Molecular Recognition by Self-Assembled Monolayers of Cyclodextrin on Ag†

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Received: November 25, 1996; In Final Form: June 17, 1997[⊗]

Self-assembled monolayer (SAM) of a thiolated cyclodextrin (CD) derivative was prepared, and stereoselective inclusional complexation of optically active azo dyes (*p*- or *o*-methyl red conjugates with chiral 1-phenylethylamine, *p*- or *o*-MR−PEA) with both free CD and the SAM of the CD derivative on silver surface was investigated by using resonance Raman (RR) and surface-enhanced resonance Raman spectroscopy (SERRS), respectively. Characteristic molecular vibrations of functional groups of MR−PEA were used to describe the complexation. To our knowledge, this is the first report which compares the complexations of free and surface-confined CD by Raman spectroscopy. Association constants for the complexation (*K*) were determined from the relative intensity of Raman scattering of the dye complexed with CD. The *K* values for the complexation between MR−PEA and free CD determined by Raman spectroscopy were quite similar to those measured by UV−visible spectroscopy, which confirmed the validity of Raman spectroscopy for the analysis of the inclusion phenomena. Though the *K* values of the SAM system were much larger than that of the free system, similar stereoselectivity was observed in both of the systems. The *R* enantiomer of *o*-MR−PEA was more preferentially included both by free and surface-confined CD than the *S*-enantiomer, whereas a slight selectivity was observed for *p*-MR−PEA. The importance of the position of optically active carbon atom with respect to the azobenzene moiety, which penetrated into the cavity of CD, was suggested. Our findings show that the enantioselectivity of CD is preserved after immobilization onto solid surface.

Introduction

Enzymes accelerate chemical reactions highly efficiently and selectively via enzyme−substrate complex. Cyclodextrins (CDs) which have a hydrophobic cavity whose dimensions are depth = 9 Å and diameters = 5, 7, and 8.5 Å for α-, *β*-, and *γ*-CD, respectively, have been investigated as an enzyme model because they form inclusional host−guest complexes with various substrates resulting in significant catalytic effects in many chemical reactions.[1] Regio- and stereoselective molecular recognition of CDs have been expected because the cavity of CDs themselves is surrounded by chiral sugars. Unmodified CDs, however, have a limit in the inclusional force and selectivity, and chemical modification of CDs with various functional groups has been carried out to improve those properties.[2] CD derivatives which have responsiveness to external stimuli,[3] and supramolecules such as catenane[4] and rotaxane (nanotube of CD)[5] were also prepared. Furthermore, modified or unmodified CDs have been applied to carriers of gas and liquid chromatography[6] or a drug delivery system.[7]

Recently, some research groups prepared self-assembled monolayers (SAMs) from thiolated CD derivatives on metal surfaces, and molecular recognition by surface-confined CDs was investigated.[8] SAMs of thiols and disulfides are spontaneously composed supramolecular assemblies on silver or gold surfaces via S−Ag or S−Au bonds, respectively.[9] The SAMs having various functionalities are expected to be applied to electronic devices and microprintings, and SAMs with long alkyl chains and hydrophilic surface are noted as biomembrane mimetics.[10]

Structure of SAMs and adsorbed molecules on the metal surfaces can be analyzed by using scanning tunneling microscopy,[11] atomic force microscopy,[12] infrared (IR) reflection absorption spectroscopy,[13] and Raman spectroscopy,[14] etc. Among them IR and Raman spectroscopies can provide very useful information because vibrational spectroscopy is sensitive to orientation and conformation of molecules. Substances adsorbed on metal surfaces are known to give rise to a strong Raman scattering (so-called surface-enhanced Raman scattering, SERS), when the surfaces are excited with a light in resonance with surface plasmon polariton of the metal.[15] SERS-active surfaces applied thus far are roughened electrode, island film and colloid, of gold, silver, and other metals.[10,14,15] Furthermore, a chromophore-containing substance causes surface-enhanced resonance Raman scattering (SERRS), which is much stronger than SERS, when the substance is excited at the resonance wavelength of the chromophore.[15] Furthermore, SERS and SERRS have a great advantage in the analysis of fluorescent molecules because of its ability to quench fluorescence, which causes trouble in normal Raman spectroscopy.[16]

In the previous study, we investigated complexation of azo dye by SAM of CD derivative[8a] as examples of analyses of molecular recognition at interface by SER(R)S. In the present work, we prepared SAM of a thiolated α-CD, 6-(2-mercaptoethylamino)-6-deoxy-α-CD (MEA-α-CD), on Ag and examined the stereoselective complexation between optically active azo dyes and the α-CD derivative by SERRS. The use of the guest molecules that have both asymmetric carbon and a chromophoric group enables us to investigate the chiral complexation phenomenon in detail by spectroscopic methods.

