Cooperative Complexation of α-Cyclodextrin with Alternating Copolymers of Sodium Maleate and Dodecyl Vinyl Ether with Varying Molecular Weights

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Received January 5, 2008; Revised Manuscript Received February 28, 2008

ABSTRACT: In this study, we investigated the effect of the polymer molecular weight on the cooperative complexation of α-cyclodextrin (α-CD) with alternating copolymers of sodium maleate and dodecyl vinyl ether ($pC_{12}M$) by ^{1}H NMR measurements. It was confirmed that the $pC_{12}M$ prepared formed micelle-like aggregates in aqueous media by steady state fluorescence and sedimentation equilibrium experiments. Using the NMR data, the concentrations of free and complexed α-CD ($C_{CD,f}$ and $C_{CD,c}$, respectively) were calculated and the $C_{CD,c}$ values were plotted as a function of $C_{CD,f}$ to prepare binding isotherms. The binding isotherms exhibited sigmoidal curves for all the polymers examined, indicative of cooperative complexation. The binding isotherms also indicated that the complexation was more cooperative for $pC_{12}M$ of a higher molecular weight. Analysis of the binding isotherms using a model proposed based on the one-dimensional lattice theory indicated that the molecular weight dependency of the cooperative complexation was due to molecular dependent attractive interactions between free dodecyl groups and between complexed α-CD molecules.

Introduction

Molecular recognition in biological systems often exhibits high selectivity to form precisely controlled supramolecular structures which express various functions necessary for maintaining living activities.[1] Since, in most cases of molecular recognition in biological systems, macromolecules recognize macromolecular or low molecular weight species, it is likely that macromolecular chains play important roles for the high selectivity. We have been aware of the importance of macromolecular chains in the biological molecular recognition for the past decade, and thus studying on the interaction of cyclodextrins (CDs) with several hydrophobic guest moieties attached to water-soluble polymers (WSPs) in order to elucidate details of the molecular recognition in biological systems and to construct artificial molecular recognition systems with high selectivity.[2–9]

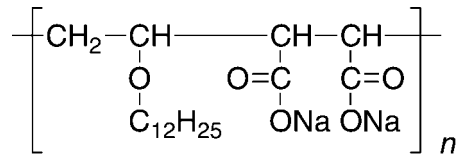
Several research groups including our group have reported a number of papers on the interaction of CDs with hydrophobically modified WSPs (HMWSPs).[5–7,10–17] Most of them dealt with changes in association properties of HMWSPs upon their complex formation with CDs. If the interpolymer hydrophobic association in HMWSPs dominates over the intrapolymer one in the absence of CDs, the formation of inclusion complexes of CDs with hydrophobes leads to dissociation of interpolymer aggregates, which sometimes results in a marked decrease in the solution viscosity.[5–7,10–17] On the other hand, if the hydrophobic association dominantly occurs intramoleculary, the formation of inclusion complexes of CDs with hydrophobes causes a transition of the polymer conformation from a folded one to an unfolded one.[18] It has been also reported that the lower critical solution temperature of thermally responsive polymers is controlled by the complexation of CDs and their derivatives.[19–23] To the best of our knowledge, however, there have been no researches from the viewpoint of the selectivity in the interaction of CDs with HMWSPs except for our studies.

In previous papers, we reported an enhancement of the selectivity in molecular recognition of CDs by the steric effect of the polymer main chain[2,3] and by the interactions at multisites (i.e., collectivity).[4] The ^{1}H NMR study on the interaction of CDs with side chains attached to poly(acrylamide) or poly(methacrylamide) backbone indicated that the selectivity of CDs toward side chains was higher than that toward low molecular weight model compounds. This may be because the steric hindrance of the polymer main chain restricted the inclusion mode of CDs.[2,3] The viscometric study on the interaction of a polymer bearing β-CD moieties with poly(acrylamide)s bearing aromatic side chains demonstrated that the formation of inclusion complexes at multisites caused a large difference in the size of interpolymer aggregates, even though the difference in the association constants for the complexation of native β-CD with the guest moieties was not very large.[4]

In the preceding paper,[24] we investigated the interaction of CDs with an alternating copolymer of sodium maleate and dodecyl vinyl ether ($pC_{12}M$ of $M_n = 6.0 \times 10^3$ in Scheme 1), which formed micelle-like aggregates in aqueous media, in order to explore the effect of the competition with self-association of hydrophobic guest groups. We demonstrated that the competition with self-association enhanced the selectivity of the polymer toward α-CD, retarded the complexation and the dissociation in the equilibrium, and caused cooperativity of the complexation.

