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Macromolecular recognition by cyclodextrins. Interaction of cyclodextrins with polymethacrylamides bearing hydrophobic amino acid residues

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Abstract

The interaction of cyclodextrins (CDs) with poly(*N*-methacryloyltryptophan) (pMTrp) and with poly(*N*-methacryloylphenylalanine) (pMPhe) was investigated as a simple model system of macromolecular recognition of proteins. The association constants (*K*) for the model compounds, sodium salts of tryptophan and phenylalanine, are not so different (i.e. 43 and 16 M^{-1} for α-CD, 59 and 69 M^{-1} for β-CD, and 12 and 3 M^{-1} for γ-CD, respectively). On the other hand, there is a significant difference in the apparent *K* values for pMTrp and pMPhe (i.e. the *K* values for pMPhe are considerably smaller than ca. 10 M^{-1}, whereas those for pMTrp are 30, 83, and 11 M^{-1} for α-, β-, and γ-CDs, respectively). These observations indicate that a subtle difference in polymer side chains can be critical in macromolecular recognition.

Keywords: Macromolecular recognition; Cyclodextrins; Hydrophobic amino acids

1. Introduction

In biological systems, macromolecules, including proteins and DNA, recognize each other or other species to form precisely controlled supramolecular structures, resulting in expression of various functions necessary for maintaining living activities [1]. Macromolecular recognition in biological systems often exhibits high selectivity, in which noncovalent bonds through polymer side chains are crucial. Thus, it is important to understand how polymer side chains enhance the selectivity of macromolecular recognition in biological systems for construction of artificial molecular recognition systems with high selectivity. For this purpose, we have chosen systems composed of cyclodextrins (CDs) and guest moieties attached to water soluble polymers as simple model systems.

The interactions of CDs with guest moieties attached to water soluble polymers have been investigated by a number of research groups to construct supramolecular structures and to manipulate association properties of polymers [2–15]. We have been studying in detail the interaction of CDs with several guest moieties attached to water soluble polymers with a focus on the importance of polymer side chains in macromolecular recognition in biological systems [16–18]. In our previous paper [16], we have reported an enhancement of the selectivity of CDs by the steric effect of the polymer main chain: the ^1H NMR study on the interaction of CDs with alkyl side chains attached to poly(acrylamide) backbone indicated that the selectivity of CDs for alkyl side chains was higher than that for low molecular weight model compounds. This may be because CDs include alkyl side chains from one direction restricted by the steric hindrance of the polymer main chain. Furthermore, we have also reported an enhancement of the selectivity of CD by collectivity (i.e. interactions at multi-sites) [17]: 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 multi-sites caused a large difference in the size of interpolymer aggregates, even though the difference in the association constants for complexation of native β-CD with the guest moieties was not very large.

Recently, we also investigated the association behavior of poly(methacrylamides) bearing hydrophobic amino acid residues, tryptophan and phenylalanine (pMTrp and pMPhe, respectively, in Scheme 1), to understand polymer structural factors which cause distinctions in their association properties [19]. Both the polymers formed hydrophobic microdomains at pH < apparent pK_a (≈5.8), and adopted a rather extended conformation at pH > apparent pK_a. At pH ≈ 5 (< apparent pK_a), pMTrp had a stronger tendency for interpolymer association than pMPhe did. These observations led us to

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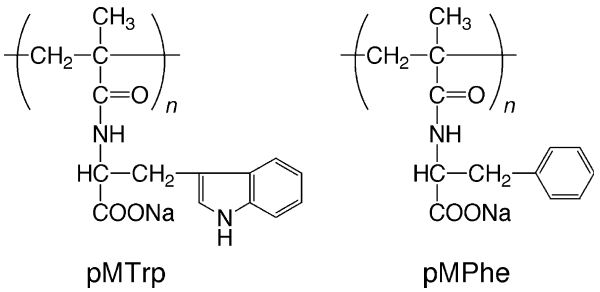

Scheme 1. Chemical structure of pMTrp and pMPhe.

conclude that the self-association properties of pMTrp and pMPhe somehow depended on the bulkiness and the hydrophobicity of the substituents of amino acid residues.

