Dynamic Aspects in Host–Guest Interactions. Part 2. Directional Inclusion Reactions of Some Azo Guest Molecules with α-Cyclodextrin

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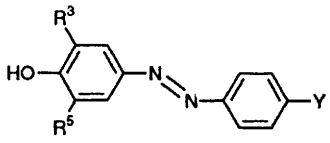
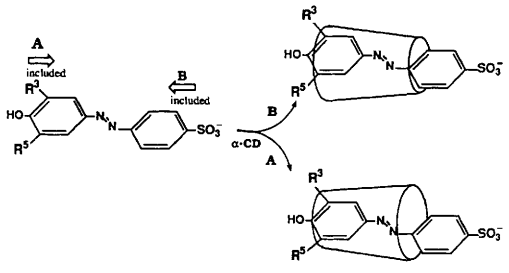
The inclusion reactions of the azo guest molecules, 3',5'-$(Me)_2$-4'-(OH)Ph–N=N–Ph–Y **1–4** and 3'-Pr-4'-(OH)Ph–N=N–Ph–Y **5–8** (Y = CO_2^-, SO_2NH_2, SO_3^- and AsO_3H^-), with α-cyclodextrin were investigated. The formation constants of the inclusion complexes ranged from 5.0×10^3 to 1.75×10^4 mol^{-1} dm^3. The rate constants for the inclusion reactions in aqueous solution at 25 °C were determined by the stopped-flow method. The rate constants and reaction mechanism were quite sensitive to the substituent group Y on C-4 of one phenyl ring. The reactions of **1** and **5** (Y = CO_2^-) were found to be very fast (millisecond or less) and to proceed as one step processes. On the other hand, the reactions of **4** and **8** (Y = AsO_3H^-) were quite slow (several tens of minutes). In the case of the reaction of



1–4; $R^3 = R^5 = Me$
5–8; $R^3 = Pr$, $R^5 = H$
1,5; $Y = CO_2^-$
2,6; $Y = SO_2NH_2$
3,7; $Y = SO_3^-$
4,8; $Y = AsO_3^{2-}$

2, **6** (Y = SO_2NH_2) and **3**, **7** (Y = SO_3^-), the inclusion clearly proceeds as a two-step process. A specific orientation and/or direction of reactant within the inclusion complex is also discussed.

Recently, some progress has been made in understanding the mechanism of the inclusion reaction with α-cyclodextrin (α-CD).[1] However, one aspect which has remained relatively unexplored is the direction and orientation of the reactant in the formation of the α-CD inclusion complex. In our series of kinetic studies, a preferential and directional (Direction **B**) binding of the hydrophilic phenyl ring with the sulfonate group of some azo guest molecules **3**, **7** and **9–12** in Scheme 1 was clearly

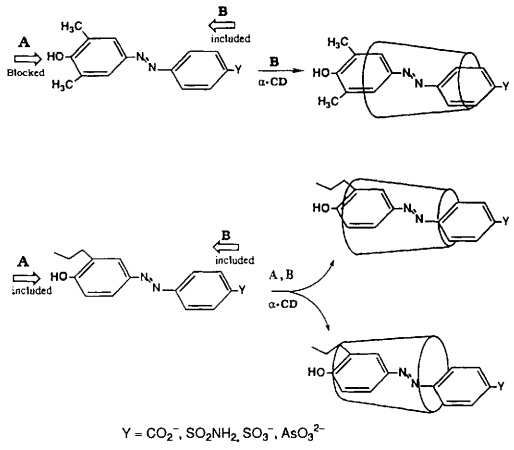


Scheme 1 **3** (R^3, R^5 = Me); **9** (R^3, R^5 = Pr^i); **10** (R^3 = Pr^i, R^5 = H); **11** (R^3 = Bu^t, R^5 = H); **12** (R^3 = Et, R^5 = H); **7** (R^3 = Pr, R^5 = H); **13** (R^3 = Me, R^5 = H); **14** (R^3, R^5 = H)

observed. In the guest systems **13** and **14** with a smaller substituent, the inclusion is possible from both the phenolic ring (Direction **A**) and the sulfonate side (Direction **B**). If the binding from the phenolic ring with α-CD occurs in this system, the substantial decrease in the forward and backward rate constants (k_f and k_b) would be observed upon ionization of the 4'-hydroxy group.[2] The inclusions of **3** and **9** with a bulky substituent are forced to proceed exclusively from the sulfonate side (Direction **B**). Charge and steric effects at the periphery of the entering site of the guest molecules are particularly important in influencing the direction and the kinetic parameters such as $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$. However, the overall equilibrium constants for the inclusion reaction are little influenced by changes in the guest substituents.

