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Solvent Isotope Effect on the Complexation of Cyclodextrins in Aqueous Solutions

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The replacement of H_2O by D_2O as a solvent resulted in an appreciable increase in the binding constants (K_b) for complexes of native and modified cyclodextrins with such typical guests as *p*-nitrophenol, the *p*-nitrophenolate ion, Methyl Orange, phenolphthalein, and inorganic anions. The observed increase in K_b was attributed mainly to a solvent H/D isotope effect on hydrophobic and electrostatic interactions between the hosts and guests.

Cyclodextrins (CD's) are doughnut-shaped molecules which include a variety of molecules and ions within the holes of the doughnuts. The binding constants (K_b) of CD complexes vary widely either with the size of the CD cavity or with the size, structure, and physicochemical properties of the guest molecules (molecular recognition). Thus, the determination of K_b values is important for studying molecular recognition in CD's. A number of methods have been reported for determining the K_b values.[1] Among them, nuclear magnetic resonance (NMR) spectrometry is very useful. The complexation of CD's with guests often causes significant changes in the chemical shifts (δ's) of the ^1H's and ^{13}C's involved in the CD's and/or guests.[2—4] The K_b values for the complexes are easily determined by curve-fitting analyses of the changes in δ's with the concentrations of the CD's or guests. However, NMR measurements for CD complexation are generally carried out in D_2O, whereas measurements by other methods are mostly carried out in H_2O. Recently, we determined the K_b values for complexes of mono-[6-(pyridinio)-6-deoxy]-α- (**1**) and β-CD with KI by UV spectrometry in H_2O[5] and by ^1H NMR spectrometry in D_2O.[6] Although both of the K_b values were in fairly good agreement with each other, the differences between them exceeded the experimental errors. The discrepancy would be ascribed to a solvent isotope effect on CD complexation. However, no work has been reported concerning this effect. The present work deals with a solvent H/D isotope effect on the K_b values for some typical complexes of native and modified CD's with such guests as *p*-nitrophenol (NP) and its phenolate ion (NP^-), Methyl Orange (MO), phenolphthalein (PP), I^-, SCN^-, and ClO_4^-. Ordinary UV-visible spectrophotometry was used for observing the complexation.

Experimental

Materials and Apparatus. Native α- and β-CD's were kindly supplied by Nihon Shokuhin Kako Co., Ltd. and Ensuiko Seito Co., Ltd. They were dried overnight in vacuo at 383 K. The HCO_3^- salt of the monopyridinio derivative, **1**, of α-CD was prepared by a previously described method.[7] Dyes such as NP, MO, and PP and inorganic salts such as KI, KSCN, $NaClO_4$, and Na_2SO_4 were of reagent grade, and were used without further purification. D_2O (Isotec Inc., 99.8 atom%) and D_2SO_4 (Aldrich, 98 wt% solution in D_2O, 99.5 atom%) were also commercially available.

The absorption spectra were recorded using a Shimadzu (Model UV-2100) spectrophotometer. The quartz cells (1.0 cm) were maintained at 298 K by means of a jacket through which water was circulated from a constant-temperature bath. The pH of an aqueous solution was measured by means of an Orion (Model 801A) digital pH/mV meter. It is well known that the pD of a D_2O solution is larger by ca. 0.4 than the reading of a pH meter. However, no such correction was made on the value for the sake of a simplified expression.

