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Cooperative Binding and Diffusion of Cyclodextrins in Aqueous Polymer Solutions

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ABSTRACT: Cooperative binding of α- and β-cyclodextrin to poly(methacrylic acid), sodium poly(styrene sulfonate), and poly(methylbenzyldiallylammonium chloride-SO_2) was discussed in terms of the variations of the differential diffusion coefficients of the cyclodextrins in the aqueous polymer solutions. Incorporation of McGhee and von Hippel's theory with the diffusion equation has given the number of consecutive residues covered by a bound cyclodextrin, the intrinsic binding constant, and the cooperative parameter for the systems investigated.

Diffusion of a small molecule in polymer solution may be suppressed for two reasons: one is the obstruction by the polymer of the diffusing path of the molecule[1] and the other is the immobilization of the molecule by a specific interaction with the polymer.[2] If the former is properly taken into account, the diffusion behavior of the molecule may be interpreted in terms of the interaction or be correlated with the binding isotherm to the polymer. From this point of view, we have discussed the diffusion of cyclodextrins (CD) in aqueous solutions of poly(methacrylic acid) (PMA), sodium poly(styrene sulfonate) (NaPSS), and their copolymers;[2] the decreases of the integral diffusion coefficients of CD's with the polymer concentration were explained by assuming the complex formation between a CD and a residue in the polymer.

β-CD, for instance, comprising seven glucopyranose rings, has the molecular weight of 1135 daltons. Therefore, it would be rather realistic to imagine that a CD molecule covers *n* consecutive residues in a polymer upon the complex formation. In the course of a continuing study of CD–polymer systems, it was found that β-CD induces PMA to give circular dichroism centered at 218 nm. The spectral change with CD concentration could not be explained by the 1:1 complex assumption. These facts prompted us to examine in more detail the diffusion behaviors of CD's in aqueous polymer solutions. The present paper reports the differential diffusion coefficient (*D*) of α- and β-CD in aqueous PMA, NaPSS, and poly(methylbenzyldiallylammonium chloride-SO_2) (BACS) solutions; the variations of *D* with CD or polymer concentration are explained by employing McGhee and von Hippel's model of cooperative ligand binding to homogeneous polymers.[3]

The variation of *D* of α-CD in PMA solution is shown in Figures 1A and 1B, where linear diminutions with the CD or PMA concentration are found. These are caused either by the decreased activity of the CD or by the increased obstruction by the polymer with their concentrations; eq 1 gives the relation empirically,

$$D_r = D_0(1 - 0.026c_p) - 1.09 \times 10^{-7}c_{CD} \qquad (1)$$

where D_0 (3.20×10^{-6} cm^2/s for α-CD) is *D* of α-CD at infinite dilution in the absence of polymer and *c* denotes the concentration in g/100 mL. Assuming the obstructive effect of a polymer to be the same for α- and β-CD, D_r of β-CD in the absence of any interaction with the polymer may be represented by the same equation by substituting 3.06×10^{-6} for D_0.[4] Figures 2A and 2B show that eq 1 holds for the α-CD–NaPSS system with the same coefficients. We assume that D_r of CD's in BACS solution for hypothetical noninteracting diffusion can be calculated by eq 1, too. For all systems, experiments were done under the conditions $c_p < 5.7$ and $c_{CD} < 1.4$.

β-CD in PMA and NaPSS solution interacts with the polymers and gives *D* dependent on both the CD and the polymer concentration. Figures 1C and 2C show D/D_r, the diffusion ratio, of β-CD against CD concentration, where characteristic S-shaped variations for the lower polymer concentration cases are found. This fact suggests intuitively that, with the CD concentration, the binding of β-CD

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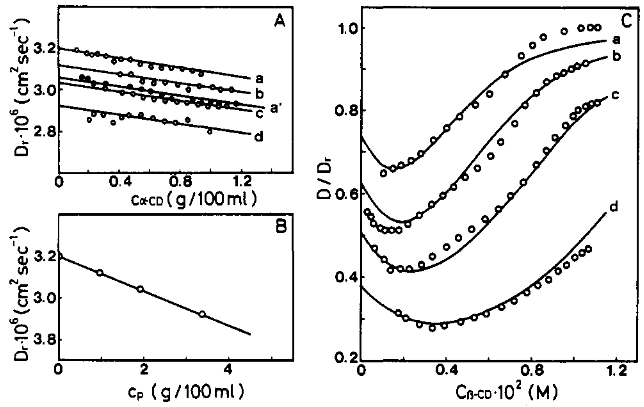

