Retardation of acetal hydrolysis by cyclodextrins and its use in probing cyclodextrin–guest binding

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Hydrolysis of benzaldehyde dimethyl acetal 1 in aqueous acid is slowed down greatly by cyclodextrins (CDs): α-CD, β-CD, hp-β-CD (hydroxypropyl-β-cyclodextrin) and γ-CD. The variations of the observed first-order rate constants (k_{obs}) with [CD] exhibit saturation behaviour, consistent with 1 : 1 binding between 1 and the CDs. In the case of β-CD and hp-β-CD, the binding is relatively strong and the CD-bound acetal is unreactive. In contrast, binding of the acetal by α-CD and γ-CD is much weaker, but only with α-CD does the CD-bound form show significant reactivity. The four CD-mediated reactions have been evaluated as probe reactions for determining dissociation constants of {CD– 'guest'} complexes. In this approach, added guests attenuate the retarding effect of CD–substrate binding and cause an increase in the rate of acetal hydrolysis. The method works well for aliphatic alcohols and ketones binding to β-CD and hp-β-CD, but it is less successful with α-CD because of the shallow dependence of k_{obs} on [α-CD] in the probe reaction. With γ-CD, the approach is not applicable at all, because added guests cause a further reduction in the rate of acetal hydrolysis, not an increase. Various implications of these findings are discussed.

Introduction

The influence of cyclodextrin (CD) hosts[1] on reactions of organic guests has been widely studied.[2,3] The vast majority of such studies have been carried out in neutral or basic aqueous solution, most probably because CDs, being glycosides, are presumed to be labile in aqueous acid media. In fact, cyclodextrins are reasonably robust, and moderately strong acids (or elevated temperatures) are required to destroy them quickly.[1] Thus, the effects of CDs on reactions which are fast in dilute acid, such as the bromination of activated aromatics,[4] can be studied without the CDs undergoing appreciable degradation.

The hydrolysis of simple acetals proceeds rapidly in aqueous acid.[5,6] As exemplified in eqns. (1*a*) and (1*b*), the reaction has

$$PhCH(OMe)_2 + H_2O \xrightarrow{H^+} PhCH(OH)OMe + MeOH \quad (1a)$$

$$PhCH(OH)OMe \xrightarrow[\text{or base}]{\text{acid}} PhCHO + MeOH \quad (1b)$$

two major steps: first, the acetal undergoes acid-catalysed conversion to the hemiacetal; second, there is elimination of a molecule of alcohol from the hemiacetal, facilitated by acid or base catalysis.[6] In the case of benzaldehyde dimethyl acetal, $PhCH(OMe)_2$ **1**, the second step is faster, as discussed later.

We have studied the hydrolysis of acetal **1** in the presence of four cyclodextrins, with three main objectives in mind. First, we wished to see whether the CDs catalyse or retard the reaction. Second, how are the CD-mediated reactions affected by the presence of other species (guests) that bind to the CD hosts? Are the reactions inhibited, or influenced in other ways? Third, can the effects of added guests on the CD-mediated acetal hydrolysis be used for estimating dissociation constants (K_G) of {CD–guest} complexes formed in acidic solution, using 'inhibition kinetics'? Most previous studies making use of inhibition kinetics to estimate K_G values have been carried out in basic solution.[7–9] Also, we were particularly eager to find a quick and convenient method for determining K_G values for the binding of aliphatic guests to γ-CD[1] because relatively few such values are available in the literature, at the present.

