Spectators in the Cleavage of *p*-Nitrophenyl Acetate by Cyclodextrins in Basic Solution. Catalysis by Potential Inhibitors

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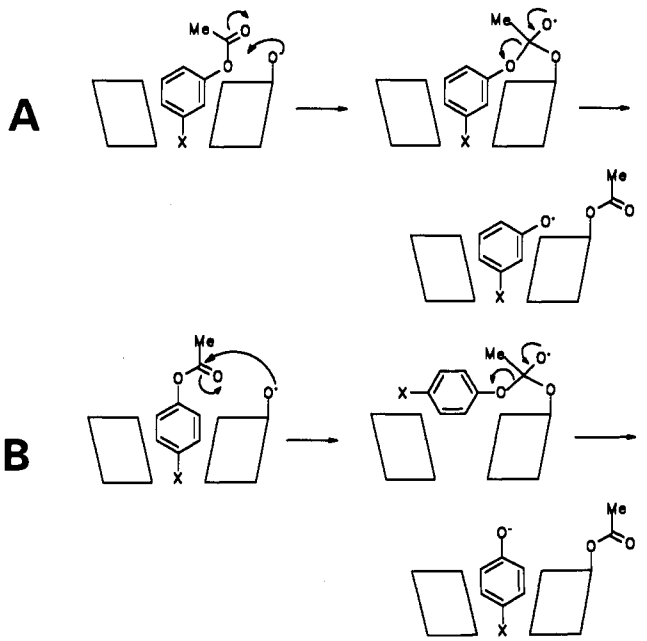
Abstract: Various species that form guest–host complexes with cyclodextrins (CDs) inhibit the cleavage of *m*-nitrophenyl acetate (mNPA) by CDs in basic aqueous solution, in quantitative agreement with their binding constants. In contrast, many alcohols, alkanesulfonate ions, and alkanoate ions do not inhibit the cleavage of *p*-nitrophenyl acetate (pNPA) by α- and β-CD to the same extent, and in several cases, actual rate enhancements are found. The observed kinetics require the presence of a process whereby *one* molecule of the potential inhibitor (PI) is involved in the ester cleavage by CD in some way. Rate constants k_a for the apparent reaction of the CD·pNPA complex with a series of PIs show a dependence on structure which is strongly correlated to the ability of the PIs to bind to CD. On the other hand, rate constants k_b for the kinetic equivalent reaction of pNPA with the PI·CD complexes have a smaller range of values, not very different from the rate constant for pNPA reacting with CD alone. Also, the logarithms of the apparent dissociation constants pK_{TS} for the loss of PI from the transition states of the PI-mediated reactions correlate very well with those of the dissociation constants pK_I of the PI·CD complexes with slopes near 1. These observations suggest that the binding of PI in the transition state of the PI-mediated reaction is not very different from that in the PI·CD complex. The cleavage of pNPA by CDs is less efficient than that of mNPA, due to poorer transition-state binding. It is proposed that in the transition state for the cleavage of pNPA, the ester moiety is largely *outside* the CD cavity, so that the cavity may be occupied by a PI, acting mainly as an inert spacer or "spectator". In some cases, the PI serves to stabilize the transition state for acyl transfer by a modest amount, so that overall catalysis is observed.

Introduction

The cleavage of phenyl acetates by cyclodextrins[2] (CDs) in basic aqueous solution has been widely studied.[2-6] In general, with both α- and β-CD,[2] the meta-substituted esters are cleaved more efficiently than their para isomers, and so it is believed that a meta substituent holds the phenyl group of the ester in the CD cavity in a geometry which is more appropriate for acyl transfer to an ionized secondary hydroxyl group of CD[3-6] (Scheme I, route A). This belief is supported by NMR studies of ester·CD complexes[7] which indicate that the ester carbonyl group is located closer to a secondary OH group when the substituent on the phenyl group is meta, rather than para, to the ester group. Moreover, there is a strong dependence of the logarithm of the rate acceleration on the inverse of the estimated C=O···HO distance.[8] In the case of para-substituted phenyl acetates, it seems that the aryl group, which is clearly bound in the initial state,[9] must come out of the CD cavity, at least partially, to attain the transition state for ester cleavage (Scheme I, route B).

Scheme I



Support for these ideas has been obtained using correlation analysis of the apparent rate constants for the reaction of the ester·CD complexes.[6] For cleavage by α-CD, the multiple linear regression equation has a positive coefficient for the steric parameter of para substituents, indicating "a deleterious effect of bulky groups in the para position".[6] In contrast, the analogous equation for β-CD has *no* steric term for para substituents, implying that the substitutent is not in the CD cavity at all, in

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(9) By virtue of the observed saturation kinetics[2-4,10,11] and NMR studies.[7]

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the transition state. The present paper provides a large amount of experimental evidence that effectively supports this view.

Both α-CD and β-CD bind to the acyl groups of *m*- and *p*-nitrophenyl *n*-alkanoates (beyond the acetate or propionate), rather than to their aryl groups, and the strength increases with the acyl chain length.[10,11] Nevertheless, cleavage of the *m*-nitro isomers takes place via aryl group binding, whereas the para isomers react through an acyl-bound transition state, i.e., with the acyl chain of the ester in the CD cavity to some extent. Only with a long acyl chain ($\sim$C10) does the efficiency of the cleavage of the *p*-nitrophenyl alkanoates approach that of the meta isomers.[11] As a result, we speculated that the presence of a medium-length, straight-chain alcohol in the CD cavity might result in more efficient binding of the acyl group of a medium-chain alkanoate (hexanoate, say), possibly through the formation of a ternary (1:1:1) complex.[12] Such binding might lead to more efficient ester cleavage,[12b] or, if the geometry was appropriate, it might promote nucleophilic attack by the anion of the alcohol on the ester.[13] In the course of the preliminary studies based on these speculations, we found that the reaction of β-CD with *p*-nitrophenyl acetate (pNPA) in basic solution is *not* inhibited to the extent expected by several species which do inhibit the cleavage of its meta isomer.[14] This finding led us to a detailed study of the effect of various potential inhibitors on the cleavage of pNPA by both α- and β-CD which is here presented. Some of our initial results for β-CD have been reported in a communication.[14]

Results

We have examined the effect of a large number of potential inhibitors (PIs) on the rates of the cleavage of some nitrophenyl esters by α- or β-CD in a basic aqueous phosphate buffer (pH 11.6). Before describing these experiments, we review some of the background information necessary to appreciate the kinetic results and our approach in analyzing of them.

A substrate (S) reacting in the medium (eq 1) and through a 1:1 complex with a CD (eq 2) gives rise to saturation-type kinetics (eq 3).[2-5] Appropriate analysis of the variation of k^{obsd} on [CD],

$$S \xrightarrow{k_u} \text{products} \tag{1}$$

$$S + CD \underset{K_S}{\rightleftharpoons} CD{\cdot}S \xrightarrow{k_c} \text{products} \tag{2}$$

$$k^{obsd} = \frac{(k_u K_S + k_c[CD])}{(K_S + [CD])} \tag{3}$$

based on eq 3, affords values of k_c and K_S, with k_u being fixed at the measured value.[2-5,11]

Addition of a PI that forms a complex with the CD (eq 4) reduces the concentration of free CD so that less CD·S is formed and k^{obsd} is reduced, assuming $k_c > k_u$, as is the case here. In the absence of any other processes, measurements of k^{obsd} at fixed [CD] and with varying [PI] can be analyzed to find the dissociation constant K_I of the PI·CD complex (see Appendix).

$$PI + CD \underset{K_I}{\rightleftharpoons} PI{\cdot}CD \tag{4}$$

This approach was used by Bender and co-workers[3] to demonstrate the competitive inhibition of the reaction of *m*-nitrophenyl acetate (mNPA) with α-CD by various carboxylate and sulfonate anions and to find their values of K_I, but they appear not to have studied inhibition of the reaction of pNPA. Likewise, we have found that the method gives reasonable values of K_I for the reaction of *m*-nitrophenyl esters with β-CD. For example, the cleavage of mNPA and of *m*-nitrophenyl hexanoate (mNPH) is inhibited by *n*-hexanol, with $K_I = 4.4 \pm 0.1$ and 4.1 ± 1.0 mM, respectively, in good agreement with the literature value of 4.6 mM, determined in an entirely different manner.[15] Thus, we have used the inhibition of mNPA cleavage as a way of estimating K_I values for a large number of PIs for comparison with literature values (obtained by other means) and of finding values where none were available already. These inhibition constants are collected in Table I.

