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pH Dependence of Inclusion Complexation between Cationic Poly(ϵ-lysine) and α-Cyclodextrin

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Introduction

Threading cyclodextrin (CD) molecules onto linear polymer chains that results in polymer inclusion complexes (PICs) has been extensively studied for constructing supramolecular structures such as polyrotaxanes and other potential applications.[1,2] In particular, specific noncovalent interaction between the polymers and CDs, represented as host−guest interactions, has been of interest for the study of enzyme−substrate interactions in biological systems. Since the first report by Harada et al. that α-CD can form a crystalline IC with poly(ethylene glycol) in aqueous solution, many kinds of linear polymers of hydrophilic or hydrophobic nature have been found to form PICs together with their corresponding CDs with specific selectivity.[3,4] The characteristic properties of PICs have been broadly investigated and considered by Tonelli et al. for several useful applications.[5] For instance, they have fabricated unique polymer−polymer composites and blends with normally incompatible polymers as well as modified the phase structure of block copolymers using PIC formation.[6]

In a previous paper, we have reported the preparation and characterization of a new PIC made up of α-CD and a biodegradable and cationic poly(ϵ-lysine) (PL), which is composed of 25−30 L-lysine residues connected via peptide linkages between α-carboxyl and ϵ-amino groups.[7] Figure 1 shows the chemical structure of PL and α-CD and the proposed structure of PL IC. The stoichiometry for complex formation has been suggested to be 1:1 (α-CD:monomeric unit of PL). X-ray diffraction measurements have revealed that the PL IC has a channel-type crystalline structure often found in typical PICs. That study has a significant meaning because PL is a cationic polymer that shows a quite different behavior during IC formation, depending on environmental conditions such as pH of aqueous media due to the existence of ionizable amino side groups. Most of PICs reported so far are accomplished from neutral polymers and thus prepared without any consideration of some significant factors such as pH and salt of aqueous media.

For the past few decades, the effect of pH and salt on inclusion complexation of CDs with various ionic guests has been studied, although CDs did not show an appreciable change in their own properties within a moderate range of pH and salt concentration.[8] On the other hand, until now there has been no report on such effect on the polymeric guests. Considering the significance of macromolecular recognition in biological systems, this study may have fundamental importance, which is relevant for not only understanding and modulating the complexation behavior of the polymeric guests but also studying analogous biological systems.

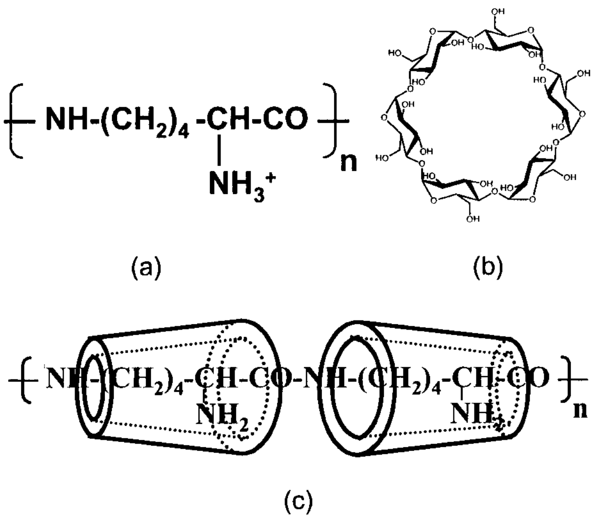

Figure 1. (a) Poly(ϵ-lysine), (b) α-cyclodextrin, and (c) the proposed structure for PL inclusion complex with α-cyclodextrin (PL IC).

In connection with the previous paper, we report here the results of further examination of the inclusion complexation between cationic PL chains and α-CD molecules under varied conditions. A significant pH dependence of inclusion complexation, which has never been observed for usual PICs so far, is demonstrated and evaluated with consideration of the characteristics of both the polymeric guest and α-CD host.

Experimental Section

Materials. Poly(ϵ-lysine) (PL, $M_n = 4060$, $M_w/M_n = 1.14$) was kindly supplied from Chisso Co., Japan, and used after drying in vacuo for 24 h. α-CD was purchased from Bio-Research Corp., Yokohama, Japan. The other chemical reagents were used as received.

Preparation of PL ICs. PL IC formation was performed by adding PL to the aqueous media containing a predetermined amount of α-CD, with initial stirring for 30 min. The mixture solutions were kept at room temperature for 24 h. The pH of the aqueous media containing α-CD was changed from 3 to 13 by using an appropriate buffer solution with the addition of HCl or NaOH, and sodium chloride was added to adjust the ionic strength. PL IC products were obtained as crystalline precipitates. The complexes were separated by centrifugation, washed with distilled water, and dried in vacuo for 24 h.