Results

The hydrolysis of benzaldehyde dimethyl acetal **1** was followed by monitoring the appearance of benzaldehyde, using stopped-flow UV spectrophotometry. In 0.10 M aqueous HCl, the reaction has a half-life of *ca.* 0.2 s and it is essentially complete in 2 s. At short times (<100 ms) the absorbance trace shows a distinct induction period which is indicative of the two-step nature of the reaction.[5,6] Furthermore, a trace (average of 5) covering the first 0.5 s was accurately described by the sum of two exponentials[10] and rate constants of 3.67 ± 0.02 s^{-1} and 75.5 ± 4.4 s^{-1} for the two steps of the reaction. On the basis of previous results for the hydrolysis of $PhCH(OMe)_2$ and related acetals,[6] the larger rate constant is ascribed to decomposition of the hemiacetal [eqn. 1(*b*)]. Thus, for the hydrolysis of **1** in dilute HCl, the first step [eqn. 1(*a*)] is largely rate-limiting, and most of the absorbance increase can be treated as a single exponential due to this step. From absorbance data collected over 2 s, non-linear analysis of the final 90% of the absorbance trace gave a rate constant of 3.55 ± 0.01 s^{-1} for hemiacetal formation. In what follows, the observed rate constants (k_{obs}) were all obtained in this manner and so they also refer to the first step (acetal ⟶ hemiacetal).

Effects of cyclodextrins

As shown in Fig. 1, all four cyclodextrins [α-CD, β-CD, hp-β-CD (hydroxypropyl-β-CD) and γ-CD][1] retard the hydrolysis of **1**, significantly. However, the effects are more pronounced with β-CD and hp-β-CD than with α-CD or γ-CD because there is stronger substrate binding by the first two CDs (*vide infra*). In all four cases, the decrease of k_{obs} with [CD] is consistent with simple, saturation kinetics, arising from 1 : 1 binding between the acetal and the CD, as shown by the fitted curves in Fig. 1. These curves correspond to the following model: reaction of the free substrate (S) in the medium [eqn. (2)] and reaction through

$$S \xrightarrow{k_u} \text{products} \quad (2)$$

a complex (S–CD) [eqn. (3)]. The variation of k_{obs} with [CD] is

Table 1 Constants for the effects of cyclodextrins on the hydrolysis of benzaldehyde dimethyl acetal, **1** [a]

Cyclodextrin	k_u/s^{-1}	K_S/mM	k_c/s^{-1}	$r(n)$
α-CD	3.70 ± 0.01	46.5 ± 3.1	0.588 ± 0.118	0.9990 (21)
β-CD	3.62 ± 0.02	2.26 ± 0.05	0 [b]	0.9992 (12)
hp-β-CD	3.60 ± 0.01	3.64 ± 0.03	0 [c]	0.9999 (12)
γ-CD	3.66 ± 0.01	51.3 ± 0.4	0 [d]	0.9997 (19)

[a] In 0.10 M aqueous HCl, at 25 °C. Values of k_u, K_S and k_c were obtained by non-linear fitting of eqn. (4); the errors cited are standard errors and r is the correlation coefficient. In each case, the data set used was derived from two or three distinct experiments, hence the large number of points (n). The data and fitted curves are shown in Fig. 1. [b] Value fixed at zero. With k_c as a parameter, fitting gave $k_c = -0.005 \pm 0.155$ s^{-1}, along with $K_S = 2.27 \pm 0.03$ mM ($r = 0.9992$). [c] Value fixed at zero. With k_c as a parameter, fitting gave $k_c = -0.0233 \pm 0.0303$ s^{-1}, along with $K_S = 3.70 \pm 0.09$ mM ($r = 0.9999$). [d] Value fixed at zero. With k_c as a parameter, fitting gave $k_c = 0.0567 \pm 0.0866$ s^{-1}, along with $K_S = 49.9 \pm 2.1$ mM ($r = 0.9997$).

