Ester Cleavage by Cyclodextrins in Aqueous Dimethyl Sulfoxide Mixtures. Substrate Binding versus Transition State Binding

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The effects of DMSO on the kinetics of cleavage of *m*- and *p*-nitrophenyl alkanoates (mNPAlk and pNPAlk) by α- and β-cyclodextrin (α-CD and β-CD) in basic aqueous solution have been studied. For the two acetates addition of up to 60% (v/v) of DMSO increases rates but overall it has little effect on substrate binding, transition state binding, or the acceleration due to complexation. By contrast, in 50% (v/v) aqueous DMF these characteristics are greatly affected such that the normal difference in reactivity of the isomers is almost removed. The cleavage of mNPAlk and pNPAlk (C2 to C10) by the CDs in 60% (v/v) aqueous DMSO have very different chain length dependences for substrate binding and transition state binding, and there are significant changes from their behavior in water. Even though hydrophobic effects seem to be largely removed in 60% aqueous DMSO, and the difference between the reactivities of the isomers is reduced, reaction of mNPAlk proceeds through aryl group inclusion and that of pNPAlk through acyl group inclusion, as in water. The cleavage of *m-tert*-butylphenyl acetate is accelerated more in 60% (v/v) aqueous DMSO than in water because the solvent change weakens substrate binding more than transition state binding.

Introduction

The complexation of organic guests by cyclodextrins[1] (CDs) in aqueous solution is partially or largely determined by hydrophobic effects.[1-3] However, other effects such as van der Waals interactions must be important since well-defined 1:1 complexes are also formed in the solid state and in solvents other than straight water. For example, the complexation of esters undergoing basic cleavage by CDs has been observed in mixtures of water with dimethyl sulfoxide (DMSO),[4,5] *N,N*-dimethylformamide (DMF),[4] ethylene glycol,[6] and acetonitrile.[3a,b] Also, in discussing solvent effects it must be borne in mind that small organic solvent molecules such as DMSO may bind to CDs[7] in a mixed aqueous organic medium, thereby contributing an additional "solvent" effect at the molecular level.

In one particular example, Siegel and Breslow[4] found that the efficiency of cleavage of *m-tert*-butylphenyl acetate by β-cyclodextrin[1] (β-CD) was enhanced most in 60% (v/v) aqueous DMSO, relative to that in water containing the same buffer. The solvent change increased the rate of the background hydrolysis by a factor of 25 and the limiting rate acceleration due to β-CD was raised from 270 to 510. Together, these factors mean that reaction of the β-CD·ester complex in 60% aqueous DMSO was 13 000 times faster than hydrolysis of the ester in water. Obviously, the aqueous DMSO mixture is more convenient for the study of many organic substrates having limited solubilities in water and so it has been used by Breslow's group and others for several studies.[5] However, the generality (or otherwise) of its effect on ester cleavage by CDs has not been explored previously.

To probe further the effect of solvent change on the binding and reactions of cyclodextrins we have studied the basic cleavage of nitrophenyl alkanoates in aqueous DMSO, a reaction that has been studied previously in wholly aqueous media.[8-10] For the most part, the present paper describes two related studies in which different parameters were varied. Firstly, we studied the effects of increasing amounts of DMSO on the rates of cleavage of *m*- and *p*-nitrophenyl acetate by α-CD and β-CD. Secondly, we looked at the chain length dependence of kinetic parameters for the cleavage of *m*- and *p*-nitrophenyl alkanoates (acetate to decanoate) in 60% (v/v) aqueous DMSO, for the purposes of comparison with the results found earlier for the reaction in aqueous solution.[8-10] It was hoped that this comparison would shed light on the binding of alkyl chains to cyclodextrins in aqueous media with and without an organic cosolvent.

In basic aqueous solution cyclodextrins react with phenyl acetates by acyl transfer from the ester to the

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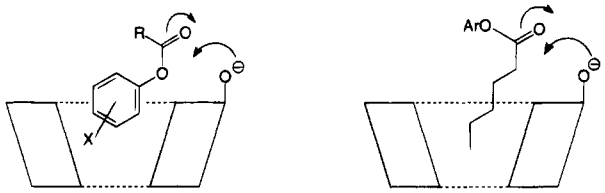
CD.[1,3,11-13] Generally speaking, cleavage by α-CD and β-CD (but not by γ-CD)[3a,14] is more efficient for *m*-substituted derivatives than for their *p*-substituted isomers. This "meta–para" distinction is ascribed to differences in transition state binding for the isomers, in particular that a meta substituent orients the phenyl group of the ester in the CD cavity in a geometry that is favorable for acyl transfer whereas a para substituent does not. Furthermore, in the case the cleavage of *p*-nitrophenyl acetate by CDs we have provided experimental evidence that the aryl group must come out of the CD cavity for attainment of the transition state,[15] consistent with an earlier analysis of steric effects on ester cleavage.[11]

