 (4 mM).

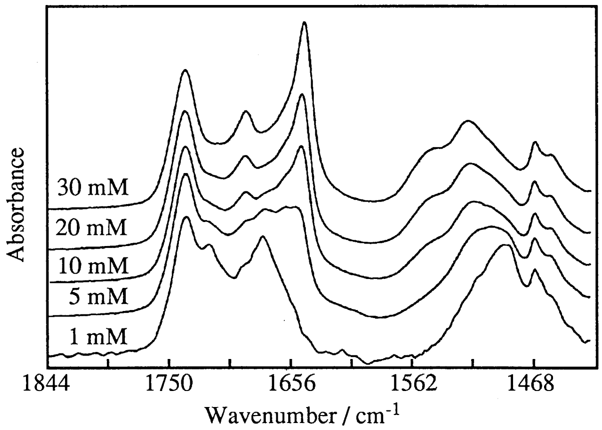


Figure 2. Change in FT-IR spectra of **2a** versus the concentration in CCl_4.

We then compared the disintegration process of **2a** with that of **1a**. As shown Figure 3, the relative ratio of absorbance at the non-bonding (1678 cm^{-1}) and the bonding amide I modes (1649 cm^{-1}) continuously changed with increasing temperature within the aggregate of **2a**. Therefore, the aggregation and disintegration of **2a** takes place stepwise, which is strongly dependent upon concentration and temperature. On the other hand, the aggregate of **1a** did not collapse till the critical temperature. The ratio of absorbance at the non-bonding (1668.5 cm^{-1}) and the bonding amide I modes (1637.5 cm^{-1}) drastically increased at 55 °C, when the amphiphile **1a** was dissolved in the $CHCl_3$-CCl_4 mixture. Because the aggregates of **1a** and **1b** are stable over a wide temperature range and formed at a very low concentration, only the aggregate should have spontaneous assemblage, while the other is the concentration-induced assemblage. The distinction between the two types of assemblages is very important when considering the driving force of the self-assembling in organic media.

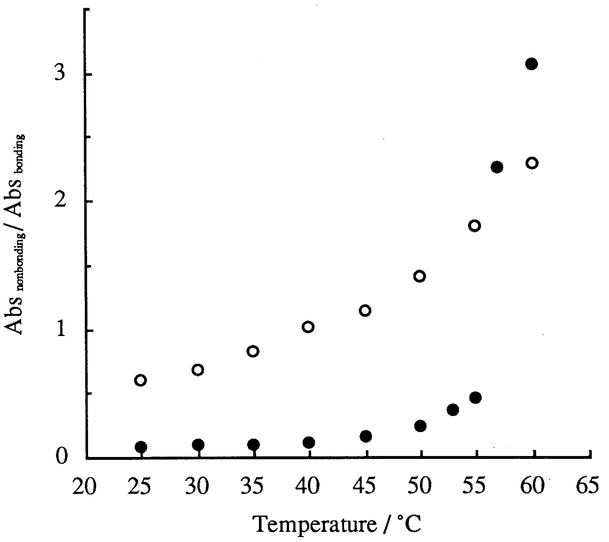


Figure 3. Temperature dependence of the ratio of absorbance ($Abs_{nonbonding\ amide\ I}$ / $Abs_{bonding\ amide\ I}$); solid circles are the ratio at 1668.5 (nonbonding) and 1637.5 cm^{-1} (bonding) for a 4 mM solution of **1a** in a 1:4 mixture of $CHCl_3$-CCl_4; open circles are the ratio at 1679 (nonbonding) and 1649 cm^{-1} (bonding) for a 10 mM solution of **2a** in CCl_4.

In conclusion, H-bonding is not the driving force for the self-assembling of molecules in organic solvents, because molecules containing the H-bonding units do not always aggregate spontaneously. In the present case, a trimethylammonium head acts as the solvophobic part and the hydrocarbon chains act as the solvophilic part, and hence amphiphilicity of the molecule should be the driving force of the self-organization. Another example can be seen in the report by Kunitake et al.[2] They used a perfluorocarbon chain and a normal hydrocarbon chain as the solvophobic and solvophilic parts, respectively. Coexistence of such different groups at both ends of a molecule should be essential for the self-organization of molecules in organic media. The H-bonding should act as a binder of adjacent molecules in these resultant assemblages and contribute to the stability of the aggregate.

References and Notes

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8 All amphiphiles were prepared in the same manner described elsewhere.[5,6] They gave the expected IR, NMR, and microanalytical data.
9 The FT-IR spectra were recorded using a Nicolet 740 spectrophotometer equipped with a TGS detector. Two hundred interferograms were co-added and Fourier transformed, giving the FT-IR spectra with 4 cm^{-1} of optical resolution. Details of the measurement are described in our previous report.[7]
10 The other amphiphile such as **1b** showed no spectral change in the $CHCl_3$-CCl_4 mixture even if the temperature was increased close to the boiling point of the solvent (60 °C), which meant that the H-bonding within the aggregate was stronger than that of the amphiphile **1a**.