The effect of alcohols on the basic cleavage of *m*-nitrophenyl hexanoate by β-cyclodextrin: allosteric reaction mode switching

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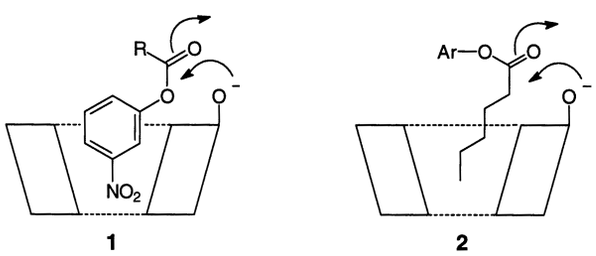
Evidence is presented of a reacting guest–host system where binding of an 'allostere' to the host inhibits its reaction with the guest by one particular mode and promotes its reaction by another. Simple aliphatic alcohols do not slow down the basic cleavage of *m*-nitrophenyl hexanoate by β-cyclodextrin (β-CD) to the extent required for competitive inhibition and so an additional, alcohol-mediated reaction must be taking place. Rate constants for this process correlate well with the ability of the alcohol to bind to β-CD, as do those for the analogous reaction of *p*-nitrophenyl hexanoate, suggesting that the alcohol is in the cavity of β-CD during the reaction. Transition state binding parameters for the alcohol-mediated reaction of the two nitrophenyl esters are very similar, and they show the same dependence on the binding ability of the alcohols. Overall, the results are consistent with a switch in the mode of reaction from cleavage of *m*-nitrophenyl hexanoate by aryl group inclusion (1) to its cleavage by acyl group inclusion (2), brought about by binding of a simple alcohol, acting as an 'allostere'.

In enzymology, the term 'allostery' is used to describe the situation in which the binding of an effector, called an 'allostere', at one site on an enzyme influences the reactivity at another site on the enzyme.[1,2] Usually, the two sites are remote from one another and transmission of the 'allosteric effect' between them is through a conformational change of the enzyme, induced by binding of the allostere. However, there is nothing inherent in the basic idea of allostery that demands this level of complexity: there may be simpler situations in which binding of an effector at a site on a host causes a reaction to take place in a different way at more or less the same site.

In previous studies,[3–5] we have shown that the binding of guests to a cyclodextrin[6] host may alter its reactivity in a particular reaction, rather than simply causing inhibition.[1] The present study was undertaken to identify a reacting system in which the binding of a non-reacting guest to a cyclodextrin host causes a distinct switch from one reaction mode to another, that is, where the binding of an 'allostere' inhibits the normal mode of reaction and promotes reaction by a different mode.

The reaction chosen for scrutiny was the basic cleavage of *m*-nitrophenyl alkanoate esters by β-cyclodextrin.[6] In general, the mode of cleavage of substituted phenyl alkanoates by cyclodextrins (CDs) in aqueous base depends on the aryl substituent, the alkanoate chain and the CD.[7–12] *m*-Nitrophenyl alkanoates react with α-CD, β-CD, and 'hydroxypropyl-β-CD' by way of aryl group inclusion (**1**), even though most of the ester substrates bind by acyl group inclusion.[9,11] In contrast, most *p*-nitrophenyl alkanoates bind and react by acyl group inclusion (**2**).[8,9,11] Reaction of *m*-nitrophenyl ethanoate by mode **1** is subject to competitive inhibition by additives which bind to the CD[3,12,13] but the cleavage of *p*-nitrophenyl ethanoate is not retarded to the same extent in most instances.[3] Moreover, the cleavage of *p*-nitrophenyl hexanoate by mode **2** can be mediated by various additives and it is actually catalysed by alcohols.[4] Taken together, these observations suggested to us that it might be possible to inhibit the cleavage of a *m*-nitrophenyl ester by reaction mode **1**, while promoting its reaction through mode **2**. As reported below, this has been achieved for the cleavage of *m*-nitrophenyl hexanoate by β-CD, using simple aliphatic alcohols as additives.



1 **2**

Background

First, we review how additives normally affect the kinetics of ester cleavage by CDs and what deviations from competitive inhibition have been found previously.[3–5] With varying CD concentration, pseudo-first-order rate constants (k_{obs}) for the reaction generally show saturation kinetics, due to 1:1 binding of the ester to the CD.[6–12] For reaction of the ester (S) in the medium [eqn. (1)] and reaction through an {ester·CD} complex

$$S \xrightarrow{k_u} \text{product} \qquad (1)$$

[eqn. (2)], or its kinetic equivalent,[9,10] the variation of k_{obs} with [CD] is given by eqn. (3), when [CD] ≫ [S].

