Substrate Specificity in the Cyclodextrin-catalyzed Cleavage of Organic Phosphates and Monothiophosphates in Alkaline Solutions

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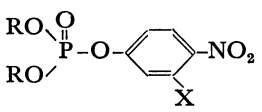
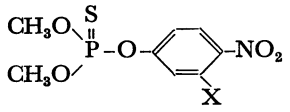
Cyclodextrin accelerates the phenol release from organic phosphates such as dialkyl 4-nitrophenyl phosphates, but it decelerates the reactions of the corresponding monothiophosphates in alkaline solutions. Each reaction proceeds *via* the prior formation of a cyclodextrin–substrate inclusion complex, followed by the nucleophilic attack of the cyclodextrin alkoxide ion or the hydroxide ion on the reaction site of the included substrate. The equilibrium and kinetic parameters involved in the reaction processes were determined and discussed in connection with the geometry of a cyclodextrin–substrate complex.

Substrate specificity is one of the most important characteristics of enzyme-catalyzed reactions. A little modification in the structure of substrates results in pronounced changes in both the binding and catalytic processes of an enzymatic reaction. A similar feature has been observed in relatively simple enzyme-model systems such as cyclodextrin.[1] For example, the cyclodextrin-induced rate acceleration of the phenol release from *meta*-substituted phenyl acetates is much greater than that from the corresponding *para*-substituted phenyl acetates.[2] The process includes the prior complexation of a substrate with cyclodextrin, and the specific spatial orientation of a substrate in a cyclodextrin complex causes a marked substrate specificity.

During the course of an investigation of the catalytic action of cyclodextrin, we found that the cyclodextrin-catalyzed cleavage of some organophosphorus pesticides in alkaline solutions reveals a characteristic substrate specificity: cyclodextrin accelerates the phenol release from the phosphates, **1a**, **1b**, and **1c**, but decelerates the release from the corresponding monothiophosphates, **2a** and **2b**.



1a: R=CH_3, X=CH_3
1b: R=CH_3, X=H
1c: R=C_2H_5, X=H



2a: X=CH_3
2b: X=H

The present study was undertaken in order to elucidate the catalytic modes of the cyclodextrin and the hydroxide ion by means of a close determination of the equilibrium and kinetic parameters involved in the reaction processes. The rate effect of cyclodextrin was also examined on the alkaline hydrolysis of a few phenyl acetates, such as *o*- and *m*-acetoxybenzoic acids (**3a** and **3b** respectively), in order to compare the equilibrium and kinetic parameters with those of the organophosphorus compounds.

Experimental

Materials. The α- and β-cyclodextrins were prepared by the method of Lane and Pirt.[3] They were separated and purified according to the directions of Cramer and Henglein.[4] Methyl α-D-glucopyranoside of a reagent grade was used without further purification. 3-Methyl-4-nitrophenol (**4**) was prepared by the method of Koelsch[5] and was purified by means of chromatography on an activated alumina column with benzene, ether, and ether–ethanol (4: 1, v/v) as eluants. The ether and ether–ethanol fractions were collected and concentrated by evaporation. The residue was recrystallized from water. Mp 127—129 °C (lit, mp 128 °C[6]). **1a** was prepared by the reaction of **4** with dimethyl chlorophosphate.[7] Crude **1a** was purified by chromatography on a silica gel column, with hexane, benzene, and ether as eluants. The ether fractions were collected and concentrated by evaporation. The residue was distilled *in vacuo*. **1b** was prepared by the reaction of sodium 4-nitrophenoxide with dimethyl chlorophosphate.[8] The **1c**, **2a**, and **2b** were provided by Sumitomo Chemical Co. and were used without further purification. The purity of these organophosphorus compounds was estimated to be 95% for **1a**, 100% for **1b**, 95% for **1c**, 97% for **2a**, and 94% for **2b** by the spectrophotometric determination at 400 nm of the phenolate anions afforded by the alkaline hydrolysis of the compounds. **3a** of a reagent grade was used without purification. **3b** was prepared by the reaction of *m*-hydroxybenzoic acid with acetic anhydride in dry pyridine and was recrystallized from chloroform.