Polymers of *N*-(meth)acryloyl-amino acids have been synthesized and studied by many research groups because of their relevance to proteins [20–28]. Since it is considered that pMTrp and pMPhe are useful model polymers to study the macromolecular recognition of proteins, we were motivated to investigate the interaction of CDs with pMTrp and with pMPhe. In the present work, we have studied the interaction of CDs with these polymers mainly by ^{1}H NMR spectroscopy, and roughly estimated the association constants (K) for the complex formation of CDs with these polymers. Comparing the K values for the polymers with those for low molecular weight model compounds, we will describe that the subtle difference in the polymer side chains is important in macromolecular recognition by CDs.

2. Experimental

2.1. Materials

α-, β-, and, γ-Cyclodextrins (α-, β-, and γ-CDs, respectively) were purified by recrystallization from water. Milli-Q water was used for all the measurements except for NMR. Other reagents were used without further purification.

The polymers used in this study, poly(*N*-methacryloyltryptophan) (pMTrp) and poly(*N*-methacryloylphenylalanine) (pMPhe) (Scheme 1), were the same as those used in our previous work [19]. Values of M_n for pMTrp and pMPhe (esterified with diazomethane) were determined to be 5.1×10^4 and 3.1×10^4, respectively, by GPC relative to polystyrene standards.

Sodium salts of tryptophan and phenylalanine (Trp and Phe, respectively), used as model compounds in this study, were prepared by neutralization with an equimolar amount of NaOH and then recovered by freeze-drying.

2.2. Measurements

Absorption spectra were recorded on a JASCO V-550 spectrophotometer at 25 °C using a 1 cm path length quartz cuvette. Steady-state fluorescence spectra were measured on a HITACHI F-4500 fluorescence spectrophotometer with excitation at 280 nm at 25 °C using a 1 cm path length quartz cuvette. The slit widths for both excitation and emission sides were kept at 2.5 nm during measurement. Circular dichroism spectra were recorded on a JASCO J-820 spectropolarimeter at 25 °C using a 1 mm path length quartz cuvette.

^{1}H NMR spectra were measured on a JEOL JNM EX270 spectrometer at 30 °C with a resolution of 0.16 Hz using D_2O as a solvent under carefully optimized conditions. Halfwidths were determined by curve fitting utilizing a JEOL ALICE2 software for Windows 98/NT4.0 (version 2.04.4). Two dimensional nuclear Overhauser effect spectroscopy (2D NOESY) data were obtained on a Varian UNITY INOVA plus 600 spectrometer at 30 °C using D_2O as a solvent. Mixing time before the acquisition of free induction decay was carefully varied and then fixed at 150 ms to obtain a genuine NOE and to avoid the effect of spin diffusion.

All the measurements were carried out at neutral pH without any adding salt, where either pMTrp or pMPhe does not form hydrophobic microdomains [19].

3. Results and discussion

Since Trp residues in pMTrp emit fluorescence, the interaction of CDs with Trp residues in pMTrp was investigated by steady-state fluorescence spectroscopy. Fig. 1 shows steady-state fluorescence spectra of 0.01 g/L pMTrp in the presence and absence of 10 mM α-, β-, and γ-CDs. The fluorescence spectrum in the presence of 10 mM α-CD is the same as that in its absence. On the other hand, in the cases of β- and γ-CDs, the fluorescence intensities in the presence of CDs are considerably higher than that in their absence, indicative of the interaction of β- and γ-CDs with Trp residues in pMTrp. Since absorption spectra of pMTrp in the presence of CDs were the same as that in their absence (data not shown), the formation of inclusion complexes of β- and γ-CDs with Trp residues in pMTrp enhances the fluorescence quantum yield. This may be because β- and γ-CDs protect the singlet-excited Trp residue from contact with water molecules upon complexation.

To determine the association constants (K) for the complex formation of CDs with Trp residues in pMTrp, steady-state

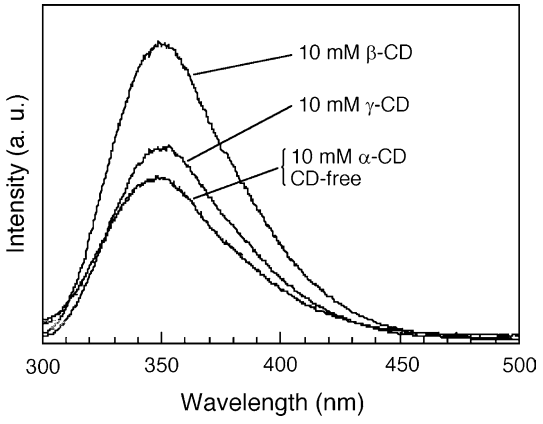

Fig. 1. Steady-state fluorescence spectra of 0.01 g/L pMTrp in the presence and absence of 10 mM α-, β-, and γ-CDs.