Figure 1. Differential diffusion coefficients of α- and β-CD in aqueous PMA solutions: (A) D_r of α-CD vs. $c_{\alpha\text{-CD}}$ for (a) 0, (b) 0.960, (c) 1.92, and (d) 3.37 g/100 mL of PMA solution and (a') D_r of β-CD vs. $c_{\beta\text{-CD}}$ in the absence of PMA; (B) D_r of α-CD at infinite dilution vs. c_p; (C) D/D_r for β-CD vs. $C_{\beta\text{-CD}}$ for (a) 3.28×10^{-2}, (b) 5.46×10^{-2}, (c) 9.10×10^{-2}, and (d) 1.517×10^{-1} M PMA solution. The straight lines in A and B were drawn according to eq 1. See the text for the abbreviations and the curves in C.

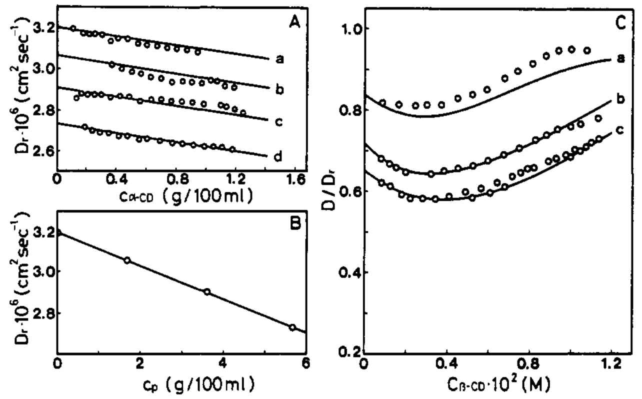

Figure 2. Differential diffusion coefficients of α- and β-CD in aqueous NaPSS solutions: (A) D_r of α-CD vs. $c_{\alpha\text{-CD}}$ for (a) 0, (b) 1.70, (c) 3.60, and (d) 5.65 g/100 mL of NaPSS solution; (B) D_r of α-CD at infinite dilution vs. c_p; (C) D/D_r for β-CD vs. $C_{\beta\text{-CD}}$ for (a) 7.91×10^{-2}, (b) 1.582×10^{-1}, and (c) 2.174×10^{-1} M NaPSS solution. The lines in A and B were drawn according to eq 1. See the text for abbreviations and the curves in C.

with these polymers is once enhanced and afterward becomes less and less influential to the diffusion. In contrast, it is found in Figures 3A and 3B that the diminution of D/D_r of both α- and β-CD by BACS is monotonically relaxed with CD concentration. A quantitative interpretation of these diffusion behaviors is given based on the following three assumptions: (1) an equilibrium between the bound and the free CD molecule is set up throughout the diffusion space, (2) the rates of the equilibrium processes are rapid, and (3) the diffusion of the bound CD or of the polymer is so slow that the real diffusion of CD is brought on by the free species. Then, by Fick's law, the flux of CD at a given position is given as,

$$J = -D_r \frac{\partial C_f}{\partial x} = -\left(D_r \frac{\partial C_f}{\partial C}\right) \frac{\partial C}{\partial x} = -D \frac{\partial C}{\partial x} \qquad (2)$$

where C_f is the molar concentration of free CD and C is the total concentration of CD at the position. D is obtained experimentally. From eq 2,

$$D/D_r = \partial C_f/\partial C \qquad (3)$$

i.e., the diffusion ratio corresponds exactly to the fraction of free CD in a minute increment of total CD under a given polymer concentration. Given the last two concentrations experimentally, any binding isotherm will give the ratio in terms of the constants included therein.

