Catalysis of the Reaction of *p*-Nitrophenyl Alkanoates with Cyclodextrins by Potential Inhibitors: Simple Allosteric Activation

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The cleavage of *p*-nitrophenyl alkanoates (acetate to hexanoate) by β-cyclodextrin (β-CD) in basic aqueous solution is catalyzed by additives (ROH, RCO_2^-, and RSO_3^-) that are expected to be inhibitors. The magnitude of the catalysis by 1-butanol increases with the acyl chain length of the ester. For RCO_2^- and RSO_3^- as the potential inhibitors (PIs), the kinetics of the cleavage of *p*-nitrophenyl hexanoate (pNPH) are analyzable in terms of reaction between the CD·ester complex and one molecule of PI. Rate constants for this process (k_a) increase systematically with the ability of PI to bind to β-CD, implying that the catalytic reaction is better viewed as being between the PI·CD complex and the ester; rate constants for the latter process (k_b) show little variation and are not very different from the second order rate constant for the ester reacting with β-CD alone. With alcohols as the PIs, saturation kinetics implicate ternary complexes, {PI·CD·ester}, and for pNPH, the dissociation constants of these complexes (K_t) strongly parallel those of the binary {PI·CD} complexes (K_I). The reactivity of the ternary complexes (k_t) varies little with the structure of the alcohol or the ester. Catalysis of the cleavage of pNPH by α-CD is more restricted; simple alcohols catalyze the reaction modestly but show no evidence of ternary complex formation. Alkanoate ions inhibit the reaction, but the limited results for RSO_3^- were equivocal. The results are discussed in terms of the Kurz approach to transition state stabilization. For the PI catalysis, there are LFERs with strong correlations between the parameters for initial state and transition state binding of the PIs, suggesting that binding is very similar in the two states. In contrast, transition state binding of *n*-BuOH is insensitive to the chain length of the ester being cleaved, implying that the ester chain is not in direct contact with the alcohol bound in the CD cavity.

Introduction

The cleavage of esters by cyclodextrins[2] (CDs) in basic solution has been the subject of numerous studies.[2–9] Many such studies have been prompted by an interest in modeling enzyme behavior, using the binding abilities of CDs.[3–6] However, ester cleavage also provides a convenient way of probing modes of binding in the initial state and the transition state[7–12] of a reaction that is reasonably well-understood,[13] and such has been the main focus of our studies in the area.[8–12]

Generally speaking, the cleavage of meta-substituted phenyl acetates by α- or β-CD[2] is more efficient than that of the para isomers.[3,7–9,12] The rationalization of this selectivity is that a meta substituent locates the ester in the CD cavity in a geometry that more readily leads to nucleophilic attack by an ionized secondary hydroxyl group of the CD, whereas a para substituent holds the ester in a less appropriate orientation. Moreover, there is evidence that a para-substituted phenyl group must partially or totally come out of the CD cavity during the acyl transfer.[7] Consistent with this view, we found that the reaction of *p*-nitrophenyl acetate (pNPA) with α- and β-CD is not totally inhibited by many species that bind to the CDs and which do inhibit cleavage of the *m*-nitro

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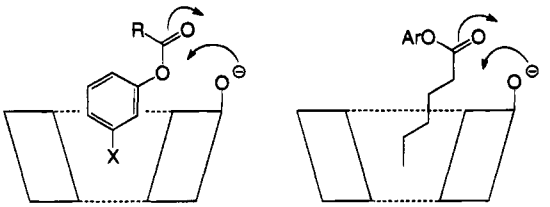
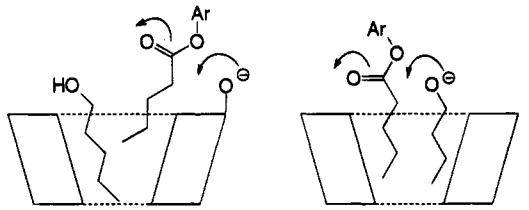
isomer.[10] Furthermore, in a few cases, the cleavage of pNPA is actually catalyzed, and the transition state stabilization afforded by the potential inhibitor correlates strongly with its ability to bind to the CD.[10]

Beyond the acetate or propionate, *m*- and *p*-nitrophenyl alkanoates bind to α-CD, β-CD, and "hydroxypropyl-β-CD" through their acyl groups, rather than through their aryloxy groups, and the strength of substrate binding increases monotonically with the acyl chain length.[8] Still, cleavage of the *m*-nitro isomers takes place via aryl group binding (**1**), while many of the para isomers react through a transition state having acyl group binding (**2**).[8b,c] Against this background, we speculated that the



1 **2**

presence of a medium-sized alcohol in the CD cavity might improve the binding of the acyl group of a medium chain alkanoate (pentanoate, say), possibly through the formation of a ternary {ROH·CD·ester} complex. Such binding might lead to more efficient ester cleavage (**3**), or if the geometry was appropriate, it might promote attack of the anion of the alcohol[14] on the ester (**4**).



3 **4**

On the basis of the foregoing, we have studied the effects of potential inhibitors, particularly alcohols, on the cleavage of *p*-nitrophenyl alkanoates by α- and β-CD in basic solution. Initial experiments with the acetate (pNPA) showed interesting behavior[10a] which has been reported in depth.[10b] The present paper deals mainly with longer esters for which we have found evidence of ternary complexes and catalysis by various potential inhibitors. Preliminary results for the effects of alcohols on the cleavage of *p*-nitrophenyl hexanoate by β-CD have been reported.[11]

Results

We have examined the effects of many potential inhibitors (PIs) on the rates of the cleavage of *p*-nitrophenyl alkanoates (acetate to hexanoate, C2–C6) by α- or β-CD in a basic aqueous phosphate buffer. For the same reasons as in our studies of pNPA cleavage,[10] we have used simple alcohols, alkanoate ions, and alkanesulfonate ions as the PIs. Before describing the results in detail, we outline the main features of ester cleavage kinetics, how the PIs alter them, and how we have analyzed the effects.

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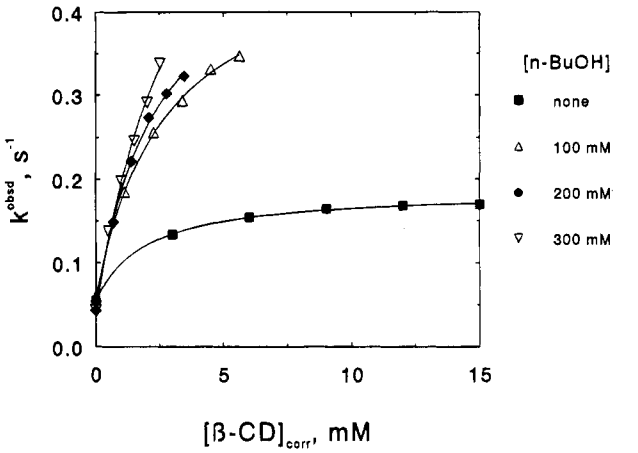

Figure 1. Effects of 1-butanol on the rates of cleavage of *p*-nitrophenyl pentanoate by β-CD at pH 11.6 for $[n\text{-BuOH}]_0$ = 0, 100, 200, and 300 mM, as indicated. Note that, for each graph, [β-CD] has been corrected for binding to *n*-BuOH (K_I = 60 mM), before which the values were $[\beta\text{-CD}]_0$ = 0, 3, 6, 9, 12, and 15 mM.