Spectrophotometric Determination of K_b's for Complexes of CD's. The complexation of CD's with NP, NP^-, and PP were spectrophotometrically observed for 0.1 $mol\,dm^{-3}$ citrate and/or carbonate buffer solutions. The dye concentration and the observed wavelength were 5.0×10^{-5} $mol\,dm^{-3}$ and 352 nm for NP, 5.0×10^{-5} $mol\,dm^{-3}$ and 418 nm for NP^-, and 2.5×10^{-5} $mol\,dm^{-3}$ and 552 nm for PP, respectively. The complexation of CD's with MO was observed at 507 nm for 0.10 $mol\,dm^{-3}$ H_2SO_4 solutions in H_2O, or for 0.10 $mol\,dm^{-3}$ D_2SO_4 solutions in D_2O, containing 1.96×10^{-5} $mol\,dm^{-3}$ MO, 0.10 $mol\,dm^{-3}$ Na_2SO_4, and various concentrations of the CD's. The complexation of **1** with I^- was observed at 301 nm for H_2O or D_2O solutions containing 8.0×10^{-4} $mol\,dm^{-3}$ **1** and various concentrations of KI.[5] The K_b values for these host–guest systems were determined by a nonlinear least-squares curve-fitting analysis of changes in the absorbance with guest or host concentrations, based upon the assumption of 1 : 1 complexation. The thus-calculated curves were well-fitted to the observed data with correlation coefficients greater than 0.999. Similar measurements and analyses were repeated several times to give the mean of K_b as well as the standard deviation of the mean. As for α-CD–NP^-, β-CD–PP, and **1**-I^- complexes, the K_b values were likewise determined in binary solvent systems comprising H_2O and D_2O, as well as in H_2O and in D_2O at various temperatures (T) ranging from 288 to 328 K. The thermodynamic parameters (ΔH and ΔS) were estimated by least-squares analyses of the relationships between $\ln K_b$ and T^{-1}. The values of ΔH and ΔS were calculated from the observed slopes and intercepts, respectively, of the obtained straight lines. The correlation coefficients of the lines were larger than 0.997.

The K_b values for complexes of α-CD with I^-, SCN^-, and ClO_4^- were spectrophotometrically determined by analyses of the inhibitory effect of the Na or K salts of these anions on the complexation of the CD with MO in a similar manner as previously described.[8] The absorption spectra were recorded for a 0.1 mol dm^{-3} H_2SO_4 solution in H_2O (or a D_2SO_4 solution in D_2O) containing 1.96×10^{-5} mol dm^{-3} MO, 1.0×10^{-3} mol dm^{-3} α-CD, and various concentrations of inorganic salts. The ionic strength of the solutions was adjusted to be 0.6 mol dm^{-3} by adding Na_2SO_4. The absorbance at 507 nm increased and approached a constant value with increasing concentration of the inorganic salts, suggesting that the salts competitively inhibit the complexation of α-CD with MO. Based on the assumption that the inorganic anions of the salts form 1 : 1 complexes with α-CD, the K_b values for the complexes were determined by a nonlinear least-squares curve-fitting analysis of the changes in the absorbance with the concentrations of inorganic salts. The calculated curves were well-fitted to the observed data with correlation coefficients greater than 0.99. Similar measurements and analyses were repeated several times to give the mean of K_b as well as the standard deviation of the mean.

Results and Discussion

Solvent Isotope Effect on the Complexation of α-CD with NP. The K_b values for the complexation of α-CD with NP were spectrophotometrically determined in acidic (pH 3—4) and basic (pH 10—11) H_2O and D_2O at 298 K (Table 1). Since the pK_a of NP is 6.89[9] at 298 K, NP is the overwhelmingly the major species at the acidic pH's, and NP$^-$ at the basic pH's. Slight changes in the pH of the acidic or basic solutions had no effect on K_b. On the other hand, the K_b values for NP$^-$ were much larger than those for NP. Similar results were reported by Cramer, et al.[10] The replacement of the H_2O solvent by D_2O resulted in a statistically significant increases in K_b at both acidic and basic pH's. Interestingly, the ratio of $K_b(H_2O)$ to $K_b(D_2O)$ remained virtually constant at ca. 0.8, irrespective of the solution pH.