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

$$k_{obs} = \frac{(k_u K_S + k_c[CD])}{(K_S + [CD])} \qquad (3)$$

In competitive inhibition,[1] an additive (PI) that binds to the CD [eqn. (4)] lowers the concentration of free CD and k_{obs} is reduced in accordance with eqn. (3) (when $k_c > k_u$). Such behaviour is observed for the cleavage of *m*-nitrophenyl ethanoate (*m*NPA) by α-CD, β-CD, and 'hydroxypropyl-β-CD', and variations of k_{obs} with $[PI]_0$, at fixed $[CD]_0$, can be analysed to estimate dissociation constants of {PI·CD} complexes.[3,12,13]

$$PI + CD \underset{K_I}{\rightleftharpoons} PI{\cdot}CD \qquad (4)$$

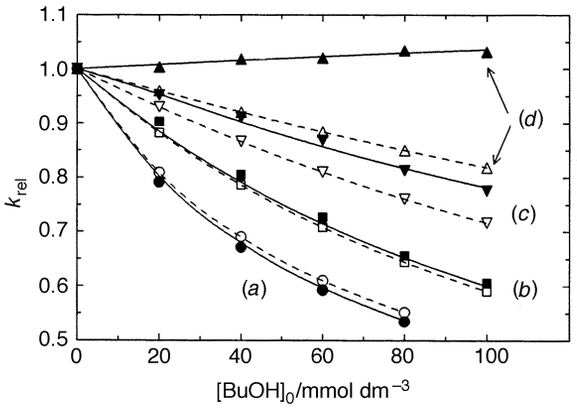


Fig. 1 Rate constants for the effects of butanol on the cleavage of *m*-nitrophenyl alkanoates in the presence of β-cyclodextrin (1.0 mmol dm^{-3} for *m*NPA; 10 mmol dm^{-3} for the others). For ease of comparison, they are presented as k_{rel}, relative to the rate constant at zero butanol. The solid symbols are the observed data and the solid lines through them are calculated for the model given in the text [eqn. (6)]. The open symbols are points calculated for strict, competitive inhibition. The symbols for the esters are: (*a*) ethanoate ●, ○; (*b*) propanoate, ■, □; (*c*) butanoate, ▼, ▽; (*d*) hexanoate, ▲, △.

Many 'potential inhibitors' (PIs) that inhibit the cleavage of *m*NPA by CDs do not retard the cleavage of *p*-nitrophenyl ethanoate (*p*NPA) to the same extent.[3,5b] This latter behaviour is accounted for by a simple process involving one molecule of the PI [eqn. (5)] which counterbalances the effects of competitive

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

inhibition.[3] With this process operative, eqn. (3) must be expanded to eqn. (6) but to facilitate analysis it is converted to

$$k_{obs} = \frac{(k_u K_S + k_c[CD] + k_a[PI][CD])}{(K_S + [CD])} \quad (6)$$

the linear form in eqn. (7). According to eqn. (7), the slope of

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

k_{corr} against [PI] affords k_a, the rate constant for the PI-mediated process.[3–5]

Results

We have studied the rates of cleavage of some *m*-nitrophenyl alkanoates by β-CD at high pH, and in the presence of aliphatic alcohols. After initial studies with the C_2–C_6 esters and butanol, the remaining work was carried out with *m*-nitrophenyl hexanoate (*m*NPH) and various alcohols, as well as a series of experiments with α-CD.

In the presence of butanol, the reaction of *m*-nitrophenyl ethanoate with β-CD shows inhibition [Fig. 1(*a*)], and analysis[3] of the variation of k_{obs} with $[BuOH]_0$ in two quite different experiments† gave K_I = 55 and 62 mmol dm^{-3}, very close to the literature value of 60 mmol dm^{-3}, given as pK_I = 1.22.[14] With the propanoate ester, the values of k_{obs} are marginally above those expected for inhibition [Fig. 1(*b*)] but for reaction of *m*-nitrophenyl butanoate they are distinctly greater than expected [Fig. 1(*c*)]. This trend is continued with the pentanoate ester, and the cleavage of *m*-nitrophenyl hexanoate (*m*NPH) is actually accelerated, albeit modestly, by the addition of butanol [Fig. 1(*d*)].