A substrate (S), at low concentration, reacting in the medium (eq 1) and through a 1:1 complex with an excess of CD (eq 2) gives rise to saturation-type kinetics (eq 3).[2,3,8,9] This equation describes the kinetic behavior of

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

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

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

a large number of substrates, not only esters, reacting in the presence of CDs.[2,6,12] Nonlinear least squares fitting of eq 3 to the k^{obsd} vs [CD] data affords values of k_c and K_S, with k_u being fixed at the measured value at zero [CD].[9,12] An example of such fitting is seen in the lowest curve in Figure 1, which is for the cleavage of *p*-nitrophenyl pentanoate in the presence of β-CD; fitted parameters for all the esters used in the present work are given in the Experimental Section.

Normally, addition to the reaction mixture of an inert species (here, PI) that forms a complex with the CD (eq 4) reduces the concentration of free CD, so that less CD·S complex is formed and k^{obsd} is decreased, in accord with eq 3. Such behavior (competitive inhibition) is observed

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

with *m*-nitrophenyl acetate, and values of k^{obsd} at fixed $[CD]_0$ with varying $[PI]_0$ can be analyzed to find the dissociation constant (K_I) of the PI·CD complex.[3a,10]

In contrast to such inhibition, simple alcohols, alkanoate ions, or alkanesulfonate ions catalyze the cleavage of *p*-nitrophenyl alkanoates by β-CD and, to a lesser extent, by α-CD. For example, as shown in Figure 1, the addition of 1-butanol increases the rate of reaction of *p*-nitrophenyl pentanoate with β-CD. The catalytic effect is particularly dramatic when values of k^{obsd} are plotted against the reduced concentrations of β-CD, corrected for complexation with *n*-BuOH (K_I = 60 mM).[7,15] Clearly,

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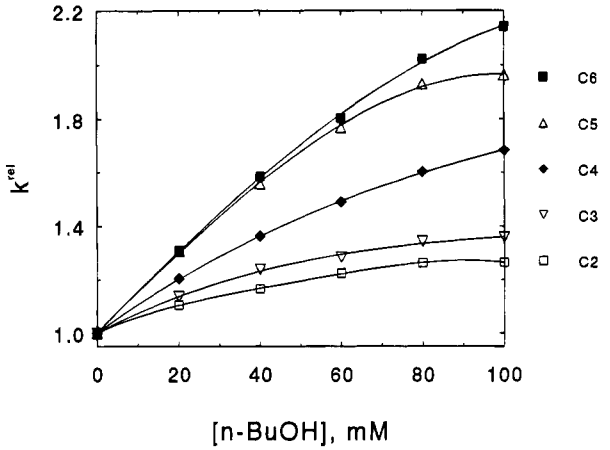


Figure 2. Effects of 1-butanol on the rates of cleavage of *p*-nitrophenyl alkanoates (acetate to hexanoate; C2 to C6) in 15 mM β-CD at pH 11.6. For presentation purposes, the data are plotted as k^{rel} vs $[n\text{-BuOH}]_0$, where k^{rel} is k^{obsd} divided by the value at zero alcohol.

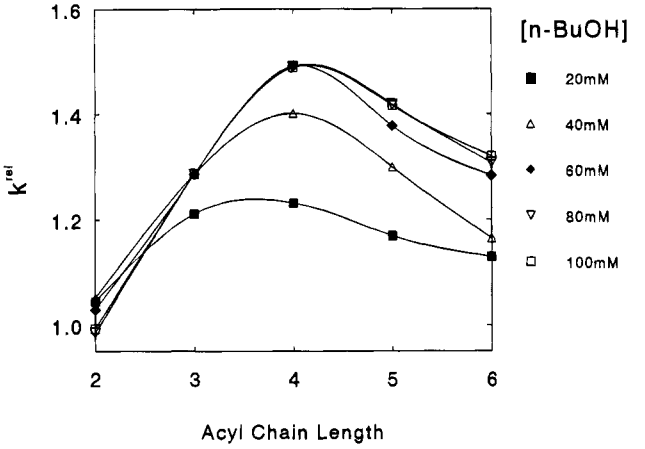


Figure 3. Effects of 1-butanol on the rates of cleavage of *p*-nitrophenyl alkanoates in 15 mM α-CD at pH 11.6. For comparative purposes, the data are plotted as k^{rel} vs acyl chain length, where k^{rel} is k^{obsd} divided by the value at zero alcohol.

the alcohol catalyzes the reaction and the effects of competitive inhibition are completely overridden.

Similar, but less pronounced, catalysis was observed for the cleavage of *p*-nitrophenyl acetate by CDs.[10] How then does the catalysis vary with the acyl chain length of the ester? The data presented in Figure 2 show that, for reaction with β-CD, the extent of catalysis by 1-butanol increases systematically as the ester is varied from the acetate (C2) to the hexanoate (C6). Catalysis is also observed for cleavage of these esters by α-CD, but the magnitude is less and the variation with acyl chain length is more complex, increasing from C2 to C4 and then decreasing (Figure 3).

Since *p*-nitrophenyl hexanoate (pNPH) promised to yield the largest catalytic effects, most of our experiments involved the reaction of it with β-CD in the presence of three classes of potential inhibitors: alcohols, alkanoate ions, and alkanesulfonate ions, all of which have been studied with pNPA.[10] The hope was that the variation of the catalysis with the structure of the PI might lead to some insight into the origin of the catalysis and the structure of the transition state. Our approach to the analysis of the results, which proved successful in earlier work with pNPA,[10] assumed that catalysis arises from reaction of the PI with the CD·ester complex (eq 5); the

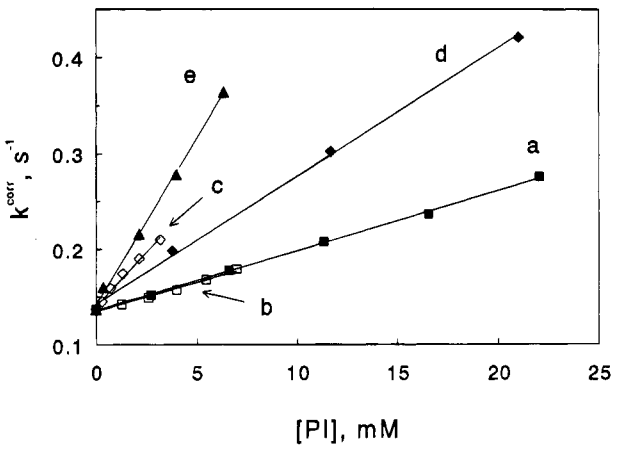


Figure 4. Examples of plots according to eq 7 for the effects of PIs on the cleavage of pNPH by β-CD: (a) $HexSO_3^-$, (b) $PenCO_2^-$, (c) $HeptCO_2^-$, (d) $HeptSO_3^-$, (e) $OctSO_3^-$. Note that [PI] and [CD], which are needed to calculate k^{corr}, have been corrected for the formation of the PI·CD complex. Similar plots have been shown in earlier published work on pNPA.[10]

kinetic equivalent of PI·CD reacting with ester is considered later in the Discussion. The involvement of the process in eq 5 leads to the expected form of k^{obsd} given by eq 6. For analytical purposes, we use the linearized form eq 7 in which the concentration variables, [CD] and [PI], are separated.