Values of K_I for the binding of many alcohols to both α-CD and β-CD have been determined by Matsui and co-workers[6] using a spectrophotometric method, based on competition with an azo

Table I. Dissociation Constants of PI·CD Complexes[a]

PI	K_I, mM: this work	K_I, mM: lit. values[ref]
	α-Cyclodextrin	
t-BuOH	240 ± 20[b]	204[15]
neo-PenOH	39 ± 5[b]	34[15]
n-$BuSO_3^-$	$22 \pm 1, 23 \pm 1$	
n-$PenSO_3^-$	6.4 ± 0.1	6.4[19]
n-$HexSO_3^-$	2.0 ± 0.1	2.6[19]
n-$HepSO_3^-$	1.03 ± 0.05	1.5[19]
n-$OctSO_3^-$	$0.33 \pm 0.02, 0.61 \pm 0.03$	0.93[19]
n-$DecSO_3^-$	0.050 ± 0.017[b,c]	0.50[19] [c]
p-$TolSO_3^-$	59 ± 14[b]	60[3]
$EtCO_2^-$	604 ± 6	570[3]
n-$PrCO_2^-$	$89 \pm 6, 64 \pm 2$	
n-$BuCO_2^-$	16 ± 1[d]	12.3[16a]
n-$PenCO_2^-$	3.9 ± 0.1	$\sim$2.5[16a]
c-$HexCO_2^-$	3.1 ± 1.5[b]	19[3]
$PhCO_2^-$	107 ± 18[b]	81,[3] 59–100[e]
	β-Cyclodextrin	
n-BuOH	56 ± 1	60[15]
n-HexOH	4.4 ± 0.1	4.6[15]
c-HexOH	$1.8 \pm 0.2, 1.49 \pm 0.08$	2.0[15]
$HO(CH_2)_6OH$	29 ± 5[b]	20[6]
n-$BuSO_3^-$	89 ± 3	
n-$PrCO_2^-$	259 ± 4	
n-$BuCO_2^-$	74 ± 6	52[16a]
n-$PenCO_2^-$	$16 \pm 1, 15.9 \pm 0.5$	$\sim$9,[16a] 29[18]
i-$PenCO_2^-$	14.7 ± 0.4	
n-$HexCO_2^-$	5.9 ± 0.2	$\sim$3.3,[16a] 9.1[18]
n-$HepCO_2^-$	$1.57 \pm 0.17, 1.41 \pm 0.11$	$\sim$1.6,[16a] 2.7[18]
c-$HexCO_2^-$	4.6 ± 0.2	
$^-O_2(CH_2)_4CO_2^-$	$232 \pm 2, 195 \pm 58$[b]	
$^-O_2(CH_2)_6CO_2^-$	$32 \pm 1, 30 \pm 3$[b]	
I^-	$215 \pm 2, 189 \pm 11, 285 \pm 30$[b]	140–260[f]
Br^-	602 ± 2	600[g]
ClO_4^-	73 ± 8	37,[h] 50,[g] $\sim$100[i]

[a] In aqueous solution at 25 °C. Values of K_I determined in this work were obtained from the inhibition of the cleavage of mNPA (except where noted otherwise) in 1 or 10 mM α- or β-CD in a 0.2 M phosphate buffer at pH 11.6. Where multiple values of K_I are given, they were obtained by different workers and/or using different conditions. Most of the values ascribed to ref 16a were estimated from a graph therein; these are indicated by $\sim$. [b] From inhibition of the cleavage of pNPA by 10 or 15 mM CD. [c] May be distorted by 2:1 (host:guest) binding. [d] Average of four separate determinations of 16.6 ± 1.2, 16.1 ± 0.4, 15.8 ± 1.2, and 15.6 ± 0.3. [e] Gelb, R. I.; Schwartz, L. M.; Radeos, M.; Laufer, D. A. *J. Phys. Chem.* **1983**, *87*, 3349. [f] The literature contain an alarming number of values for iodide ion, ranging from 56 to 690 mM, with most of them being between 140 and 260 mM. (See Table 2 in Diard, J. P.; Saint-Aman, E.; Serve, D. *J. Electroanal. Chem.* **1985**, *189*, 113.) [g] Mochida, K.; Kagita, A.; Matsui, Y.; Date, Y. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3703. [h] Rohrbach, R. P.; Rodriguez, L. J.; Eyring, E. M.; Wojcik, J. F. *J. Phys. Chem.* **1977**, *81*, 944. [i] Buvari, A.; Barcza, L. *Inorganica Chim. Acta* **1979**, *33*.

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dye.[15] This fact and the ready availability of alcohols were the principal reasons we have studied so many of them as PIs (see later). In the few cases where we have determined K_I for alcohols, we find good agreement with the literature values[6,15] (Table I).

Surprisingly, there are relatively few values of K_I for simple alkanoate ions scattered through the literature,[2,3,16] even though other surfactants have often been studied. For the most part, we have used a graph in the preprint of Ono et al.[16a] as a starting point and our values, estimated by using the inhibition method, broadly agree with theirs determined by a conductometric method. During the course of our studies, more values appeared, and some of these are added to Table 1 for comparison. One complication with longer chain alkanoates (and other surfactants) is that they tend to show 2:1 (host:guest) binding at high [CD], the extent of which depends on the alkyl chain length.[17,18] We have seen indications of this problem in the present work and tried to avoid it by working with low concentrations of CD and PI. In cases where 2:1 binding appeared to be unavoidable, the experiments were abandoned. It is partly for this reason that our results for RCO_2^- and RSO_3^- are much less extensive than those for alcohols.

In view of the substantial disagreement between the K_I values determined for alkanesulfonate ions by two research groups, using slightly different conductometric approaches,[19,20] we have redetermined many of them using the inhibition method (Table I). Our values agree fairly well with those of Satake and co-workers,[19] as opposed to the more recent values from Okubo's group which imply much weaker binding and a surprisingly shallow dependence on chain length.[20] One problem may be that some surfactants show 2:1 binding, which distorts measurements made at high [CD].[17,18] In view of these findings, we have used Satake's K_I values and our own for the analysis of the effects of RSO_3^- on the cleavage of pNPA by CDs, presented below.

In contrast to our results for mNPA and mNPH, both pNPA and *p*-nitrophenyl hexanoate (pNPH) gave poor inhibition plots with β-CD and seemingly high K_I values of 25 and 19 mM for *n*-hexanol (K_I = 4.6 mM). Similar behavior was found for many other PIs and for α-CD in that the k^{obsd} values are generally higher than expected for simple competitive inhibition; three examples are shown in Figure 1. In a few cases (smaller alcohols and β-CD), the k^{obsd} values for pNPA actually *increase*, as seen in two of the examples in Figure 1. Obviously, in such cases there is true *catalysis* by the PI, not inhibition. For the cleavage of pNPH (and homologous alkanoates) by β-CD, we have found more substantial catalysis by many alcohols,[12] details of which will be reported separately. Clearly, with the two *p*-nitrophenyl esters, there is a process involving the PI which increases in importance as [PI] rises and which negates the normal effects of competitive inhibition. This process gives rise to false, high values of K_I, a reflection of high values of k^{obsd} (Figure 1), when the data are analyzed for inhibition.

