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Complex Formation of Hexakis(2,6-di-*O*-methyl)-α-cyclodextrin with Substituted Benzenes in Aqueous Solution

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Abstract. The host–guest interaction of hexakis(2,6-di-*O*-methyl)-α-cyclodextrin (DM-α-CDx) with substituted benzenes in aqueous solution has been investigated by circular dichroism spectra. From the resemblance of the spectra, it is concluded that the guest molecules are included in the DM-α-CDx ring in a manner similar to that of corresponding α-cyclodextrin complexes. Thermodynamic parameters of the complex formation were determined on the basis of the temperature dependent intensity change of the spectra. In spite of the considerable variation in ΔH and ΔS values, the free energy does not change much among complexes because of the strong compensation effect. The compensation temperature was 309 ± 11K. Negative values of ΔH and ΔS, suggesting that the complex formation is not governed by the hydrophobic interaction, can be interpreted in terms of the tight binding of the guest molecule within the host cavity.

Key words. Dimethyl-α-cyclodextrin, induced circular dichroism, host-guest interaction, inclusion complex.

1. Introduction

Cyclodextrins are macrocylic oligosaccharides consisting of optically active D-glucose units and form host–guest inclusion complexes with a variety of molecules or ions. The internal cavity of the macrocycle provides an asymmetric field which perturbs the electronic state of the included guest and induces optical activity [1, 2]. The induced CD (circular dichroism) spectra have been investigated to elucidate the structure of host–guest interaction in the cyclodextrin complexes, especially in the complexes with optically inactive guests [3, 4]. According to the coupled oscillator theory [5], the induced optical activity is interpreted in terms of dipole–dipole interaction between cyclodextrin and guest chromophor. The sign and intensity of the induced CD are expressed as a function of $\cos 2\theta$ where θ is the angle made by the symmetry axis of cyclodextrin and the electric dipole moment of the guest chromophore [3, 6]. In our previous paper [7], we have reported that hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin (TM-α-CDx) forms a complex with substituted benzenes in a manner different from that of corresponding α-cyclodextrin (α-CDx) complexes. The macrocylic ring of TM-α-CDx is highly flexible and less symmetrical than the α-CDx ring because of the lack of intramolecular hydrogen bonds [8]. In contrast, the macrocylic conformation of hexakis(2,6-di-*O*-methyl)-α-cyclodextrin (DM-α-CDx) resembles that of α-CDx [9]. Since only O(2)H and O(6)H hydroxyl groups are methylated, DM-α-CDx can form intramolecular O(3)—H---O(2) hydrogen bonds. The cavity of DM-α-CDx is expected to be more

hydrophobic than that of α-CDx because of many methyl groups located at both ends of the cavity. Such characteristics of the molecule may affect the interaction with the guest. The present paper deals with the induced CD study of the complex formation of DM-α-CDx with substituted benzenes in aqueous solution.

2. Experimental

2.1. CD MEASUREMENTS

DM-α-CDx was purchased from Toshin Chemical Co. and recrystallized three times from ethanol and then from hot water. Benzene derivatives were purchased from Nakarai Chemicals Ltd. and used without further purification. Solutions were prepared with deionized and distilled water. CD spectra were recorded on a JASCO J-600 Spectropolarimeter. The temperature was regulated by a Tokyo Rico TC-100 thermo-controller and a water-jacketed cylindrical cell with 1 mm path length was used. The temperature inside the cell was measured by using a thermocouple. The CD spectra were measured at DM-α-CDx concentrations of 9.0 mM, 4.0 mM, 2.0 mM, and 1.0 mM, and the guest concentrations of ca. 1 mM.

2.2. DETERMINATION OF THERMODYNAMIC PARAMETERS

The CD intensity data obtained at various temperatures and host concentrations were used for the determination of molecular ellipticity (θ_m), enthalpy (ΔH), and entropy (ΔS). The θ_m value was estimated from the Rose–Drago plot [10], then the ΔH and ΔS values were obtained from the van't Hoff plot. In the next stage, molecular ellipticity and thermodynamic parameters thus obtained were refined by the least-squares curve-fitting method from the equations [11],

$$\ln K_d = \Delta H/RT_i - \Delta S/R \tag{1}$$

$$K_d = ab_j\theta_m/\theta_{ij} - a - b_j + \theta_{ij}/\theta_m \tag{2}$$

where K_d is the dissociation constant for the 1 : 1 complex, a and b_j are concentrations of the guest and DM-α-CDx, respectively, θ_{ij} is the observed CD intensity at the temperature T_i, and θ_m is the molecular ellipticity of the DM-α-CDx complex. The observed and calculated ellipticities of the benzoic acid complex are listed in Table I. The molecular ellipticity and error statistics are given in Table II. The average discrepancy between observed and calculated ellipticities, given by the R index, were in the range from 0.9% to 2.2%. The estimated standard deviation for the calculated ellipticity was distributed in the range form 0.04 to 0.08 mdeg.