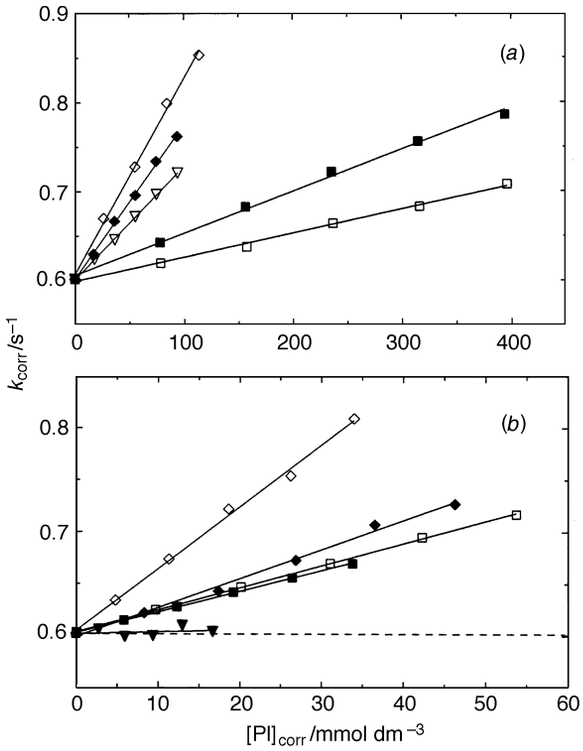


Fig. 2 Examples of data for the effects of alcohols on the cleavage of *m*-nitrophenyl hexanoate by β-cyclodextrin, plotted in accordance with eqn. (7). The concentrations of the alcohol and of β-CD were corrected for binding. In part (*a*) the symbols are: PrOH, ■; Pr^{i}OH, □; BuOH, ◆; Bu^{i}OH, ◇; 2-BuOH, ▽. In part (*b*) the symbols are: 2-PenOH, □; PenOH, ◆; Pen^{i}OH, ◇; Bu^{t}OH, ■; cycloPenOH, ▼. The behaviour of cyclopentanol is not distinguishable from competitive inhibition, which corresponds to the horizontal dashed line of zero slope (k_a = 0).

Because the cleavage of *m*NPH showed the largest effect with butanol,‡ this ester was subjected to further studies with 13 other alcohols. Analysis of the variation of k_{obs} with $[alcohol]_0$, in terms of eqn. (7) generally worked well, as shown in Fig. 2 which includes the data for butanol corresponding to Fig. 1(*d*). From the slopes of the plots of k_{corr} *vs.* [PI], the values of k_a collected in Table 1 were obtained. Two of the alcohols studied, cyclopentanol and cyclohexanol, showed only inhibition of the cleavage of *m*NPH, whereas they mediate the reaction of *p*NPH.[4] This is not necessarily a contradiction. All it may mean is that it is not possible to get enough of these two alcohols into solution to elicit a well-defined increase in k_{corr} [eqn. (7)].

Fig. 3 shows log k_a for the cleavage of *m*NPH and *p*NPH by β-CD in the presence of alcohols, plotted against pK_I values[7,14] for the binding of the alcohols to β-CD. Clearly, the plots for the two esters are essentially parallel, and analysis shows that the slopes for *m*NPH and *p*NPH are 0.72 ± 0.05 and 0.75 ± 0.03, respectively. Moreover, for the 12 alcohols they have in common, the two sets of data are correlated (r = 0.965) and the plot of log k_a for *m*NPH *vs.* log k_a for *p*NPH has a slope of 0.95 ± 0.08. Thus, the sensitivity of the alcohol-mediated process [eqn. (5)] to the structure of the alcohols is exactly the same for *m*NPH as for *p*NPH.

We have also carried out experiments on the cleavage of *m*NPH by α-CD, which has a smaller cavity than β-CD.[6] In the

† The data shown in Fig. 1(*a*), obtained with $[\beta\text{-CD}]_0$ = 1 mmol dm^{-3} and $[BuOH]_0$ = 0–80 mmol dm^{-3}, gave K_I = 55 ± 1 mmol dm^{-3}. Another experiment with $[\beta\text{-CD}]_0$ = 15 mmol dm^{-3} and $[BuOH]_0$ = 0–300 mmol dm^{-3}, gave K_I = 62 ± 4 mmol dm^{-3}. (Experiments by Mr J. J. Hoeven[3] and Dr T. A. Gadosy, respectively.)

‡ Unfortunately, experiments with *m*-nitrophenyl heptanoate and octanoate were not practical because of the low solubility of these esters in water.

Table 1 Constants for the alcohol-mediated cleavage of *m*-nitrophenyl hexanoate in the presence of β-cyclodextrin [a]