Following the initial experiments with pNPA and pNPH, we examined the effect of various concentrations of β-CD on k^{obsd} at fixed $[PI]_0$. Saturation-type kinetics were observed for the cleavage of pNPA at the various $[PI]_0$, and analysis of the data showed only minor variations of k_c values but more definite (downward) variations in K_S values (see Table II). This behavior stands in contrast to that expected for competitive inhibition in which the apparent values of K_S rise with inhibitor concentration.[21] These results also implicate the intrusion of some process involving participation of the PI and which overrides inhibition.

As a working hypothesis for such a process, we chose the reaction of the PI with the CD·pNPA complex (eq 5). With this process, in addition to those in eqs 1 and 2, eq 3 must be replaced by eq 6. The form of eq 6 is not particularly convenient for analysis,

$$PI + CD + S \underset{K_S}{\rightleftharpoons} PI + CD{\cdot}S \xrightarrow{k_a} \text{products} + PI \quad (5)$$

$$k^{obsd} = \frac{(k_u K_S + (k_c + k_a[PI])[CD])}{(K_S + [CD])} \quad (6)$$

as it is nonlinear and contains two concentration variables, but it may be rearranged to a linear form which is more tractable:

$$k^{corr} = (k^{obsd}(K_S + [CD]) - k_u K_S)/[CD] = k_c + k_a[PI] \quad (7)$$

This transformation amounts to correcting the observed rate constant for the background reaction (eq 1) and substrate binding, leaving the contributions from the two reactions of the bound ester (eqs 2 and 5) on the right-hand side. According to eq 7, the "corrected" rate constant k^{corr} should vary linearly with [PI] and the slope provides an estimate of the k_a. Note that the proper

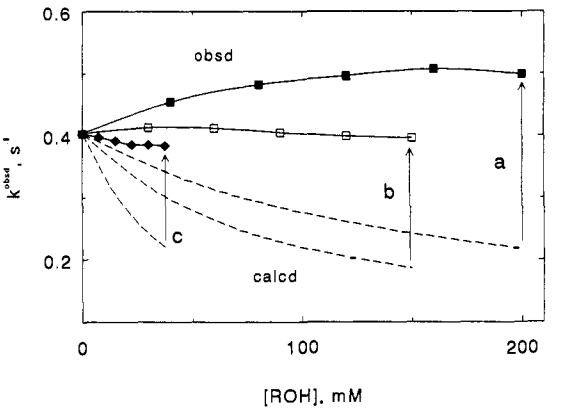

Figure 1. Examples of the effect of PIs on rate constants for the cleavage of *p*-nitrophenyl acetate by β-cyclodextrin: (a) PI = 2-butanol, (b) PI = 2-pentanol, and (c) PI = 2-hexanol. The observed rate constants have been scaled to make the points at zero CD coincide exactly (see Experimental Section). For other examples, see Figure 1 of ref 14. Note that in each case, the observed values are significantly higher than those calculated for simple inhibition.

Table II. The Effect of Two Potential Inhibitors (*n*-hexanol and cyclohexanol) on Parameters for the Saturation Kinetics of the Cleavage of pNPA by β-CD[a]

[PI], mM	K_S, mM	k_c, s^{-1}	[PI], mM	K_S, mM	k_c, s^{-1}
		n-Hexanol			
0	8.6	0.60	5.0	5.2	0.53
1.0	7.2	0.58	7.5	4.9	0.55
2.5	6.1	0.55			
		Cyclohexanol			
0	8.6	0.60	5.0	5.0	0.52
1.0	7.8	0.62	7.5	3.8	0.47
2.5	5.9	0.54			

[a] At 25 °C in a 0.2 M phosphate buffer at pH 11.6. For each, [PI] values of K_S and k_c were determined by nonlinear fitting of eq 3 to values of k^{obsd} measured at $[CD]_0$ = 0, 2, 4, 6, 8, and 10 mM.

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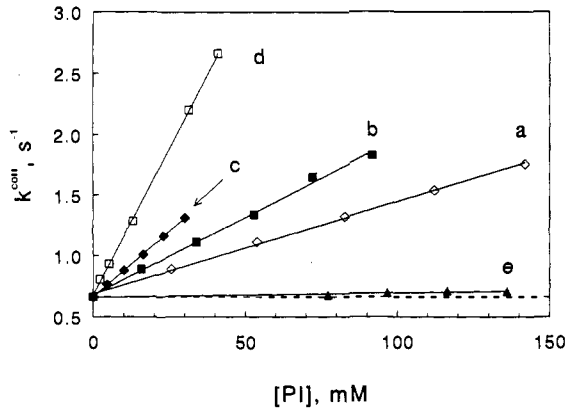


Figure 2. Examples of the linear dependence of k^{corr} on [PI] (eq 7) for the cleavage of pNPA in 10 mM β-CD. For such plots, [PI] must be corrected for the formation of PI·CD (see Appendix). The data shown are for the following PIs: (a) 2-pentanol, (b) *tert*-butyl alcohol; (c) 2-hexanol, (d) *iso*-pentyl alcohol, and (e) iodide ion (only this last one shows inhibition). For other examples, see Figure 2 of ref 14. Similar plots were obtained for the other PIs and for α-CD, and the slopes afforded values of k_a (Tables III and IV). The accessible ranges of [PI] are limited by the solubility of PI.

use of eq 7 for analytical purposes requires the actual concentrations of free CD and free PI, taking account of the formation of PI·CD (eq 4). These must be calculated from $[CD]_0$ and $[PI]_0$, knowing K_I, as described in the Appendix.

On the basis of this approach, we have examined the effect of a large number of potential inhibitors (see Tables III and IV) on the rate of cleavage of pNPA by α- and β-CDs. As seen already with the example in Figure 1, curve c, the observed data usually show some inhibition but describe a curve which is decidedly higher than expected for straight inhibition by PI. In some cases, modest rate increases were observed (e.g., Figure 1, curves a and b). In either case, treatment of the rate data according to eq 7 affords linear plots, some examples of which are shown in Figure 2; different examples were presented earlier.[14] Note that when full inhibition is operative, the slope of the plot of k_{corr} vs [PI] is indistinguishable from zero (e.g., iodide ion in Figure 2, curve e).

The range of $[PI]_0$ used in the experiments was constrained by the solubility of PI in water. Fortunately, this turns out not to be a major problem since the slopes of the plots of k^{corr} vs [PI] generally increase with the size of PI (Figure 2). The values of k_a obtained from such plots are collected in Tables III and IV.

The linearity of the plots of k^{corr} with [PI] supports the involvement of *one* molecule of PI, only, in the process responsible for the higher than expected rate constants for pNPA cleavage. With simple alcohols, the process might conceivably be due to nucleophilic attack of the alkoxide ion on the ester[13] bound to the CD. Therefore, we also studied other PIs which are unlikely to react in such a manner: alkanesulfonate ions, carboxylate ions, and perchlorate ion (Tables III and IV). In the event, reduced inhibition and an adherence to eq 7 were found with these much less nucleophilic anions, even with ClO_4^-, whereas iodide ion, a stronger nucleophile, caused inhibition.

Up to this point, we have emphasized the absence of inhibition in the reaction of pNPA with a CD, but in several cases, we have found that the reaction is inhibited by the PI, more especially for α-CD. For example, while the reaction of pNPA with α-CD is not completely inhibited by most alcohols (Table IV), it is inhibited by *tert*-butyl alcohol and neopentyl alcohol, as well as by decanesulfonate ion, *p*-toluenesulfonate ion, benzoate ion, and cyclohexanecarboxylate ion. Likewise, the reaction with β-CD is inhibited by iodide ion, 1,6-hexanediol, and dianions of adipic acid and suberic acid ($^-OOC(CH_2)_nCOO^-$, $n = 4$ and 6). No doubt, by extensive screening experiments, we could have found other PIs which fully inhibit pNPA cleavage, but instead, we concentrated our efforts on the more interesting aspect of noninhibition, bordering on catalysis.