Kinetics. The reaction rates of the esters were measured by following the appearance of the absorption of the corresponding phenoxide anions at 410 nm for the phosphates and monothiophosphates and at 300 and 310 nm for **3a** and **3b** respectively in aqueous NaOH solutions, the ionic strength (I_c) of which was maintained at 0.65 M with Na_2SO_4. In a typical run, 3.00 ml of a base solution was pipetted into a pair of 1.00-cm quartz cells, one of which was used as a reference cell and the other, as a sample cell in a Hitachi spectrophotometer, Model 124. After thermal equilibrium at 25.0±0.1 °C had been reached, 5 to 15 μl of a 8 to 55 mM ester in ethanol or methanol was added to the sample cell and the change in absorbance was followed. The results for various reaction times were treated according to the ordinary first-order rate equation. The rate constants were graphically determined. All the reactions examined obeyed good first-order kinetics with respect to substrates in both the absence and presence of cyclodextrin.

Determination of the Dissociation Constants and Rate Constants of Cyclodextrin–Substrate Complexes. As is shown in Table 1 as an example, the observed pseudo first-order rate constant (k_{obsd}) for each substrate examined approached a maximum or minimum value as the cyclodextrin concentration ([CD]) is increased. This saturation behavior, as well as the fact that methyl α-D-glucopyranoside had only a slight effect on the reaction rate, suggests that the rate process involves the prior formation of the inclusion complex of cyclodextrin with each substrate. Upon the assumption of the formation of an 1: 1

Table 1. Pseudo-first-order rate constants for the phenol release from **1a** and **2a** in alkaline solutions containing carbohydrates at 25.0 °C and I_c=0.65 M

Carbohydrate	Concn. (mM)	$k_{obsd}\times10^2$, min^{-1}	
		1a[a]	**2a**[b]
None	—	1.33	3.14
Methyl α-D-glucopyranoside	35.0	1.30	3.06
α-Cyclodextrin	1.00	1.30	2.77
	10.0	1.15	2.65
β-Cyclodextrin	1.00	1.58	2.22
	2.50	1.74	1.64
	5.00	1.96	1.22
	7.50	2.07	1.01
	10.0	2.19	0.87

a) 0.0094 M NaOH. b) 0.20 M NaOH.

cyclodextrin–substrate complex, k_{obsd} is represented by Eq. 1[1,2] if [CD] is much higher than that of a substrate ([S]):

$$k_{obsd} = K_{diss}(k_{un}-k_{obsd})/[CD] + k_c \quad (1)$$

In this equation, K_{diss} is the dissociation constant of an inclusion complex, and k_{un} and k_c, the rate constants for free and complexed substrates respectively. The plot of k_{obsd} *vs.* $(k_{un}-k_{obsd})/[CD]$ was virtually linear for each cyclodextrin–substrate system, and the values of K_{diss} and k_c were calculated from the slope and the intercept of the line respectively.

Calculation of Equilibrium and Kinetic Parameters from the pH Dependence of the Cyclodextrin-rate Effects. We postulated the reaction scheme shown below in order to explain the pH dependence of the cyclodextrin-rate effect:

Equilibrium Processes:

$$\begin{array}{ccc} CDOH + S & \overset{K_{diss}}{\rightleftharpoons} & CDOH\cdot S \\ K_a \uparrow\downarrow OH^- & & K_a \uparrow\downarrow OH^- \\ CDO^- + S & \overset{K_{diss}}{\rightleftharpoons} & CDO^-\cdot S \end{array}$$