Experimental Section

Materials. α-Cyclodextrin (α-CD), (*R*)-(+)- and (*S*)-(−)-1-phenylethylamine ((*R*)- and (*S*)-PEA) were purchased from Wako Pure Chemicals (Osaka, Japan). *p*- and *o*-methyl red (*p*- and *o*-MR) were purchased from Tokyo Chemical Co. (Tokyo, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. Other reagents were commercially available. Milli-Q grade water was used for sample solutions.

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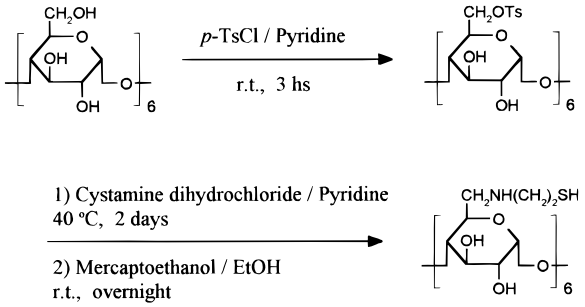
† Presented at the 45th Annual Meeting of the Society of Polymer Science, Japan, at Nagoya, in May 1996.

⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

S1089-5647(96)03916-8 CCC: $14.00

SCHEME 1. Preparation of 6-((2-Mercaptoethyl)amino)-6-deoxy-α-cyclodextrin (MEA-α-CD)



Ag colloids were prepared by reduction of $AgNO_3$ with $NaBH_4$ at 0 °C for 1 h, and finally pH of the suspension was adjusted to 7.35 with HCl. The final concentration of Ag was 3.13×10^{-4} mol/L. The absorption maximum of the colloids was 393 nm. Using a dynamic light-scattering method (DLS-7000DL, Otsuka Electronics, Hirakata, Japan; light source, Ar laser 488.0 nm), the average hydrodynamic diameter of the Ag particles was estimated to be 64 nm.

Modification of Cyclodextrin (Scheme 1). 6-((2-Mercaptoethyl)amino)-6-deoxy-α-cyclodextrin (MEA-α-CD), the thiolated α-CD, was prepared by a reduction with mercaptoethanol at room temperature overnight after a substitution of tosylated α-CD (degree of substitution of tosyl groups; 4) with excess amount of cystamine in pyridine at 40 °C for 2 days. After evaporation of the solvent, an oily reaction mixture was put into cold acetone, and a white precipitate was obtained. The precipitate was dissolved in MeOH:water = 3:1 and recrystallized in cold acetone. The precipitate was finally filtrated and dried in vacuo.

Yield 975 mg (86.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.26 (6H, C(1)*H*), 2.53 (8H, C*H*$_2$SH), 2.43 (16H, C*H*$_2$NH). Anal. Calcd for (MEA)$_4$-α-CD: C, 43.70; H, 6.67; N, 4.63. Found: C, 43.47; H, 6.81; N, 4.68.

Syntheses of Optically Active Azo Dyes. Four chiral azo dyes, methyl red−1-phenylethylamine conjugates (MR−PEAs), were synthesized by coupling of *p*- or *o*-MR with chiral PEA in chloroform at 0 °C for 3 h and at room temperature overnight by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as condensing agents. Dicyclohexylurea was filtrated off, and the filtrate was washed carefully with water, 10% $NaHCO_3$, 0.1 N HCl, and water in this order. The washed organic phase was dried with anhydrous Na_2SO_4 overnight. After Na_2SO_4 was filtrated off, the solvent was evaporated. The crude product was purified by column chromatography (Silica Gel 60 (Merck), mobile phase; solvent A (mentioned below) for *p*-MR−PEA and solvent B for *o*-MR−PEA).

All of the dyes were characterized by elemental analysis, optical rotatory power, and IR and UV−vis spectroscopy. Solvent systems used were solvent A $CHCl_3$:MeOH = 9.4:0.6, B $CHCl_3$:MeOH = 9.5:0.5, C $CHCl_3$:MeOH = 9.8:0.2, and D $CHCl_3$.

p-MR-(R)-PEA: Yield: 1.025 g (72.4%). $[\alpha]_D^{28} = -138$ mL dm^{-1} g^{-1} (in solvent C). $\epsilon_{max} = 15\,200$ cm^{-1} M^{-1} (437 nm, in solvent C). IR (KBr, cm^{-1}) 1633 ($\nu_{C=O}$, amide I), 1560 (δ_{NH}, amide II). Anal. Calcd for $C_{23}H_{24}N_4O \cdot {}^1/_3H_2O$: C, 73.08; H, 6.59; N, 14.80. Found: C, 72.75; H, 6.70; N, 14.31.

p-MR-(S)-PEA: Yield: 992 mg (70.6%). $[\alpha]_D^{28} = 142$ mL dm^{-1} g^{-1} (in solvent C). $\epsilon_{max} = 15\,400$ cm^{-1} M^{-1} (437 nm, in solvent C). IR (KBr, cm^{-1}) 1633 ($\nu_{C=O}$, amide I), 1560 (δ_{NH}, amide II). Anal. Calcd for $C_{23}H_{24}N_4O \cdot {}^1/_3H_2O$: C, 73.08; H, 6.59; N, 14.80. Found: C, 73.98; H, 6.56; N, 14.69.