Characterization. ^{1}H NMR spectra were recorded using a 300 MHz NMR spectrometer (Varian, Unity Plus) to determine the compositions of PL ICs. X-ray diffraction measurements were performed with a powder diffractometer (RINT2000, Rigaku) using graphite-monochromatized Cu Kα radiation (λ = 1.542 Å). The melting behavior of PL was traced by a differential scanning calorimeter (DSC) (DSC 120, Seiko Instruments Co., Tokyo, Japan) as a function of the amount of α-CDs introduced to the polymer.

Results and Discussion

Our previous study demonstrated that PL could form crystalline PL IC precipitates in aqueous solutions saturated with α-CD. However, β- and γ-CD could not form ICs with PL because their cavity sizes are too large

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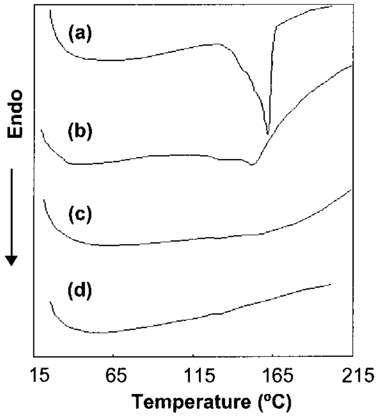

Figure 2. DSC thermograms of (a) poly(ϵ-lysine), (b) PL IC (α-CD/Lys = 0.14), (c) PL IC (α-CD/Lys = 0.27), and (d) PL IC (α-CD/Lys = 0.75).

for steric fit with the polymer chain. The stoichiometry of 1:1 (α-CD:lysine unit) was suggested, which is reasonable considering the height of the α-CD molecule and the unit length of PL. In this study, we have prepared several PL ICs containing different amounts of α-CD molecules threaded on PL by varying the feed composition between PL and α-CD. In the presence of the equivalent or excessive amount of α-CDs to the lysine units in PL, the stoichiometry of 1:1 was demonstrated for all the PL ICs obtained. Meanwhile, when the lysine units are present in an excessive amount over α-CD, the resulting PL ICs achieved the stoichiometry of less than 1:1. The properties of three kinds of PL ICs containing different amount of α-CDs (α-CD/Lys = 0.14, 0.27, and 0.75) were chosen and compared to each other.

Thermal properties of PL ICs were traced with a variation in the amount of threaded α-CD by DSC. Figure 2 shows DSC thermograms for PL homopolymer and PL ICs. A large melting endotherm was observed around 165 °C for PL homopolymer, while in the case of the PL IC with the α-CD/Lys of 0.14, the corresponding melting peak was shifted to a lower temperature region. In addition, the melting peak became considerably smaller and broader than that of PL. Presumably, the introduction of α-CD molecules to PL chains through inclusion complexation may hinder the ordering of the remaining PL chain segments. Further increase in the amount of α-CDs threaded results in a significant decrease of the melting peak, and finally a disappearance of the melting, as observed for the PL ICs with the α-CD/Lys of 0.75. It can be concluded that when the α-CD/Lys is above 0.75, most PL chains participate in the formation of new channel-type crystalline structures together with α-CD molecules threaded along the polymer chain.

The powder X-ray diffraction patterns from PL ICs with different amount of α-CDs threaded (α-CD/Lys = 1.00, 0.75, 0.27, and 0.14) are compared in Figure 3. All the PL ICs showed a strong reflection observed at $2\theta = 20.0°$ (d = 4.44 Å), which is typical of channel-type crystalline structures of usual PICs based on α-CD, suggesting the electron density distribution of the core of α-CD molecules with radius ~5 Å.[7] For the α-CD/Lys of 1.00 and 0.75, two PL ICs showed the same diffraction patterns, whereas the PL ICs with the α-CD/Lys of 0.27 and 0.14 exhibited decreased intensity in total reflections, especially for 110 reflections, in comparison with the other PL ICs. In the patterns of the PL ICs with the α-CD/Lys of 1.00 and 0.75, no diffraction from the neat PL crystal is detectable, while the patterns of IC and PL are overlapped in the cases of the α-CD/Lys of 0.27 and 0.14. The peak of $2\theta = 11°$ (d = 16 Å) is the 002 reflection from the γ form of PL, in which the PL chain conformation is not extended but slightly twisted as in the case of nylon-6. These results compare well with those of DSC studies on PL ICs.