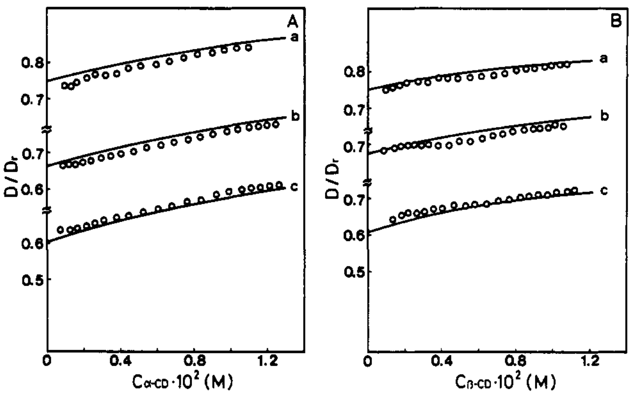

Figure 3. Diffusion ratio vs. C_{CD} in aqueous BACS solutions: (A) D/D_r for α-CD vs. $C_{\alpha\text{-CD}}$ for (a) 6.98×10^{-2}, (b) 1.081×10^{-1}, and (c) 1.428×10^{-1} M BACS solution; (B) D/D_r for β-CD vs. $C_{\beta\text{-CD}}$ for (a) 6.98×10^{-2}, (b) 1.041×10^{-1}, and (c) 1.393×10^{-1} M BACS solution. See the text for abbreviations and the curves in A and B.

McGhee and von Hippel have introduced the following equation for the cooperative binding of ligand which covers n consecutive residues of a homogeneous polymer,[3]

$$\frac{\nu}{C_f} = K(1 - n\nu) \times \left\{\frac{(2\omega - 1)(1 - n\nu) + \nu - R}{2(\omega - 1)(1 - n\nu)}\right\}^{n-1} \left\{\frac{1 - (n + 1)\nu + R}{2(1 - n\nu)}\right\}^2 \qquad (4)$$

$$R = [\{1 - (n + 1)\}^2 + 4\omega\nu(1 - n\nu)]^{1/2} \qquad (5)$$

where ν is the binding density or moles of bound ligand per mole of polymer residue, n is the number of the coverage, K is the intrinsic binding constant, and ω is the cooperativity factor between adjacently bound ligands. Differentiation of this equation gives

$$\frac{D}{D_r} = \frac{\partial C_f}{\partial C} = 1 - \frac{KC_p(ABC)^2}{KC_p(ABC)^2 + ABC - \nu(AmBC + ABmC + ABCm)} \qquad (6)$$

where

$$A = 1 - n\nu \qquad (7)$$

$$B = [\{(2\omega - 1)(1 - \mathrm{n}\nu) + \nu - R\}/\{2(\omega - 1)(1 - n\nu)\}]^{n-1} \qquad (8)$$

$$C = [\{1 - (n + 1)\nu + R\}/2(1 - n\nu)]^2 \qquad (9)$$

$$A' = -n \qquad (10)$$

$$B' = (n - 1)[\{(2\omega - 1)(1 - n\nu) + \nu - R\}/2(\omega - 1) \times (1 - n\nu)]^{n-2}\{[\{1 - (2\omega - 1)n - R'\}(1 - n\nu) + n\{(2\omega - 1)(1 - n\nu) + \nu - R\}]/[2(\omega - 1)(1 - n\nu)^2]\} \qquad (11)$$

$$C' = [\{1 - (n + 1)\nu + R\}/2(1 - n\nu)]\frac{[\{R' - (n + 1)\}(1 - n\nu) + n\{1 - (n + 1)\nu + R\}]}{2(1 - n\nu)^2} \qquad (12)$$

$$R' = [2\omega(1 - 2n\nu) - \{1 - (n + 1)\nu\}(n + 1)]/R \qquad (13)$$

where C_p is the polymer concentration in residue moles/liter. Equation 6 gives

$$\lim_{C \to 0}\left(\frac{D}{D_r}\right) = (1 + KC_p)^{-1} \qquad (14)$$

Table I
Parameters for Cooperative Binding of Cyclodextrins to Polymers

polymer	CD	n	K, M^{-1}	ω
PMA	α-CD		0	
	β-CD	14	10.5	30
NaPSS	α-CD		0	
	β-CD	29	2.5	60
BACS	α-CD	5	4.6	1
	β-CD	4	4.6	1

hence K is obtained by extrapolation of D/D_r. n and ω are estimated by the curve fitting to the experimental data. The curves in Figures 1C, 2C, 3A, and 3B show that, with appropriate n, K, and ω values in Table I, eq 6 reproduces quite satisfactorily the complex variations of D/D_r with CD or polymer concentration.