Table III. Constants for the Cleavage of *p*-Nitrophenyl Acetate by α-CD in the Presence of PIs[a]

PI	K_I, mM	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}	K_{TS}, mM
		Alcohols		
t-Bu	204	*b*		
i-Pr	200	0.33	6.7	800
n-Pr	43	1.8	7.4	150
s-Bu	38	1.9	7.1	140
i-Bu	36	1.5	5.2	180
neo-Pen	34	*b*		
c-Pen	22	2.2	4.8	120
c-Hex	15	2.5	3.8	110
i-Pen	13	4.5	6.1	59
n-Bu	11	6.0	6.7	44
2-Pen	7.4	9.1	6.7	29
n-Pen	3.1	22	6.7	12
2-Hex	2.8	28	7.9	9.5
n-Hex	1.1	75	8.3	3.6
n-Hep	0.44	145	6.3	1.8
		Alkanesulfonate Ions[c]		
C4	23	1.3	2.9	209
C5	6.4	5.9	3.7	45
C6	2.6	13	3.5	20
C7	1.5	26	3.8	10
C8	0.93	29	2.7	9.2
		Alkanoate Ions		
C3	600	0.19	11	1410
C4	89	1.8	16	148
C5	16	2.9	4.6	94
C6	3.9	12	4.6	22
		Others		
p-$TolSO_3^-$	60	*b*		
$PhCO_2^-$	81	*b*		
c-$HexCO_2^-$	3.1	*b*		

[a] At 25 °C. Values of k_a are for the reaction of CD·S with PI (eq 5) and k_b ($= k_aK_I/K_S$) are for the reaction of CD·PI with S (eq 9); $K_{TS} = k_c/k_a$ (eq 10; see text), where $k_c = 0.267$ s^{-1}. Values of K_I are taken from the literature or from Table I. [b] Very small. Within experimental error, the PI shows full inhibition. [c] The C10 ion inhibits, but it gave data which were not analyzable, perhaps due to 2:1 binding.

Discussion

The results reported above clearly support the presence of a cleavage process that involves pNPA, cyclodextrin, and *one* molecule of the potential inhibitor. Since similar behavior is observed for various, structurally different PIs, it is unlikely that they are involved in the reaction in any covalent manner. Most probably, the PI is present simply as an inert spacer or "spectator", occupying the CD cavity as acyl transfer takes place largely outside the CD cavity. This conclusion is consistent with the depiction of the ester cleavage of pNPA in Scheme I, route B, and with the views of Matsui and co-workers, based on correlation analysis of substituent effects.[6] As detailed below, our analysis of the data for the various PIs supports this interpretation.

The rate constants k_a for the apparent reaction of PIs with the CD·pNPA complex (eq 5) increase substantially and regularly with the ability of the potential inhibitors to bind to CDs (Tables III and IV). For example, with the alcohols as the PIs, there are good linear free energy relationships (LFERs) for these rate constants and the dissociation constants for the PI·CD complexes:

$$\alpha\text{-CD, } \log k_a = 1.03pK_I - 1.25,\ N = 13,\ r = 0.993 \quad (8a)$$

$$\beta\text{-CD, } \log k_a = 0.67pK_I + 0.00,\ N = 15,\ r = 0.986 \quad (8b)$$

These relationships, with slopes of 1 and 0.7, suggest that the alcohol PI is bound in the CD cavity during the cleavage reaction and in a geometry that is not very different from that in the

Table IV. Constants for the Cleavage of p-Nitrophenyl Acetate by β-CD in the Presence of PIs[a]

PI	K_I, mM	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}	K_{TS}, mM
		Alcohols		
n-Pr	269	2.8	94	240
i-Pr	263	2.9	95	230
s-Bu	65	6.1	50	110
n-Bu	60	6.3	48	105
s-Pen	32	7.7	31	86
i-Bu	24	10	31	66
t-Bu	21	13	34	51
n-Pen	16	15	30	44
s-Hex	10.5	22	29	30
c-Pen	8.3	26	27	25
i-Pen	5.6	48	34	14
n-Hex	4.6	36	21	18
c-Hex	2.0	81	21	8.1
neo-Pen	1.74	83	18	8.0
n-Hep	1.41	58	10	11
		Alkanesulfonate Ions		
C4	89	3.0	34	220
C5	16.7	11.5	24	57
C6	5.6	33	24	20
C7	2.3	60	17	11
C8	0.97	90	11	7.3
C10	0.24	260	7.8	2.5
		Alkanoate Ions[b]		
C4	260	2.2	74	300
C5	74	5.2	49	130
C6	16	21	43	31
C6*	15	25	46	26
C7	5.9	46	35	14
C7*	4.6	64	37	10
C8	1.5	130	25	5.1
		Others		
ClO_4^-	73	1.29	12	510
I^-	200	c		
adipate	232	c		
suberate	32	c		
$HO(CH_2)_6OH$	20	c		

[a] At 25 °C. Values of k_a are for the reaction of CD·S with PI (eq 5) and k_b (= k_aK_I/K_S) are for the reaction of CD·PI with S (eq 9); K_{TS} = k_c/k_a (eq 10; see text), where k_c = 0.66 s^{-1}. Some of the rate constants may differ slightly from those given earlier[14] because they have been redetermined using a wider range of [PI] or because of the use of different K_I values. Values of K_I are taken from the literature[6,15,19] or from Table I. [b] C6* is the 4-methylpentanoate ion and C7* is the cyclohexanecarboxylate ion. [c] Small and so within experimental error, the PI shows full inhibition.

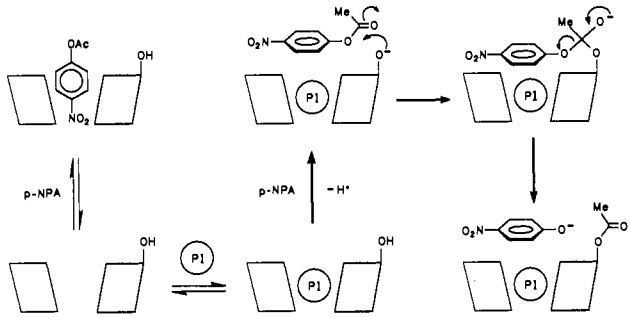
PI·CD complexes, particularly in the case of the narrower α-CD.[22] Thus, it may be more appropriate and realistic to view the PI-mediated process in terms of reaction between the PI·CD complex and the ester S:

$$PI + CD + S \underset{K_I}{\rightleftharpoons} PI{\cdot}CD + S \xrightarrow{k_b} \text{products} + PI \qquad (9)$$

The rate constants k_b are simply evaluated as k_aK_I/K_S, since the third-order rate constant for the reaction PI + CD + S → products + PI is $k_3 = k_a/K_S$ or k_b/K_I, depending on whether the PI-mediated reaction is viewed as proceeding as in eq 5 or in eq 9. Values of k_b derived in this way are also collected in Tables III and IV.