This study deals with the effect of the polymer molecular weight on the interaction of α-CD with $pC_{12}M$. Here, we report that the binding isotherms obtained exhibit that the complexation is more cooperative for $pC_{12}M$ of a higher molecular weight, and analyze the binding isotherms using a model based on one-dimensional lattice theory as a first approximation.

Scheme 1. Chemical Structure of $pC_{12}M$ Used in This Study



$pC_{12}M$

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10.1021/ma800026h CCC: $40.75
Published on Web 04/17/2008

Experimental Section

Materials. Maleic anhydride (MAnh), dodecyl vinyl ether (C_{12}VE), and 2,2′-azobis(isobutyronitrile) (AIBN) were used as received. Tetrahydrofuran (THF) and toluene used for polymerization were purified by distillation over sodium under an argon atmosphere. 1-Cyano-1-methylethyl dithiobenzoate (CMEDTB) was prepared by a slight modification of the procedure of Thang et al.,[25] and used as a chain transfer reagent to prepare two polymer samples of lower molecular weights. α-, *β*-, and *γ*-CDs were recrystallized from water. Pyrene was recrystallized from ethanol. Other reagents were used without further purification.

Preparation of Polymer Samples. The polymers (pC_{12}M) used in this study were prepared as follows: MAnh, C_{12}VE, and AIBN were dissolved in THF or toluene under an argon atmosphere. For the preparation of polymer samples of lower molecular weights, a solution of CMEDTB ([CMEDTB]/[AIBN] = 5) was added to the mixture solutions under an argon atmosphere. The reaction mixtures were warmed using an oil-bath thermostatted at 65 or 70 °C (for THF or toluene). After polymerization, the reaction mixtures were poured into a large excess of methanol or hexane to precipitate polymers. The polymers obtained were purified by reprecipitation from THF into a large excess of methanol or hexane. The alternating copolymers of MAnh and C_{12}VE were hydrolyzed with 1.0 M NaOH in THF. The polymer of the lowest molecular weight was neutralized with 1.0 M HCl, and then recovered by freeze-drying. The other three polymers were further purified by dialysis against pure water, and recovered by freeze-drying.

Measurements. ^{1}H NMR spectra were measured on a JEOL JNM EX270 spectrometer at 30 °C. Ratios of area intensities were determined by curve fitting utilizing a JEOL ALICE2 software for Windows 98/NT4.0 (version 2.04.4). For all the NMR measurements, D_2O containing 11 mM $NaHCO_3$ and 11 mM Na_2CO_3 was used as a solvent.[26] Vapor pressure osmometry (VPO) measurements were performed by a Gonotec OSMOMAT070 vapor pressure osmometer equipped with two thermistor probes using toluene as a solvent at 60 °C. Gel permeation chromatography (GPC) analyses were carried out at 40 °C on a TOSOH CCP & 8010 system equipped with two TOSOH TSKgel MultiporeH_{XL}-M columns connected in series, using THF as eluent at a flow rate of 0.8 mL/min. TOSOH UV-8010 and TOSOH RI-8012 detectors were used. Steady state fluorescence spectra were measured on a Hitachi F-2500 fluorescence spectrophotometer using a 1 cm path length quartz cuvette. Emission spectra were measured with excitation at 333 nm. The slit widths for both excitation and emission sides were kept at 2.5 nm during measurement. For fluorescence measurements, a saturated aqueous solution of pyrene was used to prepare sample solutions. Sedimentation equilibrium experiments for micelle-like aggregates formed from pC_{12}M samples were performed using a Beckman-Coulter Optima XL-I type ultracentrifuge equipped with a Rayleigh interference optical system and a diode laser operating at 675 nm at 30.0 °C, and analyzed according to the standard procedure. Specific density increments ($\partial\rho/\partial c$) were measured by an Anton-Paar DMS5000 oscillation U-tube densitometer. The details of sedimentation equilibrium instrumentation and theory are described in the literature.[27]

Results

Basic Characteristics of pC_{12}M Samples Used in This Study. Since this study is focusing on the effect of the polymer molecular weight on the interaction of CDs with pC_{12}M, four pC_{12}M samples of different molecular weights were prepared: two pC_{12}M samples of lower molecular weights (pC_{12}M-1 and 2) were prepared by reversible addition-fragmentation chain transfer (RAFT) radical copolymerization and the other two (pC_{12}M-3 and 4) were prepared by conventional free radical copolymerization, followed by hydrolysis using NaOH.[28] Values of M_n and M_w/M_n for the pC_{12}M samples are listed in Table 1. The M_n values for pC_{12}M-1, 2, and 3 were determined by VPO data measured for the esterified polymer samples in toluene at 60 °C. Since the M_n for pC_{12}M-4, the polymer of the highest M_n, was unable to be determined by VPO, it was determined by GPC measured for the esterified polymer using THF as eluent, in which the molecular weight was calibrated using the relationship between the M_n values and the GPC elution times for the other three esterified polymers. Dividing the M_n values by the mass of the repeat unit, the numbers of the repeat units (n) were calculated to be 9, 16, 48, and 239 for pC_{12}M-1, 2, 3, and 4, respectively, as also listed in Table 1.