Reaction Processes:

$$S + OH^- \xrightarrow{k_f} P$$

$$CDO^-\cdot S \xrightarrow{k_s} P$$

$$CDOH\cdot S + OH^- \xrightarrow{k_b} P$$

$$CDO^-\cdot S + OH^- \xrightarrow{k_b} P$$

where CDOH and CDO^- represent the unionized and ionized cyclodextrins respectively; S, a substrate; P, products; K_a, the first ionization constant of cyclodextrin; k_s, the first-order rate constant for the reaction of a completed substrate with the cyclodextrin alkoxide ion, and k_f and k_b, the second-order rate constants for the reactions of free and complexed substrates respectively with OH^-. We assumed that (1) the ionization of cyclodextrin has approximately no effect on the K_{diss} and k_b values; (2) the inclusion of a substrate also has little effect on the K_s value, and (3) the reaction of a substrate with the unionized cyclodextrin is negligible. Then, the rate constants, k_{un} and k_c, may be represented as is shown below:

$$k_{un} = k_f[OH^-] \quad (2)$$

$$k_c = k_s\{K_a[OH^-]/(K_a[OH^-]+K_w)\} + k_b[OH^-] \quad (3)$$

where K_w is the ion product of water and where the term, $K_a[OH^-]/(K_a[OH^-]+K_w)$, corresponds to the fraction of the ionized cyclodextrin. To calculate the values of k_s, k_b, and K_s in Eq. 3 from the $[OH^-]$ dependence of k_c observed, we used a computerized statistical technique of regression analysis. The calculated values excellently well explained the measured values of k_c at various OH^- concentrations with the correlation coefficients of 0.986 to 0.999, as is illustrated in Figs. 1 and 2.

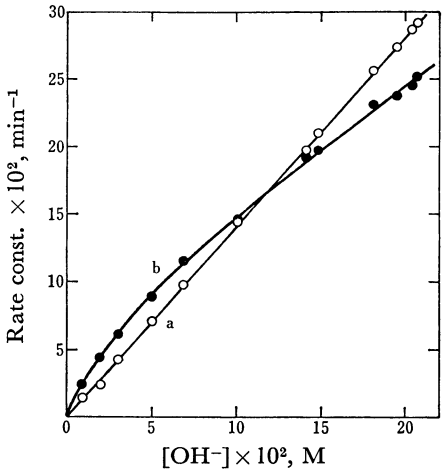


Fig. 1. Plots of k_{un} (a) and k_c (b) *vs.* $[OH^-]$ for **1a** at 25 °C and I_c=0.65 M. The solid lines were computed using the parameters in Table 3 and correspond to the best fit.

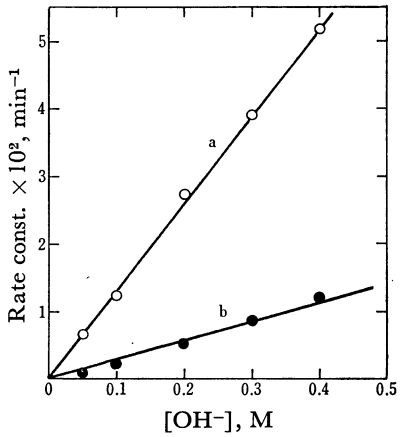


Fig. 2. Plots of k_{un} (a) and k_c (b) *vs.* $[OH^-]$ for **2a** at 25 °C and I_c=0.65 M. The solid lines were computed using the parameters in Table 3 and correspond to the best fit.