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

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

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

According to eq 7, the slopes of linear plots of k^{corr} against [PI] provide estimates of k_a. This approach accommodates a large body of data for the effects of PIs on the cleavage of pNPA by α- and β-CD.[10] Likewise, kinetic data for the cleavage of pNPH by β-CD catalyzed by alkanoate ions and alkanesulfonate ions are linearized by the use of eq 7. Some examples of the plots are given in Figure 4, and values of k_a and related quantities are collected in Table 1a,b. The good linearity of the plots indicates that only one molecule of PI is implicated in the catalysis, as was assumed in developing eqs 6 and 7.

Data for the effects of simple alcohols on the reaction of pNPH and *p*-nitrophenyl butanoate (pNPB) with α-CD are also analyzable using eq 7, giving values of k_a in Table 1c,d. For the cleavage of pNPH by β-CD, the effects are slightly different: The catalysis by alcohols is larger, and the data show more curvature than they do for reaction with α-CD. Several examples of this type of behavior are shown in Figure 5. These plots are decidely curved, and the data are not linearized when treated according to eq 7; they still show downward curvature, suggestive of the onset of saturation of an equilbrium involving the alcohol. Consequently, we propose that a ternary complex is formed from the alcohol, β-CD, and pNPH (eq 8), in which case eq 6 must be replaced by eq 9. As it stands, eq 9 is

$$\mathrm{PI} + \mathrm{CD{\cdot}S} \underset{K_t}{\rightleftharpoons} \mathrm{PI{\cdot}CD{\cdot}S} \xrightarrow{k_t} \text{products} + \mathrm{PI} \quad (8)$$

Table 1. Constants for the Cleavage of *p*-Nitrophenyl Hexanoate or Butanoate (pNPH or pNPB) by α- or β-Cyclodextrin in the Presence of Potential Inhibitors (PI)[a]

PI	K_I, mM	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}	K_{TS}, mM
(a) Alkanesulfonate Ions and β-CD with pNPH				
C5	17	2.4	26	57
C6	5.6	6.3	22	22
C7	2.3	13	19	11
C8	0.97	35	21	3.9
C10	0.24	96	14	1.4
(b) Alkanoate Ions and β-CD with pNPH				
C4	260	0.25	41	550
C5	74	1.6	74	86
C6	16	6.1	61	23
C7	5.9	5.7	21	24
C8	1.5	23	22	6.0
(c) Alcohols and α-CD with pNPH				
Et	178	0.074	3.7	1400
n-Pr	43	0.43	5.2	250
n-Bu	11	1.5	4.7	71
n-Pen	3.1	7.9	7.0	14
n-Hex	1.1	11	3.4	9.7
(d) Alcohols and α-CD with pNPB				
n-Pr	43	0.74	6.8	120
n-Bu	11	2.6	6.1	34
n-Pen	3.1	7.1	4.7	13
n-Hex	1.1	31	7.3	2.9
n-Hept	0.44	110	10	0.81

[a] At 25 °C, in a phosphate buffer (pH 11.6) containing 10 or 15 mM CD. The dissociation constants of the PI·CD complexes (K_I) are from the literature[7,15,16] and previous work.[10] Values of k_a are the slopes of k^{corr} against [PI] (eq 7); k_b values are calculated from k_aK_I/K_S, and $K_{TS} = k_c/k_a$ (see text).

$$k^{obsd} = \frac{k_uK_tK_S + k_cK_t[CD] + k_t[PI][CD]}{K_tK_S + K_t[CD] + [PI][CD]} \quad (9)$$

inconvenient for analysis because it contains two concentration variables, [PI] and [CD]. However, if experiments are carried out at high [CD] (≥10 mM), so that the terms containing [CD] are dominant in both the numerator and denominator, [CD] essentially cancels out, and eq 9 can be approximated to

$$k^{obsd} = \frac{k_cK_t + k_t[PI]}{K_t + [PI]} \quad (10)$$

Equation 10 corresponds to saturation kinetics, and it accounts for the curvature of the data in Figure 5. Nonlinear fitting of eq 10 to data for various alcohols (C3–C6) affords the values of K_t and k_t collected in Table 2. Beyond C6, the alcohols are not soluble enough to allow for determination of these constants.

Kinetic data for the effects of 1-butanol on the cleavage of other *p*-nitrophenyl alkanoates by β-CD are also curved (Figure 2), and they are not treated well by eq 7. However, when analyzed for saturation behavior using eq 10, the data give good fits (r = 0.9974–0.9998) with the parameters presented in Table 3. The curvature is less pronounced with the shorter esters, and it was not detected earlier for pNPA[10] because of the range of [*n*-BuOH] used.

In contrast to the catalytic effects presented above, the cleavage of pNPH by α-CD shows inhibition by alkanoate ions and it gave equivocal results for alkanesulfonate ions,[17] even though these ions catalyze the reaction of pNPA modestly.[10] We have not searched systematically for inhibition of the reaction of pNPH with CDs but have found it in a few other cases. Reaction with β-CD is

Table 2. Constants for the Basic Cleavage of *p*-Nitrophenyl Hexanoate by β-Cyclodextrin in the Presence of Alcohols, ROH[a]

R	K_I, mM	K_t, mM	k_t, s^{-1}	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}	K_{TS}, mM
n-Pr[b]	270	370	0.51	1.4	230	100
i-Pr	260	415	0.60	1.4	230	95
s-Bu	65	116	0.41	3.6	140	38
n-Bu	60	120	0.47	3.9	150	35
2-Pen[b]	32	81	0.31	3.8	78	36
i-Bu[b]	24	44	0.36	8.2	120	17
t-Bu	21	45	0.34	7.7	100	18
n-Pen	16	38	0.39	10	100	14
c-Pen	8.3	16	0.37	23	120	6.0
n-Hex	4.6	9.8	0.24	25	70	5.6
c-Hex	2.0	5.8	0.28	48	59	2.9
neo-Pen[b]	1.7	3.3	0.20	61	66	2.3

[a] At 25 °C, in a phosphate buffer (pH 11.6) containing 15 mM β-CD. The dissociation constants of PI·CD (K_I) are from the literature.[7,15] Values of K_t and k_t were obtained by fitting eq 10 to k^{obsd} obtained over a range of $[ROH]_0$. The other constants are $k_a = k_t/K_t$ (cf. eqs 5 and 8), $k_b = k_aK_I/K_S$, and $K_{TS} = k_c/k_a$ (eq 13). [b] Solution contained 10 mM β-CD.