ROH	$[ROH]_0$	pK_I	k_a/dm^3 mol^{-1} s^{-1}	k_b/dm^3 mol^{-1} s^{-1}	K_{TS}'/mmol dm^{-3}	pK_{TS}'
PrOH	0–400	0.57	0.470 ± 0.014	71	0.33	3.49
Pr^{i}OH	0–400 [b]	0.58	0.269 ± 0.009	40	0.58	3.24
2-BuOH	0–100	1.19	1.27 ± 0.01	46	0.50	3.30
BuOH	0–100	1.22	1.73 ± 0.04	59	0.39	3.40
2-PenOH	0–60	1.49	2.13 ± 0.03	39	0.60	3.22
Bu^{i}OH	0–150 [b]	1.62	2.20 ± 0.07	30	0.78	3.11
Bu^{t}OH	0–40	1.68	1.99 ± 0.02	24	0.99	3.01
PenOH	0–50 [c]	1.80	2.79 ± 0.11	25	0.93	3.03
2-HexOH	0–10	1.98	4.73 ± 0.14	28	0.83	3.08
cycloPenOH	0–20 [c]	2.08	— [d]			
Pen^{i}OH	0–40 [b]	2.25	5.95 ± 0.17	19	1.28	2.91
HexOH	0–10 [c]	2.34	5.97 ± 0.86	15	1.19	2.82
cycloHexOH	0–5.0 [c]	2.70	— [d]			
Pen^{neo}OH	0–5.0	2.76	18.3 ± 1.5 [e]	18	1.29	2.89
2-HexOH^f	0–10	1.98	12.3 ± 0.4 [f]	80	0.56	3.25
Pen^{i}OH^f	0–40 [b]	2.25	25.8 ± 0.4 [f]	91	0.50	3.30

[a] At 25 °C, in a phosphate buffer (0.2 mol dm^{-3}) of pH 11.6, with $[\beta\text{-CD}]_0$ = 10 mmol dm^{-3}, except where noted otherwise. $[ROH]_0$ shows the range of concentrations (usually six) used in each experiment (*cf.* Fig. 2). The pK_I values are taken from the literature.[7,14] Values of k_a were obtained as the slopes of the linear plots of k_{corr} *vs.* [ROH], based on eqn. (7). Most of the data are presented in Figs. 2(*a*) and 2(*b*). Values of k_b are calculated from k_aK_I/K_S and $K_{TS}' = k_u/k_b$ (see text). [b] $[\beta\text{-CD}]_0$ was 7.0 mmol dm^{-3}. [c] $[\beta\text{-CD}]_0$ was 5.0 mmol dm^{-3}. [d] The results obtained were not distinguishable from competitive inhibition—see Table 2. [e] Another experiment with $[ROH]_0$ = 0–50 mmol dm^{-3} and $[\beta\text{-CD}]_0$ = 2.0 mmol dm^{-3} gave k_a = 21.1 ± 2.5 dm^3 mol^{-1} s^{-1}. [f] Experiment with *p*NPH, with data treated in the manner described in ref. 4(*b*).

Table 2 Effects of alcohols on kinetics of ester cleavage by cyclodextrins, analysed for inhibition [a]

ROH	[ROH]/mmol dm^{-3}	Ester	CD [b]	K_I/mmol dm^{-3}	pK_I	K_I (lit.) [c]/mmol dm^{-3}	pK_I (lit.) [c]
EtOH	100–500	*m*NPH	α	199 ± 4	0.70	178	0.75
PrOH	80–400	*m*NPH	α	42.2 ± 2.5	1.35	42.7	1.37
BuOH	20–100	*m*NPH	α	11.3 ± 0.9	1.95	11.2	1.95
PenOH	10–50	*m*NPH	α	3.41 ± 0.28	2.47	3.09	2.51
HexOH	2.0–10.0	*m*NPH	α	1.08 ± 0.03	2.97	1.12	2.95
BuOH	20–80 [d]	*m*NPA	β	54.7 ± 0.8 [d]	1.26	60.3	1.22
BuOH	60–300 [e]	*m*NPA	β	62.4 ± 4.1 [e]	1.21	60.3	1.22
BuOH	20–100	*m*NPPr [f]	β	67.7 ± 4.9	1.17	60.3	1.22
BuOH	20–100	*m*NPBu [g]	β	90.8 ± 6.7 [g]	1.04	60.3	1.22
cycloPenOH	4.0–20 [h]	*m*NPH	β	8.48 ± 0.49	2.07	8.32	2.08
cycloHexOH	1.0–5.0 [h]	*m*NPH	β	2.04 ± 0.43	2.73	2.00	2.70

[a] At 25 °C, in a phosphate buffer (0.2 mol dm^{-3}) of pH 11.6. The entry under $[ROH]_0$ indicates the concentration range (five points, plus a point at zero ROH) used in the inhibition analysis.[3] [b] Experiments were carried out with $[\alpha\text{-CD}]_0$ = 5.0 mmol dm^{-3} or $[\beta\text{-CD}]_0$ = 10 mmol dm^{-3}, except where noted otherwise. [c] Values of K_I are calculated from pK_I values determined by Matsui *et al.*[7,14] Similar values have been reported by other workers.[15] [d] $[\beta\text{-CD}]_0$ = 1.0 mmol dm^{-3}.† [e] $[\beta\text{-CD}]_0$ = 15 mmol dm^{-3}. [f] *m*NPPr = *m*-nitrophenyl propanoate. Its behaviour may be close to the boundary between competitive inhibition and the onset of the alcohol-mediated process [eqn. (5)]. [g] *m*NPBu = *m*-nitrophenyl butanoate. The apparent inhibition constant 'K_I' is high due to the intrusion of the alcohol-mediated process. Analogous data for the pentanoate and hexanoate cannot be analysed for inhibition because k_{obs} increases with added BuOH [*e.g.* Fig. 1(*d*)]. [h] $[\beta\text{-CD}]_0$ was 5.0 mmol dm^{-3}.