fluorescence spectra were recorded at varying CD concentrations (c_{CD}). Using these spectra, the ratios (I/I_0) of the fluorescence intensities in the presence of CDs and in their absence were calculated and plotted in Fig. 2 as a function of c_{CD}. In the cases of β- and γ-CDs, I/I_0 increases with c_{CD} and shows a slight tendency for saturation at higher c_{CD}, whereas, in the case of α-CD, I/I_0 is practically independent of c_{CD}. Assuming the formation of 1:1 complexes of CDs with Trp residues, the K values were determined to be 54 and 20 M^{-1} for β- and γ-CDs, respectively, by fitting data in Fig. 2 with

$$I/I_0 = \frac{(1 + a_1 K c_{CD})}{(1 + K c_{CD})} \quad (1)$$

where a_1 is a constant [29].

The interaction of CDs with pMTrp and with pMPhe was investigated by ^{1}H NMR spectroscopy, which does not require any fluorophores. ^{1}H NMR spectra of 1 mM α-, β-, and γ-CDs were measured in the presence and absence of pMTrp and pMPhe (20 mM monomer units). As an example, Fig. 3 shows expanded spectra for the resonance band due to C_1 protons in CDs, which does not overlap the resonance bands due to protons in the polymers. In contrast to our expectation, significant peak shifts were not observed upon addition of pMTrp and pMPhe. However, it should be noted that, in the presence of pMTrp, the resonance bands due to C_1 protons are broader than those in its absence. Especially, the spectrum of the β-CD/pMTrp mixture exhibits a remarkable broadening. These broad spectra in the presence of pMTrp are ascribable to an increase in the rotational correlation time of CDs, indicating that CDs interact with pMTrp to form complexes. In the case of pMPhe, on the other hand, the spectrum of the β-CD/pMPhe mixture exhibits only a slight broadening, but the spectra of the α-CD/pMPhe and γ-CD/pMPhe mixtures do not. These observations indicate that CDs interact only weakly or do not interact with pMPhe.

When the formation of complexes considerably disturbs the rotational motion of the components, the association constant can be determined utilizing the increase in halfwidth of resonance bands in NMR spectra (i.e. excess halfwidth) [30–32]. Using ^{1}H NMR spectra of CDs/pMTrp mixtures measured at varying polymer concentrations, excess halfwidths for the resonance band due to C_1 protons were calculated and plotted in Fig. 4 as a function of the concentration of the monomer unit (c_{mu}). As shown in this figure, plots are in fairly

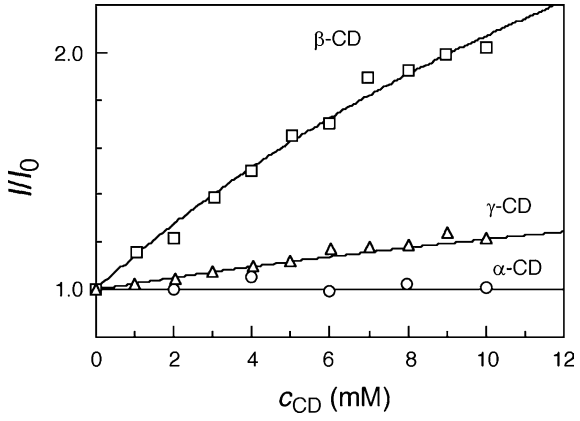

Fig. 2. I/I_0 as a function of the concentration of CD (c_{CD}) for the CDs/pMTrp systems. The best fitted curves using Eq. (1) are also drawn.