Unlike the values of k_a, which have a wide variation, the range of k_b for each series of PI is relatively small. For example, for the effect of alcohols on the reaction of pNPA with α-CD, the span is narrow (3.8–8.3 M^{-1} s^{-1}) and most values are in the range of 6–8 M^{-1} s^{-1}. These values for the reaction of pNPA with PI·α-CD (eq 9) are slightly lower than that for the second-order rate constant ($k_2 = k_c/K_S$ = 26 M^{-1} s^{-1}) for the reaction between pNPA and α-CD alone, under the same conditions. Likewise, for the analogous reactions with β-CD, the values of k_b (11–95 M^{-1} s^{-1}) are not very different from that of the rate constant for the reaction of β-CD with pNPA (83 M^{-1} s^{-1}) in the absence of any PI. These similarities are entirely consistent with the process depicted in eq 9, provided that the pNPA ester moiety is essentially outside the CD cavity in the transition for ester cleavage, so that the cavity may contain something else as long as it is not too big (Scheme II).

Scheme II



In analyzing our results further, we make use of an approach, due originally to Kurz,[23] whereby one may estimate the energy of stabilization of a transition state by a catalyst. Using Transition State Theory, one evaluates an apparent dissociation constant of the transition state of the catalyzed reaction into the transition state of the normal reaction and the catalyst. This approach, which has provided valuable insights in enzymology,[24] is useful for discussing CD-mediated reactions, particularly ones where different modes of binding are possible in the transition state.[25,26] In the present work, we use the Kurz approach to probe the binding of the PIs to the CD in the transition state for pNPA cleavage by the CD.

In the formalism of Transition State Theory, the rate of the reaction in eq 2 is rate = k_c[CD·S] = ν[TS], where ν is the effective frequency over the barrier and TS is the transition state for the acyl transfer (Scheme I, route B). Similarly, for the reaction mediated by PI (eq 5), rate = k_a[CD·S][PI] = ν[TS·PI], where TS·PI is the transition state containing a molecule of PI. Eliminating ν and [CD·S] from these two expressions, we obtain an apparent dissociation constant:

$$K_{TS} = \frac{[TS][PI]}{[TS{\cdot}PI]} = \frac{k_c}{k_a} = \frac{k_2}{k_3} \qquad (10)$$

Alternatively, we can obtain K_{TS} from the rate constants for the second-order process in eq 2 and the third-order process involving PI (eq 5). Since $k_2 = k_c/K_S$ and $k_3 = k_a/K_S$, the two formulations are exactly the same.

As discussed more fully elsewhere,[25,26] the dependence of the values of K_{TS} on structure may be used as a probe of transition-state binding.[27] Accordingly, in the present case, we look for LFERs between pK_{TS} (= $-\log K_{TS}$) for the transition-state binding of PI and pK_I for binding of the PI in the initial-state PI·CD

(22) α-Cyclodextrin (cyclohexaamylose) has six glucose units joined in a torus whereas β-cyclodextrin (cycloheptaamylose) has seven units. Thus, the sizes of their cavities differ in width (~6 and ~8 Å, respectively) but not in depth (~8 Å).[2]

(23) Kurz, J. L. *J. Am. Chem. Soc.* **1963**, *85*, 987; *Acc. Chem. Res.* **1972**, *5*, 1.

(24) (a) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10. Lienhard, G. E. *Science (WashingtonD.C.)* **1973**, *180*, 149. Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219. Schowen, R. L. In *Transition States in Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 2. (b) The approach has been reviewed critically; see: Kraut, J. *Science (Washington D.C.)* **1988**, *242*, 533. (c) The importance of transition-state stabilization has been questioned recently; see: Menger, F. M. *Biochemistry* **1992**, *31*, 5368.

(25) (a) Tee, O. S.; Du, X.-X. *J. Org. Chem.* **1988**, *53*, 1837. (b) Tee, O. S.; Du, X.-X. *J. Am. Chem. Soc.* **1992**, *114*, 620.

(26) (a) Tee, O. S. *Carbohydr. Res.* **1989**, *192*, 181. (b) Tee, O. S. *Adv. Phys. Org. Chem.* **1993**, *29*, in press.

(27) Despite their considerable potential for describing the binding of transition states to catalysts, the quantities K_{TS} and pK_{TS} have not been widely used by physical organic chemists.[26]

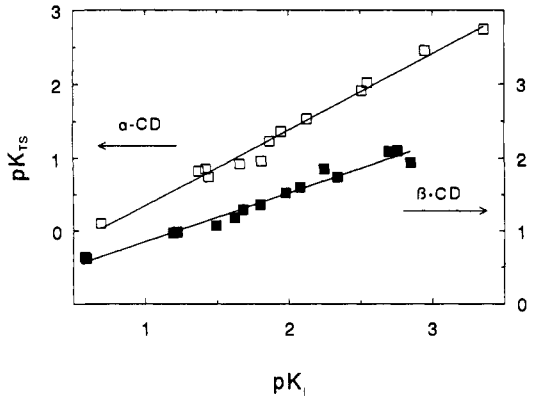


Figure 3. Correlation of transition-state binding (pK_{TS}) of alcohols with pK_I for the binding of alcohols to CDs. The slopes are 1.03 for α-CD and 0.67 for β-CD (eqs 11a and 11b; see also Table V). The vertical scales for α- and β-CD are offset for clarity.

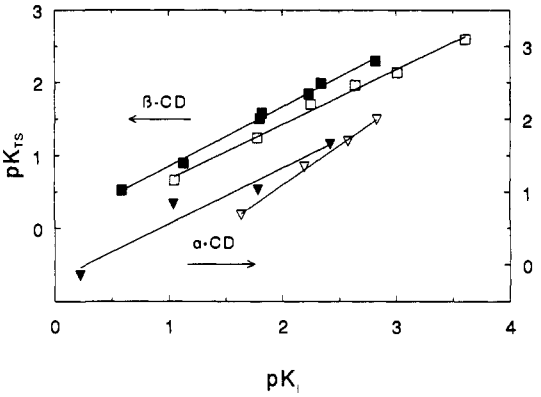


Figure 4. Correlation of transition-state binding (pK_{TS}) of carboxylate and sulfonate ions with pK_I for the binding of these ions to CDs. The slopes and correlation coefficients are given in Table V. Triangles are for α-CD and squares are for β-CD. Closed symbols are for RCO_2^-; open symbols are for RSO_3^-. The vertical scales for α- and β-CD are offset for clarity.

complexes. For the cleavage of pNPA by CDs, mediated by a large number (N) of alcohols, we find:

$$\alpha\text{-CD, } pK_{TS} = 1.03pK_I - 0.68,\ N = 13,\ r = 0.993 \quad (11a)$$

$$\beta\text{-CD, } pK_{TS} = 0.67pK_I + 0.19,\ N = 15,\ r = 0.986 \quad (11b)$$

These two correlations are shown in Figure 3. Except for their intercept terms, they are the same as those given earlier in eqs 8a,b since $K_{TS} = k_c/k_a$ (eq 10) and k_c is a constant for pNPA and a particular CD. Similar relationships have been found for the alkanesulfonate anions and alkanoate ions (Figure 4). Also, within the series of alcohols, there are subsets for the normal and secondary derivatives that show even stronger correlations. These correlations are summarized in Table V.

On the basis of these LFERs, with slopes in the range of 0.7–1.0, we infer that the mode of binding of the PI in the transition state of the PI-mediated reaction (eq 9) is generally similar to that in the corresponding PI·CD complex. This, of course, relates to the earlier observation that the rate constants k_b (for the reaction of PI·CD with pNPA) are not very different from k_2 (for the reaction of CD with pNPA, alone). Both observations are consistent with the conclusion that during cleavage, the pNPA ester moiety is sufficiently outside the CD cavity so that the PI can bind inside, more or less in its normal manner (Scheme II).