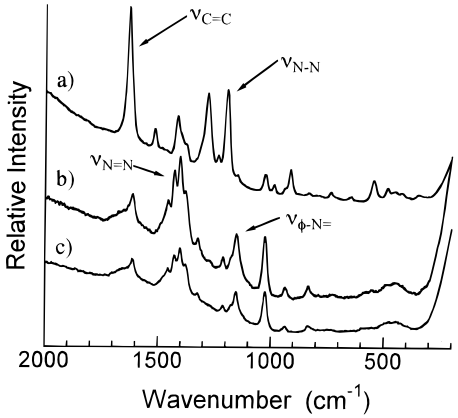


Figure 1. Resonance Raman spectra of 10 μM *p*-MR−(*R*)-PEA under various conditions. Excited at 488.0 nm: (a) 0.1 N HCl, (b) HEPES buffer (pH 7.35), and (c) 0.1 N NaOH.

o-MR-(R)-PEA: Yield: 230 mg (33.1%). $[\alpha]_D^{28} = -1056$ mL dm^{-1} g^{-1} (in solvent D). $\epsilon_{max} = 23\,200$ cm^{-1} M^{-1} (445 nm, in solvent D). IR (KBr, cm^{-1}) 1652 ($\nu_{C=O}$, amide I), 1554 (δ_{NH}, amide II). Anal. Calcd for $C_{23}H_{24}N_4O$: C, 74.20; H, 6.50; N, 15.00. Found: C; 74.42, H; 6.56, N; 15.02.

o-MR-(S)-PEA: Yield: 148 mg (21.3%). $[\alpha]_D^{28} = 1085$ mL dm^{-1} g^{-1} (in solvent D). $\epsilon_{max} = 22\,900$ cm^{-1} M^{-1} (445 nm, in solvent D). IR (KBr, cm^{-1}) 1652 ($\nu_{C=O}$, amide I), 1554 (δ_{NH}, amide II). Anal. Calcd for $C_{23}H_{24}N_4O$: C, 74.20; H, 6.50; N, 15.00. Found: C; 74.67, H; 6.59, N; 15.06.

Spectroscopic Measurements. Raman spectra were recorded on a Raman spectrophotometer (NR-1100, Japan Spectroscopic Co., Tokyo, Japan; light source, argon laser 488.0 nm) with a band resolution of 5 cm^{-1} or less in a 300 μL quartz cell. The observation cell was thermostated by a Peltier device (RT-IC, Japan Spectroscopic Co.).

UV−visible spectra were recorded on a UV−visible spectrophotometer (Ubest 35, Japan Spectroscopic Co.) equipped with a quartz cell thermostated by a Peltier device (EHC-363, Japan Spectroscopic Co.).

Preparation of Sample Solutions. To prepare SAM on Ag, MEA-α-CD dissolved in water was added to colloidal Ag and kept at ambient temperature for 1 h or more. A dye solution (0.1 mL, 270 μM in MeOH) was added to a buffer solution containing α-CD (2.6 mL) or a suspension of Ag colloid modified with MEA-α-CD for the measurements of the free CD system and the SAM system, respectively. Acidic and neutral solutions were prepared with a CH_3COONa−HCl (pH 3.00, 20 mM) and (2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES, pH 7.35, 10 mM) buffer, respectively.

Results and Discussion

Characterization of Optically Active Azo Dyes. Characterization of functional groups of *p*- and *o*-MR−PEAs in aqueous solutions of various pHs was carried out by using resonance Raman (RR) spectroscopy. Peaks attributable to the quinoid form (C═C stretching vibration ($\nu_{C=C}$) and N−N stretching vibration (ν_{N-N})) were observed under acidic conditions, and those of the azo form (N═N stretching vibration ($\nu_{N=N}$) and ϕ−N═ stretching vibration ($\nu_{\phi-N=}$)) were observed at neutral and alkaline conditions (Figure 1).[17] The pK_a's of the azo groups (pK_a(N═N)) of *p*- and *o*-MR−PEA obtained by pH titration using UV−visible spectroscopy were 4.0 and 4.2, respectively. These results suggest that these dyes exist as the azo form in neutral and alkaline solutions (pH > pK_a(N═N)), and the protonation of azo group in acidic solutions (pH < pK_a(N═N)) produces the quinoid form (Figure 2).[17]