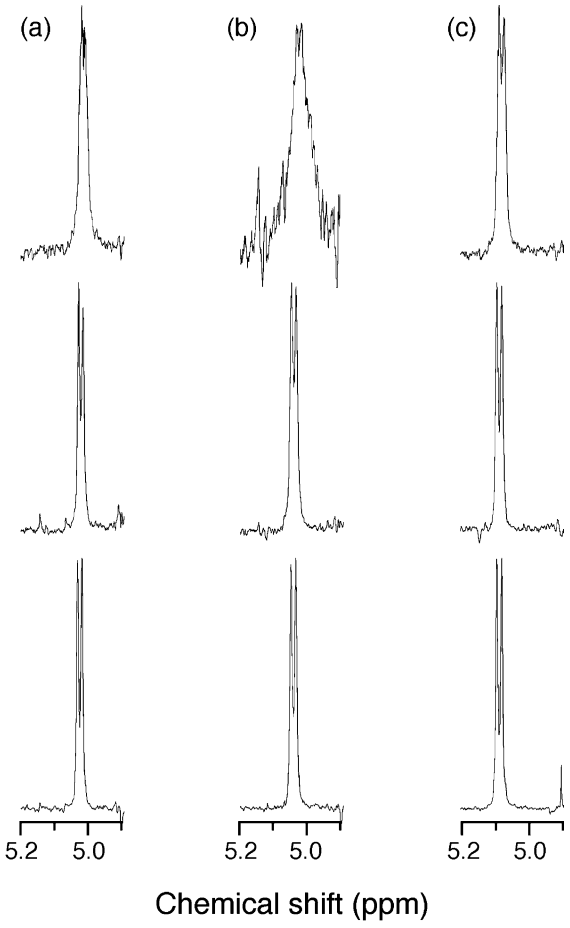

Fig. 3. ^{1}H NMR spectra of 1 mM α-, β-, and γ-CDs (a, b, and c, respectively) in the presence of pMTrp (top) and pMPhe (middle) (20 mM monomer units), and in the absence of the polymers (bottom).

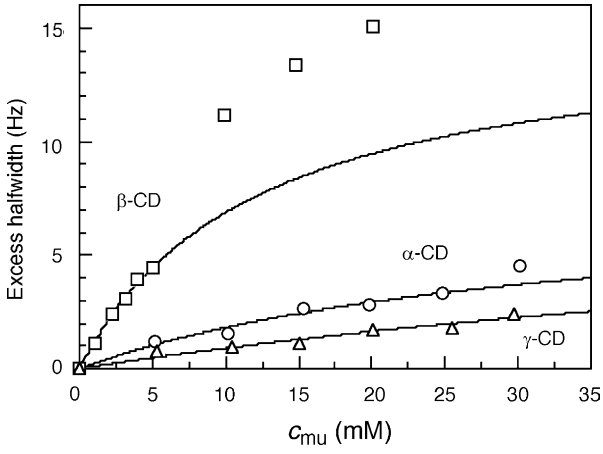

Fig. 4. Excess halfwidth as a function of the monomer unit concentration (c_{mu}) for the CDs/pMTrp systems. The best fitted curves using Eq. (2) in the c_{mu} regions 0–25, 0–5, and 0–30 mM for α-, β-, and γ-CDs, respectively, are also drawn.

Table 1
Apparent association constants (K) for complex formation of CDs with pMTrp and with pMPhe

	K (M^{-1})		
	α-CD	β-CD	γ-CD
pMTrp[a]	–[b]	54±3	20±2
pMTrp[c]	30±2	83±3	11±2
pMPhe[c]	–[d]	–[d]	–[d]

[a] Determined by steady-state fluorescence at 25 °C.
[b] The K value was unable to be determined because no increase in fluorescence was observed.
[c] Determined using change in the halfwidth of the resonance band due to C_1 protons at 30 °C.
[d] The K value was unable to be determined because of small excess halfwidths.

good agreement with the curves best fitted using Eq. (2) in the c_{mu} regions 0–25, 0–5, and 0–30 mM for α-, β-, and γ-CDs, respectively.

$$\{\text{Excess halfwidth}\} = \frac{a_2 K c_{mu}}{(1 + K c_{mu})} \quad (2)$$

where the formation of 1:1 complexes of CDs with the monomer units is assumed and a_2 is a constant. At c_{mu} higher than these regions, plots deviate from the best fitted curve presumably because of a significant effect of the solution viscosity. Especially, for the β-CD/pMTrp system, a significant increase in the solution viscosity was observed at higher c_{mu}. This may be because β-CD acts as cross-linker to form interpolymer aggregates of pMTrp. From the best fitted curves, the apparent K values were roughly estimated to be 30, 83, and 11 M^{-1} for α-, β-, and γ-CDs, respectively, as listed in Table 1. In the case of pMPhe, on the other hand, the apparent K values were unable to be estimated because the excess halfwidths were smaller than 1 Hz. It is likely that the apparent K values for pMPhe are significantly smaller than the smallest one for pMTrp (i.e. 11 M^{-1} for the γ-CD/pMTrp system).

