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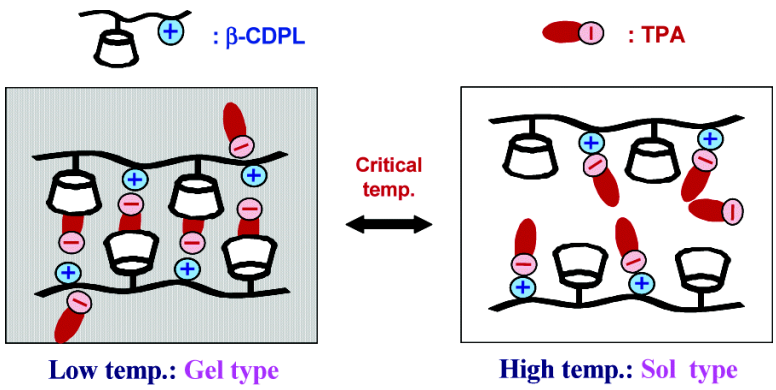

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pH- and Thermosensitive Supramolecular Assembling System: Rapidly Responsive Properties of β-Cyclodextrin-Conjugated Poly(ϵ-lysine)

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Stimuli-responsive polymers have been extensively investigated and used as smart biomaterials and drug-delivery systems.[1] The phase transition of polymers is induced by a continuous change in various conditions such as temperature,[2] pH,[3] electric field,[4] or solvent composition.[5] Our studies have focused on new stimuli-responsive polyrotaxanes as useful materials for artificial muscles or molecular shuttles, in which the location of cyclodextrin (CD) rings along a polymeric chain can be reversibly controlled in response to external stimuli.[6] On the basis of these studies, we recently designed new stimuli-responsive supramolecular-structured hydrogel systems that are constructed by unique noncovalent interactions between CDs and polymeric guests.[7] Although reversible gelation could be successfully induced by inclusion complexation, the time for gel-induction and gel-sol transition was too long and not evitable, giving possible limitations for their applications. As a different approach to overcome these problems, we demonstrated a pH-sensitive supramolecular assembling system using a specific host−guest interaction of α-CD-conjugated poly(ϵ-lysine) (α-CDPL), which showed a remarkable phase transition with a small pH change.[8]

In this study, we focused on a thermally sensitive pH-dependent gel-forming system consisting of β-CD-conjugated poly(ϵ-lysine) (β-CDPL) and 3-trimethylsilylpropionic acid (TPA). It should be pointed out here that the rapid intermolecular association and the following dramatic phase transition by pH as well as temperature may be due to dual complexation phenomena, inclusion complexation and ionic complexation.

We introduced the β-CD molecule, the internal cavity of which has enough space to completely include one TPA molecule, onto a PL backbone via chemical conjugation. This is a small but definite difference in the structure from α-CDPL because α-CD was found to partially include TPA.[8] The β-CDPL was prepared by a coupling reaction between monoaldehyde activated β-CD and PL according to a previously reported method.[8] The degree of β-CD substitution was determined by the peak integration in ^{1}H NMR spectra and calculated to be 0.48. The inclusion complexation of β-CDPL with TPA was achieved by simply adding TPA aqueous solution into β-CDPL aqueous solution with various feed molar ratio. The stoichiometric ratio of β-CD and TPA, confirmed by ESI mass spectroscopy, was found to be 1:1.

The inclusion properties of β-CD/TPA and β-CDPL/TPA were investigated by ^{1}H NMR to clarify specific interactions between the host and guest molecules. With an increase in the amount of TPA, an upfield shift of the H-3 peaks was observed until a molar ratio of 1:1 was reached and the H-5 signals of β-CD broadened and shifted downfield. The peak shifts were caused by complexation of β-CD with TPA, and the marked broadening of the signals may be due to acceleration of magnetic relaxation by van der Waals contact of the methyl groups of TPA with the β-CD cavity.[9,10]

To verify the inclusion complexation mechanism in detail, 2D-ROESY NMR measurements were carried out in terms of observing dipolar interactions (nuclear Overhauser effects).[11] As shown in Figure 1, partial contour plots of the spectra of the inclusion complex show that the signals of H-3 protons of β-CD were correlated with the resonance of methyl protons of TPA. On the other hand, the signals of H-1, H-2, and H-4 protons of β-CD, which are located outside the cavity, did not interact with the guest protons. In addition, cross-peaks between the H-5 protons of β-CD and the carboxyl groups of TPA were not observed. This result indicates that the H-3 protons of β-CD are the most prone to be affected by the inclusion process and the inclusion complexation occurs preferably from the secondary-ring side of β-CD.[12] It confirms that TPA molecules are fully enclosed into β-CD cavities with a stoichiometry of 1:1.

To verify the range of pH for the complexation-induced aggregations, the transmittance changes of the mixture solutions of α-CDPL/TPA and β-CDPL/TPA were measured by light transmittance using a UV−vis spectrophotometer (V-550, Jasco, Tokyo, Japan). From pH 3 to 12, there was no significant change in the transmittance of the mixture solutions of TPA with α-CD or β-CD (data not shown). On the other hand, clear decreases in the transmittance of α-CDPL/TPA and β-CDPL/TPA inclusion complexes were observed in the range of pH 7.0−7.5 and pH 6.0−6.5, respectively (Figure 2). These results indicate that the transmittance changes are induced by attractive ionic interactions between cationic groups of PLs and anionic end-groups of TPAs.[13] In the case of the β-CDPL/TPA system, some associations should exist below pH 6 because the solution viscosity below pH 6 was higher than over pH 6 even in the transparent conditions.[14] This may be due to the hydrophobic interaction between TPA molecules included in CD cavities; however, repulsive interactions between cationic amino groups also exist, which prevent the intermolecular aggregation of the inclusion complexes. In the basic condition, neutral conditions of PL backbone and repulsive ionic interactions between negative carboxyl groups of TPAs affected the solution, which leads to transparency and lower viscosity.