The "meta–para" distinction is maintained for longer alkanoate esters reacting with α-CD, β-CD, and "hydroxypropyl-β-CD" (Hp-β-CD).[9,10] For acyl chain lengths of 10 carbons or less, *m*-nitrophenyl alkanoates are cleaved more readily than their para isomers so that the kinetic parameters for the two series of isomers are quite dissimilar and they display different sensitivities to changes in the acyl chain length. In consequence, it has been concluded that the *m*-nitrophenyl alkanoates react through a transition state involving aryl group inclusion (**1**) whereas many of the para isomers react with acyl group inclusion (**2**).[10]



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Results

We have measured the kinetics of cleavage of *m*- and *p*-nitrophenyl alkanoate (mNPAlk and pNPAlk) esters (acetate to decanoate, C2 to C10) by α-CD and β-CD in aqueous DMSO mixtures containing a carbonate buffer. The reactions were followed as the first order production of the nitrophenolate ions and rate constants (k^{obsd}) were obtained over a range of [CD]. In all cases, saturation kinetics[1,3,13] were observed which may be ascribed to reaction of the substrate (S) in the medium (eq 1) and

$$S \xrightarrow{k_u} \text{products} \quad (1)$$

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

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

reaction via an ester·CD complex (eq 2) (or its kinetic equivalent).[9,13] With the normal condition that [S·CD] < $[S]_t \ll [CD]$, these two processes lead to the dependence of k^{obsd} on [CD] expressed by eq 3.[1,13] Equation 3 gave good to excellent fits to the observed data[16] from which were obtained the parameters k_c and K_S collected in Tables 1–3, along with the measured values of k_u.

Table 1. Constants for the Basic Cleavage of Nitrophenyl Acetates by α- and β-Cyclodextrin in Aqueous DMSO Mixtures[a]

DMSO, % (v/v)	x_{DMSO}	K_s, mM	$10^3 k_u$, s^{-1}	k_c, s^{-1}	k_c/k_u	K_{TS}, mM
	(a) α-Cyclodextrin and *m*-Nitrophenyl Acetate					
0	0	13 ± 0.3	1.6	0.38 ± 0.01	240	0.053
20	0.062	22 ± 0.6	2.3	0.59 ± 0.01	250	0.086
40	0.150	27 ± 2	4.8	1.2 ± 0.07	240	0.10
60	0.284	15 ± 2	21	1.8 ± 0.15	87	0.17
	(b) α-Cyclodextrin and *p*-Nitrophenyl Acetate					
0	0	8.2 ± 0.3	3.3	0.011 ± 0.0001	3.3	2.5
20	0.062	16 ± 1	3.7	0.014 ± 0.0004	3.9	4.2
40	0.150	8.0 ± 1.4	8.2	0.027 ± 0.002	3.3	2.5
60	0.284	9.9 ± 1.1	36	0.16 ± 0.008	4.5	2.2
	(c) β-Cyclodextrin and *m*-Nitrophenyl Acetate					
0	0	10 ± 1	1.9	0.14 ± 0.01	73	0.14
20	0.062	21 ± 2	3.7	0.25 ± 0.02	68	0.31
40	0.150	13 ± 1	5.9	0.29 ± 0.01	49	0.27
60	0.284	7.5 ± 0.7	22	0.68 ± 0.03	31	0.24
	(d) β-Cyclodextrin and *p*-Nitrophenyl Acetate					
0	0	7.6 ± 0.1	2.7	0.026 ± 0.0002	9.6	0.79
20	0.062	20 ± 1	3.7	0.062 ± 0.002	17	1.2
40	0.150	14 ± 1	8.4	0.16 ± 0.007	19	0.73
60	0.284	5.2 ± 0.1	38	0.46 ± 0.004	12	0.44

[a] At 25 °C, in a 0.01 M carbonate buffer which had a pH of 10.3 in straight water. The cited uncertainties in K_s and k_c are the standard errors obtained from nonlinear fitting of eq 3.

Table 2. Constants for the Cleavage of Nitrophenyl Alkanoates by α-Cyclodextrin in 60% (v/v) aqueous DMSO[a]

acyl	K_s, mM	k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	K_{TS}, mM
	m-Nitrophenyl				
C2	15 ± 1.8	0.021	1.8 ± 0.15	87	0.17
C4	10 ± 0.4	0.013	0.59 ± 0.01	47	0.22
C6	9.3 ± 0.9	0.013	0.60 ± 0.03	48	0.20
C8	6.0 ± 0.2	0.013	0.57 ± 0.01	44	0.14
C10	4.2 ± 0.3	0.014	0.53 ± 0.01	37	0.11
	p-Nitrophenyl				
C2	9.9 ± 1.1	0.035	0.16 ± 0.01	4.5	2.2
C4	12 ± 2	0.017	0.081 ± 0.008	4.8	2.5
C6	9.4 ± 0.5	0.015	0.15 ± 0.004	9.9	0.95
C8	5.6 ± 0.2	0.015	0.26 ± 0.004	17	0.33
C10	2.3 ± 0.3	0.016	0.29 ± 0.01	19	0.12

[a] As in Table 1.