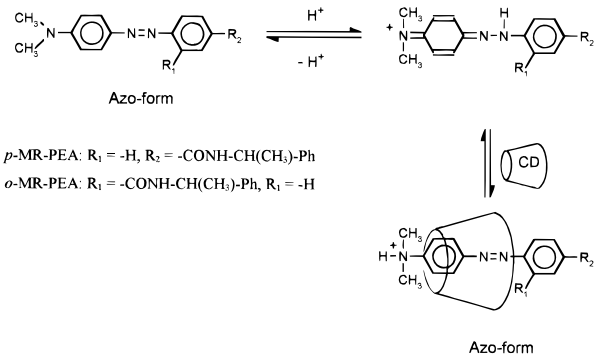


Figure 2. Schematic drawing of the structure changes of aminoazo-type dye induced by the environmental changes.

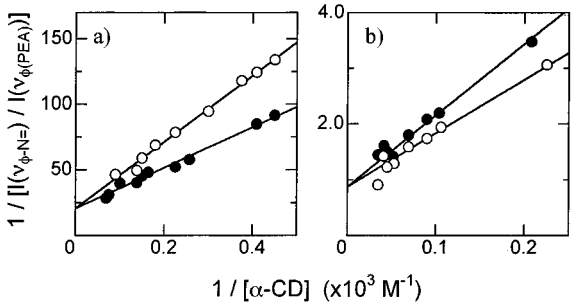


Figure 3. Double-reciprocal plot of $[I(\nu_{\phi-N=})/I(\nu_{\phi(PEA)})]$ versus the concentration of α-CD obtained by resonance Raman technique in CH_3-COONa−HCl buffer (pH 3) at 25 °C. $[I(\nu_{\phi-N=})/I(\nu_{\phi(PEA)})]$ is the difference in normalized intensities of $\nu_{\phi-N=}$ between systems with α-CD and without α-CD: (a) *o*-MR−PEA, (b) *p*-MR−PEA, (●) *R* enantiomer, and (○) *S* enantiomer.

The color of the solution of the dyes changed from red to yellow by an addition of α-CD solution under acidic conditions (pH 3.0). At the same time, in RR spectra of the solutions, the peak intensities at 1630 cm^{-1} ($\nu_{C=C}$) and 1194 cm^{-1} (ν_{N-N}) decreased, and those at 1405 cm^{-1} ($\nu_{N=N}$) and 1152 cm^{-1} ($\nu_{\phi-N=}$) increased with increasing concentration of α-CD. These spectral changes were considered to arise from the environmental change around the azo group of the dye molecules by a complexation in the hydrophobic cavity of α-CD. Consequently, the phenomenon observed suggests structural change of the dye from the quinoid to azo form due to the inclusion of the azonium group (=N−NH−) by α-CD (Figure 2).[8a,18] These spectral properties were commonly observed for all four dyes examined here.

Evaluation of the Association Constants by Using Resonance Raman Technique. The Raman spectra of MR−PEA drastically changed by the complexation with α-CD at acidic pH. The $\nu_{\phi-N=}$ band, which showed a pronounced spectral change with the inclusion by α-CD, was used to evaluate the relative population of complexed MR−PEA to free one. The intensity of the peak at 1025 cm^{-1} (attributable to a benzene ring of phenylethylamino group ($\nu_{\phi(PEA)}$)) was used as an internal standard, and the reciprocal of the relative intensity of $\nu_{\phi-N=}$ band $[I(\nu_{\phi(PEA)})/I(\nu_{\phi-N=})]$ was plotted against the reciprocal of the concentration of α-CD in Figure 3. A linear relationship in the wide concentration range of α-CD suggests that the stoichiometry of the inclusional complex between each azo dye and α-CD is 1:1. The association constants (*K*) of the complexation of these chiral azo dyes with α-CD were obtained from the *x* intercept of the plot.

On the other hand, some peaks coming from azo dyes such as methyl red and methyl orange were reported to shift by the complexation with CDs at neutral pH.[19] We used $\nu_{\phi-N=}$ band of MR−PEAs, which showed the most prominent peak shift by the complexation with α-CD under neutral conditions, for the evaluation of *K* of MR−PEAs. Stoichiometry of the inclusional complexation was 1:1, too. The *K* values obtained by using RR spectroscopy were close to those determined by UV−vis method which has been often used to analyze inclusional phenomena of CD (Table 1).[1,20] Therefore, the estimation of the association constants by Raman spectroscopy is acceptable.