Hersey and Robinson [3] have also pointed out that comparing rates of inclusion for a number of azo-guests containing two substituted phenyl groups separated by an azo-linkage enables us to determine which aromatic ring of the azo guests preferentially enters the α-CD cavity during the binding process. As α-cyclodextrin is relatively rigid, the size of the cavity is fixed, and guests that are too large will not enter the annulus. As mentioned above, two factors: steric effects and charge of the guest-inserting group can prevent the inclusion; this has been pointed out in detail by Wojcik *et al.*[4]

In order to provide a further understanding of the role of the charge and the steric effect in the binding process, we have begun systematic kinetic studies of the effect of substituents around the entering site on the direction, rate and mechanism of the inclusion reaction by α-CD with some azo guests **1–8** (Scheme 2). Preferential binding was exclusively accomplished in the guest systems **1–5** which possess bulky 3',5'-dimethyl substituents. Since inclusion from direction **A** is fully blocked owing to the steric hindrance of 3',5'-dimethyl groups,[1,5] the charge and steric effect of only the Y substituents can be examined. In the case of the guest systems **5–8** in which the entrance from direction **A** is also possible due to the smaller steric hindrance of the 3'-propyl group, it is more complicated to determine which aromatic ring of **5–8** preferentially enters the α-CD cavity during the binding process. Dynamic aspects in these inclusion reactions are significant in clarifying the



Scheme 2

preferential substrate binding step of enzyme–substrate and drug–receptor interactions.

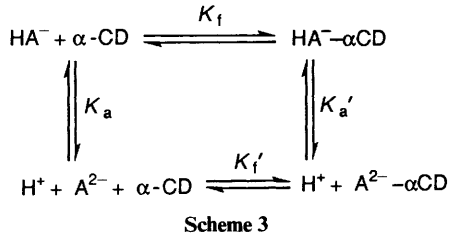
Experimental

Materials.—All the azo guest molecules **1**–**8** used in this study were synthesized by the azo coupling method and purified by column chromatography as described elsewhere.[1] Their elemental analyses showed excellent agreement with that calculated from the formulae. α-CD was purchased from Tokyo Kasei Chemicals Co. and used without further purification.

Measurements.—Acid dissociation constants (K_a) of the guests and the formation constants (K_f) of the inclusion complexes were determined spectrophotometrically using a JASCO Ubest-30 spectrophotometer as previously described.[1] The kinetic measurements were performed by using a Unisoku optical fibre type stopped-flow apparatus. Pseudo-first-order conditions of excess α-CD concentrations were maintained over guest concentrations {[guest] = 2–5 × 10^{-5} mol dm^{-3}}. The observed rate constants (k_{obsd}) were determined from the average of three replicate experiments. The reaction temperature was maintained to within 0.1 °C at 25 °C by means of an external circulating water bath. The pH of the solution in the acidic and alkaline regions was maintained with phosphate buffer (pH 4.2–4.5) and NaOH (pH 11.0–11.5), respectively. The ionic strength was maintained at 0.1 mol dm^{-3} with NaCl.

Results and Discussion

Stability and Directional Binding.—It has been shown[1] that the azo guest molecules except for **2**, **4**, **6** and **8** exist as the monovalent anion (HA^-) at pH 4.2–5 and as the divalent anion (A^{2-}) at pH 11–11.5, where the acid dissociation constant K_a in Scheme 3 corresponds to the dissociation of the 4′-hydroxy proton.



Scheme 3

Guests **4** and **8** in the alkaline pH region exist as the trivalent anion (A^{3-}) due to the acid dissociation of the $-AsO_3H^-$ group (pK_a ~ 8.5). Guest **6** precipitated in the weakly acidic pH region due to the formation of the neutral species (HA). In the alkaline pH region, compounds **2** and **6** exist as monovalent anion (A^-) with the liberation of the 4′-hydroxy proton of HA species. The acid form HA^- and the base form A^{2-} of the guest molecules were found to form 1 : 1 inclusion complexes with α-CD (Scheme 3), judging from the existence of an isosbestic point. Fig. 1 shows the change in the absorption spectra which occur when HA (**2**) binds to α-CD.