3. Results and Discussion

CD spectra of DM-α-CDx complexes with substituted benzenes are shown in Figures 1 and 2. Benzoic acid shows a strong positive CD at 228 nm. The *para*-isomers of hydroxy- and aminobenzoic acid also show positive CD peaks at 253 and 280 nm, respectively. In contrast, two positive CD peaks are observed in the 200–250 nm region in the complexes with corresponding *meta*-isomers. The DM-α-

Table I. Observed and calculated ellipticity of the DM-α-CDx complex with benzoic acid.[a]

	$[C]=9.0\times10^{-3}$ mol		$[C]=4.0\times10^{-3}$ mol		$[C]=2.0\times10^{-3}$ mol		$[C]=1.0\times10^{-3}$ mol	
T K	θ_{obs} mdeg	θ_{calc} mdeg	θ_{obs} mdeg	θ_{calc} mdeg	θ_{obs} mdeg	θ_{calc} mdeg	θ_{obs} mdeg	θ_{calc} mdeg
293	13.5	13.4	11.4	11.6	8.6	9.0	5.6	5.9
298	12.7	12.7	10.4	10.5	7.5	7.7	4.7	4.8
303	11.8	11.9	9.2	9.2	6.5	6.4	3.9	3.9
308	10.7	10.8	8.0	7.8	5.3	5.2	3.1	3.0
313	9.7	9.7	6.8	6.6	4.3	4.1	2.5	2.3
318	8.5	8.4	5.6	5.3	3.4	3.2	1.8	1.8
323	7.3	7.1	4.4	4.3	2.5	2.5	1.4	1.3
328	6.0	5.9	3.3	3.3	1.7	1.9	1.0	1.0
333	4.8	4.8	2.5	2.6	1.1	1.4	0.6	0.7

[a]The concentration of benzoic acid is 1.018×10^{-3} M. [C] is the concentration of DM-α-CDx.

Table II. Molecular ellipticity and error statistics of DM-α-CDx complexes with substituted benzenes.[a]

Guest	[C] 10^{-3} mol	λ nm	$[\theta_m]$ 10^4 deg cm^2/dmol	R^b	$\sigma(\theta_{calc})^c$ mdeg
Benzoic acid	1.018	229	1.47(1)	0.022	0.08
m-Hydroxybenzoic acid	0.938	238	1.00(1)	0.018	0.08
p-Hydroxybenzoic acid	0.952	253	1.39(1)	0.010	0.04
p-Aminobenzoic acid	0.998	280	0.83(2)	0.009	0.04
m-Nitrophenol	1.115	280	0.48(1)	0.014	0.05
p-Nitrophenol	0.868	340	0.83(1)	0.013	0.04
m-Nitroaniline	0.706	220	1.28(1)	0.011	0.04
p-Nitroaniline	0.691	400	1.24(1)	0.009	0.05

[a]Standard deviations are given in parentheses.
[b]The residual index R is defined as: $R=\Sigma\,\|\,\theta_{obs}|-|\theta_{calc}\|/\Sigma|\theta_{obs}|$.
[c]The estimated standard deviation for calculated ellipticity.

CDx complexes with *p*-nitroaniline and *p*-nitrophenol give a positive CD peak in the lower wavelength region and a negative CD peak in the 220–230 nm region. *m*-Nitrophenol shows two positive peaks at 350 nm and 280 nm, while in the *m*-nitroaniline complex positive and negative CDs are observed in the 200–300 nm region.