A reaction system comprising NP and α-CD at any pH involves both inclusion and acid-dissociation equilibria, as shown in

$$\begin{array}{ccc} \mathrm{AH} + \mathrm{CD} & \rightleftharpoons & \mathrm{CDAH} \\ \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ \mathrm{H^+} + \mathrm{A^-} + \mathrm{CD} & \rightleftharpoons & \mathrm{H^+} + \mathrm{CDA^-}, \end{array} \tag{1}$$

where AH and A$^-$ are the conjugate acid-base pair of NP and CDAH and CDA$^-$, the inclusion complexes of α-CD with AH and A$^-$, respectively. If Eq. 1 represents the total processes in the system, the following relations must hold for both H_2O and D_2O solutions:

$$K_a(\mathrm{AH}, H_2O)/K_a(\mathrm{CDAH}, H_2O) = K_b(\mathrm{AH}, H_2O)/K_b(\mathrm{A^-}, H_2O) \tag{2}$$

and

$$K_a(\mathrm{AH}, D_2O)/K_a(\mathrm{CDAH}, D_2O) = K_b(\mathrm{AH}, D_2O)/K_b(\mathrm{A^-}, D_2O) \tag{3}$$

where K_a and K_b are the acid-dissociation constants and binding constants, respectively, for the chemical species and solvents given in the parenthesis. Combining Eqs. 2 and 3 leads to

$$\frac{K_a(\mathrm{AH}, H_2O)/K_a(\mathrm{AH}, D_2O)}{K_a(\mathrm{CDAH}, H_2O)/K_a(\mathrm{CDAH}, D_2O)} = \frac{K_b(\mathrm{AH}, H_2O)/K_b(\mathrm{AH}, D_2O)}{K_b(\mathrm{A^-}, H_2O)/K_b(\mathrm{A^-}, D_2O)}. \tag{4}$$

The right-hand side of Eq. 4 is the ratio of the magnitude of the solvent isotope effect on the complexation of α-CD with AH to that on complexation with A$^-$. The ratio was experimentally shown to be virtually equal to unity, as described above (Table 1). Then, the left-hand side of Eq. 4 should be virtually equal to unity, indicating that the magnitude of the solvent isotope effect on the acid-dissociation of AH is virtually equal to that on the acid-dissociation of CDAH. This presumption was substantiated by a spectrophotometric determination of pK_a for NP in both H_2O and D_2O in the absence and presence of 5.0×10^{-5} mol dm^{-3} α-CD (Fig. 1). The observed pK_a values of NP were 7.0 in H_2O and 6.5 in D_2O in the absence of α-CD and 6.2 in H_2O and 5.7 in

Table 1. Binding Constants, K_b/mol^{-1} dm^3, for Complexes of α-CD with NP in Acidic and Basic H_2O and D_2O Solutions at 298 K

pH	$K_b(H_2O)$	$K_b(D_2O)$	$K_b(H_2O)/K_b(D_2O)$
3.0	151± 3[a]	180± 5	0.84
3.5	146± 5	186± 5	0.78
4.0	148± 2	184± 2	0.80
10.0	1860±24	2410±26	0.77
10.4	1870±20	2410±23	0.78
11.0	1860±20	2420±27	0.77

a) The mean ± the standard deviation of the mean.

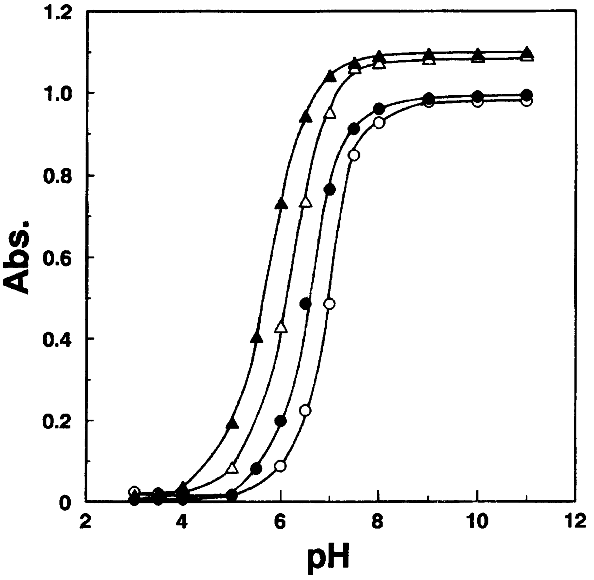

Fig. 1. Effects of pH on the absorbances (Abs) of NP at 400 nm in H_2O (○) and in D_2O (●) containing no α-CD and at 409 nm in H_2O (△) and in D_2O (▲) containing 5.00 mmol dm^{-3} α-CD at 298 K.