Table 1 Formation constants (K_f) for the inclusion complexes with α-CD of the acid form (HA^-) and the base form (A^{2-}) of the azo guest molecules **1**–**8**

Azo guest molecules	pK_a	K_f/mol dm^{-3}	K_f'/mol dm^{-3}
1	7.89	9 100	6 100
2	7.68	6 500	6 700
3	8.09	5 400	4 700
4	7.89	1 900	No inclusion
5	7.99	17 500	12 600
6	ppt.	ppt.	9 000
7	8.19	8 300	7 100
8	7.97	7 000	5 900

In Scheme 3, the following relations must hold. The

$$K_f^{-1}K_a = (K_f')^{-1}K_a' \quad (1)$$

formation constants, K_f for HA^- and K_f' for A^{2-}, of the 1 : 1 inclusion complexes were determined spectrophotometrically using a Benesi–Hildebrand method. The formation constants obtained are listed in Table 1 with the pK_a values.

In most cases, the inclusion complex of A^{2-} is less stable than the corresponding complex of HA^-. This destabilization effect has already been pointed out.[1] Since the 3′,5′-dimethylphenol derivatives **1**–**4** could enter only from direction **B**, their stability is lower than that of the corresponding 3′-propylphenol derivatives **5**–**8**. In particular, the inclusion complex of the acid

$$K_f \text{ and } K_f' \text{ for } \mathbf{1}\text{–}\mathbf{4} < K_f \text{ and } K_f' \text{ for } \mathbf{5}\text{–}\mathbf{8} \quad (2)$$

form H_2A^- of **4** is appreciably less stable than that of **8**. The base form A^{3-} of **4** does not bind α-CD, but that of **8** could form a complex with α-CD. The order of the formation constants K_f for **1**–**4** is found as **1** (Y = CO_2^-) > **2** (Y = SO_2NH_2) > **3** (Y = SO_3^-) ≫ **4** (Y = AsO_3H^-) and K_f' as **1** (Y = CO_2^-) ~ **2** (Y = SO_2NH_2) > **3** (Y = SO_3^-). This order of stability is closely related to the size and charge of the Y substituents on C-4 of one phenyl ring. The size effect changes from $-SO_3^-$ to $-AsO_3H^-$ [van der Waals radii: S (1.85 Å) and As(2.0 Å)[6]] and the charge effect from monovalent anion $-AsOH_3^-$ to divalent anion $-AsO_3^{2-}$ lower, or prevent, the formation of the inclusion complexes of **4** (Y = AsO_3H^-; H_2A^-) and **4** (Y = AsO_3^{2-}; A^{3-}), respectively. However, the stability is recovered in the 3′-propylphenol derivatives, **8** (Y = AsO_3H^-; H_2A^-) and **8** (Y = AsO_3^{2-}; A^{3-}). Perhaps the inclusion from the 3′-propylphenol side (Direction **A** in Scheme 2) would occur in this guest system. The order in stability of the inclusion complexes for **5**–**8** is also found as **5** (Y = CO_2^-) > **6** (Y = SO_2NH_2) > **7** (Y = SO_3^-) > **8** (Y = AsO_3H^- and AsO_3^{2-}).

Rate Constants and Mechanistic Aspects.—Two distinct behaviour patterns were observed among the various inclusion reactions of **1**–**8** with α-CD. Table 2 shows the relationships between the structure of the Y substituent group of the guest molecule and the mechanistic aspects of the inclusion reaction with α-CD. In most cases, the observed rate constants (k_{obsd}) increased linearly with the concentration of α-CD and are simply interpreted in the following reaction mechanism, where