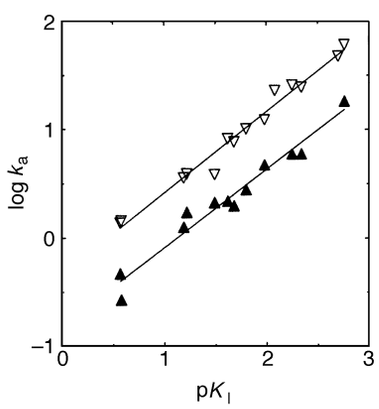


Fig. 3 Correlation of the rate constants (k_a) for the alcohol-mediated process in eqn. (5) with the strength of binding of the alcohol to β-cyclodextrin (pK_I). The two sets of data are shown for *m*NPH (▲), from this work, and for pNPH (▽), from previous work,[4b] supplemented by two additional points (Table 1).

presence of five linear alcohols the behaviour observed was not distinguishable from competitive inhibition and analysis of the data gave K_I values in good agreement with literature values.[7,14,15] These are shown in Table 2, along with the results from other experiments where inhibition analysis was performed.

Discussion

Allosteric effects, which are of great importance in metabolic regulation, can be of two basic types.[1–2] Depending on the system, the effect may be inhibitory, and turn off a behaviour, or it may be activating, and turn on a behaviour. We believe we have uncovered a model system that shows both types of effect, in that one type of behaviour is inhibited and another is promoted.

The basic cleavage of *m*-nitrophenyl ethanoate by β-CD is inhibited by butanol, and that of the analogous propanoate is hardly different. However, inhibition of the reaction of the butanoate is much less than expected, and cleavage of the hexanoate (*m*NPH) is not inhibited by butanol at all—it is slightly accelerated [Fig. 1(*d*)]. Thus, as the acyl chain length of the *m*-nitrophenyl ester is lengthened from C_2 to C_6, inhibition of ester cleavage through aryl group inclusion (mode **1**) gradually gives way to another process that is mediated by the alco-

hol.§ We believe that this process is cleavage by acyl inclusion (mode **2**) since reaction of the *p*-nitrophenyl isomers by this mode becomes more and more favourable as the acyl chain is lengthened[8,9,11] and reaction of *p*NPH by this mode can be catalysed by aliphatic alcohols.[4] In support of this belief, we have the results for the effects of 12 simple alcohols on the cleavage of *m*NPH by β-CD (Table 1). The kinetic data conform well to eqn. (7) (Fig. 2), meaning they are consistent with an alcohol-mediated process [eqn. (5)]. Moreover, the rate constants for this process show a strong parallel with analogous results for the cleavage of *p*NPH (Fig. 3).

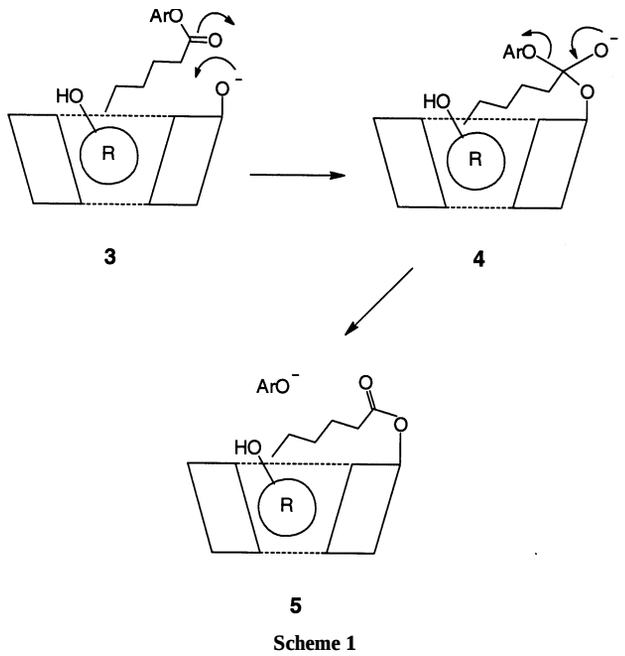
Relative reactivities (k_a and k_b)