As shown in Table 1, there are differences in the K values estimated by steady-state fluorescence and ^{1}H NMR spectroscopies (i.e. ca. 0 and 30 M^{-1} for α-CD, 54 and 83 M^{-1} for β-CD, and 20 and 11 M^{-1} for γ-CD, respectively). This may be because steady-state fluorescence spectroscopy focuses only on fluorophores, and because the conditions of measurements were different: the fluorescence measurements employed mixtures of a fixed concentration (0.01 g/L) of the polymer and excess CDs, whereas the ^{1}H NMR measurements employed mixtures of a fixed concentration (1 mM) of CDs and excess polymers.

As model systems, the interaction of CDs with Trp and with Phe was also investigated by ^{1}H NMR spectroscopy. Fig. 5 shows ^{1}H NMR spectra for 1 mM α-, β-, and γ-CDs measured in the presence and absence of 30 mM Trp and Phe. These spectra contain the resonance bands due to C_2 and C_4 protons in CDs in the region 3.5–3.7 ppm, those due to C_3, C_5, and C_6 protons in the region 3.7–4.0 ppm, and that due to C_1 protons in the region 5.0–5.1 ppm. In contrast to the cases of the CDs/pMTrp mixtures (Fig. 3), the spectra in Fig. 5 do not exhibit peak broadening, indicating that the rotational correlation time of CDs does not increase very much even upon complexation. However, the resonance bands due to C_1, C_3, C_5, and C_6 protons exhibit considerable upfield shifts upon addition of Trp and Phe (it is difficult to see peak shifts for the resonance bands due to C_2 and C_4 protons because these bands overlap the resonance bands due to the C_α methine protons in Trp and Phe). These upfield shifts may be caused by the ring current of the

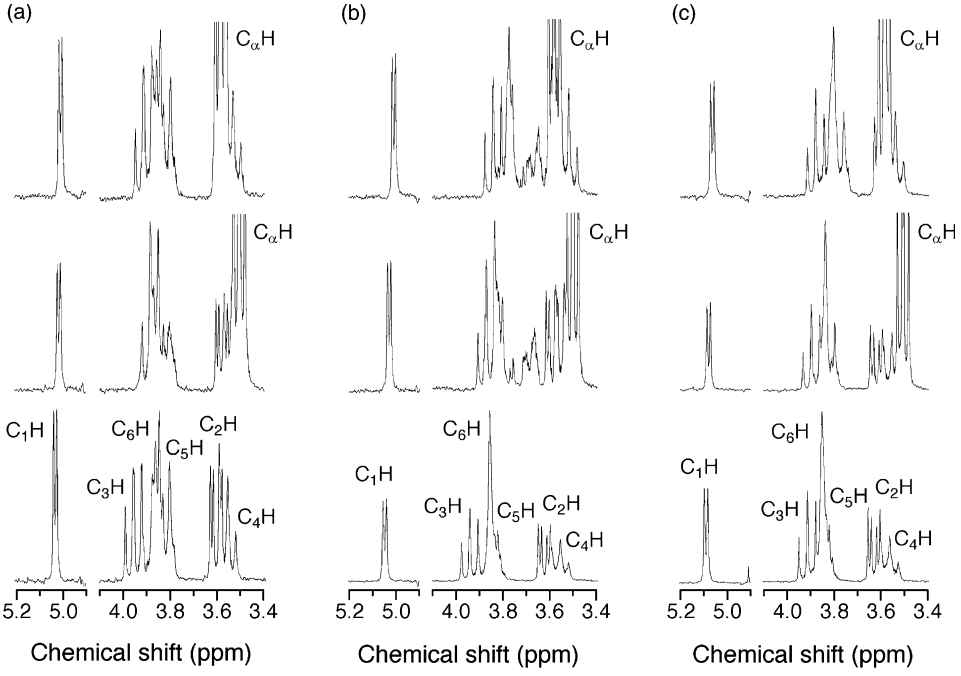

Fig. 5. ^{1}H NMR spectra of 1 mM α-, β-, and γ-CDs (a, b, and c, respectively) in the presence of 30 mM Trp (top) and Phe (middle), and in the absence of the model compounds (bottom).

aromatic rings in Trp and Phe, indicative of the formation of inclusion complexes.