In addition to the pH-responsiveness, the β-CDPL/TPA system showed a rapid phase transition in response to a small temperature change across their upper critical solution temperature. On a decrease in temperature from 50 to 10 °C at pH 6, the β-CDPL/TPA system underwent a sudden phase transition with the temperature change of 1−2 °C, while the α-CDPL/TPA system did not show any critical point at pH 7. Lo Nostro and Ito's groups reported that the temperature increase is likely to induce dissociation of the guest molecules from the CDs with a restoration of its intrinsic entropy from random conformations in solution and vice versa.[15] From these reports, we assumed that increasing temperature in the β-CDPL/TPA system dissociates presumably the inclusion complex rather than the ionic interaction. This is why this system could show a phase transition under the critical temperature, where

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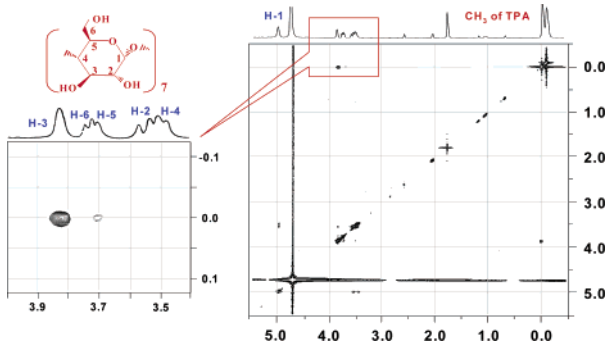

Figure 1. Partial contour plots of the 2D-ROESY NMR spectrum of the β-CDPL/TPA inclusion complex in D_2O at pH 7.

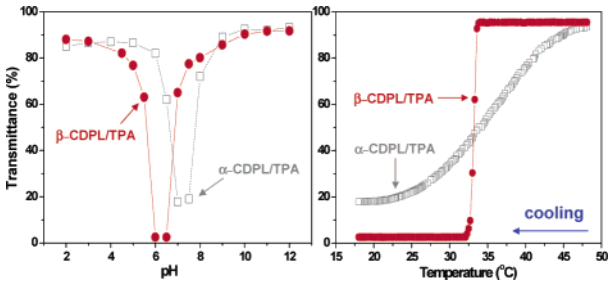

Figure 2. pH-responsive behaviors of inclusion complexes at 20 °C (left) and thermoresponsive behaviors of α-CDPL/TPA at pH 7 and β-CDPL/TPA at pH 6 (right) in 0.1 M PBS. The total concentration was 1 wt %, and the degree of substitution of α-CDPL was 0.41.

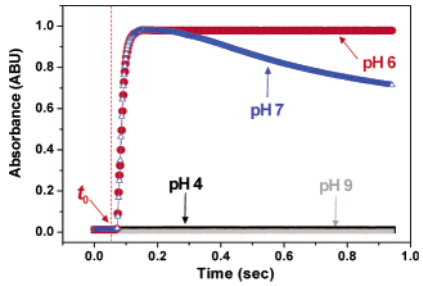

Figure 3. Typical time-course of absorbance changes observed at 500 nm after mixing of [β-CDPL] (4.7×10^{-4} M) and [TPA] (7.3×10^{-3} M) solutions (stoichiometry 1:1). The samples were prepared in 0.1 M PBS at 20 °C. The mixing time and the dead time were 100 and 60 ms, respectively, and t_0 is the start point of the measurement.

the interaction of CD with TPA is strong and the complexes tend to associate each other through intermolecular interactions such as hydrogen bonding, van der Waals force, and hydrophobic interactions.

To confirm the kinetics of the instantaneous aggregation phenomena of the β-CDPL/TPA system, we used a stopped-flow spectrophotometer.[16] As shown in Figure 3, when the two solutions are mixed together, the absorbance at 500 nm of the mixture solutions dramatically increased at pH 6 and pH 7 during 100 ms. On the other hand, such an increase was not observed at the other pH range. This result reconfirms that multifaceted interactions participated in the aggregation phenomena simultaneously. In the case of pH 7, the molecular recognition occurred clearly as a two-step process: a rapid binding process of TPA by a specific host−guest interaction followed by a subsequent intermolecular-structural transformation of the complexes to reach equilibrium conditions with stable final aggregation.[17]

Our current study has revealed several important consequences of inclusion complexation of CD-conjugated polymers, in particular, the rapid assembly of β-CDPL and the guest molecule at only physiological pH and the temperature-sensitive character of this inclusion complex system, allowing for a sharp phase transition upon a small amount of cooling. We have furthermore demonstrated a clear interdependence of pH and temperature, both of which significantly affect dramatic aggregation. On the basis of these results, we conclude that the rapid response of this system originates from dual cooperative interactions, specific host−guest interaction and intermolecular ionic interaction, which play a key role in dramatic aggregation phenomena.

These results indicated that the β-CDPL inclusion complex system can be considered as a potential carrier for drug-delivery systems and may be used especially for local therapeutic applications of ionic drugs. More detailed studies on the mechanism and the kinetics of hydrogel formation are now in progress.

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Supporting Information Available: Synthetic procedure, ESI-mass, ^{1}H NMR spectroscopies, and viscosity changes for the inclusion complexes of β-CDPL with TPA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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