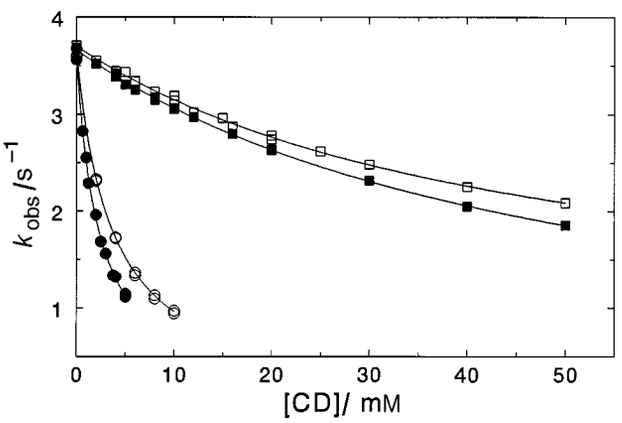


Fig. 1 Effects of cyclodextrins on the hydrolysis of 50 μM benzaldehyde dimethyl acetal **1** in 0.10 M aqueous HCl: β-CD, ●; hp-β-CD, ○; α-CD, □; γ-CD, ■. The curves were calculated with eqn. (4) and the fitted parameters given in Table 1.

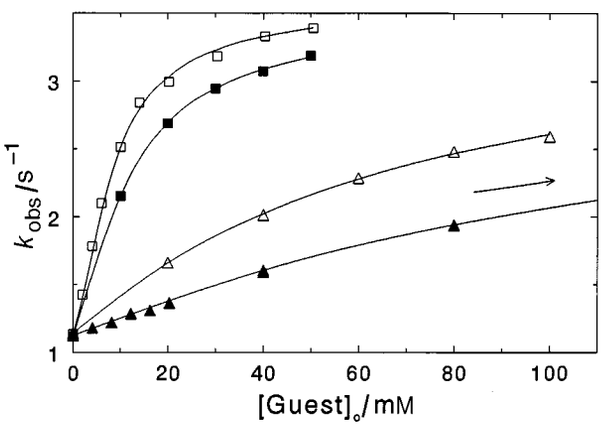


Fig. 2 Examples of the effects of guests on the hydrolysis of acetal **1** retarded by the presence of 5.0 mM β-CD with: cyclohexanol, □; cyclohexanone, ■; 2-methylpropan-2-ol (Bu^{t}OH), △; butan-1-ol, ▲ (the data extend out to 200 mM). The curves are calculated with the appropriate K_G values in Table 2. Comparable results were obtained for other guests and for hp-β-CD.

$$S + CD \rightleftharpoons S\text{–}CD \xrightarrow{k_c} \text{products} \qquad (3)$$

given by eqn. (4),[2,3] assuming that [CD]≫ [S–CD] < $[S]_o$, which was valid for all experiments.

$$k_{obs} = \frac{(k_u K_s + k_c[CD])}{(K_s + [CD])} \qquad (4)$$

Eqn. (4) is normally associated with reactions where k_{obs} increases with [CD] because $k_c > k_u$ but it is equally applicable for rate retardation ($k_c < k_u$) and for outright inhibition ($k_c = 0$). As long as the observed data corresponding to eqn. (4) have sufficient curvature, values of K_S and k_c can be estimated with reasonable confidence. In the present work care was taken to vary [CD] over a wide enough range to make the curved dependence of k_{obs} quite evident (Fig. 1).

Table 1 contains the fitted parameters used to generate the curves in Fig. 1. Two points should be noted. First, the binding of **1** to β-CD and hp-β-CD is about 20 times stronger than that to α-CD or γ-CD, causing the different curvatures seen in Fig. 1. Second, the CD-bound forms of the acetal are unreactive ($k_c = 0$) within experimental error (see Table 1, footnotes *b–d*), except in the case of α-CD, and even there the reactivity of the acetal is reduced appreciably ($k_c/k_u = 0.16$). In effect, for β-CD, hp-β-CD and γ-CD, eqn. (4) could be replaced by: $k_{obs} = k_u K_S/(K_S + [CD])$.