The associations of α-CD with both *p*- and *o*-MR−PEA were pH dependent, and the *K* values in acidic solutions were larger than those in neutral solutions. This may be mainly due to difference in the flexibility of the azo and quinoid forms. The quinoid form (dominant in acidic solutions) is considered to be more flexible than the azo-form because the degree of rotational freedom around azonium bond (=N−NH−) is larger than that of azo bond (−N=N−). Accordingly, the quinoid form may penetrate into the cavity of α-CD more deeply and fit the inner wall of the CD cavity better than the azo form does. Association constant is the ratio of reaction rates for an association to a dissociation. The larger *K* value at acidic pH may be due to slower dissociation rate induced by the deeper inclusion.

The stereoselectivity was judged from the ratio of the *K* values for *R* and *S* enantiomers (*R*/*S* in Table 1). *o*-MR−PEA showed noticeable stereoselectivity at neutral pH and the selectivity slightly decreased under acidic conditions (*R*/*S* = 1.86 (pH 7.35) → 1.68 (pH 3.00)). The reduction of the selectivity of *o*-MR−PEA may be due to the increase in the nonspecific interaction of the MR moiety relative to the stereospecific interaction of the chiral PEA moiety. As for *p*-MR−PEA, the stereoselectivity was not observed at neutral pH (*R*/*S* = 1.06 (pH 7.35)), whereas at acidic pH a slightly selective complexation with the *S* enantiomer was realized (*R*/*S* = 0.76 (pH 3.00)). *p*-MR−PEA is presumed to be included more deeply, and the effect of the chiral center becomes more significant under acidic conditions. In short, when the inclusional force is strong, *p*-MR−PEA shows the enhanced stereoselectivity, whereas *o*-MR−PEA does not.

Recently, stereoselective complexation of α- and β-CD with chiral isomers was examined by titration calorimetry.[21] Stereoselectivity was observed in the complexations with ephedrine and pseudoephedrine,[21b] whereas no noticeable selectivity was detected for phenylalanine and chiral aliphatic alcohols.[21a] It is generally agreed that the existence of two bulky substituents on an asymmetric carbon atom is necessary for the stereoselective complexation with unmodified CDs and that spatial arrangements of the substituents are important for the selectivity.[1b] The present results are not inconsistent with the former one.

Thermodynamic Analysis. The temperature dependence of the inclusional complexation was examined by UV−vis spectroscopy at pH 7.35 (Table 1). The *K* values for both *p*- and *o*-MR−PEA decreased with the rise of temperature. Furthermore, the values of *R*/*S* for *o*-MR−PEA increased with the rise of temperature, whereas the stereoselectivity for *p*-MR−PEA did not change. The enhancement of selectivity for *o*-MR−PEA at higher temperature can also be rationalized with the similar mechanism stated above, that is, the reduction of the nonspecific MR moiety−CD interaction which relatively weakens the specific interaction of the chiral moiety improves the stereoselectivity.

Thermodynamic parameters for the inclusional complex formation were given in Table 2. With respect to *p*-MR−PEA, the driving force of the inclusional complexation is the large negative ΔH ($\Delta H = -34$ kJ/mol) for both of the optical isomers. On the other hand, the association of *o*-MR−PEA with α-CD is governed by entropy change (ΔS = 13 J/K mol) for the *R*

TABLE 1: Association Constants (*K*) and the Stereoselectivity (*R*/*S*) for the Complexation of *p*-MR−PEA and *o*-MR−PEA with Free α-CD and MEA-α-CD on Ag

			p-MR−PEA			*o*-MR−PEA		
			K (M^{-1})			K (M^{-1})		
	pH	temp. (°C)	(*R*)	(*S*)	*R*/*S*[d]	(*R*)	(*S*)	*R*/*S*[d]
free α-CD	3.00[a]	25	58 ± 23	73 ± 22	0.79 ± 0.37	138 ± 21	84 ± 12	1.64 ± 0.28
	3.00[b]	25	68 ± 8	90 ± 9	0.76 ± 0.10	140 ± 9	83 ± 7	1.68 ± 0.15
	7.35[a]	15	30 ± 4	30 ± 9	1.01 ± 0.29	91 ± 12	62 ± 11	1.47 ± 0.27
	7.35[a]	25	18 ± 4	17 ± 8	1.03 ± 0.21	84 ± 21	45 ± 16	1.85 ± 0.42
	7.35[b]	25	15 ± 4	14 ± 5	1.06 ± 0.23	90 ± 11	48 ± 8	1.86 ± 0.32
	7.35[a]	35	12 ± 3	12 ± 4	1.02 ± 0.38	76 ± 7	40 ± 10	1.92 ± 0.41
SAM[c]	7.35	25	(20 ± 0.2) × 10^4[e]	(23 ± 0.1) × 10^4[e]	0.86 ± 0.08[f]	(2.0 ± 0.1) × 10^4	(1.3 ± 0.2) × 10^4	1.61 ± 0.36

[a] Measured by UV−vis. [b] Measured by RR. [c] Measured by SERRS with SAM of MEA-α-CD on colloidal Ag. [MEA-α-CD] = 16 μM. [d] Ratio of the association constants (*K*) for *R* and *S* enantiomers. [e] The values of 1/*P* estimated from Freundlich isotherm are shown. [f] The ratio of 1/*P* for *R* and *S* enantiomers is shown.