Table 1. Basic Characteristics of the Polymers Used in This Study

polymer code	$M_n \times 10^{-3}$ [a]	M_w/M_n [b]	n [c]	$M_{w,m} \times 10^{-4}$ [d]	m [e]
pC_{12}M-1	3.3	1.4	9	7.6	16
pC_{12}M-2 [f]	6.0	1.5	16	8.0	8
pC_{12}M-3	18	1.9	48	6.7	2
pC_{12}M-4	89 [g]	3.0	239	19	0.7

[a] Calculated using M_n determined by VPO for the esterified samples. [b] Determined by GPC for the esterified samples. Molecular weights were calibrated using polystyrene standards. [c] The number average degree of polymerization calculated using M_n and the molar mass of the repeat unit (372.4). [d] Determined by sedimentation equilibrium for aqueous solutions including 11 mM $NaHCO_3$ and 11 mM Na_2CO_3 [e] Aggregation number (i.e., the number of polymer chains per micelle-like aggregate) calculated by $M_{w,m}/(M_n \times M_w/M_n)$. [f] This polymer was the same as that used in the previous study.[24] [g] Calculated using M_n determined by GPC for the esterified samples. Molecular weight was calibrated using the relationship between the M_n values and the GPC elution times for the other three esterified polymers.

It is reported that alternating copolymers of sodium maleate and alkyl vinyl ether form micelle-like aggregates through hydrophobic association of alkyl hydrophobes in aqueous media.[29] However, since it remains unknown how the association behavior of pC_{12}M depends on the molecular weight, we have roughly characterized the micelle-like aggregates formed from the pC_{12}M samples of different molecular weights.

The formation of micelle-like aggregates was confirmed by steady state fluorescence measurements using molecular pyrene as a fluorescence probe. It is known that the fluorescence of pyrene is strongly dependent on the polarity of microenvironment around pyrene. The ratio of the third to first vibronic peaks (I_3/I_1) in fluorescence spectra is larger in more hydrophobic media.[30] Figure 1A shows fluorescence spectra normalized at the first vibronic peak for pyrene solubilized in aqueous solutions of pC_{12}M-3 containing 11 mM $NaHCO_3$ and 11 mM Na_2CO_3 at varying polymer concentrations (C_p). As C_p is increased, I_3/I_1 gradually increases. These spectra indicate that the polarity of microenvironment around pyrene probes becomes more hydrophobic with increasing C_p, indicative of the formation of micelle-like aggregates. As can be seen in Figure 1B, I_3/I_1 values were plotted against C_p for all the polymers used in this study. This figure indicates that I_3/I_1 commences to increase markedly at $C_p \approx 2 \times 10^{-3}$ g/L (5×10^{-6} M) for all the polymers, indicative of the formation of micelle-like aggregates. Whereas I_3/I_1 for pC_{12}M-3 and 4 exhibits a saturation tendency at higher C_p, which is normal behavior, that for pC_{12}M-1 and 2 does not. In the case of pC_{12}M-1 and 2, the fluorescence from pyrene probes was remarkably quenched at $C_p \geq 1$ g/L. This is because pC_{12}M-1 and 2, prepared by the RAFT technique, contain fragments derived from the chain transfer agent, which quench pyrene fluorescence.[31] From the I_3/I_1 values for pC_{12}M-3 and 4, the ratio of the concentrations of pyrene solubilized in micellar and bulk water phases ($[Py]_m/[Py]_w$) can be calculated using[32]

$$\frac{[Py]_m}{[Py]_w} = \frac{(I_3/I_1) - (I_3/I_1)_{min}}{(I_3/I_1)_{max} - (I_3/I_1)} = \frac{K_v\chi(C_p - cmc)}{1000\rho} \quad (1)$$

Here, $(I_3/I_1)_{min}$ and $(I_3/I_1)_{max}$ are the minimum and maximum values of I_3/I_1 that can be estimated from the data for pC_{12}M-3