Table 2. Binding Constants, $K_b/\mathrm{mol}^{-1}\,\mathrm{dm}^3$, for Complexes of CD's with Some Typical Guests in H_2O and D_2O at 298 K

Host	Guest	$K_b(H_2O)$	$K_b(D_2O)$	$K_b(H_2O)/K_b(D_2O)$
α-CD	MO[a)]	1020±12	1190±14	0.86
α-CD	NP^-[b)]	1870±20	2410±23	0.78
β-CD	MO[a)]	352± 4	428± 2	0.82
β-CD	PP[c)]	24800±100	32400±100	0.77
	PP[d)]	23200±100	30600±300	0.76
	PP[e)]	23300±100	30000±150	0.78
1	I^-[f)]	163± 1	219± 1	0.74
α-CD	I^-[a)]	9.1±0.3	13.5±0.5	0.67
α-CD	SCN^-[a)]	22.4±0.3	25.3±0.3	0.89
α-CD	ClO_4^-[a)]	26.4±0.4	32.0±0.5	0.83

a) In 0.1 $\mathrm{mol\,dm}^{-3}$ H_2SO_4 (or D_2SO_4). b) pH 10.4. c) pH 11.0. d) pH 11.4. e) pH 11.6. f) In unbuffered H_2O (or D_2O).

Table 3. Thermodynamic Parameters, ΔG, ΔH, and ΔS, for Host–Guest Complexation in H_2O and in D_2O at 298 K

Host	Guest	$-\Delta G/\mathrm{kJ\,mol}^{-1}$		$-\Delta H/\mathrm{kJ\,mol}^{-1}$		$-\Delta S/J\,\mathrm{mol}^{-1}\,\mathrm{K}^{-1}$	
		H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
α-CD	NP^-[a)]	18.7	19.3	34.6	39.2	53.6	67.5
β-CD	PP[b)]	24.9	25.6	45.6	45.2	69.6	65.1
1	I^-	12.6	13.4	34.2	33.2	72.1	66.4

a) pH 10.4. b) pH 11.5.

D_2O in the presence of α-CD. The pK_a's of NP in D_2O were seemingly smaller by 0.5 than the corresponding values in H_2O, irrespective of the presence of α-CD. This fact indicates that the magnitudes of the solvent isotope effect on the acid-dissociation of free and complexed NP are virtually equal to each other. Furthermore, taking into account the fact that the pD value of a D_2O solution is larger by ca. 0.4 than the reading of the pH meter, we could regard the magnitudes of the solvent isotope effect on the acid-dissociation of free and complexed NP as being small. In any event, the above results clearly show that the replacement of H_2O by D_2O as a solvent results in an appreciable increase in the K_b values for α-CD complexes with NP and its conjugate base.

Comparison of K_b's in D_2O with Those in H_2O for Various Host–Guest Systems. The K_b values in H_2O and D_2O were spectrophotometrically determined for such typical host–guest systems as α-CD complexes with MO, I^-, SCN^-, and ClO_4^-, β-CD complexes with MO and PP, and a complexes of **1** with I^- (Table 2). The obtained K_b values in H_2O were in fairly good agreement with those thusfar reported,[3,5,9—12)] though they were measured under conditions not strictly identical with each other. Interestingly, the K_b values in D_2O were always larger than the corresponding K_b values in H_2O. A student *t*-test of the data showed that the difference between them are statistically significant with a significance limit of 0.1%. The $K_b(H_2O)/K_b(D_2O)$ ratio varied from 0.67 to 0.89, the mean being 0.80. It thus seems that a significant increase in K_b upon the replacement of H_2O by D_2O as a solvent is a general phenomenon.