TABLE 2: Thermodynamic Parameters for the Complexation of *p*-MR−PEA and *o*-MR−PEA with α-CD in 10 mM HEPES Buffer (pH 7.35)

	p-MR−PEA		*o*-MR−PEA	
	(*R*)	(*S*)	(*R*)	(*S*)
Δ*H* (kJ/mol)	−33.9 ± 2.5	−34.2 ± 2.9	−6.6 ± 3.8	−16.7 ± 5.8
Δ*S* (J/K mol)	−90 ± 1.3	−90 ± 1.4	13 ± 2.1	−26 ± 3.0
Δ*G* (kJ/mol)	−7.0 ± 0.46	−7.0 ± 1.04	−11.0 ± 0.52	−9.0 ± 0.76

enantiomer and governed by enthalpy change (Δ*H* = −16.7 kJ/mol) for the *S* enantiomer. As was often observed in the complexation of CDs with guest molecules, a linear relationship (the compensation effect) between Δ*H* and Δ*S* is present, and the slope of the straight line (isoequilibrium temperature) is 275 K, which is good agreement with the literature values (265[22] and 274 K[21a]).

The thermodynamic analysis of the complexation of CD has been performed by many research groups.[21−23] The formation of the inclusional complex of CD is considered to include the contribution of various factors such as van der Waals interaction, release of the water molecules from the cavity (high-energy water), the break of a water cluster, and the conformational change of the CD ring. The large favorable Δ*S* indicates that an apolar binding has a large contribution to the stability of the complex formation between *o*-MR−(*R*)-PEA and α-CD in the same way as the complexation between α-CD and hydrophobic molecules such as 1-adamantanecarboxylate.[24] One of reasons for the favorable Δ*S* of *o*-MR−PEA is a large break of water cluster around the guest molecule in the course of complexation compared with *p*-MR−PEA. On the other hand, the unfavorable Δ*S* for *p*-MR−PEA is due to an absence of interaction between the benzene ring of phenylethylamino group and α-CD rim. The decrease in the degree of rotational freedom of PEA moiety of *p*-MR−PEA might give a small negative Δ*S*, whereas a favorable Δ*S* due to the break of water cluster around PEA moiety of *p*-MR−PEA would be much smaller than that of *o*-MR−PEA. These factors (the break of a water cluster and the change in degree of freedom) are considered to lead to the small negative or favorable ΔS for *o*-MR derivatives and the large negative ΔS for *p*-MR derivatives.

Mechanism of the Stereoselectivity in the Inclusional Complex Formation of CD. Dye−α-CD inclusional complexes were schematically drawn in Figure 5. X-ray crystallographic study on α-CD−methyl orange complex showed that the azobenzene moiety can pass through the cavity of α-CD, and the azo group is located near the center of the cavity.[25] Electronic spectra indicated that this is also true in aqueous solutions. Taking Raman spectral change of MR−PEA induced by the complexation with α-CD into consideration, the MR moiety of MR−PEA seems to interact with the cavity of α-CD

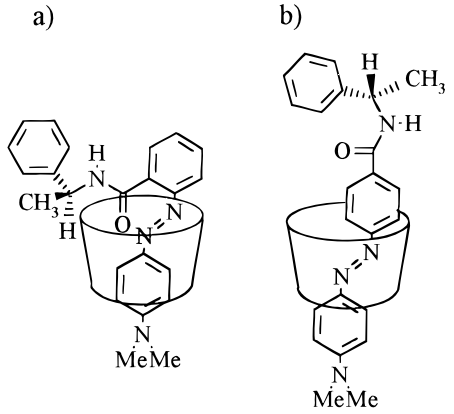


Figure 4. Schematic drawings of the inclusional complex of MR−PEA and α-CD: (a) *o*-MR−(*S*)-PEA, (b) *p*-MR−(*S*)-PEA.

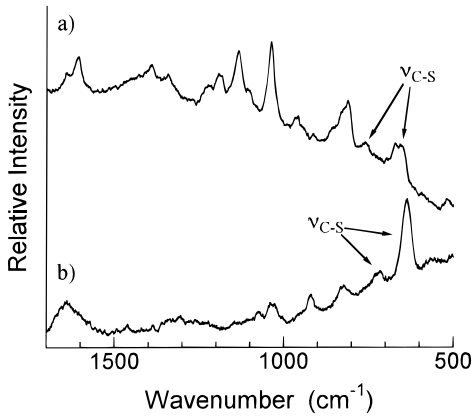


Figure 5. Raman spectra of MEA-α-CD excited at 488.0 nm (a) solid state and (b) SAM on colloidal Ag (pH 7.35).