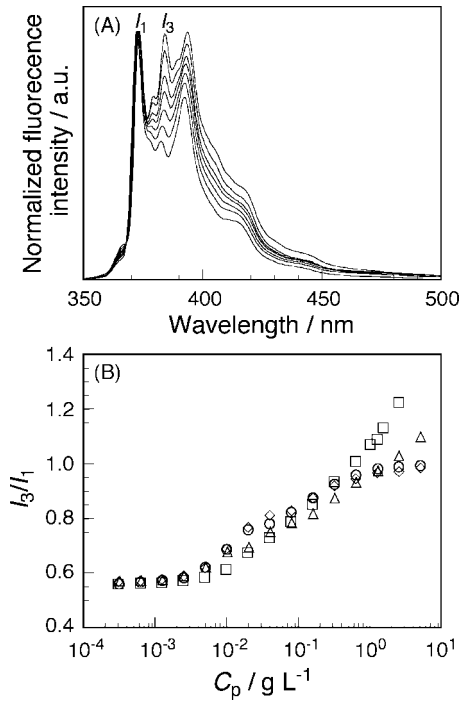

Figure 1. Normalized steady state fluorescence spectra for pyrene solubilized in aqueous solutions containting varying concentrations of $pC_{12}M$-3 (A) and I_3/I_1 as a function of C_p for $pC_{12}M$: $pC_{12}M$-1 (square), $pC_{12}M$-2 (triangle), $pC_{12}M$-3 (circle), and $pC_{12}M$-4 (diamond) (B).

and 4 in Figure 1B. K_v, χ, cmc, and ρ denote the partition coefficient of pyrene between the micellar and water phases, the weight fraction of the dodecyl (C_{12}) group in the polymer, the critical micelle concentration, and the density of the hydrophobic microdomain, respectively. As shown in Figure S1 in the Supporting Information, plots of $[Py]_m/[Py]_w$ against C_p did not exhibit any clear onset C_p at which $[Py]_m/[Py]_w$ increased abruptly. These observations indicated that the cmc values were too low to be determined for $pC_{12}M$-3 and 4. The cmc values for $pC_{12}M$-1 and 2 were unable to be estimated because $(I_3/I_1)_{max}$ for these polymers could not be determined. On the basis of the I_3/I_1 data in Figure 1B, however, it is likely that these polymers also form micelle-like aggregates at $C_p \geq 2 \times 10^{-3}$ g/L.

The molar masses of micelle-like aggregates formed from $pC_{12}M$ were determined by sedimentation equilibrium measurements in the C_p range, where $pC_{12}M$ formed micelle-like aggregates. Figure 2 shows the reciprocal of apparent molar mass (M_{app}^{-1}) as a function of C_p. For all the $pC_{12}M$, plots exhibit good linear relationships. From the intercepts of these straight lines, the weight-average molar masses ($M_{w,m}$) of micelle-like aggregate were calculated as listed in Table 1. Using the $M_{w,m}$ and M_w ($= M_n \times M_w/M_n$) values, the numbers of polymer chains forming a micelle-like aggregate (m) were estimated to be 16, 8, 2, and 1 for $pC_{12}M$-1, 2, 3, and 4, respectively, as also listed in Table 1. These data indicate that $pC_{12}M$ shows a strong tendency for closed (or intramolecular) association.

Interaction of CDs with $pC_{12}M$ Samples. The interaction of α-, β-, and γ-CDs with $pC_{12}M$ was investigated by ^{1}H NMR spectroscopy. Figure 3 shows an example of ^{1}H NMR spectra for $pC_{12}M$-3 in the absence and presence of CDs. The ^{1}H NMR spectrum in the absence of α-CD (Figure 3A) shows the signals due to methyl and methylene protons in the C_{12} groups of $pC_{12}M$-3 at 0.96 and 1.38 ppm, respectively. In the presence of a large excess of α-CD (35 mM) (Figure 3B), these signals exhibit downfield shifts, indicative of the complexation of α-CD

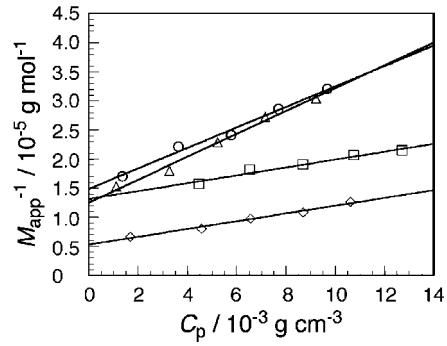

Figure 2. Concentration dependencies of M_{app}^{-1} for $pC_{12}M$: $pC_{12}M$-1 (square), $pC_{12}M$-2 (triangle), $pC_{12}M$-3 (circle), and $pC_{12}M$-4 (diamond) measured in an aqueous solution containing 11 mM $NaHCO_3$ and 11 mM Na_2CO_3 at 30 °C.