Effects of added guests

We have studied the effects of 'guests' on the CD-retarded hydrolysis of **1** in the hope that such effects might be used to estimate dissociation constants of {CD–guest} complexes. This approach is usually referred to as an 'inhibition method', or the use of 'inhibition kinetics',[7–9] but in the present case there is a twist since the probe reaction, the hydrolysis of **1**, is slowed down by CDs, not accelerated. Thus, addition of a guest (an 'inhibitor'), which binds to the CD and lowers the free CD concentration, should lead to an increase in k_{obs}, rather than a decrease. Such behaviour (*e.g.* Fig. 2) was observed for the hydrolysis of **1** in the presence of α-CD, β-CD and hp-β-CD, *but not* γ-CD.

For the purposes of the 'inhibition' analysis, the equation that describes saturation kinetics [eqn. (4)] is rearranged to eqn. (5). Thus, knowing k_u, k_c and K_S, from prior experiments,

$$[CD] = (k_{obs} - k_u)K_S/(k_c - k_{obs}) \qquad (5)$$

measurement of k_{obs} under the same conditions can be used to calculate [CD] for several $[guest]_o$, from which one can estimate the dissociation constant (K_G) for the {CD–guest} complex [eqn. (6)], as described in previous work.[8,9]

$$CD + G \rightleftharpoons CD\text{–}G \quad ; \quad K_G = [CD][G]/[CD\text{–}G] \qquad (6)$$

Fig. 2 shows some examples of data for the effects of added guests on the rate of hydrolysis of **1** in the presence of β-CD, along with curves calculated for 1:1 (CD:guest) binding. Comparable data were obtained for hp-β-CD. With increasing $[guest]_o$ more and more of the CD is bound up by the guest [eqn. (6)], lowering the concentration of free CD, and so k_{obs} *increases* in accord with eqn. (4), since $k_u > k_c$. Analysis of the data in the manner used previously[8,9] for 'inhibition kinetics' gives values of the dissociation constants K_G (Table 2) that are in fair to excellent agreement with literature values determined in other ways.[8,9,11,12] An appreciation of the agreement between the two sets for β-CD and hp-β-CD can be gained from Fig. 3, in which values of pK_G (= $-\log K_G$) obtained from the literature are plotted against those determined in the present work.

Experiments on the effects of guests on the hydrolysis of **1** retarded by α-CD gave data sets which did not analyse particularly well and they provided variable K_G values (Table 2). In consequence, fewer experiments were carried out with α-CD. As

Table 2 Dissociation constants of CD–guest complexes obtained from the effects of guests on the CD-retarded hydrolysis of acetal **1**, and by other methods, at 25 °C

	K_G/mM			
Guest	This work[a]	Lit.[b]	Lit.[c]	Lit.[d]
(a) β-Cyclodextrin				
propan-1-ol	216 ± 7	269		241 ± 9
butan-1-ol	54.7 ± 4.2	60.3	56 ± 1, 62 ± 4	
	48.7 ± 1.1		55 ± 1	
pentan-1-ol	15.3 ± 0.1	15.9		14 ± 1
hexan-1-ol	4.00 ± 0.05	4.57	4.4 ± 0.1	4.84 ± 0.18
heptan-1-ol	1.38 ± 0.24	1.41		
propan-2-ol	218 ± 8	263		246 ± 16
hexan-3-ol	17.2 ± 0.6		17.7 ± 0.3	
tert-butyl alcohol	21.5 ± 0.4	20.9		
cyclohexanol	1.44 ± 0.14	2.00	1.8 ± 0.2	2.10 ± 0.38
	1.47 ± 0.23		1.49 ± 0.08	1.4–2.2[e]
	1.52 ± 0.21[f]		2.0 ± 0.4	
hexan-3-one	19.7 ± 0.02		21.2 ± 0.4	
cyclohexanone	2.82 ± 0.05		2.51 ± 0.05	
(b) Hydroxypropyl-β-cyclodextrin				
propan-1-ol	334 ± 10		319 ± 7	173 ± 18
butan-1-ol	77.5 ± 2.0		64.0 ± 1.0	
pentan-1-ol	23.5 ± 0.6		16.6 ± 1.1	15.8 ± 0.5
hexan-1-ol	6.22 ± 0.27		4.37 ± 0.13	5.41 ± 0.10
heptan-1-ol	1.44 ± 0.14		1.51 ± 0.13	
hexan-3-ol	23.9 ± 0.7		20.8 ± 1.6	
cyclohexanol	3.97 ± 0.09		2.19 ± 0.04	3.66 ± 0.23
hexan-3-one	27.3 ± 0.5		27.3 ± 1.3	
(c) α-Cyclodextrin				
propan-1-ol	58.3 ± 11.5	42.7	42.2 ± 2.5	
butan-1-ol	21.7 ± 8.1	11.2	11.3 ± 0.9	
	13.5 ± 0.9			
	9.67 ± 2.02			
pentan-1-ol	4.40 ± 1.28	3.09	3.41 ± 0.28	
	2.28 ± 0.27			
hexan-1-ol	*g*	1.12	1.08 ± 0.03	