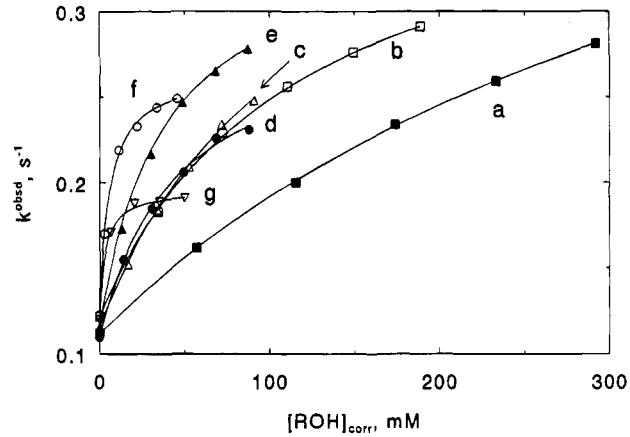


Figure 5. Effects of various alcohols on the rate of cleavage of pNPH by β-CD at pH 11.6: (a) *i*-PrOH, (b) *s*-BuOH, (c) *n*-BuOH, (d) *t*-BuOH, (e) *n*-PenOH, (f) c-HexOH, (g) *neo*-PenOH. The strongly curved plots are indicative of the formation of ternary complexes {ROH·β-CD·pNPH}. The calculated curves were generated by eq 10. Note that, for each set of data, [ROH] has been corrected for the formation of the appropriate {ROH·β-CD} complex.

Table 3. Constants for the Basic Cleavage of *p*-Nitrophenyl Alkanoates by β-Cyclodextrin in the Presence of 1-Butanol[a]

ester	K_t, mM	k_t, s^{-1}	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}	K_{TS}, mM
C2	48	0.65	14	100	49
C3	47	0.46	9.8	120	38
C4	98	0.45	4.6	110	46
C5	72	0.46	6.4	210	28
C6	120	0.47	3.9	150	35

[a] See Table 2. From experiments with $[\beta\text{-CD}]_0$ = 15 mM and k^{obsd} at $[n\text{-BuOH}]_0$ = 0, 20, 40, 60, 80, and 100 mM. The actual data are plotted in Figure 2, on a relative basis.

(16) (a) Satake, I.; Ikenoue, T.; Takeshita, T.; Hayakawa, K.; Meda, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2746. (b) Satake, I.; Yoshida, S.; Hayakawa, K.; Meda, T.; Kusumoto, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3991.

(17) Initial results for the effects of alkanesulfonate ions on the cleavage of pNPH by α-CD gave inconsistent results, on the border of weak catalysis and inhibition. Since we were having technical difficulties with equipment at the time, the experiments were discontinued.

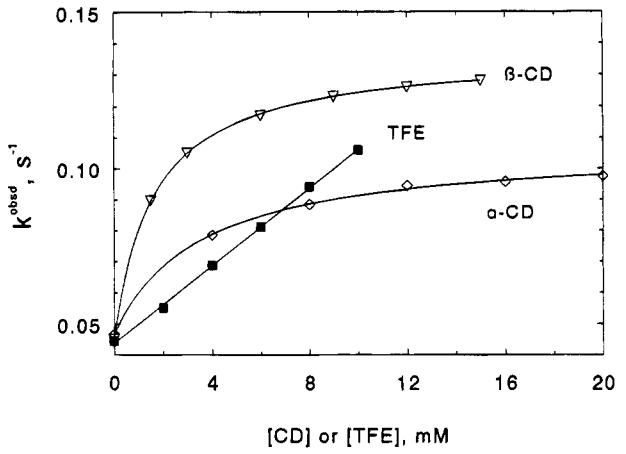


Figure 6. Comparison of the reactivity of trifluoroethanol (TFE), α-CD, and β-CD toward *p*-nitrophenyl hexanoate in a 0.2 M phosphate buffer at pH 11.6. The second order rate constants (k_2, M^{-1} s^{-1}) are 6.2 for TFE, 30 for α-CD, and 86 β-CD.

inhibited by the suberate dianion ($^{-}O_2C(CH_2)_6CO_2^{-}$), which also inhibits pNPA cleavage, and by perchlorate ion, which is a weak catalyst for pNPA.[10]

As discussed for the cleavage of pNPA,[10b] it is possible that binding of the ester in the CD cavity does not particularly help the acyl transfer. If the ester moiety is outside the CD cavity during the reaction, then perhaps the anion of the CD is simply behaving as an oxyanion nucleophile[14] of comparable pK_a (12.2 for α-CD; 12.3 for β-CD).[18] To explore this possibility, we studied the effect of trifluoroethanol (TFE, pK_a = 12.4)[14] on the cleavage of pNPH and found that values of k^{obsd} increase linearly with [TFE], with k_2 = 6.2 M^{-1} s^{-1}. As shown in Figure 6, TFE reacts more slowly than α-CD or β-CD at the same pH, and so the two CD anions are more reactive toward pNPH than one would expect solely on the basis of their pK_as.

Discussion

The results presented above show that the catalysis by PIs, first observed for the cleavage of pNPA by CDs,[10] is more pronounced with longer alkanoate esters (Figures 2 and 3). For all the esters studied, catalysis is greater with β-CD, presumably because its larger cavity[2,19] can more easily accommodate a PI and part of the ester at the transition state. With the narrower α-CD[19] and 1-butanol as PI, the catalytic effect is maximal for the C4 ester, suggesting that the combination of the *n*-butyl group of the alcohol and the butanoyl group of the ester can fit snugly in the α-CD cavity, which seems reasonable from inspection of CPK space-filling models. Alkanoate ions inhibit the cleavage of pNPH by α-CD, even though they catalyze reaction with β-CD and the cleavage of pNPA by α-CD.[10] Clearly, these ions bind to α-CD differently than do alcohols and in such a way as to preclude reaction with pNPH.

For a more quantitative view of the results, we look at trends in the constants that characterize the catalytic effects. Much of the data is satisfactorily analyzed using eq 7, giving values of k_a for the reaction in eq 5. For the reaction involving pNPH, β-CD, and alcohols (Table 2), where ternary complexes are implicated, k_a is evaluated from k_t/K_t (cf. eqs 5 and 8). In general, values of k_a rise sharply as the stability PI·CD increases (as K_I decreases) (Tables 1 and 2). Moreover, for a series of PIs and a given ester (pNPA,[10] pNPB, or pNPH), there are good linear correlations[20] of log k_a with pK_I with slopes of 0.75−1.0, strongly suggesting that the PI is bound in the CD cavity in the transition state of the reaction in eq 5. Given this observation, it seems more appropriate to consider the PI-catalyzed reaction as being between the ester and a CD bound to PI :

$$PI + CD + S \underset{K_I}{\rightleftharpoons} PI{\cdot}CD + S \xrightarrow{k_b} \text{products} + PI \qquad (11)$$

Rate constants for this process are evaluated from $k_b = k_aK_I/K_S$, since the rate constant for the overall reaction $k_3 = k_a/K_S$ or k_b/K_I, depending on whether the PI-catalyzed reaction is viewed as proceeding as in eq 5 or in eq 11. Values of k_b show only slight variations (Tables 1 and 2) and are not markedly different from the rate constant for reaction between S and the CD alone ($k_2 = k_c/K_S$, eq 2). For example, the reaction of pNPH with β-CD has k_2 = 86 M^{-1} s^{-1}, whereas k_b values range from 60 to 240 M^{-1} s^{-1} for alcohols as the PIs (Table 2), from 15 to 25 M^{-1} s^{-1} for RSO_3^{-}, and from 21 to 72 M^{-1} s^{-1} for RCO_2^{-} (Table 1). Thus, the reactivity of β-CD toward pNPH is modestly attenuated or amplified by binding of these PIs. Obviously, in those few cases where full inhibition has been observed, k_b is drastically reduced relative to k_2.