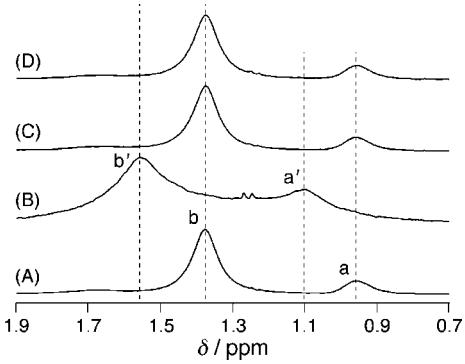

Figure 3. ^{1}H NMR spectra for $pC_{12}M$-3 (1.1 mM in C_{12} unit) in the absence of CDs (A) and in the presence of 35 mM α-CD (B), 15 mM β-CD (C), and 35 mM γ-CD (D) measured in D_2O containing 11 mM $NaHCO_3$ and 11 mM Na_2CO_3. Signals observed in 0.8–1.8 ppm are assignable to methyl (a and a′) and methylene (b and b′) protons in free (a and b) and complexed (a′ and b′) C_{12} groups.

with C_{12} groups in $pC_{12}M$-3. On the other hand, no peak shift was observed even upon addition of a large excess of β- or γ-CD (Figures 3C and 3D), indicating that neither β- nor γ-CD interacted significantly with $pC_{12}M$-3. NMR spectra confirmed that the other three polymers also interacted with α-CD to form inclusion complexes and did not interact significantly with either β- or γ-CD.[33] It is reported that all α-, β-, and γ-CDs interact with C_{12} groups in poly(acrylamide) modified with a small amount of C_{12} groups. The association constants were determined to be 9.9×10^2, 6.6×10^2, and 2.5×10^2 M^{-1} for α-, β-, and γ-CDs, respectively.[2] On the other hand, in the case of micelles of sodium dodecyl sulfate (SDS), all α-, β-, and γ-CDs also interact with SDS molecules to form inclusion complexes as can be seen in Figure S2 in the Supporting Information. Therefore, it is likely that the selectivity of complexation is enhanced by the competition with self-association of C_{12} groups in $pC_{12}M$. The C_{12} groups in $pC_{12}M$ associate with each other to be stabilized. When an α-CD molecule includes a C_{12} group in $pC_{12}M$, the association of the C_{12} group included with other C_{12} groups should be dissociated. Since α-CD interacts most strongly with the C_{12} group among the three CDs examined, only α-CD may induce the dissociation of self-association of C_{12} groups in $pC_{12}M$ to form inclusion complexes. Therefore, the formation of micelle-like aggregates via predominant intrapolymer self-association of C_{12} groups is responsible for the enhanced selectivity.

In order to elucidate the complexation equilibrium between α-CD and $pC_{12}M$ samples, ^{1}H NMR spectra were measured in the presence of varying concentrations of α-CD. Figure 4 shows an example of the ^{1}H NMR spectra obtained for $pC_{12}M$-3. The noteworthy is that these spectra exhibit the signals due to the

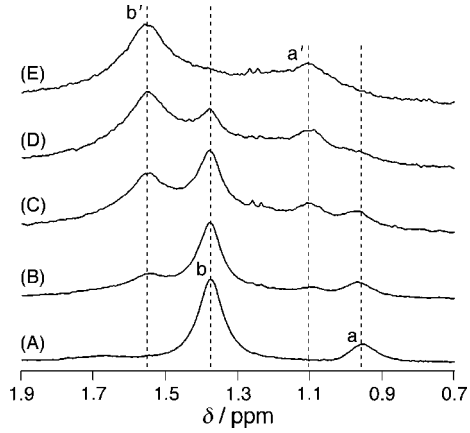

Figure 4. ^{1}H NMR spectra for $pC_{12}M$-3 (1.1 mM in C_{12} unit) in the presence of varying concentrations of α-CD measured in D_2O containing 11 mM $NaHCO_3$ and 11 mM Na_2CO_3: $C_{CD} = 0$ (A), 16.5 (B), 19.4 (C), 22.1 (D), and 33.0 mM (E). Signals observed in 0.8–1.8 ppm are assignable to methyl (a and a′) and methylene (b and b′) protons in free (a and b) and complexed (a′ and b′) C_{12} groups.