Table 1 summarizes the results from studying the cleavage of *m*- and *p*-nitrophenyl acetate (mNPA and pNPA) by α-CD and by β-CD in mixtures of water and DMSO, ranging from 0 to 60% (v/v). The last of these mixtures sounds as though it contains a lot of DMSO but the mole fraction of DMSO (x_{DMSO}) is only 0.284.[18] Table 2 contains appropriate constants for the cleavage of *m*- and *p*-nitrophenyl alkanoates (C2 to C10) by α-CD in 60% aqueous DMSO, and Table 3 has the corresponding constants for cleavage by β-CD.

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Table 3. Constants for the Cleavage of Nitrophenyl Alkanoates by β-Cyclodextrin in 60% (v/v) Aqueous DMSO[a]

acyl	K_s, mM	k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	K_{TS}, mM
		m-Nitrophenyl			
C2	7.5 ± 0.7	0.022	0.68 ± 0.03	31	0.24
C4	6.9 ± 0.3	0.011	0.26 ± 0.003	24	0.29
C6	5.6 ± 0.4	0.013	0.28 ± 0.009	22	0.25
C8	4.7 ± 0.3	0.013	0.29 ± 0.008	21	0.22
C10	4.6 ± 0.5	0.013	0.38 ± 0.02	30	0.15
		p-Nitrophenyl			
C2	5.2 ± 0.1	0.038	0.46 ± 0.004	12	0.44
C4	8.4 ± 0.7	0.017	0.25 ± 0.01	15	0.57
C6	6.8 ± 0.4	0.016	0.28 ± 0.008	18	0.38
C8	5.2 ± 0.2	0.017	0.31 ± 0.005	19	0.28
C10	4.6 ± 0.5	0.015	0.40 ± 0.02	26	0.18

[a] As in Table 1.

Table 4. Constants for the Cleavage of mNPA and pNPA by CDs in 50% (v/v) Aqueous DMF[a,b]

ester	CD	K_s, mM	10^3k_u, s^{-1}	10^2k_c, s^{-1}	k_c/k_u	K_{TS}, mM
mNPA	α	11 ± 2	5.5	1.5 ± 0.1	2.8	3.9
pNPA[c]	α	1.3 ± 0.1	40	5.2 ± 0.1	1.3	1.0
mNPA[d]	β	7.0 ± 1.1	6.1	1.8 ± 0.1	3.0	2.3
pNPA[c]	β	5.0 ± 0.7	40	5.3 ± 0.1	1.3	3.8

[a] As in Table 1. [b] Experiments in 50% aq ACN were not possible due to the low solubility of CDs in this medium. [c] Since the rate increases are so small (k_c/k_u = 1.3), the fitted parameters may not be as reliable as the errors suggest. [d] For comparison, mNPA reacting in the presence of β-CD in 50% aq DMSO gave k_u = 0.011 s^{-1}, k_c = 0.55 ± 0.02 s^{-1}, K_S = 13.8 ± 0.6 mM, from which k_c/k_u = 50 and K_{TS} = 0.28 mM. These values fit well between those for 40% and 60% aq DMSO, in Table 1.

Table 5. Dissociation Constants of Complexes formed between Cyclodextrins and Small Organic Solvent Molecules in Aqueous Solution[a]

molecule	K_d, mM α-CD	K_d, mM β-CD
DMSO	2440[7b]	536 ± 14[b,c]
DMF	337 ± 5[b]	403 ± 4[b]
ACN	180[7b]	>1000[b,d]
MeOH	1070[7a]	3090[7a]
EtOH	178,[7a] 208[7b]	1070[7a]

[a] At 25 °C. Literature values are from Matsui and Mochida[7a] and from Gelb et al.,[7b] as indicated. [b] Determined in this work, from the kinetics of inhibition of cleavage of mNPA.[15b] [c] In excellent agreement with a literature value of 555 mM, determined in a different manner.[7c] [d] Must be large since the addition of ACN up to 0.5 M had virtually no effect on k^{obsd} for the cleavage of mNPA by β-CD.

We also tried to study the cleavage of mNPA and pNPA by the two CDs in aqueous mixtures containing 50% (v/v) of DMF or acetonitrile (ACN). Unfortunately, the CDs are not sufficiently soluble in 50% aqueous ACN to allow the experiments, but satisfactory results were obtained for 50% aqueous DMF, yielding the constants presented in Table 4.

Since we were concerned about the complexation of organic solvent molecules by CDs in water, we attempted to estimate some of the dissociation constants for DMSO, DMF, and ACN binding to the CDs, using inhibition kinetics.[3a,15b] The results are presented in Table 5, along with other relevant values. Both DMSO and DMF showed kinetic behavior that analyzed extremely well for inhibition due to 1:1 binding but experiments with ACN were inconclusive. Addition of up to 500 mM had essentially no effect on rate constants for the cleavage of mNPA by β-CD. From this observation we infer that ACN binds very weakly to β-CD (K_d > 1 M), even though