[a] From the effect of guests on the retardation of the hydrolysis of **1** by CDs in 0.10 M aqueous HCl. Multiple entries for the same guest are the results from separate experiments carried out with different $[CD]_o$ or ranges of $[guest]_o$ (see Experimental). [b] Calculated from pK_d values, obtained by a dye displacement method: for β-CD, pH 6.4, citrate buffer, $I = 0.05$ M; for α-CD, pH 1.2 (H_2SO_4), $I = 0.50$ M.[11a] [c] From various studies of the inhibition of cleavage of esters by CDs in a 0.2 M phosphate buffer at pH 11.6–11.7, carried out in this laboratory.[8,9] [d] From displacement of a fluorescent probe, at pH 11.6 (0.2 M phosphate buffer).[9b] [e] Other literature values, obtained by various methods.[12] [f] From the combined data of two separate experiments, immediately above. [g] Experiments unsuccessful.

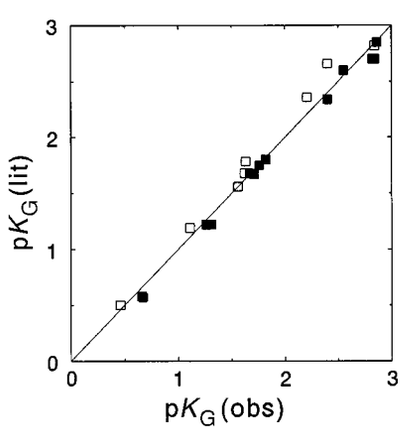


Fig. 3 Comparison of K_G values for CD–guest binding, plotted as pK_G (lit.) *vs.* pK_G (obs.), where the latter are from the present work (Table 2). The diagonal line through the origin, with a slope of one, corresponds to pK_G (lit.) = pK_G (obs.). The symbols are for: β-CD, ■; hp-β-CD, □. The literature values for β-CD are taken from Matsui and co-workers,[11] with three additional points from our work;[9c] those for hp-β-CD are all from previous work in this laboratory[9b,c] (see Table 2).

discussed later, the difficulties with α-CD most probably result from the shallow dependence of k_{obs} on [α-CD], which means that small errors in k_{obs} give rise to larger errors in [α-CD] and in K_G, when eqn. (5) is used in the data analysis.

Our results for the effects of alcohol guests on the hydrolysis of **1** in the presence of γ-CD could not be analysed for simple, CD–guest binding. Such 1 : 1 binding should lead to increases in k_{obs}, as observed for the other CDs (*e.g.* Fig. 2), but we found that k_{obs} is *lowered* even more by added guests (butan-1-ol, hexan-3-ol and cyclohexanone). Conceivably, there is cooperative (1 : 1 : 1) binding of the acetal **1**, the guest and γ-CD which lowers the amount of free **1** even further, and so k_{obs} is depressed beyond that due to the binding of **1** to γ-CD, alone. In support of this notion, numerical simulations showed that a ternary complex of **1**, the guest and γ-CD which has low or negligible reactivity in acetal hydrolysis could account for observed data.