The parameters k_t and K_t which characterize the cleavage of pNPH via ternary complexes (eq 8) show interesting sensitivities to structural variation in the alcohols, ROH. Going from *n*-PrOH to *neo*-PenOH, k_t gradually diminishes from 0.60 to 0.20 s^{-1} (Table 2), meaning that the ternary complex becomes somewhat less reactive as the alcohol becomes larger. Still, the ternary complexes {ROH·β-CD·pNPH} are more reactive than the binary complex {β-CD·pNPH} lacking a molecule of ROH (k_c = 0.14 s^{-1}). The dissociation constants K_t decrease substantially and in parallel with K_I (Table 2) even though the alcohols are comprised of several types (primary, secondary, tertiary, cyclic, etc). In fact, there is a very good linear free energy relationship (LFER) between the two sets of constants:

$$pK_t = 0.92pK_I - 0.17 \qquad N = 12 \qquad r = 0.995 \qquad (12)$$

Numerically, K_t is 1.4−2.9 times larger than K_I, indicating weaker binding of ROH in the ternary complexes {ROH·β-CD·pNPH} than in {ROH·β-CD}, but the slope of 0.92 of the LFER and the high quality of the correlation are strong evidence that binding of ROH in the two types of complexes is geometrically very similar.

The parameters for the ternary complexes formed from *n*-BuOH, β-CD, and five different alkanoate esters also show interesting variations (Table 3). As the acyl chain length increases (C2 to C6), the binding of *n*-BuOH generally weakens (K_t increases), presumably because it becomes harder for the CD to accommodate two species. For the same structural change, however, k_t is essentially

(18) 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.

(19) α-CD has six glucose units joined in a torus, and β-CD has seven units. Thus, their cavities differ in width (~6 and ~8 Å, respectively) but not in depth (~8 Å).[2]

(20) For example, the data in Table 2 give log k_a = 0.75pK_I − 0.33 (N = 12, r = 0.989). Except for their intercepts, such linear free energy relationships are the same as the correlations presented later in Table 4.

Table 4. Correlation of the Binding of PIs in the Transition State for the Cleavage of pNPH by CDs (pK_{TS}) with Their Binding in PI·CD Complexes (pK_I)[a]

PI	CD	slope	N	r
alcohols	α	1.02	5	0.991
alcohols[b]	α	1.08	5	0.992
alcohols	β	0.75	12	0.989
primary[c]	β	0.74	6	0.999
$R\text{-}SO_3^-$	β	0.88	5	0.998
$R\text{-}CO_2^-$	β	0.81	5	0.967

[a] Based on the data presented in Tables 1 and 2; r is the correlation coefficient for pK_{TS} vs pK_I. Most of these data are shown in Figure 7. [b] For the cleavage of *p*-nitrophenyl butanoate. [c] Includes *n*-Pr, *n*-Bu, *n*-Pen, *n*-Hex, *i*-Bu, and *neo*-Pen.

constant (Table 3), particularly when one bears in mind that the C2 and C3 esters are normally 1.5–2 times more reactive than the rest for simple steric reasons.[8,21] Probably, for related reasons, values of k_b also show little variation (100–210 M^{-1} s^{-1}) with the ester chain length, similar to, but slightly greater than, k_2 values (70–100 M^{-1} s^{-1}). As discussed later, the insensitivity of kinetic parameters to the ester chain length suggests that the acyl chain is not bound in the CD cavity during the transition state of the PI-catalyzed reaction.

In much of our recent work,[8b,c,9-12] we have made use of an approach, due to Kurz,[22] for estimating the stabilization of a transition state by a catalyst. This approach, which is a quantification of earlier ideas of Pauling's[23] and which has become incorporated into modern enzymology,[24] is useful for analyzing the kinetics of reactions mediated by CDs, particularly ones where different modes of transition state binding are possible.[12] In the present case, we use the approach to probe the binding of potential inhibitors to the CD in the transition state for the cleavage of esters by the CD.

From simple transition state theory,[25] the rate of the reaction in eq 2 = k_c[CD·S] = ν[CD·TS], where ν is the effective frequency over the barrier and CD·TS is used to symbolize the transition state for the acyl transfer to the CD. Likewise, for the same reaction catalyzed by a PI (eq 5), the rate = k_a[PI][CD·S] = ν[PI·CD·TS], where PI·CD·TS symbolizes the transition state containing PI. Division of the two rate expressions, to eliminate ν and [CD·S], leads to an apparent dissociation constant:

$$K_{TS} = \frac{[PI][CD\cdot TS]}{[PI\cdot CD\cdot TS]} = \frac{k_c}{k_a} \qquad (13)$$

which is a measure of the stabilization of the cleavage transition state by the PI. Values of K_{TS} for various combinations of ester, CD, and PI are given in Tables 1–3.

Variations of K_{TS} with structure, particularly in the form of LFERs, can be used to probe the transition state binding of catalysts.[12] For the present results, there are LFERs between pK_{TS} (= $-\log K_{TS}$) for the transition state binding of PI and pK_I for the binding of the PI in the initial {PI·CD} complexes. These correlations are summarized in Table 4.

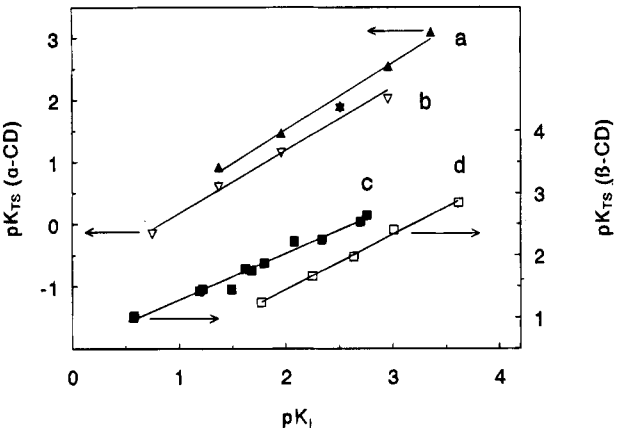


Figure 7. Linear free energy relationships between transition state binding (pK_{TS}) and initial state binding (pK_I). The systems are (a) pNPB/α-CD/ROH, (b) pNPH/α-CD/ROH, (c) pNPH/β-CD/ROH, and (d) pNPH/β-CD/RSO_3^-. These LFERs are summarized in Table 4.

For the reaction of pNPH with both CDs, catalyzed by alcohols, the correlations are

$$\alpha\text{-CD: } pK_{TS} = 1.02pK_I - 0.84 \qquad N = 5 \qquad r = 0.991 \qquad (14a)$$

$$\beta\text{-CD: } pK_{TS} = 0.75pK_I + 0.53 \qquad N = 12 \qquad r = 0.989 \qquad (14b)$$

These two correlations and others are shown in Figure 7. Except for their intercept terms, they are the same as those of log k_a with pK_I, referred to earlier, since pK_{TS} = $\log(k_a/k_c)$ (eq 13) and k_c is a constant for a given ester and CD. Similar relationships have been found for the effects of RSO_3^- and RCO_2^- on the cleavage of pNPH by β-CD (Table 4). Also, catalysis of the reaction of pNPB with α-CD by linear alcohols exhibits a good correlation, and within the series of 12 alcohols which catalyze the cleavage of pNPH by β-CD, the 6 primary alcohols show a very strong correlation (Table 4). Several analogous correlations, with similar slopes, were found earlier for the cleavage of pNPA by both CDs.[10]

The slopes of the LFERs in Table 4, which range from 0.74 to 1.08, are strong indications that the mode of binding of PI in the transition state of the PI-catalyzed reaction is similar to that in the corresponding PI·CD complex. This evidence may be coupled with the earlier observation that the rate constants (k_b) for the reaction of pNPH with PI·CD are quite similar to k_2 for the reaction of pNPH with CD, alone. Both observations support the conclusion that the PI-catalyzed cleavage takes place with the ester largely outside the CD cavity, so that the PI can be inside it, and bound more or less normally.