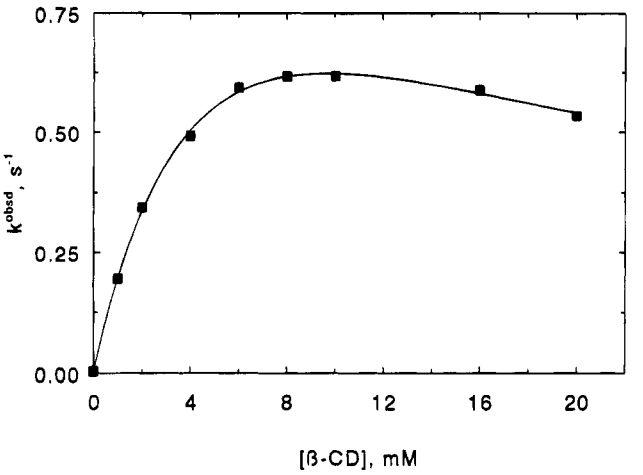

Figure 1. Rate constants for the basic cleavage of *m-tert*-butylphenyl acetate in the presence of β-CD in 60% aqueous DMSO. The calculated curve was generated from eq 5 with parameters given in Table 6.

it binds fairly well to α-CD (K_d = 180 mM),[7b] since it is quite normal for small linear molecules to bind more strongly to α-CD than to β-CD (e.g., MeOH and EtOH, Table 5).[7a,11] By contrast, DMSO binds more weakly to α-CD than to β-CD, possibly because it is more angular. Interestingly (and surprisingly), DMF binds almost equally well to both CDs (Table 5).

Lastly, we studied the cleavage of *m-tert*-butylphenyl acetate by β-CD in 60% aqueous DMSO to corroborate and complement the earlier findings.[4] We were surprised to find that rate constants measured in the range of [β-CD] = 0−10 mM did not conform particularly well to eq 3, with deviations at high [CD] that suggested the onset of 2:1 (CD:ester) binding.[16] As seen in Figure 1, this behavior was confirmed by extending [β-CD] up to 20 mM. The decreases in k^{obsd} at high [CD] can be accomodated by the equilibrium formation of a nonproductive 2:1 complex (eq 4) in which case eq 3 is replaced by eq 5. Fitting of this equation to the data gave the constants given in Table 6 which also contains constants for reaction in aqueous solution for the purposes of later discussion.

$$S{\cdot}CD + CD \underset{K_2}{\rightleftharpoons} S{\cdot}CD_2 \qquad (4)$$

$$k^{obsd} = \frac{(k_u K_S + k_c[CD])K_2}{(K_S K_2 + K_2[CD] + [CD]^2)} \qquad (5)$$

Discussion

Solvent effects on reaction kinetics are generally discussed in terms of the separate effects on the initial state and the transition state of the reaction.[19,20] In the context of a CD-mediated process, this requires consideration of the differential effects of solvent change on substrate binding to the CD as opposed to those on transition state binding. The former may be discussed in terms of the values of K_S obtained from the saturation kinetics (eq 3) and for the latter we will use "dissociation

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Table 6. Constants for the Cleavage of *m-tert*-Butylphenyl Acetate by β-CD in Water and in 60% (v/v) Aqueous DMSO

medium	K_S, mM	10^3k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	K_{TS}, μM
water[a]	0.13	0.49	0.122	250	0.52
water[b]	0.10	0.03	0.008	270	0.37
60% aq DMSO[b]	(5)[c]	0.75	0.38	510	9.8
60% aq DMSO[d]	6.3 ± 0.7	3.5	1.43 ± 0.10	410	15

[a] From the data of VanEtten et al.[3a] In a carbonate buffer of pH 10.6. [b] From the data of Siegel and Breslow.[4] In a buffer having a pH of 9.5 in water. [c] A value was not reported by Siegel and Breslow. We assumed a value of 5 mM for the purposes of an analysis given earlier.[13b] [d] This work. In a carbonate buffer having a pH of 10.3 in water. The constants K_S and k_c were obtained from fitting eq 5 to the data, along with $K_2 = 15 \pm 2$ mM for the second binding. The data and calculated curve are shown in Figure 1.

constants" (K_{TS}) obtained by an application of transition state theory.[13] Following an approach developed by Kurz,[21] we define K_{TS} as the apparent dissociation constant of the transition state of the CD-mediated reaction (TS·CD) into the transition state of the normal reaction (TS) and the CD, as in eq 6.

$$K_{TS} = \frac{[TS][CD]}{[TS\cdot CD]} = \frac{k_u K_S}{k_c} \qquad (6)$$

Values of K_{TS} can be useful for distinguishing between different modes of transition state binding and for a recent review we calculated many such values for reactions mediated by CDs (and other species).[13b] Variations of K_{TS} with structure, sometimes expressible as linear free energy relationships (LFERs), can serve as probes of the binding of the CD in the transition state. In particular, a significant increase of pK_{TS} (= $-\log K_{TS}$) with chain length may provide evidence of acyl group binding during the cleavage of alkanoate esters by CDs.[9,10,13,14,17]

Increasing DMSO Content. We consider first the effect of adding DMSO on the cleavage of mNPA and pNPA by CDs. Since the results for the two CDs are broadly similar (Table 1) they do not merit separate discussion and even though DMSO binds more strongly to β-CD than to α-CD (Table 5) there are no particular differences in the constants in Table 1 that could be ascribed to this fact.