These CD spectra resemble those of corresponding α-CDx complexes [6] but differ from those of the TM-α-CDx complexes [7]. The CD spectra of the benzoic acid complexes with three α-CDxs are shown in Figure 3. The shape of the CD spectra is almost the same between the α-CDx complex and DM-α-CDx complex. But, the TM-α-CDx complex shows a positive CD peak at 280 nm and a negative CD peak at 244 nm which is not observed in the α-CDx and DM-α-CDx complexes. Therefore, the guest molecules can be expected to be included in a manner similar to that found in the α-CDx complexes. The CD spectra also reflect the change of the macrocylic conformation. The X-ray structure analysis of DM-α-CDx

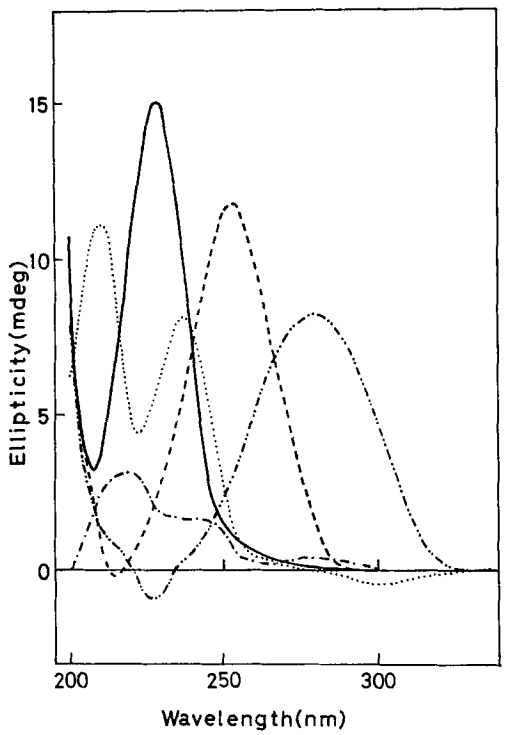

Fig. 1. CD spectra of DM-α-CDx complexes with benzoic acid (——), *m*-hydroxybenzoic acid (· · · ·), *p*-hydroxybenzoic acid (- - -), *m*-aminobenzoic acid (- · · · -), and *p*-aminobenzoic acid (- · · · · · -) at 20°C with the concentrations of 1.018×10^{-3} M, 0.938×10^{-3} M, 0.952×10^{-3} M, 0.900×10^{-3} M, and 0.998×10^{-3} M, respectively. The concentration of DM-α-CDx was 0.90×10^{-2} M.

complexes has shown that the macrocyclic conformation of DM-α-CDx is similar to that of α-CDx [9]. The major difference betewen α-CDx and DM-α-CDx is that the cavity of DM-α-CDx is deeper than the cavity of α-CDx because of the methyl groups attached to O(2) and O(6). Since the macrocylic ring of TM-α-CDx is considerably distorted, the guest molecule may be included in a different way [8].

The CD spectra of each complex were measured at 20°C, 40°C, and 60°C. The spectral shape did not change and only an intensity change was observed at different temperatures or host concentration. This suggests that the structure of the complex is not affected by the temperature. Plots of CD intensity of the benzoic acid complex against the temperature are shown in Figure 4. The CD intensity decreased monotonously with the increase of temperature at various DM-α-CDx concentrations. A similar temperature-dependent intensity change was observed in other DM-α-CDx complexes. The molecular ellipticity (θ_m), enthalpy (ΔH) and entropy (ΔS) of the complex formation were determined by the least-squares curve-fitting method. The observed data were well fitted to the calculated ellipticity for the 1 : 1 complex formation within the experimental error (Table I and Figure 5) and systematic deviation which implies disagreement with the 1 : 1 stoichiometry

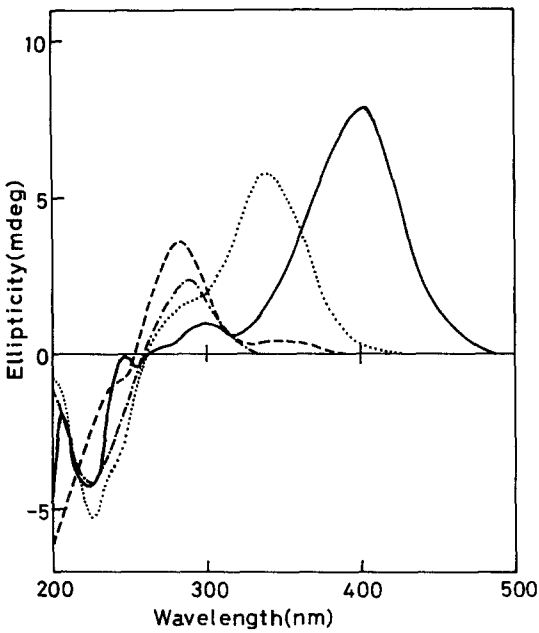

Fig. 2. CD spectra of DM-α-CDx complexes with *p*-nitroaniline (——), *m*-nitroaniline (- · · · · · -), *p*-nitrophenol (· · · ·), and *m*-nitrophenol (- - - -) at 20°C with the concentrations of 0.691×10^{-3} M, 0.706×10^{-3} M, 0.868×10^{-3} M, and 1.115×10^{-3} M, respectively. The concentration of DM-α-CDx was 0.90×10^{-2} M.