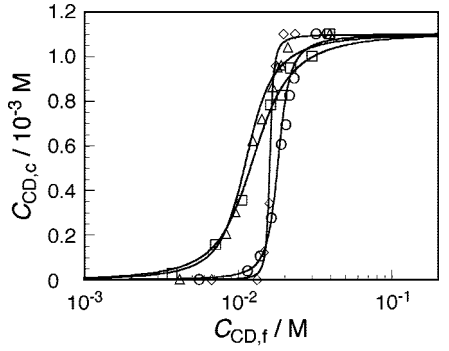

Figure 5. Binding isotherms for mixtures of α-CD and $pC_{12}M$: $pC_{12}M$-1 (square), $pC_{12}M$-2 (triangle), $pC_{12}M$-3 (circle), and $pC_{12}M$-4 (diamond). The best-fitted curve using eq 6 were also drawn.

free and complexed C_{12} groups (i.e., methyl and methylene protons) separately in the intermediate region of the concentration of α-CD added (C_{CD} = 16.5–22.1 mM). These spectra are indicative of an exchange between the free and complexed species slower than the time scale of NMR (∼milliseconds). NMR spectra for mixtures of α-CD with polymers bearing a small amount of C_{12} groups[2,5] and with SDS exhibited gradual shifts of the signals due to C_{12} groups with increasing C_{CD} but did not exhibit the separate signals due to the free and complexed C_{12} groups (data not shown). These observations indicate that the formation of micelle-like aggregates via predominant intrapolymer association of C_{12} groups causes the slow exchange. It should be noted here that the NMR spectra for mixtures of α-CD and $pC_{12}M$-1, the polymer of the lowest molecular weight, exhibit the separate signals due to the free and complexed C_{12} groups (data not shown), indicating that the number of repeat unit (n) of 9 is large enough for the slow exchange.

The concentrations of the free and complexed C_{12} groups were determined from the area intensities by curve fitting. From the concentrations of the free and complexed C_{12} groups, the concentrations of the free and complexed α-CD ($C_{CD,f}$ and $C_{CD,c}$, respectively) were calculated, assuming the formation of one-to-one inclusion complexes.[34,35] Values of $C_{CD,c}$ were plotted against $C_{CD,f}$ to prepare binding isotherms for the α-CD/$pC_{12}M$ system as shown in Figure 5. For all the polymers, binding isotherms exhibit sigmoidal curves, indicative of cooperative complexation of α-CD with $pC_{12}M$ samples. It is important to note that $C_{CD,c}$ increases more steeply with increasing $C_{CD,f}$ for $pC_{12}M$ of a higher molecular weight, indicative of more cooperative complexation of α-CD with $pC_{12}M$ of a higher molecular weight.

Discussion

The present α-CD/$pC_{12}M$ system exhibited that the cooperative complexation of α-CD with $pC_{12}M$ was dependent on the polymer molecular weight (or the number of the repeat units, n). In order to explain the cooperative complexation, it should be important to discuss the complexation of α-CD taking into account the structure of micelle-like aggregates formed from $pC_{12}M$. In the absence of α-CD, $pC_{12}M$ polymers form micelle-like aggregates, in which the polymer chains take a highly folded conformation because of hydrophobic association of C_{12} groups,[36] and thus there may be only a few C_{12} groups available for the complexation of α-CD. As C_{CD} is increased, α-CD starts to form complexes with C_{12} groups to form inclusion complexes at a certain C_{CD}. Once the complexation starts, the polymer chains are unfolded because of dissociation of the hydrophobic association of C_{12} groups,[18] and the C_{12} groups thus become more available for the complexation of α-CD, resulting in the cooperative complexation. The cooperative complexation causes dissociation of micelle-like aggregates.[24] In addition, when there are some attractive interactions between neighboring complexed α-CD molecules, it is also responsible in part for the cooperative complexation. To the best of our knowledge, however, there have been no specific models which can be applicable to the cooperative complexation of the present α-CD/$pC_{12}M$ system.

In the case of cooperative binding of a small molecule to a polymer bearing a number of binding sites, such as the binding of an ionic surfactant molecule to an oppositely charged polyelectrolyte, the binding of the small molecule at one site increases the affinity for the molecule at adjacent sites. To account for such binding cooperativity, the one-dimensional lattice theory is usually applied.[37–39] Since the one-dimensional lattice theory cannot deal with the formation of micelle-like aggregates, it is not apparently suitable to apply to the cooperative complexation of α-CD with $pC_{12}M$. However, when we attempted to analyze the present α-CD/$pC_{12}M$ system using a model proposed below on the basis of the one-dimensional lattice theory, we found surprisingly good fits as can be seen in Figure 5. Therefore, in this study, we would like to discuss the analysis of the present system using the model based on the one-dimensional lattice theory as a first approximation.