Determination of the Dissociation Constant of a Cyclodextrin–4 Complex. A 0.20 M NaOH solution (I_c=0.65 M) containing 1.33×10^{-5} M **4** and 0.0, 1.0, 2.5, 5.0, 7.5, or 10.0 mM α- or β-cyclodextrin was added to a 1.00-cm quartz cell. After the cell had been maintained at 25.0±0.1 °C, the absorption spectrum of each solution was measured by means of a Hitachi Model 124 spectrophotometer. The K_{diss} value was determined on the basis of the well-known equation:[9]

$$[CD][S]/\Delta Abs = K_{diss}/\Delta\varepsilon + [CD]/\Delta\varepsilon \quad (4)$$

where ΔAbs is the decrease in the absorbance at 380 nm of a **4** solution on the addition of cyclodextrin, and $\Delta\varepsilon$, the difference in the molar absorption coefficient at 380 nm between the free and the complexed **4** anions. The plot of $[CD][S]/\Delta Abs$ *vs.* [CD] gave an approximately straight line for a β-cyclodextrin–**4**

system, and the $\Delta\varepsilon$ and K_{diss} values were determined to be 2.8×10^{3} $M^{-1}cm^{-1}$ and 6.5×10^{-3} M respectively from the slope and the intercept of the line. The spectrum of **4** was not changed by the addition of α-cyclodextrin up to 10.0 mM.

Results

The dissociation constants (K_{diss}) of cyclodextrin–substrate-inclusion complexes and the first-order rate constants, k_{un} and k_c, of the free and included substrates respectively were measured or evaluated by the use of Eq. 1; they are summarized in Table 2. The rate effect of α-cyclodextrin on the reactions of **1a**, **2a**, and **2b** was not large enough for the corresponding k_c and K_{diss} values to be determined.

Table 2. Rate constants and dissociation constants for cyclodextrin–organic ester systems at 25.0 °C and I_c=0.65 M

Compound	[OH⁻], M	k_{un}, min^{-1},	k_c, min^{-1},	K_{diss}, mM
		α-Cyclodextrin		
1b	0.019	0.0457	0.076	4.0
1c	0.020	0.0123	0.053	18
3a	0.010	0.116	2.3	23
3b	0.0092	0.686	6.6	10
		β-Cyclodextrin		
1a	0.0094	0.0135	0.024	3.4
1b	0.021	0.0496	0.085	6.3
1c	0.019	0.0120	0.036	11
2a	0.20	0.0314	0.0046	1.9
2b	0.24	0.0591	0.011	1.1
3a	0.010	0.114	0.87	12
3b	0.0069	0.515	2.2	4.4

To learn the role of OH^- in the reactions of the esters, the reaction rates were measured at various alkaline concentrations in the absence and in the presence of cyclodextrin. The resulting rate constants, k_{un} and k_{obsd}, as well as the K_{diss} values given in Table 2, were used to calculate the k_c values at various OH^- concentrations on the basis of Eq. 1. The rate constants, k_c, thus obtained, together with the k_{un} values, were then plotted against [OH^-], as is illustrated in Figs. 1 and 2 for the β-cyclodextrin–**1a** and –**2a** systems respectively. The plot of k_{un} *vs.* [OH^-] for each substrate gave a good straight line through the point of origin. On the other hand, the plot of k_c *vs.* [OH^-] gave a hyperbolic curve for each phosphate or acetate system: The k_c values at lower OH^- concentrations were larger than the corresponding k_{un} values, but the slope of the curve decreased with an increase in the OH^- concentration until it reached a constant value. In some cases, the k_c value became smaller than the corresponding k_{un} value in a high OH^- concentration region (Fig. 1). The k_c values for the monothiophosphates were always smaller than the corresponding k_{un} values and increased linearly with an increase in the OH^- concentration over the whole [OH^-] range studied.