was not found between observed and calculated ellipticities. The 1 : 1 stoichiometry of the complex was also confirmed by the Rose–Drago plot. A common way to determine the thermodynamic parameters from the spectral data has been the graphical estimation of the dissociation constants followed by the van't Hoff plot. The least-squares method is a direct evaluation of the molecular ellipticity, enthalpy, and entropy values from the observed data and can precisely determine these parameters. The precision and reliability of the results can be evaluated by the associated error function, in which the standard deviation is calculated as a function of observed ellipticity.

The thermodynamic parameters are given in Table III with those of α-CDx and TM-α-CDx. The complex formation of DM-α-CDx with benzene derivatives is characterized by the negative change in enthalpy and entropy. The free energy of the complex formation is found in the range from −3.48 to −4.40 kcal/mol at 298 K. In contrast, the ΔH values are distributed in a wider range, from −4.4 to −14.3 kcal/mol. The suppression of the free energy within a narrow range is due to the compensation between ΔH and ΔS, which is widely observed in aqueous media [13]. The compensation effect has been also observed in α- and β-cyclodextrin complexes [6, 12] and in TM-α-CDx complexes [7]. ΔH and ΔS are linearly correlated (Figure 6) and the compensation temperature for the DM-α-CDx complex is calculated to be 309 ± 11 K, which is in good agreement with 302 ± 19 K of the TM-α-CDx complex [7] but lower than 375 ± 36 K of the α-CDx

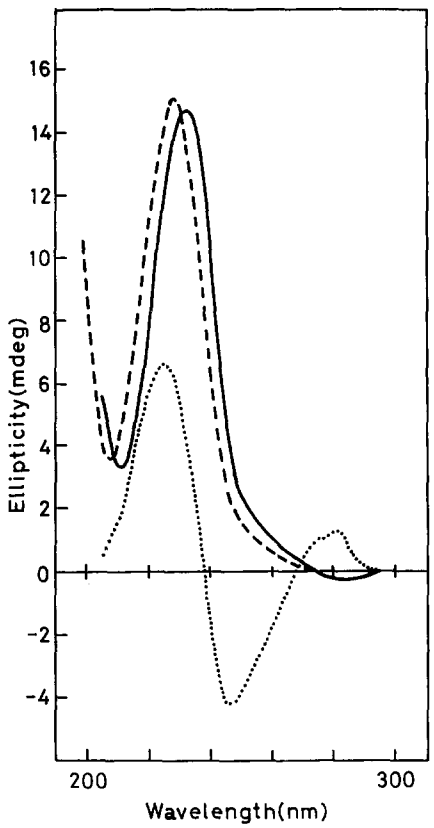

Fig. 3. CD spectra of benzoic acid complexes with α-CDx (——), DM-α-CDx (- - -), and TM-α-CDx (· · · ·) at 20°C.

complex [6]. The ΔH and ΔS values are distributed over a wider range from those of α-CDx complexes, while their absolute values are smaller than those of the corresponding TM-α-CDx complexes.

The absolute value of ΔH and ΔS of the complexes with benzoic acid derivatives are considerably larger than those of the complexes with nitrobenzene derivatives. The geometry of the host–guest interaction, the electronic state of the guest, and the hydration of the guest may be involved in such a change in thermodynamic parameters. A similar tendency has also been observed in the complexes of α-CDx and TM-α-CDx. In α-CDx complexes, the nitrophenyl and carboxyphenyl groups are included in the same manner [14]. The nitro group and carboxyl group are inserted into the α-CDx cavity from the secondary hydroxyl side and penetrate to the primary hydroxyl end of the cavity. On the other hand, in the TM-α-CDx complex with *p*-nitrophenol, the hydroxyphenyl group is included in the crystalline state [15]. In solution, however, an inclusion geometry similar to that of the α-CDx complex has been proposed [16].