In order to determine K values for the CDs/Trp and CDs/Phe systems, ^{1}H NMR spectra were measured at varying concentrations of the model compound (c_{mc}). Using these spectra, differences between the chemical shifts of the resonance bands due to the C_1 and C_3 protons were calculated to avoid an uncertainty in chemical shift values, and changes in the difference ($\Delta(\delta_{C1H}-\delta_{C3H})$) were plotted in Fig. 6 as a function of c_{mc}. Given the formation of 1:1 inclusion complexes, K values were determined by fitting data in Fig. 6 using

$$\Delta(\delta_{C1H}-\delta_{C3H}) = \frac{a_3 K c_{mc}}{(1+Kc_{mc})} \quad (3)$$

where a_3 is a constant (Table 2). These K values for Trp and Phe are in fairly good agreement with those determined by calorimetry [33,34].

Here we compare the K values for the polymers with those for the model compounds, although it should be noted that the K values for the polymers are apparent ones because monomer units are extremely localized on the polymer chains in the interaction of CDs with the polymers. As listed in Table 2, the K values for Trp and Phe are not so different (i.e. 43 and 16 M^{-1} for α-CD, 59 and 69 M^{-1} for β-CD, and 12 and 3 M^{-1} for γ-CD, respectively). However, there is a significant difference in the apparent K values roughly estimated by ^{1}H NMR for pMTrp and pMPhe (i.e. the K values for pMPhe are considerably smaller than ca. 10 M^{-1}, whereas those for pMTrp are 30, 83, and 11 M^{-1} for α-, β-, and γ-CDs, respectively). In our previous study [19], the ^{1}H NMR and 2D NOESY spectra indicated that a significant fraction of aromatic rings in pMTrp and pMPhe were located in the close proximity of the polymer main chain presumably because of hydrophobic and CH–π interactions. Furthermore, since the benzyl group in pMPhe is smaller and more hydrophobic than the (3-indolyl)methyl group in pMTrp, it is likely that benzyl groups in pMPhe interact more strongly with the polymer main chain than do (3-indolyl)methyl groups in pMTrp. Therefore, whereas the Trp residues in pMTrp are readily recognized by CDs, the Phe residues in pMPhe are not because the relatively stronger interaction of Phe residues with the polymer main chain hinders the interaction of CD.

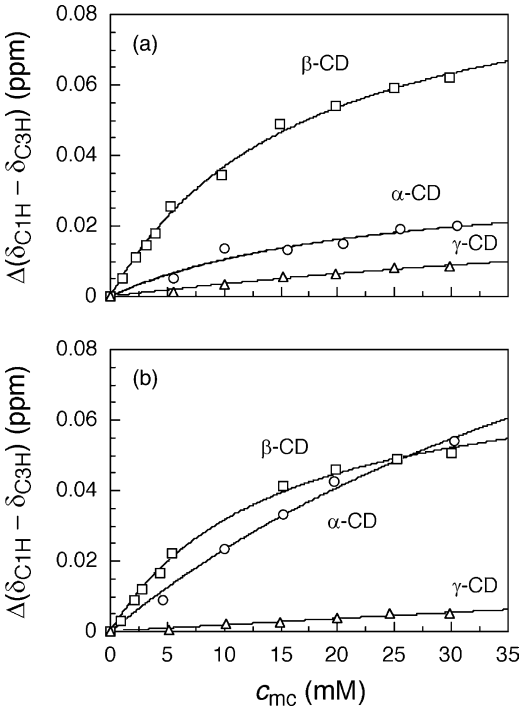

Fig. 6. $\Delta(\delta_{C1H}-\delta_{C3H})$ as a function of the concentration of the model compounds (c_{mc}) for the CDs/Trp and CDs/Phe systems. The best fitted curves using Eq. (3) are also drawn.

Table 2
Association constants (K) for complex formation of CDs with Trp and with Phe

	K (M^{-1})		
	α-CD	β-CD	γ-CD
Trp	43±6	59±5	12±6
Phe	16±5	69±7	3±1

Determined using the difference between chemical shifts of the resonance bands due to C_1 and C_3 protons in CD ($\Delta(\delta_{C1H}-\delta_{C3H})$) at 30 °C.