Discussion

The primary finding in the results presented above is that hydrolysis of benzaldehyde dimethyl acetal **1** is slowed down substantially by each of the four CDs studied (Fig. 1). Of course, it would have been more interesting (and intriguing) to have found that the reaction is catalysed by CDs but, in truth, we had no *a priori* reason to expect such catalysis. Regardless, the observed saturation kinetics provide information relating to substrate–CD binding and they have implications with respect to transition state binding, as well, even though such binding must be unfavourable.

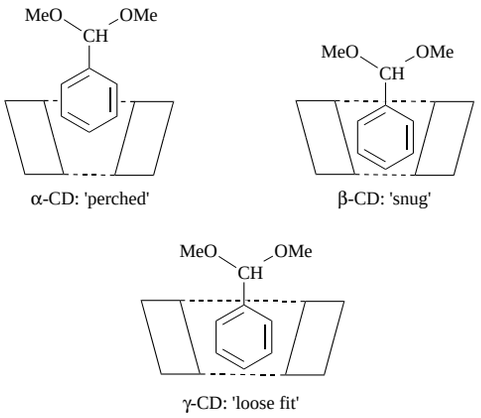


Fig. 4 Substrate–cyclodextrin binding

Substrate binding

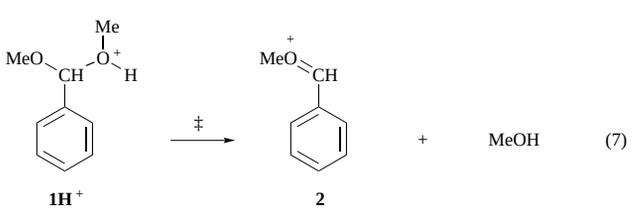
Analysis of the saturation kinetics afforded estimates of K_S for the binding of the acetal $PhCH(OMe)_2$ to the four CDs (Table 1). From these values, the strength of binding varies as α-CD < β-CD ≈ hp-β-CD > γ-CD. This order is quite normal for simple aromatic compounds, as has been found for indan-2-one,[13] 1- and 2-naphthyl acetates,[14] *m*- and *p*-*tert*-butylphenyl acetates.[15] Moreover, the binding of many simple phenyl derivatives is stronger to β-CD than it is to α-CD.[1,3b,7a,9c,11b] These trends are attributable to the cavity sizes of the CDs, which increase as: α-CD < β-CD ≈ hp-β-CD < γ-CD.[1]

With α-CD, the cavity is too narrow (~0.6 nm) to encapsulate all of the phenyl ring with ease and so the aromatic substrate is more or less constrained to be 'perched' on top of the CD cavity (Fig. 4), and the binding is relatively weak. In contrast, the cavity of β-CD and of hp-β-CD (width ~0.8 nm) is large enough that the acetal can sit inside the CD snugly and be bound more strongly. On the other hand, the cavity of γ-CD (width ~1.0 nm) is more than large enough to accommodate the aromatic substrate which can sit deep in the cavity but with a looser fit, so that it is bound less strongly.[16]

Transition state binding

It is not surprising that binding of the acetal **1** to CDs slows down its hydrolysis. What *is* surprising is that the rate reduction is so substantial in three out of the four cases (Table 1); it means that binding of the hydrolysis transition state to CDs must be very much less favourable than binding of the substrate.[3]

In the first instance, we suggest that the unfavourable transition state binding arises because the transition state is *cationic*, being intermediate in structure between the protonated acetal $\mathbf{1H}^+$ and the α-methoxybenzyl cation **2** plus methanol [eqn. (7)].[5,6] Whereas the binding of anions of many types by CDs



has been observed, and it can be quite strong,[1,4,7,8,9a,9b,17] the binding of cations has rarely been observed, and then only for large organic dyes,[1b] long chain surfactants[18] and metal ions with organic ligands.[19] In contrast, the binding of simple cations appears to be relatively unfavourable, for example, the anilinium ion ($PhNH_3^+$) binds only weakly or in combination with a counterion.[1a,20]