(21) Tee, O. S.; Enos, J. A. *Can. J. Chem.* **1988**, *66*, 3027 and references therein.

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

(23) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375.

(24) (a) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10. Lienhard, G. E. *Science* (Washington, D.C.) **1973**, *180*, 149. Jencks, W. P. *Adv. Enzymol. Relat. Subj. Biochem.* **1975**, *43*, 219. Schowen, R. L. In *Transition States in Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978. Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985. Wolfenden, R.; Frick, L. In *Enzyme Mechanisms*; Page, M. I., Williams, A., Eds.; Royal Soc. Chem.: London, 1987. Wolfenden, R.; Kati, W. M. *Acc. Chem. Res.* **1991**, *24*, 209. (b) The Kurz approach has been reviewed critically: Kraut, J. *Science* (Washington, D.C.) **1988**, *242*, 533. (c) Recently, the importance of transition state stabilization has been questioned: Menger, F. M. *Biochemistry* **1992**, *31*, 5368.

(25) Laidler, K. J. *Chemical Kinetics*, 3rd ed.; Harper & Row: New York, 1987. For a very recent modification, see: Albery, W. J. *Adv. Phys. Org. Chem.* **1993**, *28*, 139.

The different slopes of the correlations for α-CD and β-CD (Table 4) must be related to the widths of their cavities since their depths are the same.[2,19] The higher slopes for α-CD probably denote more restrictive binding that results from the tighter fit of the alkyl chains of the PIs in the narrower α-CD cavity.[7-9,15,16] Since β-CD has a wider cavity,[19] the fit can be looser,[7,12] allowing the PI and the ester to more easily assume a reactive geometry. This difference in binding by the two CDs may also account for the larger catalysis by β-CD (Tables 1 and 2). Likewise, the fact that alkanoate ions catalyze cleavage of pNPH by β-CD, but not for reaction with α-CD, may have similar geometrical origins.

For the reactions involving the ternary complexes {ROH·β-CD·pNPH}, both pK_t and pK_{TS} correlate well with pK_I for {ROH·β-CD} (eqs 12 and 14b) so these two parameters are strongly correlated with each other (r = 0.995), and for pK_{TS} against pK_t, the slope is 0.82. Thus, it seems clear that the geometry of the binding of ROH in the transition state of the ROH-catalyzed reaction is similar to that in the ternary complex preceding it (eq 8) which, in turn, is not very different from that in the binary {ROH·β-CD} complex.

From the constants for dissociation of ROH from the complexes {ROH·β-CD·pNPH} (K_t, Table 2), one can calculate K_t' for dissociation of pNPH from these complexes:

$$\text{PI·CD·S} \overset{K_t'}{\rightleftharpoons} \text{PI·CD} + \text{S} \qquad (15)$$

$$K_t' = [\text{PI·CD}][\text{S}]/[\text{PI·CD·S}] = K_tK_S/K_I \qquad (16)$$

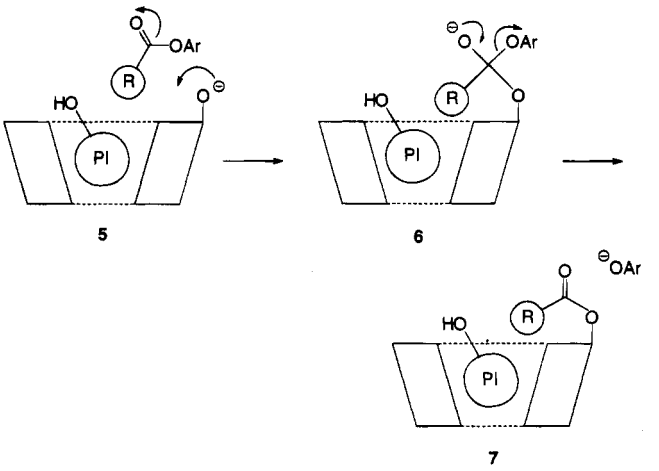
Values of K_t' vary only from 2.2 to 4.7 mM, with the trend being to weaker binding for the larger, bulkier alcohols, compared to K_S = 1.6 mM for the dissociation of the {β-CD·pNPH} complex. Thus, binding of the ester in the ternary complexes {ROH·β-CD·pNPH} is marginally weaker than in the binary {β-CD·pNPH} complex.

Using the Kurz approach,[12,22] we also consider the binding of PI·CD in the transition state for PI catalysis using the constant:

$$K_{TS}' = [\text{PI·CD}][\text{TS}]/[\text{PI·CD·TS}] = k_u/k_b = k_uK_S/k_aK_I \qquad (17)$$

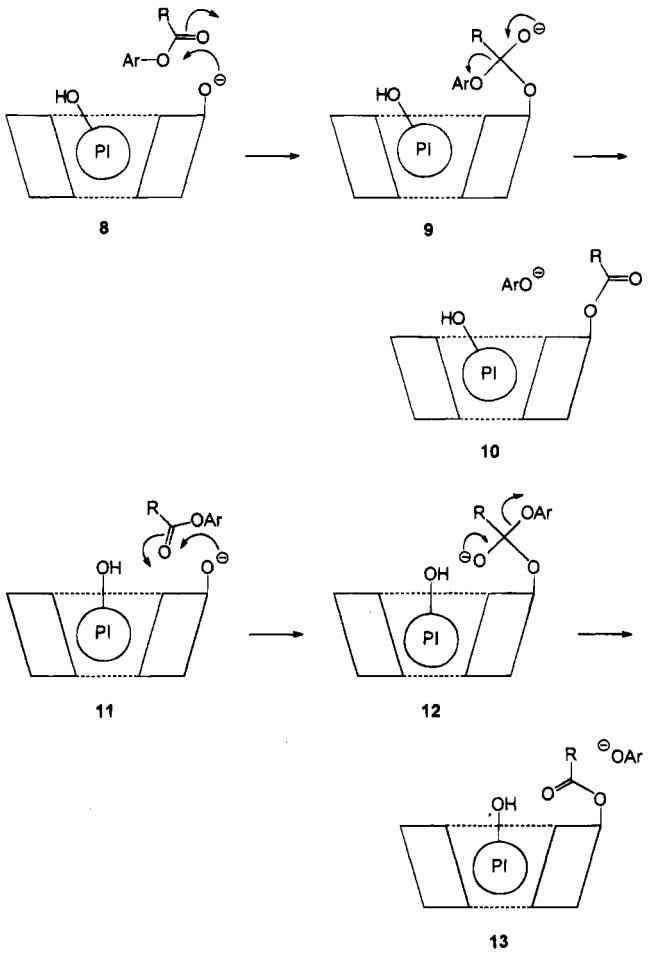
For alcohols, β-CD, and pNPH (Table 2), values of K_{TS}' gradually increase from 0.19 to 0.76 mM (*n*-PrOH to c-HexOH), whereas for transition state binding in the absence of PI, K_{TS} = [CD][TS]/[CD·TS] = k_uK_S/k_c = 0.53 mM. Thus, with the smaller alcohols (C3 and C4), the transition state is stabilized, with most of the C5 alcohols, it is hardly affected, and with the large or bulky ROH, it is slightly destabilized.[26] Overall then, the catalysis by alcohols of the cleavage of pNPH by β-CD arises from weaker substrate binding (previous paragraph) and in some cases from stronger transition state binding as well.