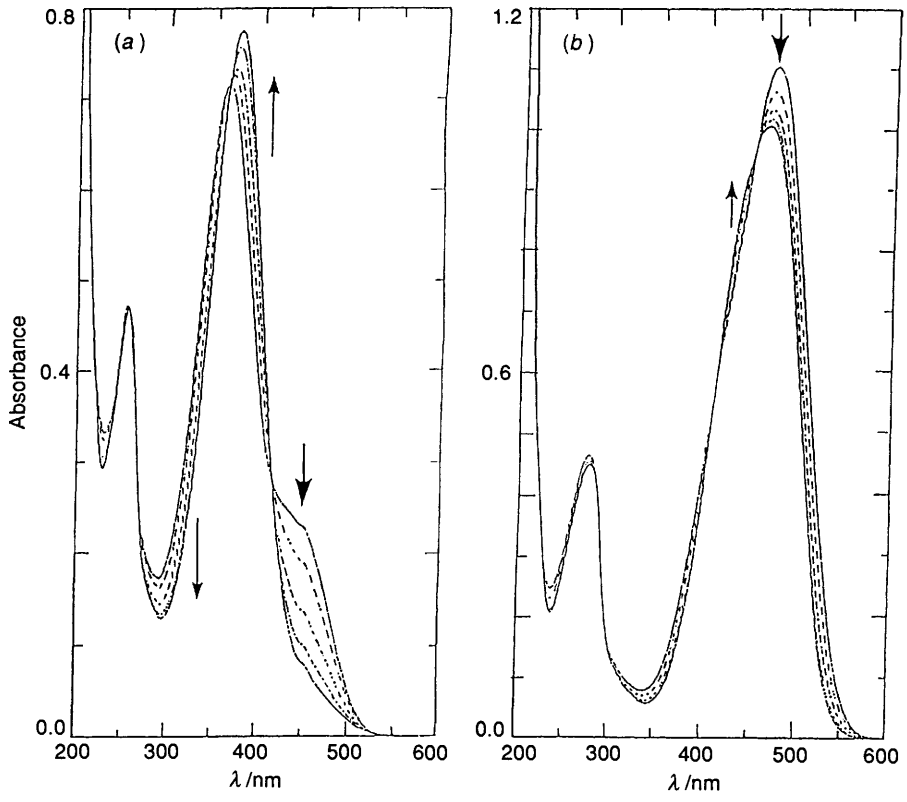


Fig. 1 Spectral changes of the acid (HA) and the base (A^-) forms of **2** at various α-cyclodextrin concentrations from 0 to (0.2, 0.6, 1, 2) × 10^{-3} mol dm^{-3} at 25 °C. [**2**] = 3.79 × 10^{-5} mol dm^{-3}. pH (*a*) 5.37, (*b*) 11.21.

Table 2 Relationship between the reaction time for the inclusion by α-CD and the structure of the Y substituents of the guest molecules, **1–8**

Guest molecules	Y	Number of steps	Reaction time: HA^- Fast	HA^- Slow	A^{2-} Fast	A^{2-} Slow
1	$-CO_2^-$	1	very fast		~ 40 ms	
2	$-SO_2NH_2$	2	~ 8 ms	~ 200 ms	~ 200 ms	~ 2 s
3	$-SO_3^-$	2	~ 100 ms	~ 5 s	~ 100 ms	~ 5 s
4	$-AsO_3H^-$	1	~ 30 min			
	$-AsO_3^{2-}$	1			No Inclusion	
5	$-CO_2^-$	1	~ 10 ms		~ 100 ms	
6	$-SO_2NH_2$	2	ppt.		~ 200 ms	~ 2 s
7	$-SO_3^-$	2	~ 100 ms	~ 5 s	~ 100 ms	~ 5 s
8	$-AsO_3H^-$	2	~ 500 ms	~ 10 s		
	$-AsO_3^{2-}$	1			~ 200 s	

$$\text{Guest (G)} + \alpha\text{-CD} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \text{G-}\alpha\text{CD} \quad (3)$$

$$k_{obsd} = k_{+1}[\alpha\text{-CD}]_T + k_{-1}, \quad (4)$$

$[\alpha\text{-CD}]_T$ is the total concentration of α-CD added. This case was observed in the guest systems of **1** (Y = CO_2^-; HA^- and A^{2-}), **4** (Y = AsO_3H^-; HA^-), **5** (Y = CO_2^-; HA^- and A^{2-}) and **8** (Y = AsO_3^{2-}; A^{3-}). On the other hand, the kinetic study of the inclusion reactions of **2** (Y = SO_2NH_2; A^-), **3** (Y = SO_3^-; HA^- and A^{2-}), **6** (Y = SO_2NH_2; A^-) and **7** (Y = SO_3^-; HA^- and A^{2-}) showed clearly the presence of two relaxation processes. Fig. 2 shows typical stopped-flow signals observed at 473 nm for the inclusion reaction of **6** (Y = SO_2NH_2; A^-) with α-CD.