The difference in the slopes of the correlations for α-CD and β-CD must be related to the widths of their cavities since their depths are the same.[2,22] The higher slopes for α-CD presumably reflect more restrictive binding that is a consequence of the tighter fit of the alkyl chains of the PIs in the narrower α-CD cavity.[11,25,26]

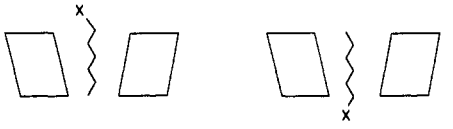
Table V. Correlation of the Binding of PIs in the Transition State for Cleavage of pNPA by CDs (pK_{TS}) with Their Binding in PI·CD Complexes (pK_I)[a]

PI	CD	slope	std error	N	r
alcohols	α	1.03	±0.04	13	0.993
normal	α	0.986	±0.04	5	0.998
secondary[b]	α	1.02	±0.02	4[b]	0.999
RSO_3^-	α	1.09	±0.04	4[c]	0.999
RCO_2^-	α	0.77	±0.13	4	0.973
alcohols	β	0.67	±0.03	15	0.986
normal	β	0.60	±0.02	5	0.997
secondary[d]	β	0.69	±0.05	6[d]	0.990
$R–SO_3^-$	β	0.75	±0.03	6[e]	0.996
$R–CO_2^-$	β	0.82	±0.03	7[f]	0.998

[a] Graphs of pK_{TS} vs pK_I for the alcohols are shown in Figure 3. [b] R = *i*-Pr, *s*-Bu, 2-Pen, and 2-Hex. Points for cyclopentanol and cyclohexanol fall slightly below the line for these (cf. β-CD). [c] Excludes C8. The C10 anion is inhibitory. [d] The correlation *includes* cyclopentanol and cyclohexanol (cf. α-CD). [e] Includes C8 and C10 (cf. α-CD). [f] The correlation *includes* the 4-methylpentanoate and cyclohexanecarboxylate ions.

In contrast, β-CD has a wider cavity[2,22] so that the fit can be looser and pNPA and PI may more easily adopt a reactive arrangement. This factor may account for the generally higher values of k_b that are observed for β-CD than for α-CD (Tables III and IV).

At the present time, we do not know the geometry of binding of the alcohols in the CD cavities: Is it with the hydroxyl group directed toward the secondary rim of the CD cavity (**1**) or toward the primary rim (**2**)? Quite possibly, the energy difference



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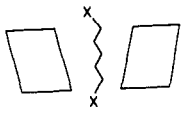
between the two orientations is not large for small PIs, so that small structural changes might bring about a switch from one mode of binding to the other. Preliminary NMR experiments with alcohols clearly showed that binding takes place, but the guest–host NOE signals were insufficient to enable us to judge the orientation of the alcohol molecule in the CD cavity. We suspect that free and bound alcohol molecules are exchanging very quickly, as do other small, unencumbered molecules,[17,28] so that the residence time in the CD cavity is too short.

As reported in the Results section, some PIs do inhibit the reaction of pNPA with CDs. Interestingly, both *tert*-butyl alcohol and neopentyl alcohol inhibit cleavage by α-CD but not cleavage by β-CD. Evidently, these two alcohols, as well as $DecSO_3^-$, *p*-$TolSO_3^-$, benzoate ion, and cyclohexanecarboxylate ion, bind to α-CD in such a way as to preclude the reaction, whereas with β-CD, their binding is sufficiently different (either looser or in another orientation) to allow the acyl transfer reaction to occur. Inhibition of the reaction with β-CD was found with 1,6-hexandiol and the adipate and suberate dianions. These last three were expected to be true inhibitors since, from the inspection of space-filling models, they appeared to be capable of completely penetrating the CD cavity, in the manner of a rotaxane,[29] with their solvated end groups blocking access to both portals of the cavity (**3**).

If, as we propose, the pNPA ester moiety is basically outside the CD cavity during the acyl transfer (Schemes I, route B, and

(28) Cramer, F.; Saenger, W.; Spatz, H.-Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14. Rohrbach, R. P.; Rodriguez, L. J.; Eyring, E. M.; Wojcik, J. F. *J. Phys. Chem.* **1977**, *81*, 944. Yoshida, N.; Fujimoto, M. *Chem. Lett.* **1980**, 1377. Hersey, A.; Robinson, B. H. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 2039.

(29) Rotaxanes based on CDs have been prepared; see: Isnin, R.; Kaifer, A. E. *J. Am. Chem. Soc.* **1991**, *113*, 8188. Wenz, G.; Keller, B. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 197. Wylie, R. S.; Macartney, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 3136 and references therein.



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II), the following question arises: Is the anion of the CD simply behaving as a nucleophile toward pNPA, like any other oxy anion of comparable pK_a?[13] To answer this question, we studied the effect of 0–10 mM trifluoroethanol (TFE) on the cleavage of pNPA, under the same conditions as our experiments with CDs. As presented in Figure 5, TFE reacts with pNPA at almost the same rate as α-CD, with β-CD being about four times more reactive than α-CD. Thus, the two CDs do not seem to be unusually reactive toward pNPA, given that the pK_as of their secondary hydroxyls (12.2 and 12.3)[2,30] are close to that of TFE (12.37).[13]

One can go a step further and estimate the second-order rate constants for the attack of various nucleophiles on pNPA and mNPA, so as to look for the effect of "productive" binding, if any. Such values are collected in Table VI. As seen there, most of the values for pNPA do not appear to be very unusual, bearing in mind that hydroxide ion is a weaker nucleophile than the pK_a of water would suggest[13] (cf. $CF_3CH_2O^-$ and EtO^-, Table VI). The apparent rate constant for the reaction of OH^- with pNPA·α-CD is only three times larger than that for pNPA alone, and it is attributable to the reaction of pNPA with the anion of α-CD, as concluded in the previous paragraph. With β-CD, the rate constants are slightly larger which may indicate a small effect of the CD cavity (or some other structural feature) in helping the reaction. By contrast, the reaction of mNPA with the CD anions is 100–200 times faster than the reaction with hydroxide ion, consistent with the view that binding of the *m*-nitrophenyl group in the CD cavity (Scheme I, A) contributes significantly to the reactivity of the CD oxy anion toward mNPA.

The reaction of pNPA with ethoxide ion has a rate constant of 250 M^{-1} s^{-1};[13] we presume that the anion of propanol reacts at a very similar rate. Compared to this value, rate constants for the apparent reactions of the pNPA·α-CD and pNPA·β-CD complexes with the anion of propanol are extraordinarily high (36 000 and 70 000 M^{-1} s^{-1}). On the other hand, rate constants for the equivalent reaction of pNPA with the CD anions, bound to propanol, are more modest and close to those for reaction with the CD anions, alone. Thus, the depiction of the PI-mediated reaction given in Scheme II is again supported.

Conclusions

The present paper provides a large body of evidence that indicates that the basic cleavage of *p*-nitrophenyl acetate by cyclodextrins can occur with a spectator molecule in the CD cavity. Even though pNPA forms inclusion complexes with CDs, as evidenced by saturation kinetics[2-4,10,11] and NMR studies,[7] it is in no way mandatory that inclusion be present in the transition state for esterolysis;[31] reaction may ensue from another, more reactive geometry.[11,26] Therefore, if the CD cavity is essentially vacant in the transition state, save for a few water molecules, it is possible for other molecules to occupy the space, as long as their presence is not too disruptive. However, the ability of spectator molecules to function in a benign, nondisruptive role in the transition state is not an all-or-nothing proposition; it depends on their structure, and it spans the whole gamit from total disruption (inhibition: $k_b \ll k_2$) through modest disruption ($k_b < k_2$) to actual catalysis ($k_b > k_2$), albeit modest. Furthermore, for the series of alcohols, carboxylate ions, and alkanesulfonate ions which do not inhibit pNPA cleavage, the kinetic parameters show structural dependences related to the ability of these PIs to bind to the CDs. In particular, there are strong correlations (LFERs) between pK_{TS} and pK_I (Figures 3 and 4 and Table V) that constitute convincing evidence that the modes of binding of the PIs in the transition state of the PI-mediated reaction are quite similar to those in the PI·CD complexes, themselves. Thus, with respect to the pNPA substrate, the modes of initial-state binding and transition-state binding are decidedly different, whereas with respect to the spectator (PI) they are probably virtually the same. This behavior stands in contrast to that of mNPA (and other *m*-nitrophenyl esters) for which substrate binding and transition-state binding are quite similar[3,4,6,8,11,26] so that spectators are not tolerated and the PIs act as true inhibitors.