Table I shows that a bound β-CD covers 14 and 29 residues of PMA and NaPSS, respectively, while it covers 4 residues of BACS of which residue length is considerably longer than the former two. n for α-CD to BACS is found to be 5. It is found that positive cooperativity[3] is at work for the binding of β-CD to PMA and NaPSS. BACS interacts with α- and β-CD lacking this factor. Although details of such interactions are of great interest, we postpone the discussion until results sufficient for any convincing interpretation are obtained.

The cooperative binding of β-CD to PMA is further evidenced by the induced circular dichroism found for the relevant solutions. Figure 4A shows the reciprocal of molar ellipticity of PMA residue at 218 nm against that of the CD concentration. By extrapolating $C_{\beta\text{-CD}}$ to ∞, Θ_{218} of PMA under full β-CD binding, $\Theta^{\infty}{}_{218}$, is estimated. Then ν can be obtained by dividing $\Theta_{218}/\Theta^{\infty}{}_{218}$ by n which is taken as 14. The Scatchard plot obtained from these spectral changes is shown in Figure 4B, where the points constitute a curve convex upward, which is a definite indication of the positive cooperativity in the binding.[3] The curve given by eq 4 with the constants given in Table I seems to be in fair agreement with these data.

α-CD supplied by Wako Pure Chemicals as analytical grade and β-CD which was gifted by Teijin Co. and was recrystallized three times from the aqueous solution were used. Preparation and purification of PMA ($M_w = 3.2 \times 10^5$ daltons) and NaPSS ($M_w = 1.17 \times 10^6$ daltons) were described in the previous report.[2] BACS (M_w is over 2.0×10^4 daltons) was a gift by Nitto Boseki Co. as the best commercial grade and was used after extensive dialysis in water. The diffusion profile of CD in aqueous polymer solution was photographed as $d\bar{n}/dC$, where $\bar{n}$ is the refractive index, vs. distance curve by using a Hitachi Tiselius HTB-2 apparatus after three intervals of diffusion time. The differential diffusion coefficient of CD from 0 to ca. 1.4 g/100 mL was calculated according to the standard procedure[5] as

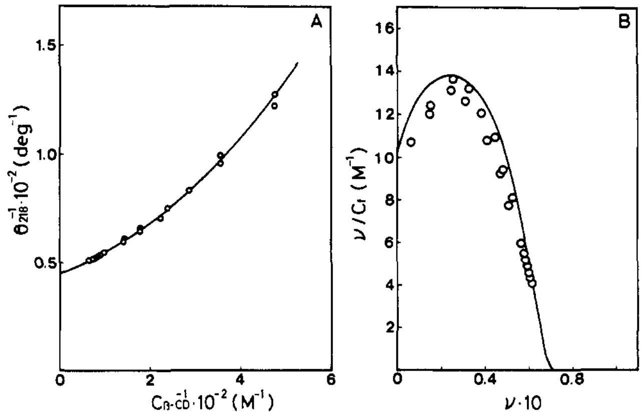

Figure 4. Induced circular dichroism of β-CD–PMA systems: (A) reciprocal plot of Θ_{218} vs. $C_{\beta\text{-CD}}$; (B) Scatchard plot. See the text for the curve.

$$D = -\frac{1}{2t}\left(\frac{dc}{dx}\right)\int_{\infty}^{x} x\left(\frac{dc}{dx}\right)dx \qquad (15)$$

by choosing the initial interface so as to satisfy the condition[6]

$$\int_{-\infty}^{+\infty} x\left(\frac{dc}{dx}\right) = 0 \qquad (16)$$

D values at both ends of the concentration were discarded because of the large experimental uncertainty. The circular dichroism spectra were obtained by a JASCO J-20A spectropolarimeter. The dichroism centered at 218 nm was found for β-CD–PMA systems but not for α-CD or glucose–PMA systems. Diffusion and dichroism measurements were performed at 25.0 ± 0.01 °C.

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