Rate constants (k_a) for the reaction of the {*m*NPH·β-CD} complex with alcohols (Table 1) increase systematically with the strength of binding in {ROH·β-CD} complexes and there is a good correlation ($r = 0.980$) of log k_a with pK_I, with a slope near one (0.72 ± 0.05). Most significantly, this slope is the same as that for cleavage of *p*NPH by the same pathway, under the same conditions (0.75 ± 0.03) (Fig. 3). In both cases, the fact that the slope is close to one is good evidence that the alcohol is largely in the CD cavity during the ROH-mediated process, suggesting that the reaction is better viewed as being between the {ROH·CD} complex and the ester [eqn. (8)].¶

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

Rate constants for the process in eqn. (8) show relatively little variation with structure (Table 1), after binding of the alcohol to β-CD is accounted for. A plot of log k_b against pK_I has a shallow slope of −0.28 for *m*NPH, and −0.25 for *p*NPH, indicating a gradual decline in reactivity as the alcohol in the {ROH·β-CD} complex becomes larger. This behaviour is quite reasonable for reaction between an ester and β-CD with a molecule of an alcohol in its cavity (**3** ⟶ **4** ⟶ **5**, Scheme 1) if the reaction is made less facile by a larger, bulkier alcohol. The fact that mediation by cyclopentanol and cyclohexanol was not observed (Tables 1 and 2) may simply mean that these alcohols bind in the β-CD cavity in such a way that the reaction **3** ⟶ **5** is very difficult or impossible. Likewise, the absence of an alcohol-mediated process for the reaction of *m*NPH with α-CD (Table 2) suggests that the geometries of {ROH·α-CD} complexes are much less suitable for the reaction, which is not unreasonable.‖ In the case of *p*NPH, reaction with {ROH·α-CD} complexes was detected but it is 14–50 times slower than the reaction with {ROH·β-CD} complexes.[4b] If the reactivity of *m*NPH with {ROH·α-CD} complexes is comparable, it would not be detectable against the background of the very efficient cleavage of *m*NPH by α-CD, reacting by aryl inclusion.

For the reaction of *p*NPH with {alcohol·β-CD} complexes, values of k_b vary from 60 to 240 dm^3 mol^{-1} s^{-1}, compared to $k_2 = 86$ dm^3 mol^{-1} s^{-1} for the reaction of *p*NPH with β-CD on its own.[4b] Thus, the presence of a simple alcohol in the cavity of β-CD modestly increases or decreases its reactivity towards *p*NPH. In contrast, the k_b values for reaction of *m*NPH with {ROH·β-CD} complexes, which vary from 15 to 70 dm^3 mol^{-1} s^{-1} (Table 1), are appreciably smaller than $k_2 = 340$ dm^3 mol^{-1} s^{-1} for the reaction of *m*NPH with β-CD, meaning that a simple alcohol in the cavity of β-CD significantly reduces its reactivity towards this ester. This difference arises mainly because the reaction of *m*NPH with β-CD by aryl inclusion (mode **1**) is much more efficient, for geometric reasons,[7,10] whereas *p*NPH reacts less readily through mode **2**,[8,9,11a] with or without a PI in the cavity of β-CD.[4] Also, it seems that reaction of *m*NPH with {ROH·β-CD} complexes is approximately three times slower than that of *p*NPH.



Scheme 1

Transition state binding (pK_{TS})

The best way of probing the mechanism of a reaction is to look at kinetic parameters that directly reflect the transition state structure but are independent of any prior choice of a mechanism.[10] In the present context, one may consider the variations with alcohol structure of the third-order rate constants for the reaction between the ester, β-CD and the alcohol; these are given by $k_3 = k_a/K_S$ or k_b/K_I [eqns. (5) or (8)]. Since values of log k_a correlate well with pK_I for the alcohols (Fig. 3) and K_S is constant for each ester, the values of log k_3 for *m*NPH and *p*NPH also correlate with pK_I, and with slopes of 0.72 and 0.75, respectively. Again, these slopes approaching one afford evidence of significant inclusion of ROH in the β-CD cavity during the ROH-mediated reactions, in keeping with the mechanism **3** ⟶ **4** ⟶ **5** (Scheme 1), and along the lines of eqn. (8).

In our view, the best parameter for probing the binding of transition states to catalysts is a quasi-equilibrium constant that provides a measure of the stabilisation of the transition state by the catalyst.[10,16,17] For the cleavage of an ester by a CD this constant is given by: $K_{TS} = [\mathrm{CD}][\mathrm{TS}]/[\mathrm{CD{\cdot}TS}] = k_u/k_2 = k_u K_S/k_c$, where K_{TS} is the apparent constant for dissociation of the transition state containing the cyclodextrin (symbolised by CD·TS) into the CD and the normal transition state (TS). Variations of K_{TS} (and p$K_{TS} = -\log K_{TS}$) with structure have been used as probes of transition state binding by CDs in ester cleavage[5a,9–11] (and many other reactions).[10,16,17] For the efficient cleavage of *m*NPH by β-CD, reacting through aryl group inclusion (**1**), p$K_{TS} = 4.17$, which is appreciably higher than p$K_{TS} = 3.28$ for the less efficient cleavage of *p*NPH, proceeding by acyl inclusion (**2**).[8–10] Presumably, if the cleavage of *m*NPH by β-CD were to occur with acyl inclusion it would have a similar value of pK_{TS} near 3.