In the few cases where there is true, formal catalysis of the cleavage of pNPA by PIs ($k_b > k_2$, Table IV), the presence of the PI in the CD cavity must have a modest stabilizing effect on the transition state for acyl transfer. Presumably, the PI in the cavity assists the approach of pNPA toward the oxy anion site, perhaps through a hydrophobic effect.[32] For the cleavage of

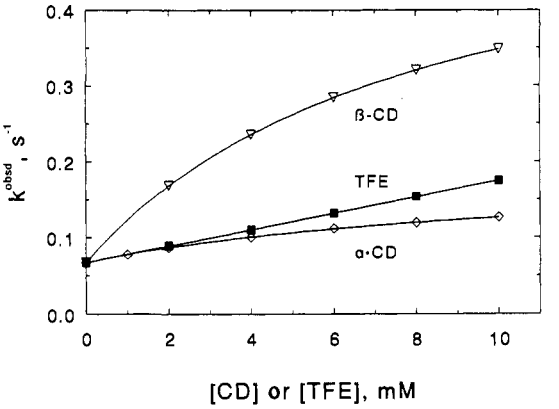

Figure 5. Comparison of the reactivity of TFE, α-CD, and β-CD toward *p*-nitrophenyl acetate (in a 0.2 M phosphate buffer at pH 11.6). The second order rate constants are TFE, 10.8 M^{-1} s^{-1};

Table VI. Apparent Second-Order Rate Constants for Reactions of pNPA and mNPA with Various Nucleophiles[a]

reaction	pK_a	k_2, M^{-1} s^{-1}
pNPA + HO^-	15.74	19 (15)[b]
pNPA + EtO^-	16.0	250[b]
pNPA + $CF_3CH_2O^-$	12.37	64 (64)[b]
pNPA + α-CDO^-	12.2	79
pNPA + β-CDO^-	12.3	420
pNPA·α-CD + HO^-	15.74	53
pNPA·β-CD + HO^-	15.74	155
pNPA·α-CD + n-PrO^-	16.0[c]	36000
pNPA·β-CD + n-PrO^-	16.0[c]	70000
pNPA + *n*-PrOH·α-CDO^-	12.2[d]	22
pNPA + *n*-PrOH·β-CDO^-	12.3[d]	470
mNPA + HO^-	15.74	17
mNPA·α-CD + HO^-	15.74	4900
mNPA·β-CD + HO^-	15.74	1230
mNPA + α-CDO^-	12.2	3000
mNPA + β-CDO^-	12.3	1580

[a] At 25 °C. The pK_as are those of the conjugate acids of the nucleophilic anions; they are taken from refs 13 and 30. [b] From ref 13. [c] The pK_a for propanol is assumed to be the same as that for ethanol. [d] Assumed to be the same as that of CD alone.

(30) Gelb, R. I.; Schwartz, L. M.; Bradshaw, J. J.; Laufer, D. A. *Bioorg. Chem.* **1980**, *9*, 299. Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *Bioorg. Chem.* **1982**, *11*, 274.

(31) It is noted that Komiyama has found that basic cleavage of cytidine-2′,3′-cyclic monophosphate by α-CD is not totally inhibited by two PIs, suggesting that reaction takes place outside the cavity. However, in this case, the substrate ester binds across the top of the CD by hydrogen bonding to the secondary hydroxyl groups; it is not bound in the CD cavity like pNPA. See: Komiyama, M. *Chem. Lett.* **1988**, 1121; *J. Am. Chem. Soc.* **1989**, *111*, 3046.

(32) Hansch, C. *Drug Des.* **1971**, *1*, 271. Leo, A.; Hansch, C.; Elkins, D. *Chem Rev.* **1971**, *71*, 525. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980.

pNPH (and other alkanoates) by β-CD, alcoholic PIs bring about much more significant catalysis and saturation kinetics indicate the presence of discrete ternary (PI·CD·ester) complexes.[12b]

We suggest that our findings for the effects of additives on the cleavage of pNPA (and pNPH)[12b] by CDs provide a paradigm for primitive allosteric effects[21,33] because they provide simple examples where the binding and reactivity of a substrate with a catalyst can be improved by the concomitant binding of a third, chemically inert species. This behaviour we have termed "spectator catalysis".[12b] With enzymes having more complex binding regions, one can readily imagine that more dramatic effects are possible with a spectator (allostere)[33] of the appropriate shape and structure to optimize the fit of the transition state to the catalyst. Under such circumstances, the concentration level of the spectator might be used as a switch: to turn the enzyme "on" or "off", as required.[21,33]

Experimental Section

The *p*-nitrophenyl esters were purchased from Sigma, and the *m*-nitrophenyl esters were prepared as in earlier work.[11] Substrate solutions (5×10^{-5} M for pNPA, 2×10^{-4} M for mNPA) were made up from strong (0.1 M) stock solutions in spectral grade methanol. The buffer solution was made by mixing appropriate amounts of dibasic sodium phosphate, 1.0 M NaOH, and the CD to give a 0.4 M buffer of pH 11.6 (which becomes 0.2 M after mixing in the stopped-flow apparatus).

The alcohols employed as PIs were the best grades available from Aldrich. In some cases, secondary alcohols were found to contain peroxides which can give spectacular, but anomalous, catalysis. To test for peroxides, a few milliliters of the alcohol were mixed with an equal volume of saturated aqueous KI solution. If a yellow color (due to I_2 and I_3^-) was observed, the alcohol was purified by distillation. Carboxylic acids and alkanesulfonic acids (or their salts) were the best grades obtainable from commercial suppliers (Aldrich, Eastman Kodak, and Sigma).

Kinetic measurements were made using an Aminco DW2a UV–visible spectrophotometer and stopped-flow apparatus, as in other recent studies.[5,11,25] The spectrophotometer was interfaced to an Olivetti M24 (8086-based) microcomputer via a Metrabyte DASH-16F A/D card, and data acquisition was carried out with software written in-house. Ester cleavage was monitored by the appearance of the nitrophenoxide ion at 405 nm (para) or 390 nm (meta). First-order rate constants were estimated from least-squares analysis of $\ln(A - A_\infty)$ vs time, for absorbance measurements covering about 90% reaction, with A_∞ being taken after 10 half-lives. Five to ten determination were made and averaged to give the values of k^{obsd} used in further analysis.

Inhibition analysis was carried out on the basis of the considerations set out in the Appendix. Typically, the effects of various $[PI]_0$, spanning the anticipated K_I, on the cleavage of mNPA by CD (1 or 10 mM) were studied. In some cases (see Results and Discussion sections), inhibition of the cleavage of pNPA was found and analyzed accordingly. Accumulated values of K_I are given above in Table I.

For the analysis of kinetic behavior in terms of eq 7, the range of alcohols amenable to study was limited by considerations of solubility. Beyond *n*-heptanol, it is not possible to get sufficient concentrations of the PI into solution to provide enough of a variation in k^{corr} (eq 7) to give a reliable estimate of k_a. At the other extreme, we chose not to study either methanol or ethanol, both of which bind very weakly to the CDs,[6,15] since they would require large concentrations to elicit a significant response. Such amounts might exert a solvent effect[34] which could obscure the effect under investigation. Similar considerations apply to the use of small or large carboxylate and sulfonate ions as PIs.