Another possibility stems from the assertion made by Jensen and Yamaguchi that diffusional separation of the molecule of alcohol from the alkoxycarbocation [*e.g.* eqn. (8)] may be the

$$PhCH(OMe)_2 + H^+ \rightleftharpoons \{PhCH{=}O^+{-}Me/MeOH\} \longrightarrow PhCH{=}O^+{-}Me + MeOH \quad (8)$$

rate-limiting step in acetal hydrolysis.[21] If such is the case for **1** then the inhibitory effect of CDs could result because binding of the initially-formed 'encounter complex', {$PhCH{=}O^+{-}Me$/MeOH}, severely impedes departure of the molecule of methanol and effectively promotes reformation of the starting acetal. The resulting retardation could be most severe when the 'encounter complex' is sequestered in the large cavities of β-CD, hp-β-CD and γ-CD, but less so when the binding involves α-CD, consistent with the observed variations in k_c.

It should be noted that the two possibilities just put forward are not in conflict; both factors could be operating to give rise to the observed rate reductions since the transition state in the second case [eqn. (8)] would also have cationic character.

Guest binding

When 'inhibition kinetics' are used to assess CD–guest binding the results cannot always be taken at face value because it may happen that the probe reaction takes place at an appreciable rate with a guest in the CD–host cavity.[8,22] In such cases, the CD-mediated reaction appears to be less inhibited than required for competitive inhibition, giving rise to anomalously low values of K_G, as we have found for several reactions.[8,9d,13,14,15,22] In the present case, however, this behaviour is not a significant factor for guest binding to β-CD, hp-β-CD or α-CD, since the estimated values of K_G generally agree with those obtained from earlier studies by a variety of methods, and under different conditions of pH and ionic strength (Table 2). As shown in Fig. 3, the agreement is particularly good for β-CD and hp-β-CD.

In the case of β-CD, the present values of K_G obtained in 0.1 M HCl tend to be slightly lower than those determined by a dye displacement method, at pH 6.4 (citrate buffer, $I = 0.05$ M)[11a] but they are not significantly different from ones determined in strongly basic solution (pH 11.6, 0.2 M phosphate buffer) from the inhibition of ester cleavage by β-CD.[8,9] On the other hand, the values of K_G found for hp-β-CD are marginally larger than those derived from the inhibition of ester cleavage in basic solution.[9] These apparent discrepancies, which are generally small, may simply reflect systematic differences between the different methodologies used.

In contrast to the experiments carried out with β-CD and hp-β-CD, those with α-CD afforded data which analysed less well and which gave inconsistent K_G values (Table 2). These difficulties are attributable to the shallow dependence of k_{obs} on [α-CD] (Fig. 1) which is such that small errors in k_{obs} are amplified to larger errors in [α-CD] and in K_G during the data analysis. For example, in an experiment with $[butanol]_o = [\alpha\text{-CD}]_o = 30$ mM, a seemingly trivial error of 1% in k_{obs} would result in an error of 5.5% in the estimate of [α-CD], and an even more serious error of 14.5% in the estimated value of K_G. Regardless of this problem, the results obtained with α-CD are largely consistent with competitive binding by simple guests which counteracts the retarding effect of α-CD on the hydrolysis of **1**.

Our results for guest binding to γ-CD were disappointing inasmuch as we were seeking a convenient method for estimating the relevant K_G values. Since the acetal **1** does not bind to γ-CD very strongly (Table 2), and it may sit deeply in the CD cavity (Fig. 4), we were hopeful that similar 'deep' binding of an added guest would compete effectively with acetal binding, and simple kinetics would be observed. In the event, addition of a guest caused a further lowering of the rate of acetal hydrolysis, suggesting that γ-CD is large enough to accommodate the acetal *and* a guest, and to bind them strongly, causing an additonal decrease in the concentration of free acetal.