The polymer, $pC_{12}M$, can be considered as a one-dimensional chain bearing binding sites, i.e., C_{12} groups, apart from each other with the same distance. When a C_{12} group is either free or complexed, the state is defined as 0 or 1, respectively. When the i- and $(i + 1)$-th C_{12} groups are free, i.e., in the 00 state, nearest neighboring C_{12} groups may be stabilized because of hydrophobic interaction. The free energy of the hydrophobic interaction is defined as ΔG_{HP}. This energy corresponds to that required for transformation from the 0′0 state where nearest neighboring C_{12} groups do not associate and are exposed to the water phase to the 00 state. When C_{12} groups in $pC_{12}M$ are exposed to the water phase, these C_{12} groups can be included by α-CD molecules to be stabilized. The free energy for the stabilization due to the formation of inclusion complexes is defined as ΔG_{IC}, corresponding the difference in energy between the 0′0 state and the 10 state. When the i-th C_{12} site is free and the $(i + 1)$-th C_{12} site is included by α-CD, i.e., the 01 state, the energy evolved upon complex formation of α-CD with the i-th C_{12} group may include ΔG_{IC} and the interaction energy between the nearest neighboring α-CD molecules. The free energy of the interaction between the nearest neighboring α-CD molecules is defined as ΔG_{CD}. When $\Delta G_{CD} < 0$, the interaction is attractive, whereas, when $\Delta G_{CD} > 0$, the interaction is

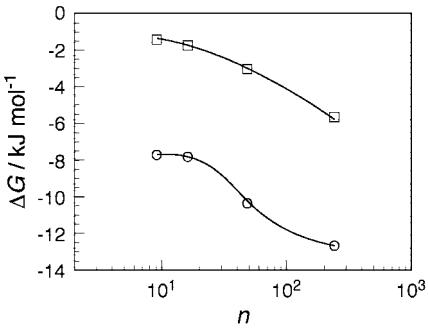

Figure 6. ΔG_{HP} (circle) and ΔG_{CD} (square) as a function of n.

repulsive. Therefore, the energy $\Delta G_{IC} + \Delta G_{CD}$ is evolved when the state is transformed from the 01 state to the 11 state. The energy diagram is shown in Figure S3 in the Supporting Information.

The statistical weight matrix **M** can be defined as

$$\mathbf{M} = \begin{pmatrix} \rho_{00} & 1 \\ \rho_{10} & \rho_{11} \end{pmatrix} \tag{2}$$

where ρ_{00}, ρ_{10}, and ρ_{11} are statistical weights. The statistical weights, ρ_{00}, ρ_{10}, and ρ_{11}, are expressed using ΔG_{HP}, ΔG_{IC}, ΔG_{CD}, and $C_{CD,f}$ as

$$\rho_{00} = \exp\left(-\frac{\Delta G_{HP}}{RT}\right) \tag{3}$$

$$\rho_{10} = C_{CD,f} \exp\left(-\frac{\Delta G_{IC}}{RT}\right) \tag{4}$$

$$\rho_{11} = C_{CD,f} \exp\left(-\frac{\Delta G_{IC} + \Delta G_{CD}}{RT}\right) \tag{5}$$

where R and T denote the gas constant and the absolute temperature, respectively. As described in the Supporting Information, the concentration of the complexed α-CD, $C_{CD,c}$, is expressed as

$$C_{CD,c} = C_{C12} D \frac{1 + R'R^n - \dfrac{(R+R')(1-R^n)}{n(1-R)}}{1 + \dfrac{D}{1-D} R'R^n} \tag{6}$$

where C_{C12} denotes the concentration of C_{12} group and $D = (\lambda_0 - \rho_{00})/(\lambda_0 - \lambda_1)$, $R' = \lambda_1/\lambda_0$, and $R' = (\lambda_1 - \rho_{00} + 1)/(\lambda_0 - \rho_{00} + 1)$. Here λ_0 and λ_1 are the eigenvalues of **M**:

$$\lambda_0 = \frac{(\rho_{00} + \rho_{11}) + \sqrt{(\rho_{00} - \rho_{11})^2 + 4\rho_{10}}}{2} \tag{7}$$

$$\lambda_1 = \frac{(\rho_{00} + \rho_{11}) - \sqrt{(\rho_{00} - \rho_{11})^2 + 4\rho_{10}}}{2} \tag{8}$$

As reported previously, the association constant for the formation of inclusion complexes of α-CD with a polymer bearing C_{12} groups exposed to the water phase was determined to be ca. 10^3 M^{-1},[2] corresponding to -17.4 kJ mol^{-1} at 30 °C. Thus, in this study, ΔG_{IC} is fixed at -17.4 kJ mol^{-1} to reduce the number of fitting parameters into two, i.e., ΔG_{HP} and ΔG_{CD}. When the values for ΔG_{HP} and ΔG_{CD} are selected appropriately, the binding isotherms calculated using eq 6 fitted well with the data obtained as can be seen in Figure 5.