The properties of liquid H_2O and D_2O are very similar to each other. However, D_2O forms stronger hydrogen bonds than does H_2O, and there is more structural order in liquid D_2O than in liquid H_2O at a given temperature.[13)] Therefore, hydrophobic interactions between apolar solutes are stronger in D_2O than in H_2O.[14)] The dielectric constant (77.937) of D_2O is slightly smaller than that (78.304) of H_2O,[13)] indicating that electrostatic interactions, including ion–ion, ion–dipole, and dipole–dipole interactions, are slightly larger in D_2O than in H_2O. The solvent isotope effect on hydrogen bonding between solutes has been regarded as being small.[15)] On the other hand, several intermolecular interactions are responsible for the complexation of native CD's:[16—19)] Hydrophobic interactions, van der Waals interactions including dipole–dipole interactions and London dispersion forces, and hydrogen bonding are typical. In addition, electrostatic ion–ion interactions and charge-transfer interactions are responsible for the complexation of a modified CD, **1**, with I^-.[5)] Thus, the observed increase in K_b upon replacing H_2O by D_2O as solvents may be mainly attributable to the strengthening of hydrophobic interactions and electrostatic ion–ion, ion–dipole, or dipole–dipole interactions between the hosts and the guests.

The K_b values [K_b(B)] in binary solvent systems comprising H_2O and D_2O were also determined at 298 K for

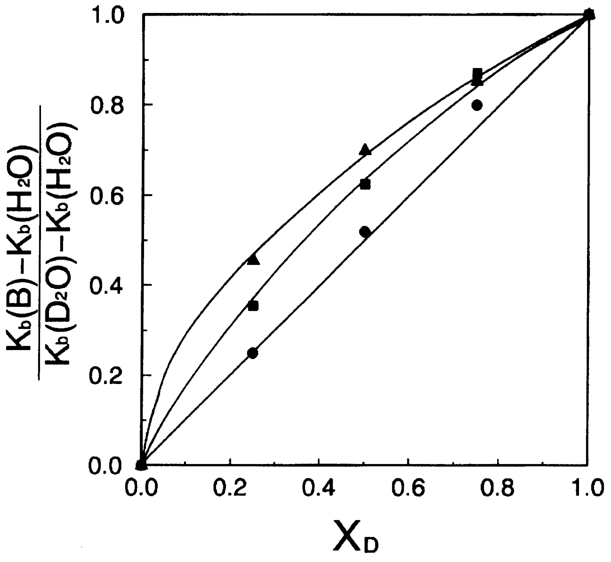

Fig. 2. Plots of $[K_b(B) - K_b(H_2O)]/[K_b(D_2O) - K_b(H_2O)]$ vs. X_D for the complexes of α-CD–NP⁻ (▲), β-CD–PP (■), and **1**-I⁻ (●) at 298 K.

α-CD–NP⁻, β-CD–PP, and **1**-I⁻ complexes. In all of the cases examined, the $K_b(B)$ values increased along with increasing the mole fraction (X_D) of D_2O in solution. Plots of $[K_b(B)-K_b(H_2O)]/[K_b(D_2O)-K_b(H_2O)]$ vs. X_D gave convex curves for α-CD–NP⁻ and β-CD–PP complexes, and virtually a straight line for a **1**-I⁻ complex (Fig. 2), suggesting that the mechanism of the solvent isotope effect on **1**-I⁻ complexation is somewhat different from that on the complexation of α-CD–NP⁻ and β-CD–PP. Electrostatic ion–ion interactions and charge-transfer interactions, in addition to hydrophobic and van der Waals interactions, are responsible for the formation of a **1**-I⁻ complex,$^{5)}$ but not for the formation of α-CD–NP⁻ and β-CD–PP complexes.

Among the binding forces responsible for the complexation of the CD's with guests, only hydrophobic interactions are governed by entropy. The thermodynamic parameters, ΔG, ΔH, and ΔS, determined for α-CD–NP⁻, β-CD–PP, and **1**-I⁻ complexes, are summarized in Table 3. Taking into account the experimental errors, we could find no appreciable changes in the thermodynamic parameters upon replacing H_2O by D_2O as solvents, mostly due to the small differences between K_b in H_2O and in D_2O. More accurate and precise determinations of the thermodynamic parameters are necessary for a discussion of the interactions responsible for the solvent isotope effect on the basis of the parameters.

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