It is impossible to explain these [OH^-] dependencies of k_c in terms of the reaction scheme proposed by Van-Etten *et al.*[10] for cyclodextrin–phenyl benzoate systems. They neglected the contribution of the k_b term to k_c in Eq. 3 and thought $k_c=k_a\{K_a[OH^-]/(K_a[OH^-]+K_w)\}$. Then, k_c should asymptotically approach a constant value of k_a with an increase in the OH^- concentration. This is not the case at present. On the contrary, the [OH^-] dependencies of k_c observed can be well explained by the use of Eq. 3 which involves the k_b term, too. Thus, when [OH^-] is low enough to be $K_a[OH^-]\ll K_w$, k_c in Eq. 3 becomes proportional to [OH^-]:

$$k_c = [(k_aK_a/K_w)+k_b]\cdot[OH^-] \quad (5)$$

When [OH^-] is high enough to be $K_a[OH^-]\gg K_w$,

$$k_c = k_a + k_b[OH^-] \quad (6)$$

This may be the case for the phosphate and acetate systems. If the k_a term is negligibly small compared with the k_b term in Eq. 3, k_c is given by:

$$k_c = k_b[OH^-] \quad (7)$$

This may be the case for the monothiophosphate systems. The actual values of K_a, k_a, and k_b for each reaction system were calculated by means of regression analysis with a computer (Table 3). The solid lines in Figs. 1 and 2 were drawn on the basis of the values in Table 3. They are in excellent agreement with the experimental values with correlation coefficients above 0.999.

Table 3. Equilibrium and kinetic parameters for cyclodextrin–organic ester systems at 25.0 °C and I_c=0.65 M

Compound	pK_a	k_f ($min^{-1}M^{-1}$)	k_a (min^{-1})	k_b ($min^{-1}M^{-1}$)	k_a/k_f (M)	k_b/k_f
			α-Cyclodextrin			
1b	11.4	2.42	0.024	2.67	0.001	1.11
1c	12.4	0.625	0.105	0.38	0.17	0.61
3a	12.1	11.6	6.52	0.00	0.56	0.00
3b	12.3	74.6	21.0	17.0	0.28	0.23
			β-Cyclodextrin			
1a	12.6	1.41	0.087	0.86	0.062	0.61
1b	12.4	2.42	0.113	1.32	0.047	0.55
1c	12.1	0.625	0.051	0.23	0.081	0.36
2a	—	0.130	0.000	0.029	0.000	0.22
2b	—	0.233	0.000	0.047	0.000	0.20
3a	12.3	11.6	2.83	4.45	0.25	0.38
3b	12.3	74.6	8.70	42.3	0.12	0.57

Discussion

The rates of the phenol release from the phosphates, monothiophosphates, and acetates in the absence of cyclodextrin showed a first-order dependence on the substrates and also on OH^-. The reactions may involve a nucleophilic attack of OH^- at the phosphorus atoms of the phosphates and monothiophosphates or at the carbonyl carbon atoms of the acetates. The water-catalyzed hydrolysis of the esters may be negligible under the present reaction conditions.

Both α- and β-cyclodextrins significantly affected the reaction rates of the esters, while methyl α-D-glucopyranoside did not. This fact, as well as the fact that the rate effect is saturated with an increase in the cyclodextrin concentration, indicates that the prior formation of an inclusion compound between cyclodextrin and a substrate is essential to the rate effect.

A marked substrate specificity was found for the cyclodextrin-catalyzed cleavage of the esters studied. Thus, β-cyclodextrin accelerates the phenol release from the **1a**, **1b**, and **1c** phosphates and the **3a** and **3b** acetates at low OH^- concentrations, while it decelerates the corresponding reactions of the monothiophosphates, **2a** and **2b**. α-Cyclodextrin also accelerates the phenol release from the **1b** and **1c** phosphates and the **3a** and **3b** acetates, whereas it only slightly decelerates the corresponding reactions of **1a**, **2a**, and **2b**.