Two distinct absorbance changes are observed in the fast (100–400 ms) and the slow (1–4 s) time regions. The plots of k_{obsd}(fast) and k_{obsd}(slow) against $[\alpha\text{-CD}]_T$ in the inclusion reaction of **3** (Y = SO_3^-; HA^-) are shown in Fig. 3. The plot of k_{obsd}(fast) *vs.* $[\alpha\text{-CD}]_T$ is linear and that of k_{obsd}(slow) *vs.* $[\alpha\text{-CD}]_T$ is curved and approaches a saturated value in the higher $[\alpha\text{-CD}]_T$ concentration range. Generally, the concentration-dependence of k_{obsd} could be interpreted in terms of the following two-step inclusion mechanism,[1,3,4] where the first step is a fast-

$$\text{G} + \alpha\text{-CD} \underset{\substack{k_{-1}\\ \text{fast}}}{\overset{k_{+1}}{\rightleftharpoons}} \text{G-}\alpha\text{CD}^* \underset{\substack{k_{-2}\\ \text{slow}}}{\overset{k_{+2}}{\rightleftharpoons}} \text{G-}\alpha\text{CD}, \quad (5)$$

binding process of the guest G with α-CD and the second step a subsequent slower intramolecular structural transformation of the intermediate species G-αCD* which is in slower equilibrium with a more stable final inclusion complex G-αCD. If the first

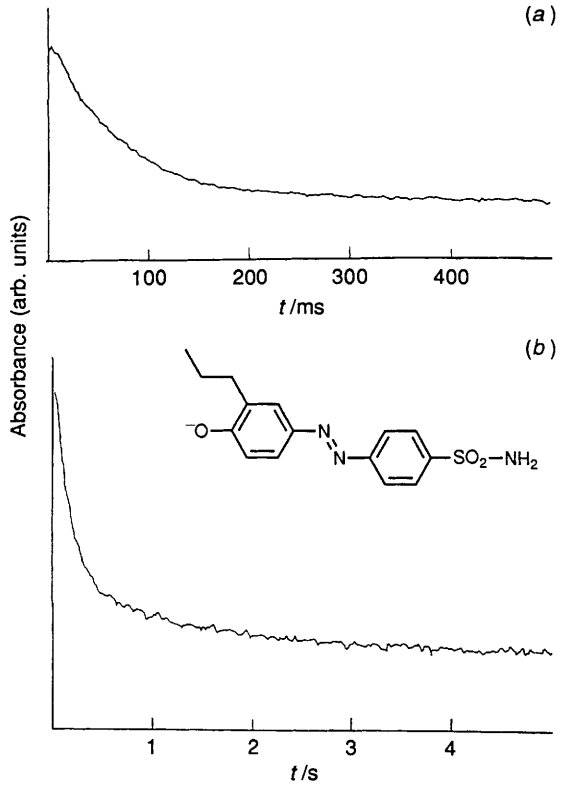


Fig. 2 Typical stopped-flow signals for the change in absorbance of the fast (upper) and the slow (bottom) processes at 473 nm. [**6**(A^-)] = 3.60×10^{-5} mol dm^{-3} and [α-CD_x] = 2.0×10^{-3} mol dm^{-3}. At pH = 11.21

step is very fast as compared with the second step, the observed rate constants are expressed by eqns. (6) and (7), where K_1 is

$$k_{obsd}(fast) = k_{+1}[\alpha\text{-CD}]_T + k_{-1} \quad (6)$$

$$k_{obsd}(slow) = \frac{K_1 k_{+2}[\alpha\text{-CD}]_T}{(1 + K_1[\alpha\text{-CD}]_T)} + k_{-2} \quad (7)$$

equal to k_{+1}/k_{-1}. Plotting and fitting the rate data to the above mechanism allow the calculation of k_{+1}, k_{-1}, k_{+2}, k_{-2} and K_1. The values of k_{+1}, k_{-1}, k_{+2}, k_{-2} and K_1 for **3** ($Y = SO_3^-$; HA^-) are (1.1 ± 0.2) × 10^4 mol^{-1} dm^3 s^{-1}, 4.0 ± 0.1 s^{-1}, 0.47 ± 0.1 s^{-1}, 0.28 ± 0.05 s^{-1} and 2.8 × 10^3 mol^{-1} dm^3, respectively. The rate constants thus obtained are summarized in Table 3.