The fitting parameters, ΔG_{HP} and ΔG_{CD}, are plotted against n in Figure 6. As can be seen in this figure, ΔG_{HP} and ΔG_{CD} take negative values in the whole n range examined, indicating that there are attractive interactions not only between the nearest neighboring free C_{12} groups but also between the nearest neighboring complexed α-CD molecules. It is supposed that the attractive interaction between α-CD molecules is due to hydrogen bonding based on the ΔG_{CD} values (-1.5 to -5.7 kJ mol^{-1}). The absolute values of ΔG_{HP} are larger than those of ΔG_{CD}, indicating that the cooperativity observed in the present system is caused dominantly by the hydrophobic interaction of free C_{12} groups. It should be noted here that ΔG_{HP} and ΔG_{CD} decrease from -7.8 to -12.7 kJ mol^{-1} and from -1.5 to -5.7 kJ mol^{-1}, respectively, with increasing n from 9 to 239. These observations are responsible for the molecular weight dependency of the cooperativity.

The n dependency of ΔG_{HP} may come from the molecular weight dependent association behavior of pC_{12}M. The present model based on the one-dimensional lattice theory considers only hydrophobic interaction between nearest neighboring C_{12} groups. However, since pC_{12}M forms micelle-like aggregates, in which the polymer main chains are highly folded,[36] the real system should include not only the hydrophobic interaction between nearest neighboring C_{12} groups but also intrapolymer hydrophobic interaction among C_{12} groups not nearest neighboring and interpolymer hydrophobic interaction. Furthermore, the structure of the micelle-like aggregate should be fluctuated with time. Therefore, the ΔG_{HP} values may be certain statistical and time average values for the hydrophobic interaction in micelle-like aggregates. As listed in Table 1, the number of polymer chains forming a micelle-like aggregate, m, decreases with increasing the polymer molecular weight, and, in the case of pC_{12}M-4, the polymer of the highest molecular weight, a micelle-like aggregate is formed from a single polymer chain. It is likely that intrapolymer hydrophobic interaction is more favored entropically than interpolymer hydrophobic interaction. The n dependency of ΔG_{CD} may come from the molecular weight dependent ability for the formation of hydrogen bonding between complexed α-CD molecules. At this point, however, we have no clear explanation for the molecular weight dependent ability for hydrogen-bonding formation, but it may be important to consider the fluctuation of the hydrogen bonding formation between complexed α-CD molecules, which may be dependent on the polymer molecular weight.

A more realistic model taking into account the formation of micelle-like aggregates of pC_{12}M should be proposed in the future.

Conclusion

The complexation of α-CD with pC_{12}M of different molecular weights, which form micelle-like aggregates in aqueous media, was investigated by ^{1}H NMR measurements to explore the effect of the polymer molecular weight on the complexation. The binding isotherms obtained from the ^{1}H NMR data exhibited sigmoidal curves, indicative of cooperative complexation of α-CD with pC_{12}M. The binding isotherms also indicated that the complexation was more cooperative for pC_{12}M of a higher molecular weight. The binding isotherms were analyzed using a model proposed based on the one-dimensional lattice theory as a first approximation, in which the hydrophobic interaction between next neighboring C_{12} groups and the interaction between next neighboring complexed α-CD molecules were taken into account. When the interaction energies, ΔG_{HP} and ΔG_{CD}, were selected appropriately, the binding isotherms calculated fitted well with the experimental binding isotherms. The fitting parameters, ΔG_{HP} and ΔG_{CD}, decreased from -7.8 to -12.7 kJ mol^{-1} and from -1.5 to -5.7 kJ mol^{-1}, respectively, with increasing the number of repeat units, n, from 9 to 239. These observations are responsible for the molecular weight dependency of the cooperativity.

Acknowledgment. The authors are grateful to Professor Takahiro Sato, Department of Macromolecular Science, Graduate

School of Science, Osaka University, for allowing us to use the ultracentrifuge instrument and densitometer and for fruitful discussion and valuable comments. One of the authors (D. Taura) expresses his special thanks for The Global COE (center of excellence) Program "Global Education and Research Center for Bio-Environmental Chemistry" of Osaka University. This work has been partially supported by a Grant-in-Aid No. A19205014 for Scientific Research and has been conducted with financial support from the "Stress and Symbiosis on Supramolecules" program of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. This research was also supported in part by "Special Coordination Funds for Promoting Science and Technology: Yuragi Project" of the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Supporting Information Available: Figures showing plots of pyrene concentration vs polymer concentration, ^{1}H NMR spectra, and an energy diagram of a model of the complexation and text showing the derivation of that model. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA800026H