dominantly. The asymmetric carbon of *p*-MR−PEA which has a linear configuration is, therefore, considered to exist at the position where it feels little attractive or repulsive force from α-CD. On the other hand, *o*-MR−PEA has a bent form, and the PEA moiety seems to locate on the edge of α-CD. Therefore, the chiral PEA moiety of *o*-MR−PEA is able to interact with the CD rim. Of course, α-CD is chiral and the interaction could be stereoselective. Further investigation is necessary to decide the interacting points on both CD and MR−PEA for the occurrence of stereoselectivity. The favorable Δ*S*, however, suggests that the selectivity to the *R* enantiomer of *o*-MR−PEA by α-CD resulted from more desirable interaction between the phenyl group and the α-CD rim than that of the *S* enantiomer. The water cluster around the PEA moiety would be destroyed by contact with the CD rim, and therefore, the effective break of water cluster of the *R* enantiomer compared

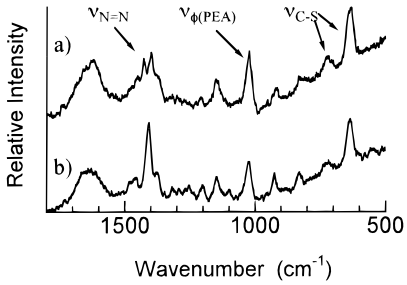


Figure 6. SERRS spectra of *p*- and *o*-MR−PEA included into SAM of MEA-α-CD on colloidal Ag (pH 7.35) excited at 488.0 nm, 25 °C; [dye] = 4 μM. (a) *p*-MR−(*R*)-PEA and (b) *o*-MR−(*R*)-PEA.

with the *S* enantiomer in the course of formation of the inclusional complex with α-CD may lead to the favorable Δ*S*.

Molecular Recognition by SAM of CD at the Solid−Liquid Interface. The SAM of α-CD was prepared from MEA−α-CD to investigate the molecular recognition at the solid−liquid interface. In Figure 5, SERS spectrum of SAM of MEA-α-CD on Ag was shown with Raman spectra of MEA−α-CD at the solid state. Key features of SERS are a shift of the C−S stretching vibration band (ν_{C-S}) at a region of 600−750 cm^{-1} and disappearance of a S−H stretching vibration band (ν_{S-H}) observed at about 2580 cm^{-1} in the solid state. These features are reported to be characteristic of the cleavage of S−H bonds followed by formation of S−Ag bonds.[26] These results suggested that MEA−α-CD was adsorbed to the Ag surface via a S−Ag bond.

We investigated the stereoselective complexation of *o*- and *p*-MR−PEA by the SAM composed of MEA−α-CD on the surface of colloidal Ag. Addition of the dye solution to the colloidal Ag modified with MEA−α-CD gave rise to Raman bands from MR−PEA together with SERS of MEA−α-CD (Figure 6). The concentration of the dye was too low to observe RR scattering from free dye in the solution with our experimental setup. Moreover as described before MR−PEA exists as the quinoid form in the solution whose pH is lower than pK_a(N═N) of the dye, whereas MR−PEA complexed with CD exists as the azo form. Characteristic bands for the azo form observed in the Raman spectrum of MR−PEA in MEA−α-CD/Ag system at pH 3.0 strongly suggested the bands were due to SERRS from MR−PEA included in the cavity of surface-confined CD. Our electrochemical study on the same system showed that surface coverage of MEA−α-CD was fairly good (97% of the calculated maximum density)[27] and SERRS from MR−PEA which directly adsorbed to Ag surface might be negligible. After all, Raman bands of the dye observed in Figure 6 were considered to at least mainly, if not exclusively, contain SERRS from the dye which was included by CD bound to Ag surface.

When the dye concentration was increased in the region where the contribution of RR scattering from free dye can be ignored, the intensities of SERRS from the dye increased correspondingly, probably because the number of dye molecules that were included in the cavity of CD to give SERRS increased. With the assumption that the intensities of SERRS per one MR−PEA molecule (α) is constant, which may be influenced by the distance from the surface and orientation of axis of the dye molecule to surface normal, the total intensities of SERRS (*I*) are proportional to the number of adsorbed molecules. When the relative intensities of the $\nu_{\phi(PEA)}$ band of the dye normalized by the intensity of ν_{C-S} band of SAM were plotted against the concentrations of the dyes, *o*-MR−PEA obeyed the adsorption isotherm of the Langmuir type (eq 1), whereas the plots for