As seen from the values of K_S in Table 1, the strength of binding of the two esters to the two CDs is hardly affected by the solvent change. After a modest rise in K_S at low DMSO content, there is a comparable decrease, so that in 60% aqueous DMSO the value of K_S is close to that in water. This does not necessarily mean that the orientation of the inclusion of the esters (nitro in; acetoxyl out)[22] is unaffected, but in the light of the relative invariance of k_c/k_u (see below) a switch in the mode of binding seems unlikely. The slight increase in K_S values at low DMSO content, which is seen for both esters and both CDs, may arise because DMSO molecules bind to CDs in a largely aqueous medium[7b,c] (Table 5).

The addition of DMSO to water significantly accelerates the basic hydrolysis of the two esters, as expected from earlier studies.[18,23-25] The increases of $\log k_u$ with the mole fraction of DMSO (x_{DMSO}) are nearly linear with slopes of about 4, comparable to the slopes of ~3 found for the cleavage of various alkyl benzoates in aqueous DMSO mixtures.[23b,26] Increasing the DMSO content also causes rises in k_c, for the reaction of the ester·CD complexes (eq 2): for pNPA the slopes of $\log k_c$ against x_{DMSO} are also near 4 but those for mNPA are only 2.4. As a consequence, the ratios k_c/k_u, which denote the limiting acceleration due complexation, decrease with added DMSO for the cleavage of mNPA but for pNPA they hardly change (Table 1). By virtue of the form of eq 6, these trends are also reflected in the values of K_{TS} so that for mNPA cleavage addition of DMSO increases K_{TS}, implying a reduction of transition state stabilization, whereas for pNPA cleavage there is either no change in K_{TS} (α-CD) or a slight decrease in K_{TS} (β-CD).

This difference between the two isomeric esters presumably results from their dissimilar transition state structures.[3,11-13,15] In the case of mNPA (and most other *m*-substituted phenyl acetates) the aryl group is decidely in the CD cavity during the reaction in aqueous solution but for pNPA (and probably other *p*-derivatives) the aryl group and its substituent apparently must come out of the cavity.[11,15] As a result, cleavage of mNPA is susceptible to inhibition by molecules which bind in the CD cavity whereas the reaction of pNPA is quite tolerant of potential inhibitors and can be catalyzed by some of them.[15] Conceivably for this reason added DMSO has more of a destabilizing effect on the transition state for mNPA cleavage than it does on that for pNPA cleavage.

The effects of 50% (v/v) aqueous DMF on the cleavage of mNPA and pNPA are very different from those of aqueous DMSO (Table 4). Firstly, the effect on the rate of hydrolysis (k_u) is not the same for the two esters: for pNPA k_u is elevated by 14, relative to water, but for mNPA the factor is only 3. The other remarkable difference is that the normal "meta–para" distinction that is observed in water (see Introduction), and in aqueous DMSO (Table 1), is virtually absent in 50% aqueous DMF and the acceleration ratios k_c/k_u are very small (1.3–3.0) for *both* mNPA and pNPA reacting with either α- or β-CD. Conceivably, DMF occupies the cavities of the CDs in such a way that the favorable transition state geometry which leads to efficient cleavage of mNPA in water and aqueous DMSO is not attainable in 50% aqueous DMF, and mNPA is constrained to react more like pNPA. In support of this idea, note that low levels of DMF (up to 0.5 M) in water inhibit mNPA cleavage, consistent with DMF binding to the CDs: K_d = 340 mM (for α-CD) and 400 mM (for β-CD). For the cleavage of pNPA by CDs in water, binding of part of the ester in the CD cavity does not seem to be significant in the transition state.[11,15] The present results for 50% aqueous DMF may mean that a similar situation applies to the reaction of both mNPA and pNPA with CDs in this medium.

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(26) For ethyl acetate reacting with sodium hydroxide in aqueous DMSO the kinetic data[18] show biphasic behavior. For x_{DMSO} = 0–0.28, the plot of $\log k^{obsd}$ against x_{DMSO} is reasonably linear with a slope of 0.86 whereas for x_{DMSO} = 0.28 to 0.70 the slope is 1.72. Other simple ethyl alkanoates show similar shallow slopes.[23a]

Our results for the reaction of *m-tert*-butylphenyl acetate with β-CD in 60% aqueous DMSO basically agree with those of Siegel and Breslow[4] (Table 6), with the added observation of 2:1 (CD:ester) binding (Figure 1). Consequently, the analysis that we presented earlier,[13b] which required the assumption of a value of K_S, is supported. In brief, addition of 60% DMSO weakens substrate binding (K_S is increased about 60-fold) but transition state binding is weakened less (K_{TS} is raised 30-fold). As a result, the acceleration k_c/k_u is elevated from ~250 to ~500.