The negative ΔH and ΔS values indicate that the complex formation is not regulated by the hydrophobic interaction. The theoretical estimation of thermody-

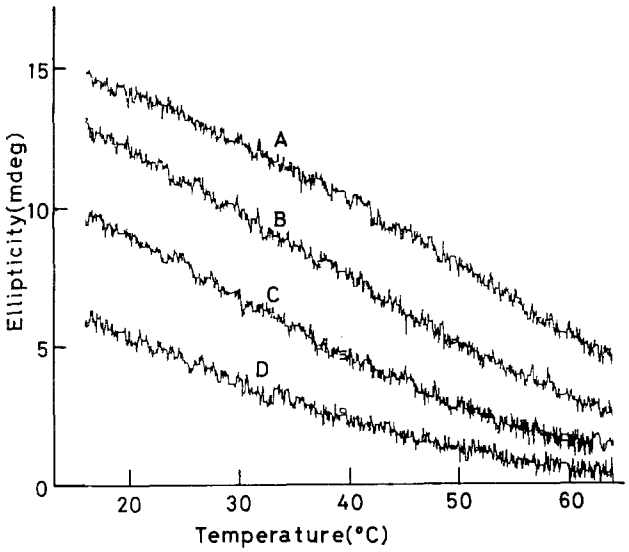

Fig. 4. Plots of CD intensity of the DM-α-CDx complex with benzoic acid at 228 nm against the temperature. The concentration of benzoic acid was 1.018×10^{-3} M and those of DM-α-CDx were 0.90×10^{-2} M (A), 0.40×10^{-2} M (B), 0.20×10^{-2} M (C), and 0.10×10^{-2} M (D).

namic parameters has suggested that the host–guest interaction, such as, van der Waals interaction and dipole–dipole interaction, mainly contributes to the negative ΔH [17, 18]. When the guest molecule is bound to the host cavity, the translational and rotational freedom of the guest molecule is restricted because of the limited size and shape of the cavity. The complex formation may also impose a restriction on the macrocyclic conformation of the host molecule so as to provide a best fit to the shape of the guest molecule. These may be the reasons for the negative ΔS [18]. It should be noted that methyl groups do not seem to enhance the hydrophobic interaction between host and guest. Methyl groups point away from the center of the macrocylic ring and are at such a distance that they do not have a close contact with the guest molecule. Therefore, the hydration around methyl groups may not be affected by the inclusion of the guest molecule.

A hydrophobic interaction is observed in the β-CDx complexes with some alcohols [19]. A small guest molecule, which is loosely bound to the host cavity, may still have rotational freedom. Moreover, such a weak host–guest interaction does not affect the macrocylic conformation of the host molecule. As a result, the major part of ΔH and ΔS may be derived from the change in hydration of the host cavity and around the guest molecule. In contrast, the large negative values of ΔH and ΔS in the TM-α-CDx complexes with benzene derivatives can be ascribed to the strong host–guest interaction. The TM-α-CDx molecule is flexible in the macrocyclic conformation and binds the guest molecule tightly via induced-fit conformational change [8]. The conformation of DM-α-CDx is less flexible than that of TM-α-CDx because of the formation of intramolecular O(3)—H---O(2) hydrogen bonds and the macrocylic conformation seems to be less affected by the inclusion of the guest molecule. This may be the reason for the fact that CD spectra

Table III. Thermodynamic parameters of the complex formation

Guest[a]	ΔG(298K)/kcal mol^{-1}			ΔH/kcal mol^{-1}			ΔS/K^{-1} cal mol^{-1}		
	α-CDx[b]	DM-α-CDx	TM-α-CDx[c]	α-CDx	DM-α-CDx	TM-α-CDx	α-CDx	DM-α-CDx	TM-α-CDx
BA	−3.45	−3.89(2)	−3.73	−10.0(2)	−14.3(3)	−16.4(4)	−22.0(7)	−34.9(8)	−43(2)
m-HB	−3.32	−3.65(1)	−4.17	−11.3(2)	−13.6(3)	−17.3(5)	−26.9(6)	−33.5(8)	−44(2)
p-HB	−3.60	−4.09(1)	−4.07	−9.7(2)	−11.6(1)	−14.4(5)	−20.6(7)	−25.1(4)	−34(2)
m-AB	−2.36			−7.8(1)			−18.1(2)		
p-AB	−3.82	−4.39(1)		−10.4(2)	−13.6(1)		−22.1(6)	−30.8(4)	
m-NP	−2.90	−3.48(2)	−3.85	−8.4(1)	−7.8(1)	−10.2(3)	−18.6(3)	−14.5(4)	−21(1)
p-NP	−3.15	−3.59(2)	−3.91	−7.6(1)	−6.7(1)	−14.9(4)	−15.0(4)	−10.3(3)	−37(1)
m-NA	−3.64	−3.66(5)	−4.16	−6.9(1)	−4.4(1)	−11.5(2)	−14.6(2)	−2.5(3)	−27(1)
p-NA	−3.64	−4.40(2)	−4.47	−10.1(1)	−9.7(1)	−14.4(2)	−21.7(2)	−17.8(5)	−33(1)