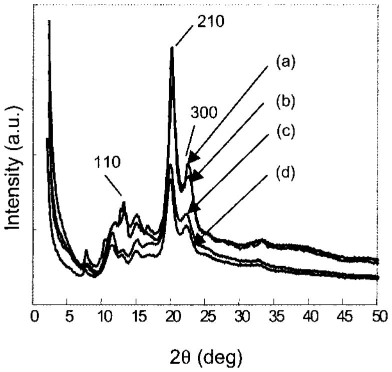

Figure 3. X-ray diffraction profiles of PL ICs. α-CD/Lys = 1.00 (a), 0.75 (b), 0.27 (c), and 0.14 (d).

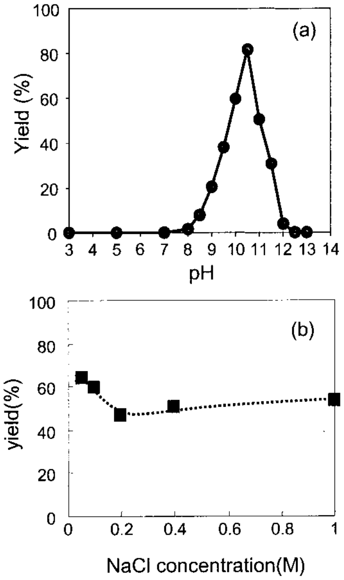

Figure 4. Change in the yield of PL ICs in PBS solutions as a function of pH (a) and ionic strength at the pH of 10.5 (b).

PL has ionizable amino groups in acid and neutral conditions. The α-amino groups can play an important role for controlling the complexation behavior or stability in response to a change in the pH and the salt concentration of aqueous media. The effect of pH and salt addition on the IC formation between cationic PL and α-CD was observed, and the results are shown in Figure 4.

The pH of the aqueous media containing α-CD was changed from 3 to 13 by using an appropriate buffer solution with the addition of HCl or NaOH. A striking change in the yield of PL ICs was observed around pH 8.5 and 11.5, as shown in Figure 4a. While the inclusion complexation could be observed in the pH range from 8.5 to 12, there was no IC formation observed in acidic and highly alkaline conditions. The yield exhibited a maximum at the pH of 10.5. It can be considered that this pH-dependent complexation is closely related to the protonation of amino side groups ($pK_a = 9$) in the PL and the dissociation of hydroxyl groups ($pK_a = 11.3$) of

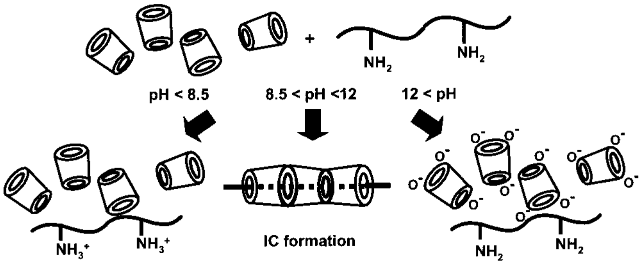

Figure 5. Schematic illustration for pH-dependent inclusion complexation.

α-CD. Probably, α-CD cannot include any PL chains containing the protonized amino side groups in acidic conditions, and also the ionized form of α-CD in highly alkaline conditions above pH = 11.5 cannot work as a host for any guests. The schematic illustration of pH-dependent PIC formation between PL and α-CD is represented in Figure 5. When an ionic salt is added to the aqueous media, the equilibrium constant for inclusion complexation may be altered by a salting-out effect. Whether the equilibrium constant is decreased or increased by the addition of salts depends on the nature of the guest as well as the host. The effect of NaCl addition on the PL IC formation was observed as a function of ionic concentration, as shown in Figure 4b. It was found that the addition of NaCl led to a slight decrease in the yield of PL ICs, which is an opposite result to the one reported in the other paper for complexation between CDs and low molecular weight guests.[8] This may be explained by the fact that the anion can interact with α-CD competing with polymeric guests for complexation.

This study is of significant importance, considering that there has been no report on the pH-dependent PIC formation. Our findings clearly suggest that the ionization of the side groups controls the threading of CD molecules and that an inclusion complexation behavior of a polyelectrolyte such as PL with CDs can be a good model for understanding noncovalent interactions in biological systems.

Conclusion

The unique inclusion complexation between cationic poly(ϵ-lysine) and α-CD and the resulting crystalline structures were observed by changing various parameters, such as the molar composition between guest and host, the pH, and the ionic strength of aqueous media. The yield of PL ICs was significantly affected by the pH of aqueous media, due to the protonation of amino side groups of PL and the ionization of hydroxyl groups of α-CD. PL ICs would be very useful as building blocks for novel supramolecular structures that can respond to environmental conditions such as pH. PL ICs also would be good models for the study of noncovalent interactions in enzyme-function-directed chemistry where the pH dependence of the activity and denaturation is very important.

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