These effects of 60% DMSO on the reaction of *m-tert*-butylphenyl acetate with β-CD are not the same as the effects found for the cleavage of mNPA and pNPA (Table 1). For mNPA, K_S is reduced marginally and K_{TS} is increased, so that the acceleration k_c/k_u is reduced (from 73 to 31); for pNPA, K_S and K_{TS} are affected to about the same extent, meaning that k_c/k_u is hardly changed (from 10 to 12). Clearly then, the effects of added DMSO on ester cleavage by CDs can vary significantly with the ester substrate. This assertion is borne out by the results for other alkanoate esters, discussed below.

Changing the Acyl Chain Length. For the basic cleavage of nitrophenyl alkanoates by CDs in water, various parameters are sensitive to the acyl chain length (N) of the esters, depending on the position of the nitro group and the CD.[8-10,14] With α-CD, β-CD[8,9] and "hydroxypropyl-β-CD"[10] the values of pK_S for substrate binding of both the *m*- and *p*-nitrophenyl isomers in water increase linearly with N, compatible with acyl group binding in the initial state. A similar strong dependence on chain length N was found for the pK_{TS} values of the longer *p*-isomers, implying that acyl binding is involved in the transition state for their cleavage (**2**), also. By contrast, the larger values of pK_{TS} for the more efficient cleavage of the *m*-nitro isomers show little variation with N, consistent with aryl group binding in the transition state (**1**). Before comparing these features to those revealed by the present studies, we review some of the background which led to the conclusions just outlined.

As we have pointed out,[9,13a] plots of pK_S against chain length for the binding of simple *n*-alkyl compounds to CDs are essentially linear up to chain lengths of about 8, with slopes in the range of 0.2–0.6. These sensitivities to alkyl chain length closely resembles those shown by various measures of hydrophobicity[27-30] which means that plots of pK_S against such measures are tolerably linear. Thus, it is quite reasonable to argue that the strength of binding of alkyl chains to CDs is determined, at least partially, by hydrophobic effects. At the same time, it must be recognized that the volumes and surface areas of *n*-alkyl chains also increase monotonically with N, so that any contributions from van der Waals (or similar) interactions[1-3,31-33] will grow likewise. On this basis, we treat plots of pK_S against N as LFERs and we use their slopes as criteria of the mode of substrate binding to CDs. In the same way, we use the slopes of plots of pK_{TS} versus N as criteria of transition state binding.[9,10,13,14]

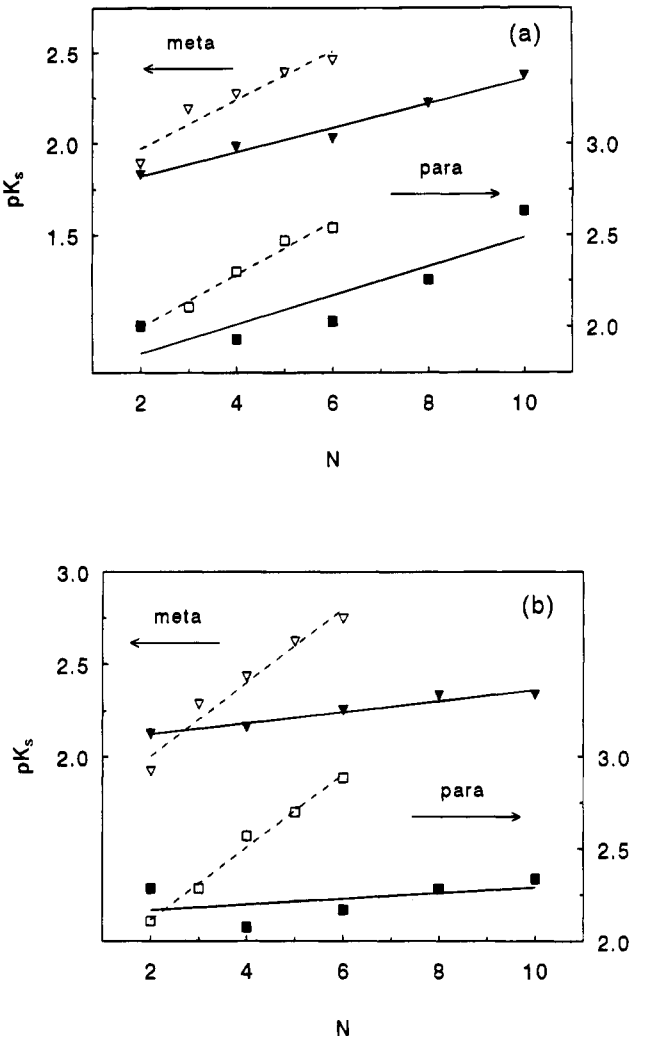

Figure 2. Dependence of substrate binding (pK_S) on acyl chain length (N) for nitrophenyl alkanoates: (a) for binding to α-CD; (b) for binding to β-CD. The symbols are as follows: mNPAlk (▽,▼); pNPAlk (□, ■); in water (open symbols, ▽, □); in 60% aqueous DMSO (closed symbols, ▼, ■). The data for aqueous solution are from earlier work;[9,10] those for 60% aqueous DMSO are from this work (Tables 2 and 3). Note that the scales for the *meta* and *para* isomers are offset for clarity, otherwise their data sets would be almost coincident.