In the ^{1}H NMR spectra of the CDs/pMTrp systems, no significant peak shifts were observed, even though CDs interact with pMTrp. These observations suggest that CDs include Trp residues in pMTrp shallowly. This proposition was confirmed by the observation that the circular dichroism spectra of 0.01 g/L pMTrp in the presence and absence of 10 mM α-, β-, and γ-CDs are almost the same (data not shown). Since Trp residues in pMTrp interact with the polymer main chain presumably through hydrophobic and CH-π interactions in the absence of CDs, it is likely that CDs include Trp residues shallowly. In order to know how α-, β-, and γ-CDs interact with pMTrp, the 2D NOESY spectra were measured for CDs/pMTrp mixtures, as shown in Fig. 7. All the spectra show correlation peaks between the resonance bands due to CDs (3.7–4.0 ppm) and the resonance bands due to the indolyl group in pMTrp (6.5–8.0 ppm), indicative of the interaction of CDs with Trp residues in pMTrp. As described above, the interaction of α-CD with Trp residues in pMTrp was not detected by steady-state fluorescence spectroscopy, although the NOESY spectrum was indicative of the interaction. These observations indicate that α-CD cannot protect the singlet-excited Trp residue efficiently from contact with water molecules even upon complexation. It is noteworthy that the NOESY spectrum of the β-CD/pMTrp mixture (Fig. 7(b)) also exhibits correlation peaks between the resonance bands due to β-CD and the resonance bands in the region 0.0–2.5 ppm, indicating that β-CD interacts not only with Trp residues but also with α-methyl groups. Since an α-methyl group is too small to be included in a β-CD cavity, an α-methyl group may interact with a β-CD molecule, together with neighboring Trp residue or α-methyl group. It is likely that the relatively stronger interaction of β-CD with pMTrp observed in the

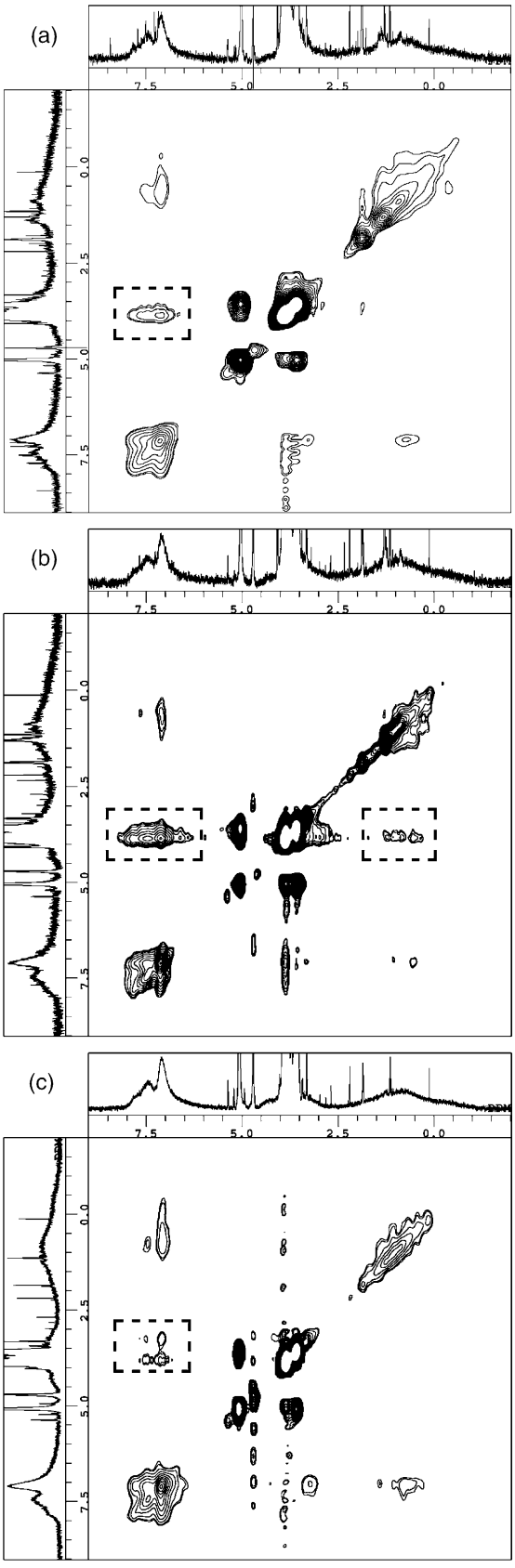

Fig. 7. NOESY spectra of mixtures of 1 mM α-, β-, and γ-CDs (a, b, and c, respectively) with pMTrp (3 mM monomer units).