For ester cleavage by a CD that is mediated by a PI, we

§ The ROH-mediated process does not arise from reaction between the ester and CD-bound alkoxide ion, present in very low concentration. As discussed for *p*NPH,[4b] rate constants for this process would have to be extraordinarily large to account for the observed data and they should show an appreciable sensitivity to the type of alcohol (primary, secondary, tertiary) that is not apparent. Also, other much less nucleophilic anions (RCO_2^- and RSO_3^-) mediate the cleavage of *p*NPH.[4]

¶ Since the third-order processes in eqns. (5) and (8) are kinetically equivalent, $k_3 = k_a/K_S = k_b/K_I$, and values of k_b are obtainable from $k_a K_I/K_S$.

‖ Because α-CD has a narrower cavity than β-CD,[6] the {ROH·α-CD} complexes are probably tighter (more rigid) and therefore less able to accommodate the reaction **3** ⟶ **4** ⟶ **5**.

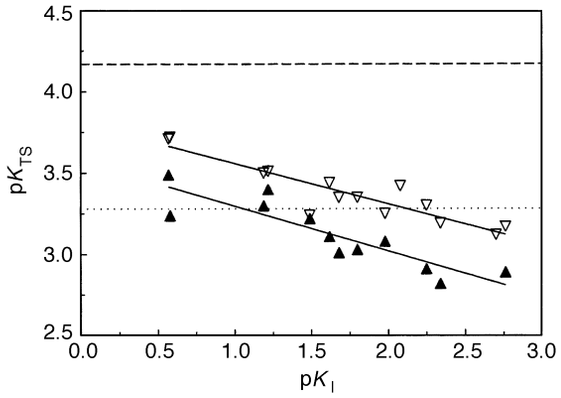


Fig. 4 Correlation of transition state binding (pK_{TS}') with alcohol binding (pK_I) for alcohols which mediate the cleavage of *m*- and *p*-nitrophenyl hexanoate by β-cyclodextrin. The symbols are: *m*NPH, ▲; *p*NPH, ▽. The slopes of the lines are −0.28 for *m*NPH and −0.25 for *p*NPH. The horizontal dashed line at $pK_{TS} = 4.17$ is dissociation of the transition state for *m*NPH reacting with β-CD alone by aryl inclusion (mode **1**). The dotted line at $pK_{TS} = 3.28$ is for *p*NPH reacting with β-CD alone by acyl inclusion (mode **2**).

can use the transition state parameter $K_{TS}' = [PI{\cdot}CD][TS]/[PI{\cdot}CD{\cdot}TS] = k_u/k_b$, defined so as to correspond to the apparent dissociation of the termolecular transition state {PI·CD·TS} into the complex {PI·CD} and TS.[4] Values of K_{TS}' (and pK_{TS}') for the cleavage of *m*NPH by β-CD in the presence of aliphatic alcohols are given in Table 1, along with two new values for *p*NPH; other values for *p*NPH were obtained in previous work.[4]

Fig. 4 shows the values of pK_{TS}' for the alcohol-mediated cleavage of *m*NPH and *p*NPH by β-CD, plotted against pK_I for alcohol binding to β-CD. In the case of *p*NPH, pK_{TS}' values range from 3.12 to 3.72, around the value of $pK_{TS} = 3.28$ for the cleavage of *p*NPH by β-CD with acyl inclusion (mode **2**); they are consistent with the reaction occurring with alcohol binding (**3**). In contrast, the pK_{TS}' values for the ROH-mediated cleavage of *m*NPH, which fall between 2.82 and 3.49, are about one p*K* unit lower than $pK_{TS} = 4.17$ for *m*NPH reacting with β-CD by aryl group inclusion (mode **1**) but they are very close to those for *p*NPH reacting by mode **2**. Thus, the addition of simple aliphatic alcohols suppresses the cleavage of *m*NPH by mode **1**, and pK_{TS}' values drop to a range that is appropriate for ester cleavage taking place with alcohol binding (**3**), as in Scheme 1.

Finally, we note that the slopes of the plots of pK_{TS}' *vs.* pK_I for *m*NPH and *p*NPH in Fig. 4 are −0.28 and −0.25, respectively, as they are for log k_b *vs.* pK_I (*vide supra*). Thus, the transition state stabilisation for cleavage by β-CD with an alcohol in its cavity gradually diminishes as the alcohol becomes larger, which is perfectly reasonable for the mechanism shown in Scheme 1.