[a]BA: Benzoic acid. *m*-HB:*m*-Hydroxybenzoic acid. *p*-HB:*p*-Hydroxybenzoic acid. *m*-AB:*m*-Aminobenzoic acid. *p*-AB:*p*-Aminobenzoic acid. *m*-NP:*m*-Nitrophenol. *p*-NP:*p*-Nitrophenol. *m*-NA:*m*-Nitroaniline. *p*-NA:*p*-Nitroaniline.

[b]See Ref. 6.

[c]See Ref. 7.

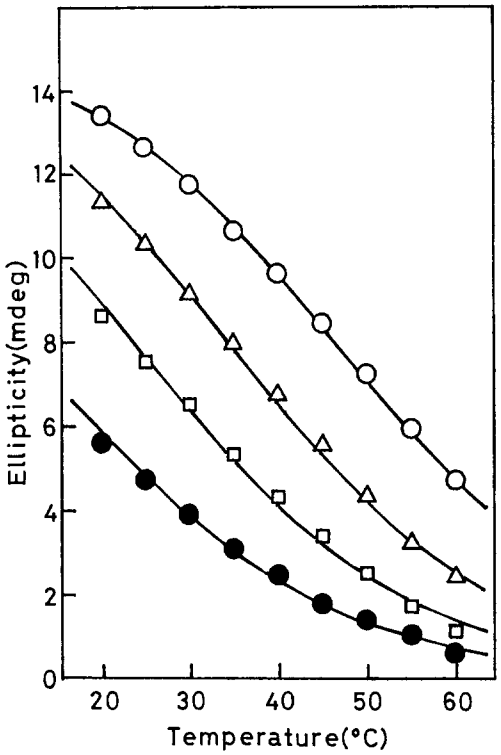

Fig. 5. Observed and calculated CD intensity change of the benzoic acid complex at the DM-α-CDx concentrations of 0.90×10^{-2} M (○), 0.40×10^{-2} M (△), 0.20×10^{-2} M (□), and 0.10×10^{-2} M (●). The calculated CD intensity is shown with solid lines.

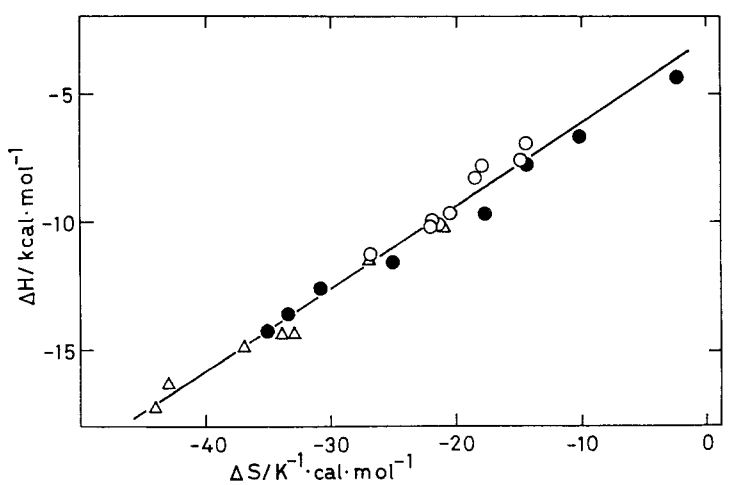

Fig. 6. Plots of ΔH against ΔS for the complexes of α-CDx (○), DM-α-CDx (●), and TM-α-CDx (△).

and thermodynamic behavior of the DM-α-CDx complex resemble those of α-CDx complexes rather than those of TM-α-CDx complexes.

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