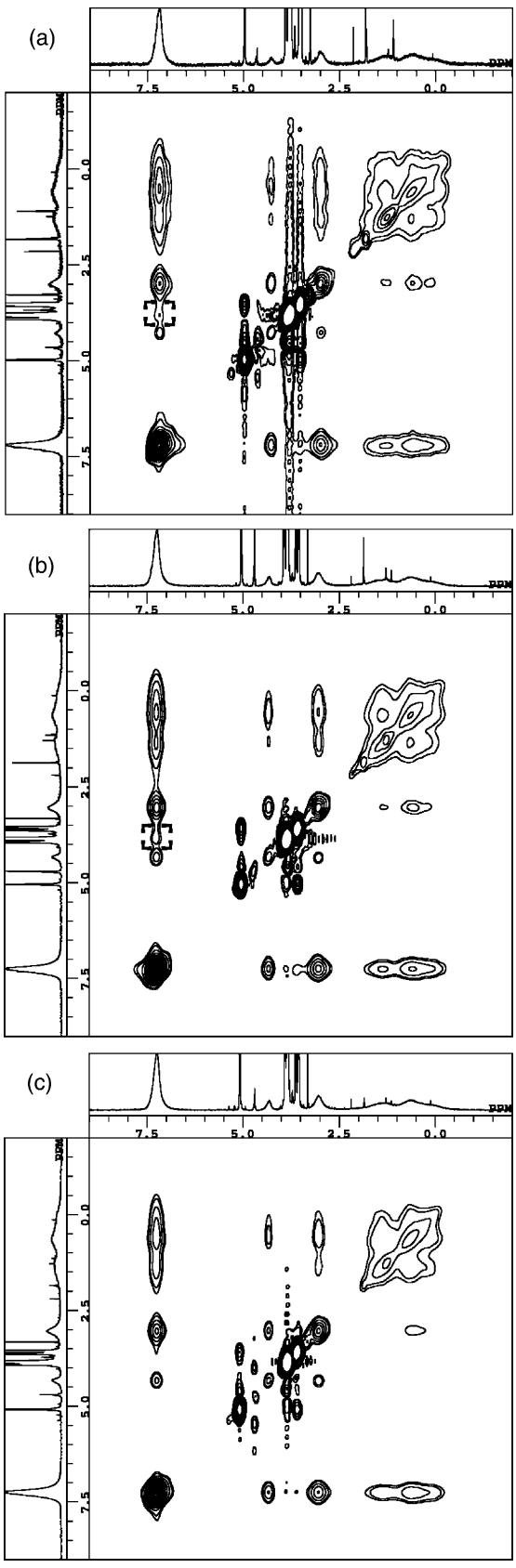

Fig. 8. NOESY spectra of mixtures of 1 mM α-, β-, and γ-CDs (a, b, and c, respectively) with pMPhe (3 mM monomer units).

^{1}H NMR measurements is caused by dual interactions of β-CD with Trp residues and with α-methyl groups.

We also measured 2D NOESY to investigate whether or not CDs interact with pMPhe (Fig. 8). The NOESY spectra for the α-CD/pMPhe and β-CD/pMPhe mixtures show weak correlation peaks between the resonance bands due to CDs (3.7–4.0 ppm) and the resonance bands due to the phenyl group in pMPhe (ca. 7.2 ppm) (Fig. 8(a) and (b)), indicating that α- and β-CDs do interact with Phe residues in pMPhe with apparent K values significantly smaller than ca. $10\ M^{-1}$. However, the interaction of γ-CD with pMPhe was not detected by the NOESY spectrum for the γ-CD/pMPhe system, neither (Fig. 8(c)).

4. Conclusion

The interaction of α-, β-, and γ-CDs with pMTrp and with pMPhe was investigated mainly by ^{1}H NMR spectroscopy as a simple model system for macromolecular recognition of proteins. Although K values for the model compounds, Trp and Phe, are not so different, there is a significant difference in apparent K values for pMTrp and pMPhe: the K values for pMPhe are considerably smaller than those for pMTrp. Since the benzyl group in pMPhe is smaller and more hydrophobic than the (3-indolyl)methyl group in pMTrp, it is likely that benzyl groups in pMPhe interact more strongly with the polymer main chain, resulting in the considerable difference in the K values. These observations indicate that a subtle difference in polymer side chains can be critical in macromolecular recognition. The polymers used in this study, pMTrp and pMPhe, take a relatively extended conformation under the present conditions, which is different from the higher order structures of proteins. However, we believe that the present system is useful as a first approximation because proteins recognize each other or other chemical species through their side chains.

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