The rate constants, k_{+1}, k_{-1}, k_{+2} and k_{-2}, correspond to those for the acid form HA^- (HA) and k_{+1}', k_{-1}', k_{+2}' and k_{-2}' to those for the base form A^{2-} (A^-, A^{3-}). Since in the case of **1**–**4** the inclusion from direction **A** is fully blocked, the values of the rate constants, k_{+1} and k_{-1}, are dependent only on the nature of the Y substituent group. The order in k_{+1} and k_{-1} is simply as **1** ($-CO_2^-$) > **2** ($-SO_2NH_2$) > **3** ($-SO_3^-$) ≫ **4** ($-AsO_3H^-$). This was mainly due to the size effect of the Y group. A reasonable van der Waals contact is observed between the inner wall of α-CD and the $-SO_3^-$ group as the inserting Y substituent (Fig. 4). In particular, a drastic decrease in k_{+1} and k_{-1} (1.1 × 10^4 mol^{-1} dm^3 s^{-1}→1.38 mol^{-1} dm^3 s^{-1} and 4.0 s^{-1}→1.6 × 10^{-3} s^{-1}) is observed when the Y substituent varies from $-SO_3^-$ to $-AsO_3H^-$. Size effects of the guest-inserting substituent Y mainly dominate the rate for the inclusion with α-CD. The order in k_+' and k_-' is almost the same as that in k_+ and k_- [**1** ($-CO_2^-$) > **2** ($-SO_2NH_2$) > **3** ($-SO_3^-$)]. However, the $-AsO_3^{2-}$ group of **4** ($Y = AsO_3^{2-}$; A^{3-}) inhibits completely the inclusion due to both the size and the charge effect. Therefore, the guest **4** ($Y = AsO_3^{2-}$; A^{3-}) does not bind α-CD. The hydration

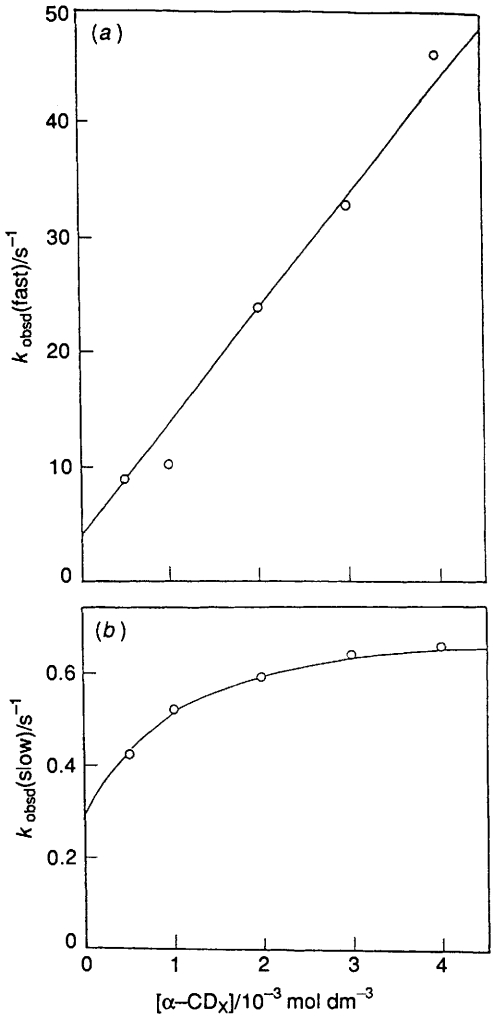


Fig. 3 Plots of the observed rate constants, k_{obsd}(fast) and k_{obsd}(slow) *vs.* [α-CD] for the inclusion reaction of **3** (HA^-) with α-CD. Solid line through the data points denotes the theoretical plot using eqns. (6) and (7).

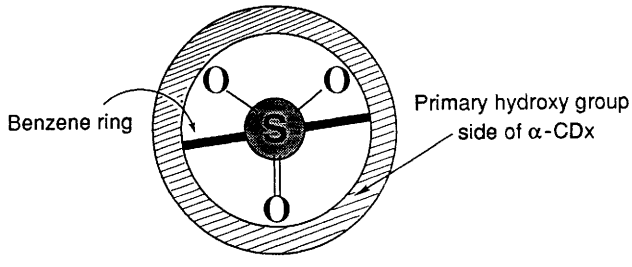


Fig. 4 The top view with the sulfonate group of the guest molecule **3** included in α-cyclodextrin