We consider first the binding of mNPAlk and pNPAlk to α- and β-CD in 60% aqueous DMSO, and compare it to that in water. As shown in Figure 2, the plots of pK_S against N for substrate binding in the two media are quite different: in 60% aqueous DMSO pK_S values hardly vary with acyl chain length (N) whereas in water they increase more systematically with N.[9,10] For binding to α-CD in water the slopes of the plots are ~0.14 whereas for binding in 60% aqueous DMSO they are only ~0.08 (Figure 2a). In the case of β-CD, the contrast is even greater: for water the slopes are 0.2 whereas for the aqueous DMSO they are ~0.02 (Figure 2b). Since the strong dependences of pK_S on N found in water are

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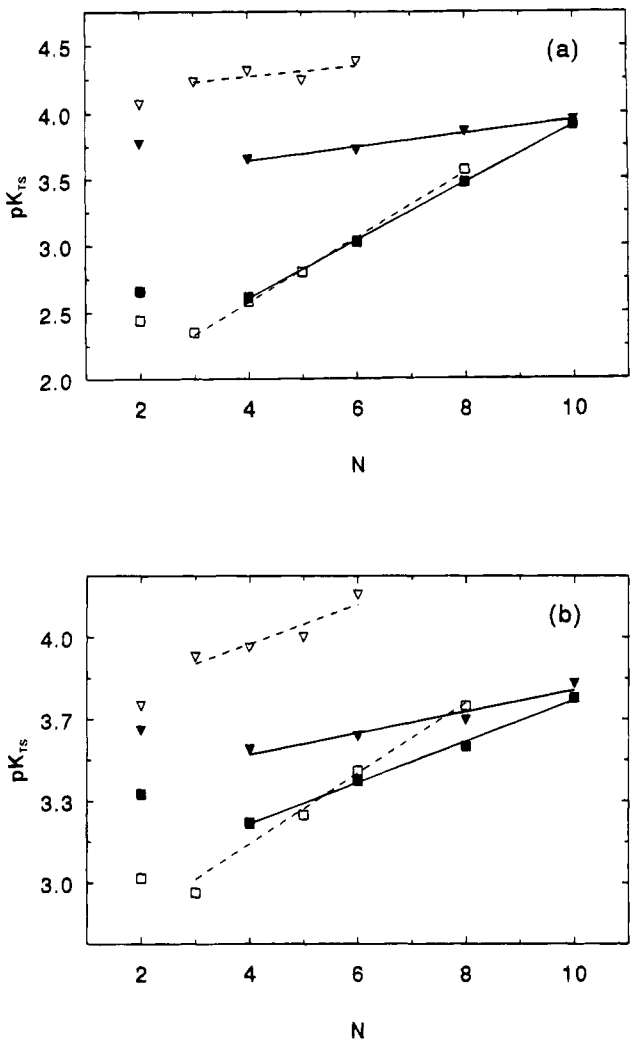

Figure 3. Dependence of transition state binding (pK_{TS}) on acyl chain length (N) for nitrophenyl alkanoates: (a) for binding to α-CD; (b) for binding to β-CD. The symbols have the same significance as in Figure 2. The data for aqueous solution are from earlier work;[9,10] those for 60% aqueous DMSO are from this work (Tables 2 and 3).

attributable to acyl group binding,[9,10,13] it is natural to infer that such binding is not dominant in 60% aqueous DMSO and that binding of the nitrophenyl groups of the esters prevails. This situation is understandable if 60% aqueous DMSO is sufficiently unlike water that solvophobic effects exhibited by alkyl groups in water[27-29] are largely absent, so that the preference for acyl inclusion is subverted.

The findings for transition state binding (Figure 3) differ substantially for the isomeric series of esters and in significant ways from those for substrate binding (Figure 2). Consider first the plots of pK_{TS} vs N for cleavage of the esters by α-CD (Figure 3a). Ignoring the points for the acetates which deviate on all plots, the slopes for the mNPAlk esters are shallow and very close for the two media (0.04 in water; 0.05 in aqueous DMSO) and much less than those for the pNPAlk esters (0.24, 0.22), consistent with aryl binding for the former (**1**) and acyl binding for the latter (**2**) in both media. For the *meta* isomers transition state stabilization (pK_{TS}) is significantly reduced in aqueous DMSO, compared to water, but *for the para isomers reacting with* α-CD transition state stabilization in water and 60% aqueous DMSO is essentially identical (Figure 3a). Since normal hydrophobic effects seem to be largely absent for initial state binding of the esters in 60% aqueous DMSO (see above, Figure 2), we conclude that other interactions (such as van der Waals forces)[2e-h,32,33] dominate and that the strong dependence of pK_{TS} on N derives from the monotonic increase in surface area of the acyl group in contact with the interior of the CD cavity,[32,33] as the ester chain is lengthened. This concluson is perfectly compatible with the view that snugness of fit contributes positively towards inclusion, and in particular that *n*-alkyl chains fit snugly into the narrow cavity of α-CD.[1c,7a,11,33,34]