Values of k_c and K_S were obtained from kinetic data by nonlinear least squares fitting[36] of eq 3, keeping k_u fixed at the observed value. This fitting was carried out using in-house software based on the Marquardt algorithm[35] and written in TurboPascal or QuickBasic. Essentially identical results were obtained later with commercial software (e.g., SigmaPlot and Inplot) which use a similar algorithm. The fitted parameters, which are required for inhibition analysis or PIANALYSIS (see below), are collected in Table VII.

Table VII. Parameters for the Cleavage of mNPA and pNPA by CDs[a]

ester	CD	k_u, s^{-1}	k_c, s^{-1}	K_S, mM	k_2, $M^{-1}\,s^{-1}$
mNPA	α	0.0858	24.6	25.0	984
pNPA	α	0.0956	0.267	10.1	26.4
mNPA	β	0.0858	6.14	15.5	396
pNPA	β	0.0772	0.660	7.92	83.3

[a] Values of k_c and K_S were estimated by nonlinear fitting[35] of eq 3. In a previous work,[11] we obtained such values using an Eadie–Hofstee approach.[2-5] The first three entries are based on the original data of Du,[11,36] measured at pH 11.7; the last was based on measurements at pH 11.6.

Table VIII. Example of Inhibition Calculation—Expt MB105[a,b]

$[PI]_0$, mM	k^{obsd}, s^{-1}	k^{scal}, s^{-1}	[CD]	[PI]	K_I, mM	X
0	0.830	1.040	1.00	0.00		24.69
2	0.602	0.754	0.70	1.70	3.99	35.67
4	0.478	0.599	0.53	3.53	4.06	46.77
6	0.394	0.494	0.42	5.42	3.98	59.10
8	0.338	0.424	0.35	7.35	3.94	71.59
10	0.291	0.365	0.29	9.29	3.75	86.92
					avg K_I =	3.94 ± 0.10 mM

graphical calculation

plot of $[PI]_0$ against X (r = 0.9982) gave K_I = 3.75 ± 0.25 mM

[a] At 25 °C in 0.2 M aqueous phosphate buffer of pH 11.6. $k^{scal} = k^{obsd}(1.040/0.830)$ and $X = (k_c - k^{scal})/(k^{scal} - k_u)$. See Appendix. [b] PI = hexanoate ion; ester = mNPA; $[\alpha\text{-CD}]_0$ = 1.00 mM. Relative to: k_u = 0.0858 s^{-1}; k_c = 24.6 s^{-1}; K_S = 25.0 mM. At this $[CD]_0$, k^{calc} = 1.04 s^{-1}; k^{obsd} scaled to this.

The analysis of k^{obsd} values for the purposes of inhibition studies (see Appendix) or for calculating k_{corr} and the estimation of k_a using eq 7 (so-called PIANALYSIS) both require the use of known values of k_u, k_c, and K_S. However, because of the pH dependence of the first two, the effect of pH variations between different experiments must be minimized. This was achieved by scaling the observed rate constants of each experiment to master runs for each ester and CD (Table VII), based on the value of k^{obsd} at zero PI. An example for inhibition is given in Table VIII, and one for PIANALYSIS, the estimation of k_a, is presented in Table IX. As pointed out earlier, for the purposes of analysis, in terms of eq 7, the actual concentrations of CD and PI were calculated using eq A3, as outlined in the Appendix.

Preliminary NMR experiments with simple alcohols showed that they bind in the cavities of CDs since appropriate chemical shifts of both host and guest were altered upon complexation. However, NOE experiments gave very small signals from which we were unable to decide which parts of the host and the guest were in close proximity.

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Table IX. Example of PIANALYSIS—Expt MB14[a,b]

$[PI]_0$, mM	k^{obsd}, s^{-1}	k^{scal}, s^{-1}	[CD], mM	[PI], mM	k^{corr}, s^{-1}
0	0.321	0.403	10.0	0	0.660
40	0.362	0.454	6.41	36.41	0.920
80	0.384	0.482	4.65	74.65	1.170
120	0.395	0.496	3.64	113.64	1.406
160	0.404	0.507	2.98	152.98	1.648
200	0.398	0.499	2.52	192.52	1.824

plot of k^{corr} vs [PI] slope = k_a = 6.09 ± 0.20 s^{-1} (r = 0.9978)

thus $k_b = k_a K_I/K_S$ = 50.0 s^{-1} and $K_{TS} = k_c/k_a$ = 108 mM

[a] At 25 °C in 0.2 M aqueous phosphate buffer of pH 11.6. $k^{scal} = k^{obsd}(0.403/0.321)$. k^{corr} is defined in eq 7. [CD] and [PI] were calculated as described in the Appendix. The scaled rate constants are plotted in Figure 1, curve a. [b] PI = 2-butanol; K_I = 65.0 mM; ester = pNPA; $[\beta\text{-CD}]_0$ = 10.0 mM. Relative to: k_u = 0.0772 s^{-1}; k_c = 0.660 s^{-1}; K_S = 7.92 mM. At this $[CD]_0$, k^{calc} = 0.403 s^{-1}; k^{obsd} scaled to this.

Appendix

Dissociation constants for the CD·PI complexes are defined as

$$K_I = [CD][PI]/[CD\cdot PI] \quad (A1)$$

Proper use of eq A1 requires the free concentrations of CD and PI, which are related through [CD·PI] and the equations for mass balance: $[CD] = [CD]_0 - [CD\cdot PI]$ and $[PI] = [PI]_0 - [CD\cdot PI] = [PI]_0 - ([CD]_0 - [CD])$, ignoring the minimal effects of low $[ester]_0$. Substitution from these equations into A1 yields

$$K_I = \frac{[CD]([PI]_0 - ([CD]_0 - [CD]))}{([CD]_0 - [CD])} \quad (A2)$$

Expansion of eq A2 gives a quadratic in [CD] whose solution is

$$[CD] = \frac{[CD]_0 - [PI]_0 - K_I + (([PI]_0 - [CD]_0 + K_I)^2 + 4K_I[CD]_0)^{1/2}}{2} \quad (A3)$$

For the use of eq 7 in data analysis, [CD] was calculated from eq A3 and [PI] was obtained by difference from $[PI] = [PI]_0 - ([CD]_0 - [CD])$.

When $[PI]_0 \gg ([CD]_0 - [CD])$, then eq A2 simplifies to

$$K_I = \frac{[CD][PI]_0}{([CD]_0 - [CD])} \quad (A4)$$

which can be rearranged to

$$[CD] = \frac{[CD]_0 K_I}{(K_I + [PI]_0)} \quad (A5)$$

Another expression for [CD], making use of k^{obsd}, is obtained by rearrangement of eq 3:

$$[CD] = \frac{(k_{obsd} - k_u)K_S}{(k_c - k^{obsd})} \quad (A6)$$

Elimination of [CD] between eqs A5 and A6 followed by rearrangement leads to the following relationship which is suitable for analysis of the inhibition data:

$$[PI]_0 = \frac{K_I[CD]_0(k_c - k^{obsd})}{(k^{obsd} - k_u)K_S} - K_I \quad (A7)$$

From the intercept of the line defined by $[PI]_0$ against $(k_c - k^{obsd})/(k^{obsd} - k_u)$ one can obtain an estimate of K_I.[3]

The above method only works when $[PI]_0 \gg ([CD]_0 - [CD]) = [CD\cdot PI]$. In many experiments with the larger, less soluble PI, this condition was not accessible and so a different treatment was used. From k^{obsd} obtained at different $[PI]_0$, values of [CD] were calculated using eq A6. Substitution of these [CD] values into eq A2 afforded estimates of K_I for each $[PI]_0$ which were then averaged. These calculations were conveniently carried out using a LOTUS 123 spreadsheet. An example is shown in Table VIII.