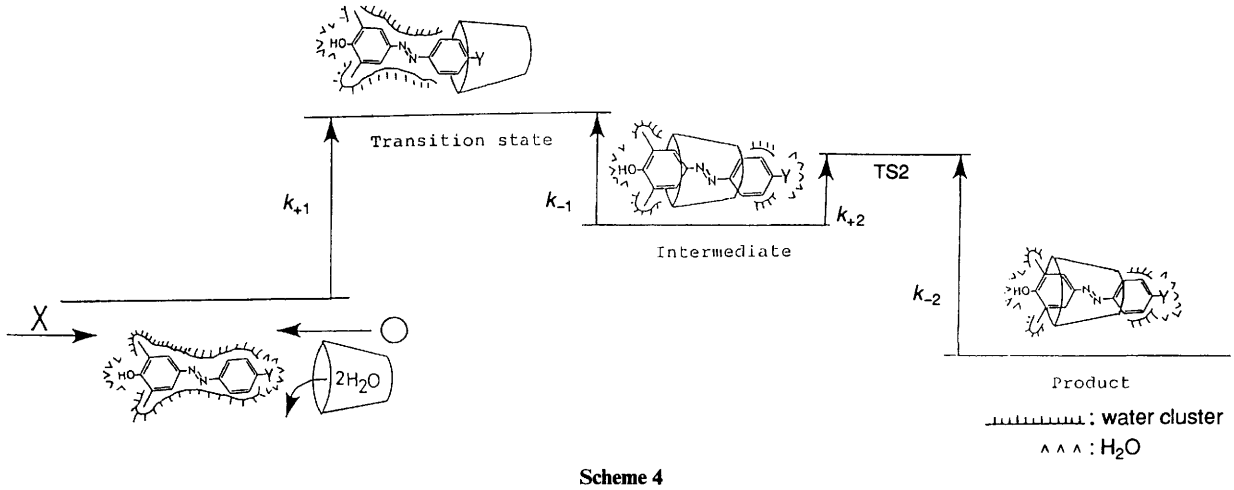
structure at the periphery of highly charged anionic substituents may be also significant in determining the rate and the mechanism for inclusion. The large values of K_1 and K_1' in Table 3 indicate the formation of the strong complex in the first stage of the reaction. The inclusion mechanism by α-CD for **2** and **3** is schematically shown in Scheme 4. Thus, the rate and mechanism for the inclusion reactions of **1**–**4** are closely related to the size and charge of the Y substituents (Table 2 and Scheme 4).

In the case of the guest systems **5**–**8**, it is more complicated to determine which aromatic ring of **5**–**8** preferentially enters the α-CD cavity. The values of the rate constants, k_+' and k_-', for **5** ($Y = CO_2^-$; A^{2-}) are almost the same as those for **1** ($Y = CO_2^-$; A^{2-}); hence the $-CO_2^-$ group must be the preferred group for inclusion. Steric and charge effects of the 3′-

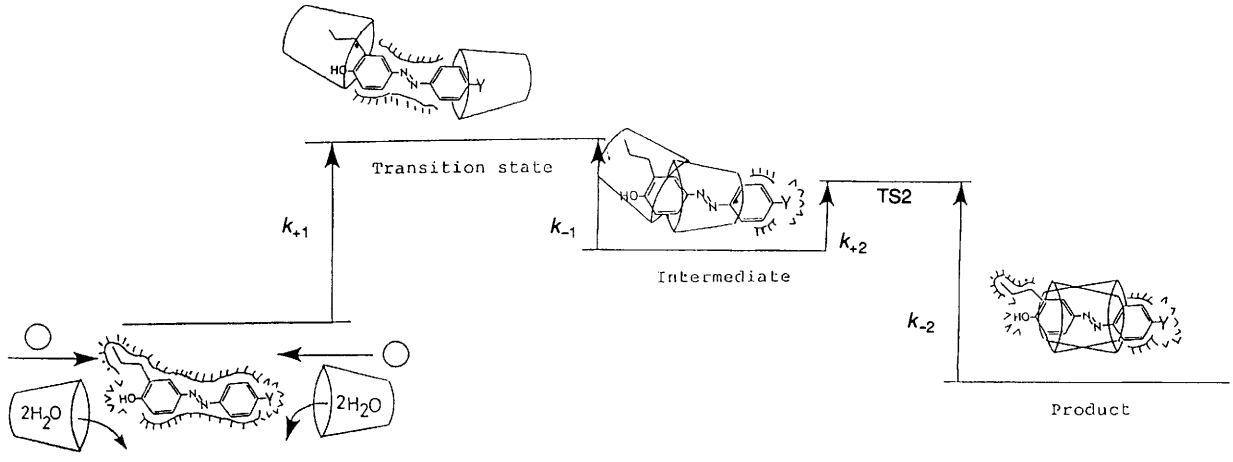
Table 3 Rate and equilibrium constants[a] for the inclusion reaction of α-cyclodextrin with the guest molecules, **1–8**