Because of the strong dependence of appropriate parameters on chain length, it has been concluded that the cleavage of many *p*-nitrophenyl alkanoates by α- and β-CD occurs with their acyl chains bound in the CD in the initial state and in the transition state (**2**).[8] In sharp contrast, parameters for reaction via ternary complexes (k_t, k_b, and K_{TS}) are insensitive to chain length (Table 3) which suggests that the acyl group is not situated in the β-CD cavity in the transition state. The seeming conflict between these conclusions is resolved if the PI in the ternary complex forces the ester to come out of the CD cavity but still leaves it in a position where it can react with an ionized hydroxyl group (**5** → **6** → **7**). Moreover,



overall catalysis will result if the new configuration in the ternary complex is more reactive, as is the case for pNPH in Table 2 (k_t = 0.20–0.60 > k_c = 0.14 s^{-1}).

If the acyl group of the alkanoate ester is not directed toward the CD cavity during the PI-catalyzed reaction, then either the *p*-nitrophenoxyl group (**8** → **9** → **10**) or the developing oxyanion of the tetrahedral intermediate (**11** → **12** → **13**) must be so directed. In the former case,

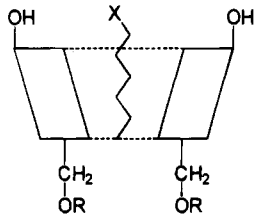


there could be some transition state stabilization due to a hydrophobic attraction[27] between the aryl group and

(26) Similar trends are found in the data for the effect of alcohols, RCO_2^-, and RSO_3^- on pNPA cleavage by β-CD since k_b values decrease as the PIs becomes larger. However, for the cleavage of pNPA by α-CD, there are no trends since k_b values are essentially constant.[10b]

the alkyl portion of the alcohol (**9**). Alternatively, the incipient anionic oxygen might be stabilized hydrophilically, by hydrogen bonding to the solvated hydroxyl of the alcohol (**12**). Either of these two situations could give rise to the observed sensitivities of the kinetic parameters to the structure of ROH, depending on the normal geometry of the ROH·β-CD complex.

Until very recently, we were unsure of the geometry of the binary {alcohol·β-CD} complexes. However, we have found that the dissociation constants for such complexes, as well as the complexes formed by alkylamines, ketones, alkanoate esters, and alkanesulfonate ions (**14**), are virtually the same as the dissociation constants for the analogous complexes formed by hydroxypropyl-β-cyclodextrin (**15**) in which the primary hydroxyls on the narrower rim of β-CD are functionalized.[28] As a result, we have concluded that the alcohols (and the



14 : β-CD; R = H

15 : "Hydroxypropyl-β-CD"; R = $CH_2CH(OH)CH_3$

X = OH, NH_2, SO_3^-, $COOC_6H_4NO_2$

other aliphatics) bind to both CDs from the wider, "secondary" side of the CD cavity (**14** and **15**).[29] It is for this reason that in structures **3**−**13** we have depicted the hydroxyl of the alcohol as protruding from the secondary side of β-CD.

We now turn to the question: In the absence of a PI, does binding of the acyl chain of the *p*-nitrophenyl alkanoate to the CD help the acyl transfer reaction? We have concluded that it is of little help in the cleavage of pNPA by α-CD, but it may make a small contribution for reaction with β-CD.[30] Judging by the second order rate constants collected in Table 5, binding to the CD makes a more substantial contribution for the cleavage of pNPH. Nucleophilic attack of hydroxide ion on pNPA is about twice as fast as on pNPH, as a result of normal steric effects,[21] and a similar difference is observed for attack by trifluoroethoxide ion on the two esters (Table 5). In contrast, the anions of both α-CD and β-CD are more reactive toward pNPH than pNPA (Table 5), and the difference must be larger for longer homologues which are cleaved even more readily by CDs,[8] clearly indicating that binding of the acyl chain of these alkanoates to the CD assists nucleophilic attack by an ionized hydroxyl group, in the absence of PI.

Table 5. Comparison of Apparent Second Order Rate Constants for the Reactions of pNPA and pNPH with Various Nucleophiles[a]

		k_2, M^{-1} s^{-1}	
nucleophile	pK_a	pNPA	pNPH
HO^-	15.7	19	11
EtO^-	16.0	250[b]	
$CF_3CH_2O^-$	12.4	64	37
α-CDO^-	12.2	79	120
β-CDO^-	12.3	420	430
EtOH·α-CDO^-	12.2[c]	15	
n-PrOH·α-CDO^-	12.2[c]	22	21
n-PrOH·β-CDO^-	12.3[c]	470	1200
α-CD·EtO^-	16.0[d]		93000[e]
α-CD·*n*-PrO^-	16.0[d,f]	190000[e]	130000[e]
β-CD·*n*-PrO^-	16.0[d,f]	2400000[e]	5900000[e]

[a] At 25 °C. The pK_as are for the conjugate acids of the nucleophilic anions; they are taken from refs 14 and 18. Rate constants for pNPA are from ref 10. [b] Reference 14. [c] Assumed to be the same as that of the CD alone. [d] Assumed to be the same as that of unbound ethanol. [e] For comparative purposes only; we do not believe the reaction proceeds this way (see text). [f] The pK_a of CD-bound propanol is assumed to be the same as that for unbound ethanol.

As discussed above, this effect of acyl chain inclusion may be modified by the presence of PI in the CD cavity. Binding of an alcohol (e.g. *n*-PrOH) to the anion of α-CD reduces its reactivity toward pNPH, from 120 to 15 M^{-1} s^{-1} (Table 5). Since alkanoate ions inhibit the cleavage of pNPH by α-CD, binding of such anions must reduce the reactivity of the CD anion even more and a contributing factor may well be that the binding of RCO_2^- to α-CD disfavors anion formation. For the cleavage of pNPH by β-CD, the effects of RCO_2^- and RSO_3^- ions are slightly retarding ($k_b < k_2 = 86$ M^{-1} s^{-1}, Table 1) but most alcohols are catalytic ($k_b > k_2$, Table 2). Thus, the binding of 1-propanol to the β-CD anion increases its reactivity 3-fold (Table 5) so that the {*n*-PrOH·β-CDO^-} complex is 110 times more reactive toward pNPH than OH^-.[31]

In the discussion above, we have deliberately ignored the possibility that the effects observed with alcohols are due to their anions, bound to a CD, acting as nucleophiles. Attractive though this is, we have discounted it for three reasons: (i) the generally similar behavior of alkanoate ions and alkanesulfonate ions, particularly for pNPA cleavage;[10] (ii) nucleophilic attack at ester carbonyl groups is sensitive to steric hindrance[13,21] and so attack by an alkoxide ion should be sensitive to the nature of the alcohol (primary, secondary, tertiary, etc.) which is not the case for pNPH (Table 2) or for pNPA;[10b] (iii) for such reactions, the rate constants would be extraordinarily large (up to 6×10^6 M^{-1} s^{-1}) and the CD-bound alkoxide ions would have to be 10^3 to 5×10^4 times as reactive as the free ions (Table 5). So, without any specific evidence to support attack by CD-bound alkoxide ions, we adhere to the belief that alcohols (and other PIs) affect the reaction of *p*-nitrophenyl alkanoates with CDs by modifying the reactivity of the CDs by binding in their cavities.