Conclusions

The results and analysis presented above show a reacting guest–host system where binding of an additive (an 'allostere') to the host inhibits reaction by the mode that is normally favoured and promotes reaction by a different mode. Thus, binding of the allostere brings about a switch from one reaction mode to another (from **1** to **3**). As far as we are aware, these results have no obvious enzymological analogue but they do provide an example of a particular type of allosteric effect.

Experimental

The *m*-nitrophenyl esters were synthesised in previous work.[9,11] *p*-Nitrophenyl hexanoate was obtained from Sigma and β-CD and the various alcohols from Aldrich. All reagents were of the best grades available. Reactions were carried out in water that had been doubly distilled from glass.

Table 3 Reference parameters for the cleavage of nitrophenyl alkanoates by cyclodextrins, used in data analysis[a]

Ester	CD	k_u/s^{-1}	k_c/s^{-1}	K_S/mmol dm^{-3}
*m*NPA[b]	β	0.0858	6.14	15.5
*m*NPPr[c]	β	0.0648	4.11	8.82
*m*NPBu[d]	β	0.0274	0.903	3.53
*m*NPPen[d]	β	0.0284	0.658	2.37
*m*NPH[d]	α	0.0238	1.96	3.49
*m*NPH[d]	β	0.0232	0.602	1.77
*p*NPH[e]	β	0.0451	0.137	1.60

[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 non-linear fitting of eqn. (3), whereas in earlier work[9] an Eadie–Hofstee approach was used. [b] Ref. 3(*b*). [c] Determined in this work. [d] Obtained by non-linear fitting of eqn. (3) to the original data of Du.[9,18] [e] Ref. 4(*b*).

Ester cleavage was initiated by 1:1 stopped-flow mixing of a dilute solution of the ester and a basic phosphate buffer (0.4 mol dm^{-3}, pH 11.6), containing the CD. When required, the alcohol was introduced in the ester solution, where it assists in solubilisation. For high concentrations of β-CD (>7 mmol dm^{-3}), the CD was present in both reactant solutions because it is not very soluble in water.[6] After mixing, the phosphate buffer concentration was reduced to 0.2 mol dm^{-3} and ester concentrations were in the range of 35 μmol dm^{-3} (for *m*NPH and *p*NPH) to 100 μmol dm^{-3} (for *m*NPA), according to solubility. The ranges of $[ROH]_0$ used in experiments, which are given in the Tables 1 and 2, were governed partly by the alcohol solubility.

Reactions were monitored by the increase in the absorption of the nitrophenoxide ion at 390 nm (*meta*) or 405 nm (*para*), using a stopped-flow spectrophotometer with its observation cell kept at 25.0 ± 0.1 °C. Initial experiments were carried out with a Tri-Tech Dynamics Instrument interfaced to a microcomputer[11a] and the later ones with an Applied Photophysics SX17MV stopped-flow spectrophotometer.[11b] Observed pseudo-first-order rate constants (k_{obs}), averaged over 5 to 12 determinations, were obtained from these two systems as detailed previously.[11]

The experiments with alcohols as potential inhibitors were conducted in the same way as in earlier studies,[3,4,11a] with $[\beta\text{-CD}]_0 = 1.0$–10.0 mmol dm^{-3}, as detailed in Tables 1 and 2. Values of k_{obs} were determined over a range of $[ROH]_0$ and k_a was estimated from the variation of k_{corr} with [ROH], according to eqn. (7). For this analysis, one must use [CD] and [ROH] which have been corrected for binding between the CD and ROH. These concentrations were calculated by solving the appropriate quadratic in CD, using a known K_I, as described in detail previously.[4] Values of K_I for the alcohols were calculated from pK_I values given by Matsui and co-workers.[7,14] Analysis of results for competitive inhibition (Table 2) was carried out with the approach introduced earlier.[3]

Both the analysis based on eqn. (7), and the analysis for inhibition, require known values of k_u, k_c and K_S for the particular ester. Because of the pH dependence of k_u and k_c (but not K_S), the effects of pH variations between different experiments must be minimised. As previously,[3–5] this was achieved by scaling the actual observed rate constants of each experiment to a reference or 'master run' for the ester and the CD (in Table 3), according to the value of k_{obs} in the buffer (+CD), and in the absence of the PI. The requisite constants k_c and K_S were estimated by non-linear least-squares fitting of eqn. (3) to k_{obs} values obtained for $[CD]_0 = 0$–10 mmol dm^{-3}, as presented in Table 3.

The data for the effects of alcohols on the cleavage of *p*NPH by β-CD, used for comparison with *m*NPH (Figs. 3 and 4), were taken from earlier work,[4] supplemented by two additional

points for isopentyl alcohol and hexan-2-ol, determined in this work (last two entries in Table 1).

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