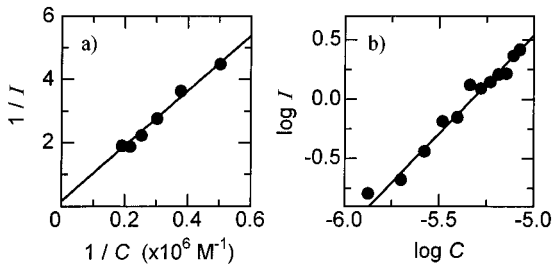


Figure 7. Langmuir and Freunflich plots for the adsorption isotherms of MR−PEA to SAM of MEA-α-CD on Ag colloid at 25 °C. The relative intensities of the $\nu_{\phi(PEA)}$ band of MR−PEA to the intensity of ν_{C-S} band of SAM (*I*) are plotted. (a) Langmuir plot for *o*-MR−(*R*)-PEA, (b) Frendlich's plot for *p*-MR-(R)-PEA.

p-MR−PEA showed a sigmoidal shape and the adsorption did not obey Langmuir's isotherm (data not shown).

Langmuir's isotherm is

$$n_a/b_L = KC/(1 + KC) \tag{1}$$

where n_a is the number of adsorbed molecules, b_L is the number of sites for adsorption, *K* is the association constant, and *C* is the concentration of MR−PEA added to the system.

In this case n_a is given by *I*/α (*I*: intensity of $\nu_{\phi(PEA)}$ band normalized by ν_{C-S} from SAM), and therefore

$$\frac{1}{I} = \frac{1}{\alpha}\left(\frac{1}{Kb_L}\frac{1}{C} + \frac{1}{b_L}\right) \tag{2}$$

The association constant is obtained from the *x* intercept of 1/*I* versus 1/*C* plots (Figure 7a).

Complexation of *p*-MR−PEA with surface-confined α-CD obeyed Freundlich's equation (eq 3):

$$n_a = K_F C^{1/n_F} \tag{3}$$

where K_F and n_F are constants. Substitution of n_a by *I*/α gives

$$\log I = \log K_F + \log \alpha + \frac{1}{n_F}\log C \tag{4}$$

The *x* intercept of log *I* versus log *C* plots gives a constant as eq 6 (Figure 7b):

$$1/P = 10^{n_F \cdot \log(K_F \alpha)} \tag{5}$$

Though 1/*P* given by eq 6 is not equal to the association constant, this value can be used to estimate relative stereoselectivity.

The equilibrium constants for the association of MR−PEAs and SAM of MEA−α-CD are given at the bottom of Table 1. The *K* value of *o*-MR−PEA with CD of SAM system is much larger than that of the free system. Taking the chemical structures into consideration, MR−PEAs are considered to penetrate into the cavity of surface-confined α-CD toward the Ag surface from the dimethylamino group side. Electrostatic interaction between the dimethylamino group of the dye and colloid surface may strongly stabilize the complex. Therefore, in the molecular recognition by the SAM composed of CD on the Ag surface, the association constant is much larger than that of free CD. In addition, the high surface concentration of CD may induce a change in local environment and have some effects on the association.

The stereoselectivity (*R*/*S*) was slightly decreased compared with the free system for *o*-MR−PEA, whereas the stereoselectivity of *p*-MR−PEA with surface-confined CD was higher than

that with free CD probably because of the deeper inclusion and the enhancement of nonspecific interaction with Ag surface. These are probable reasons for the increase in the stereoselectivity of *p*-MR−PEA (the deeper inclusion promotes the contact of chiral center with the CD rim) and reduction in that of *o*-MR−PEA (the enhanced nonspecific interaction weakens the specific effect of chiral center) on Ag surfaces.

In conclusion, investigation of the inclusional complexation of free and surface-confined CD was carried out by Raman spectroscopy for the first time. To study the stereoselectivity of the molecular recognition by CD, optically active azo dyes were used. The association constants for free CD and MR−PEA obtained by Raman spectroscopy were almost the same with those obtained by UV−vis spectroscopy. The association constants between surface-confined CD and MR−PEA were measured by surface-enhanced resonance Raman spectroscopy. *o*-MR−PEA formed more a stable inclusional complex than *p*-MR−PEA (regioselective) due to the contribution of the destruction of water cluster around PEA moiety. The *R* enantiomer of *o*-MR−PEA associated with both free and surface-confined CD more preferentially than did the *S* enantiomer (stereoselective). Furthermore, the stereoselectivity for *o*-MR−PEA decreased at interfaces due to the enhancement of the nonspecific interaction, whereas that for *p*-MR−PEA increased by the contribution of interaction with the chiral center which was induced by the deeper inclusion.

Although the stereoselectivity observed here was not satisfactorily high, our findings strongly suggest the possibility of SAM of CD derivatives as stereoselective chemical sensor.

Acknowledgment. This work was supported by Grant-in-Aid (08246222, 09240213, 09232223) from Ministry of Education, Science and Culture, Japan. Y.M is grateful for the financial support from the Nissan Science Foundation (Tokyo).

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