These specific rate effects of α- and β-cyclodextrins may be related to the stereochemistry of cyclodextrin–substrate complexes.[1,2] Thus, a spectrophotometric study (see the Experimental section), together with a study with the Corey-Pauling-Koltum scale molecular models, suggests that the 3-methyl-4-nitrophenyl moiety of **1a** or **2a** is too large to be included in the α-cyclodextrin cavity, but it is small enough to be included in the β-cyclodextrin cavity. The 4-nitrophenyl moiety of **1b**, **1c**, or **2b** and the carboxyphenyl moiety of **3a** or **3b** are small enough to be included in both the α- and β-cyclodextrin cavities.[2] Therefore, the relatively small effect of α-cyclodextrin on the rates of the reactions of **1a** and **2a** may be due to the binding to the α-cyclodextrin cavity of the parts other than the 3-methyl-4-nitrophenyl moiety of the substrate molecules. In such cases, the reaction sites of the substrates may be located too far from the catalytic site of α-cyclodextrin for them to react with each other.

The kinetic and equilibrium parameters determined by the regression analysis of the $[OH^-]$ dependency of k_c also serve as a basis for speculation about the geometry of a cyclodextrin–substrate complex. As VanEtten *et al.*[2] suggested, the ratio of k_a/k_f may be a measure of the proximity between the reaction site of a substrate and the catalytic site (the alkoxide ion) of cyclodextrin in a cyclodextrin–substrate complex. Furthermore, the ratio of k_b/k_f may depend on how much the reaction site of an included substrate is protected from the nucleophilic attack of OH^- compared with the free substrate.

The k_a/k_f values for β-cyclodextrin–monothiophosphate systems are virtually zero, and the k_b/k_f values for these systems are significantly smaller than those for β-cyclodextrin–phosphate systems. This fact suggests that the phosphorus atoms of the monothiophosphates are located in the middle of the cyclodextrin cavity. The electronegativity of sulfur (2.44[11]) is less than that of oxygen (3.50[11]), and the atomic radius of sulfur (1.27 Å[11]) is larger than that of oxygen (0.6 Å[11]). Therefore, the monothiophosphate group may be more hydrophobic than the corresponding phosphate group, and the former may be included more deeply in the β-cyclodextrin cavity than the latter. This presumption is supported by the fact that the inclusion complexes of β-cyclodextrin with monothiophosphates are more stable than those with the corresponding phosphates (compare the K_{diss} values in Table 2). On the other hand, the phosphorus atoms of the phosphates or the carbonyl carbon atoms of the acetates may be located in the vicinity of the secondary hydroxyl groups which are arranged around one edge of the torus of cyclodextrin. Hence, the k_a/k_f values are appreciably large for the phosphates and the acetates. The k_a/k_f values for α-cyclodextrin–phosphate and –acetate systems are generally larger than those for the corresponding β-cyclodextrin systems. This fact may be a result of the size of α-cyclodextrin being smaller than that of β-cyclodextrin.[2]

The pK_a values determined for various cyclodextrin–organic ester systems varied within a relatively small range of 12.1 to 12.7, with the one exception of pK_a= 11.4 for an α-cyclodextrin–**1b** system. VanEtten *et al.*[10] and Van Hooidonk and Groos[12] also estimated the pK_a value of α-cyclodextrin to be 12.1 and 12.6 respectively. The small variation in the pK_a value may be due either to experimental error or to a substrate-induced pK_a change, such as has often been observed for enzyme systems. Further accurate experiments may be necessary to determine which of them is the case.

Only in the case of an α-cyclodextrin–**1b** system is the pK_a value unusually small and the k_b/k_f value unusually large. A study with the scale molecular models suggested the possibility that the phosphonyl oxygen (>P→O) of **1b** included in α-cyclodextrin is located in the vicinity of one of the secondary hydroxyl groups of α-cyclodextrin close enough to form the hydrogen bonding between them. Upon the formation of such bonding, the acid dissociation of the cyclodextrin hydroxyl group may be facilitated, and at the same time the ester linkage of the substrate may be weakened to make it susceptible to the nucleophilic attack of OH^-. Cramer *et al.*[13,14] have also postulated the formation of hydrogen bonding between cyclodextrin and a substrate to explain the catalytic effect of cyclodextrin. However, the definite meaning of k_b/k_f remains to be ascertained.

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