It seems that the large cavity of γ-CD presents a general problem since we have encountered difficulties in trying to determine K_G values, using inhibition kinetics and various

probe reactions, because the probe reactions were incompletely inhibited or not inhibited at all by added alcohols.[15] In retrospect, this finding is not so surprising because it has been found that even with β-CD, which has a smaller cavity, there may be binding of a molecule of a simple alcohol and an aromatic probe.[23] Clearly, the assessment of alcohol binding to γ-CD alone is not straightforward and requires further careful study.[24]

Experimental

The cyclodextrins were purchased from the Aldrich Chemical Company or Wacker-Chemie (Munich, Germany) and used as supplied. Hydroxypropyl-β-cyclodextrin is available with different degrees of substitution: we used the Wacker product (Beta W 7 HP 0.9), with an average molecular weight of 1500, corresponding to alkylation of six of the seven primary hydroxyl groups of β-CD by 2-hydroxypropyl groups. Benzaldehyde dimethyl acetal, alcohols and ketones were of the best grade available from Aldrich. Hydrochloric acid solutions were made by dilution of standard 1.00 M solutions obtained from American Chemicals Ltd (Montreal).

Reactions were initiated by 1:1 mixing in a stopped-flow spectrophotometer. Since the acetal hydrolyses slowly but appreciably in water, the substrate solutions were made fresh for each set of kinetic runs. These solutions were made up by 1000-fold dilution of a 0.10 M stock solution of the acetal in spectral grade acetonitrile, so as to give $[acetal]_o = 50$ μM, after 1:1 mixing. For experiments with varying [CD] (Fig. 1), one syringe of the stopped-flow apparatus contained 0.20 M aqueous HCl and the other had the substrate and the CD at twice the concentrations desired in the reaction. For the competition experiments, with fixed $[CD]_o$ and varying $[guest]_o$, one syringe contained $2 \times [CD]_o$ and 0.20 M aqueous HCl, and the other had the acetal (100 μM) and $2 \times [guest]_o$. The values of $[CD]_o$ (in mM) were: α-CD, 20.0 or 30.0; β-CD, 5.00; hp-β-CD, 5.00; γ-CD, 20.0. The concentrations of the guests were varied between zero and the following *maximum values* (in mM): propan-1-ol, 200; butan-1-ol, 200; pentan-1-ol, 50; hexan-1-ol, 15; heptan-1-ol, 2.2; propan-2-ol, 200; hexan-3-ol, 20; 2-methylpropan-1-ol (*tert*-butyl alcohol), 100; cyclohexanol, 50; hexan-3-one, 20; cyclohexanone, 50 (*e.g.* Fig. 2).

Hydrolysis of the acetal was followed by the appearance of the benzaldehyde chromophore at λ 252 nm, using an Applied Photophysics SX17MV Stopped-flow Apparatus. Normally, 400 absorbance values, spanning 7–12 half-lives, were collected, and the first 10–20 points were ignored to allow for the induction period (see Results). A first-order rate constant was estimated from non-linear least squares fitting of an exponential increase, using computer software supplied with the apparatus. The recorded rate constants (k_{obs}) were taken as the averages of 5–10 determinations differing by less than 5%. The observation cell of the apparatus was kept at 25.0 ± 0.1 °C.

Non-linear fitting of eqn. (4) to k_{obs} *vs.* [CD] data was carried out with commercial or in-house software based on the Marquardt Algorithm.[26] The determination of dissociation constants using inhibition (competition) kinetics has been described in detail in previous publications.[8,9]

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24 It should, perhaps, be emphasized that the binding of simple, aliphatic alcohols to γ-CD is expected, by analogy with that of n-alkylamines,[9b,25] n-alkanes[26] and long chain surfactants.[18e]
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