Conclusions

Not surprisingly, most studies of reactions mediated by CDs have dealt with ones where only one reactive species (substrate or reagent) is bound to the CD host

(27) 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.

(28) (a) Tee, O. S.; Gadosy, T. A.; Giorgi, J. B. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1705. (b) Tee, O. S.; Gadosy, T. A.; Giorgi, J. B. Submitted for publication in *Can J. Chem.*, 1995.

(29) So far, we have been unsuccessful in our attempts to diagnose the orientation of binding using NMR experiments.[10b]

(30) Obviously, in the case of cleavage of *m*-nitrophenyl alkanoates by α-CD, β-CD, and hydroxypropyl-β-CD, binding of the acyl chain of the ester, while it is present in the dominant {ester-CD} complex, does not help the transition state because a more efficient reaction proceeds through binding of the *m*-nitrophenyl group.[8b,c]

(31) The deprotonation of β-keto esters is catalyzed by CDs, and the CD anions are 25−10 000 times more reactive than hydroxide ion, depending on the ester and the CD: Tee, O. S.; Iyenger, N. R.; Takasaki, B. K. *Can. J. Chem.* **1993**, *71*, 2139.

during the reaction.[2,6,12] Only a few reactions have been identified in which two species (substrate and reagent) are included in the CD cavity.[32] The present paper provides data pertinent to a more esoteric point: the binding of a nonreacting partner that can facilitate the reaction of a bound substrate, rather than inhibit it. Such catalysis can arise in two ways: by weakening of the substrate binding or by strengthening the binding of the transition state. For the catalytic effects of alcohols on the cleavage of pNPH by β-CD, where sufficient data is available to evaluate the issue, both factors appear to be operative.

As pointed out already,[10b] our findings may be viewed as primitive models for allosteric effects.[33] Such effects are of two types: allosteric inhibition or allosteric activation. In the first case, binding of the allostere sharply reduces the reactivity of an enzyme toward its substrate, while in the second case, the allostere enhances its reactivity. The present results for the cleavage of pNPH, along with those for that of pNPA,[10] provide examples of both of these extremes and of the range of behavior in between. Seen in this light, the catalysis by alcohols and other potential inhibitors that we have observed may be taken as a simple example of allosteric activation.[33]

Experimental Section

The *p*-nitrophenyl esters were purchased from Sigma. Substrate solutions (10−50 μM) were made up from strong stock solutions in spectral grade methanol. As previously described,[10] the basic medium was an aqueous phosphate buffer solution which was 0.2 M and pH 11.6, after 1:1 mixing in the stopped-flow apparatus. The alcohols, carboxylic acids, and alkanesulfonic acids (or their salts) were of the best grades available from Aldrich, Eastman Kodak, or Sigma. Some secondary alcohols were found to contain peroxides,[10b] and so they were purified by distillation.

Kinetic methods, apparatus, and data analysis were as in the previous studies of pNPA cleavage.[10] With the longest *p*-nitrophenyl esters, which are less soluble in water and which must be used at low concentrations (~10 μM), the quality of the absorbance data is not as high and sometimes it was necessary to estimate A_∞ by the Kezdy−Swinbourne method.[34] Five to ten determinations of the rate constants were made and averaged to give the values of k^{obsd} used in further analysis.

The origins and values of K_I used in the present work are the same as previously described.[10] Values of k_c and K_S were obtained from kinetic data by nonlinear fitting of eq 3 using software based on the Marquardt algorithm,[35] keeping k_u fixed at the observed value. Such values, which are required for the use of eq 7 (see below), are collected in Table 6. The same software was used to fit eq 10 and to obtain k_t and K_t values (Tables 2 and 3).

Table 6. Reference Parameters for the Cleavage of *p*-Nitrophenyl Alkanoates by Cyclodextrins[a]

ester	CD	k_u, s^{-1}	k_c, s^{-1}	K_S, mM	*r*	note
C4	α	0.0461	0.089	4.69	0.9997	*b*
C6	α	0.0467	0.107	3.52	0.9995	*c*
C2	β	0.0773	0.662	7.94	0.9999	*d*
C3	β	0.0793	0.369	5.08	0.9999	*b*
C4	β	0.0457	0.210	2.57	0.9997	*b*
C5	β	0.0466	0.179	1.86	0.9991	*b*
C6	β	0.0451	0.137	1.60	0.9999	*c*

[a] At 25 °C, in a 0.2 M phosphate buffer of pH 11.6. Values of k_c and K_S were estimated by nonlinear fitting of eq 3, with *r* being the correlation coefficient. In earlier work,[9] we used an Eadie−Hofstee approach. [b] Based on original data of Du,[8b,36] measured at pH 11.7, with k_u and k_c scaled for pH 11.6. [c] This work. [d] Reference 10b.

Analysis of k^{obsd} as a function of [PI], by calculating k^{corr} and estimating k_a using eq 7, requires known values of k_u, k_c, and K_S. Because of the pH dependence of k_u and k_c, the effect of pH variations between different experiments must be minimized. This was carried out by adjusting the actual observed rate constants of each experiment to a "master run" for the ester and the CD (Table 6), according to the value of k^{obsd} at [PI] = 0.[10b] For the effective use of eq 7 and of eq 10 for analytical purposes, one needs the actual concentrations of CD and PI; these were calculated as outlined below.

The dissociation constants for the PI·CD complexes are defined as

$$K_I = [PI][CD]/[PI{\cdot}CD]$$

Proper use of this definition requires the free concentrations of CD and PI, which are related through [PI·CD] and the equations for mass balance as follows:[10]

$$[CD] = [CD]_0 - [PI{\cdot}CD] \qquad [PI] = [PI]_0 - ([CD]_0 - [CD]) \quad (18)$$

Substitution of these relationships into the above expression for K_I and expansion leads to a quadratic in [CD] whose solution is

$$[CD] = \{-b + (b^2 + 4K_I[CD]_0)^{1/2}\}/2 \quad (19)$$

where $b = ([PI]_0 - [CD]_0 + K_I)$. For the purposes of data analysis, [CD] was calculated from eq 19 and [PI] was obtained by difference (eq 18).

For reasons given in the main text, we measured the effect of trifluoroethanol on the rate of cleavage of pNPH and found that k^{obsd} increased linearly (r = 0.9996, 6 points), corresponding to $k_2 = 6.23 \pm 0.08\ M^{-1}\ s^{-1}$, in the phosphate buffer at pH 11.6 (Figure 6).

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