Guest molecules	K_1/mol^{-1} dm^3	k_{+1}/mol^{-1} dm^3 s^{-1}	k_{-1}/s^{-1}	k_{+2}/s^{-1}	k_{-2}/s^{-1}	K'_1/mol^{-1} dm^3	k'_{+1}/mol^{-1} dm^3 s^{-1}	k'_{-1}/s^{-1}	k_{+2}'/s^{-1}	k_{-2}'/s^{-1}
1	—	*b*	*b*	*c*	*c*	5 400	97 000	18	*c*	*c*
2	—	> 10^7	> 10^3	18.5	4.5	1 800	14 000	7.8	1.9	0.3
3	2 750	11 000	4.0	0.47	0.28	960	9 630	10	0.64	0.14
4	850	1.36	0.0016	*c*	*c*			No Inclusion		
5	—	> 10^5	~ 10^2	*c*	*c*	4 600	82 000	18	*c*	*c*
6		ppt.				6 500	11 000	1.7	0.7	0.65
7	3 300	20 000	6.0	0.87	0.55	1 900	6 900	3.6	0.25	0.08
8	~ 100	~ 1 000	~ 10	~ 0.3 (k_{+2} + k_{-2})		1 400	14	0.01	*c*	*c*

[a] Error limits are estimated to be not larger than ± 10% and ± 20% in any of determination of k_{+1}(k_{+1}') and k_{-1}(k_{-1}'), respectively. In the case of determination of k_{+2}(k_{+2}') and k_{-2}(k_{-2}'), the error is very large (± 50%) because of the poor S/N ratio of a small signal amplitude and the small concentration dependence of the rate constants. [b] Too fast to measure the reaction rate by the stopped-flow method. [c] Not observed.



Scheme 4



Scheme 5

propylphenolate (–O$^-$) moiety of **5** (Y = CO_2^-; A^{2-}) would block the inclusion from direction **A**. In the case of **6** (Y = SO_2NH_2; A^{2-}), the assignment is more difficult, but comparing the rate data supports the preferential inclusion from direction **B**. The slight decrease in the rate constants (k_{+1}, k_{-1} ⟶ k_{+1}', k_{-1}') also supports the preferential binding from direction **B**. The rate constants, k_{+1}, k_{-1}, k_{+2} and k_{-2}, of the inclusion reaction for **7** (Y = SO_3^-; HA^-) are in almost the same order as those for **3** (Y = SO_3^-; HA^-). This is also the case for the base form (A^{2-}) of **7** (Y = SO_3^-; A^{2-}). Due to the larger steric hindrance of the 3'-propylphenol side of **7**, the inclusion from the sulfonate group (Direction **B**) would be preferred in the **7** (Y = SO_3^-; HA^- and A^{2-}) guest system.

In the case of **8** (Y = AsO_3H^-; HA^-), the rate constants, k_{+1} and k_{-1}, are very much larger than those of **4** (Y = AsO_3H^-; HA^-). This fact indicates that the 3'-propylphenol side (Direction **B**) must be the preferred group for inclusion due to the larger steric hindrance of the –AsO_3H^- group. Although the base form (A^{3-}) of the guest **4** (Y = AsO_3^{2-}; A^{3-}) does not bind α-CD, the base form (A^{3-}) of the guest **8** (Y = AsO_3^{2-}; A^{3-}) could be included by α-CD from the direction **A**. The drastic decrease in the rate constants (k_{+1}, k_{-1} ⟶ k_{+1}', k_{-1}') which supports the inclusion from direction **A** is also observed. The inclusion mechanism by α-CD for **6–8** (one step mechanism from direction **A**) is shown in Scheme 5. In conclusion, the preferential inclusion from direction **A** or **B** is very sensitive to the nature of both the 3'-propylphenol side and the Y substituent group of **1–8**.

References

1 N. Yoshida and M. Fujimoto, *Chem. Let.* 1980, 231 and 1377. N. Yoshida, A. Seiyama and M. Fujimoto, *J. Phys. Chem.*, 1990, **94**, 4246 and references therein.
2 N. Yoshida and M. Fujimoto, *J. Phys. Chem.*, 1987, **91**, 6691.
3 A. Hersy and B. H. Robinson, *J. Chem. Soc., Faraday Trans. 1*, 1984, **80**, 2039.
4 R. P. Rohrbach and J. F. Wojcik, *Carbohydr. Res.* 1981, **92**, 177. A. Örstan and J. F. Wojcik, *Carbohydr. Res.*, 1985, **143**, 43.
5 F. Cramer, W. Saenger and H.-Ch. Spatz, *J. Am. Chem. Soc.* 1967, **89**, 14.
6 L. Pauling, *The Nature of the Chemical Bond*, 3rd edn., Cornell University Press, New York, 1960.

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