The chain length dependences of pK_{TS} values for reaction with β-CD (Figure 3b) are generally similar to those for α-CD but with quantitative differences. The slopes of pK_{TS} vs N for the mNPAlk esters (0.08 in water; 0.04 in aqueous DMSO) are less than those for the pNPAlk esters (0.14, 0.08), broadly consistent with aryl binding for the *meta* isomers (**1**) and acyl binding for the *para* isomers (**2**) in the transition state, although the differentiation is less clear-cut than with α-CD. The "meta–para" distinction that is so evident in water[3,9,10,13] is much less marked in 60% aqueous DMSO mainly because pK_{TS} values for the mNPAlk esters are decreased significantly. Values of pK_{TS} for the pNPAlk esters are similar in the two solvents but with a reduced slope vs N (0.14 → 0.08) in 60% aqueous DMSO. These shallower slopes for pNPAlk reacting with β-CD, as opposed to those of ~0.2 for reaction with α-CD, presumably reflect the wider cavity of β-CD which permits a looser fit of the acyl chain of the reacting ester so that it is less demanding of a strict geometry for inclusion in the transition state. This same factor may also account for the lower selectivity between the isomeric esters shown by β-CD in both media but particularly in aqueous DMSO.

Conclusions

The most remarkable feature of the effect of DMSO on the cleavage of nitrophenyl alkanoates is the dramatic contrast between its effect on substrate binding (Figure 2) and on transition state binding (Figure 3). Also, it is noteworthy that the effects of DMSO on transition state binding are not the same for the two series of esters. These dissimilarities must mean that the interactions leading to stabilization of the transition states differ appreciably for the *meta* and *para* isomers and that they differ from those responsible for substrate binding. This situation is understandable if, as we suggest, the modes of substrate binding and transition binding are not the same in many instances. In water, both series of esters (beyond C2 or C3) bind to the CDs with their acyl groups[9,10] whereas in 60% aqueous DMSO they probably are bind by their nitrophenyl groups (Figure 2). By contrast, in both water and 60% aqueous DMSO the reaction of *m*-nitrophenyl alkanoates with α-CD and β-CD involves aryl inclusion (**1**) whereas reaction of most of the *p*-nitro isomers proceeds with acyl group inclusion (**2**) (Figure 3).

Added DMSO has relatively minor effects on the cleavage of mNPA and pNPA by CDs, leading to only modest changes in substrate binding, transition state binding and acceleration (Table 1). On the other hand, in 50% aqueous DMF the reactions of both mNPA and

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pNPA with the CDs are greatly affected so that the accelerations (k_c/k_u) due the CDs are diminished and the distinction between the two isomers is greatly reduced (Table 4). This behavior, which is largely due to transition state destabilization, may arise from the binding of DMF in the CD cavities (Table 5) in such a way that the esters are largely excluded from the CD cavity during the reaction, as in the case with the cleavage of pNPA by CDs in water.[15]

The effects of 60% aqueous DMSO on the cleavage of *m-tert*-butylphenyl acetate by β-CD are much larger (Table 6) than those on the reactions of mNPA or pNPA (Table 1b). Both substrate and transition state binding are greatly weakened, presumably due to the removal of the major hydrophobic interaction of the *tert*-butylphenyl group. However, the transition state binding is weakened less so that the acceleration due to complexation is doubled.

Overall, the range of the effects observed in the present work serve to emphasize that the consequences of solvent change on reactions involving complexing agents can be manifold and they vary considerably with the structure of the reactants, even when they are structurally very similar.

Experimental Section

The *p*-nitrophenyl esters were obtained from Sigma and the *m*-nitro isomers were prepared as previously.[9,10] *m-tert*-Butylphenyl acetate was synthesized by reaction of the phenol with acetic anhydride in strong aqueous base, using a textbook procedure for phenyl acetate.[35] The solid product, after recrystallization from aqueous methanol, gave satisfactory NMR spectra and an appropriate uv spectral change and rate upon hydrolysis in base.[4] Spectral grade solvents (methanol, DMSO, DMF and ACN) and the cyclodextrins were purchased from Aldrich and used as received.

The carbonate buffer in mixed solvent was made up as follows. For X% (v/v) aqueous DMSO, 2 mL of 1.0 M aqueous $NaHCO_3$, 1 mL of 1.0 M NaOH and (197 − 2X) mL of water were combined with 2X mL of DMSO to give a nominal volume of "200 mL", before the volume changes that occur on mixing. For the complementary ester/CD solution, a stock CD solution was prepared by weighing out the CD and dissolving it in 2X mL of DMSO and (200 − 2X) mL of water. This stock was diluted with mixed solvent to the desired concentration and a few μL of a strong stock solution of the ester in spectral grade methanol or acetonitrile were added. Solutions in 50% (v/v) aqueous DMF were made up in a similar manner. Studies of the cleavage reactions in 50% aqueous acetonitrile were not possible due to the low solubility of CDs in ACN.[36]

Kinetic procedures largely followed established practices.[9,10,14,37] Reactions were carried out by 1:1 stopped-flow mixing of the carbonate buffer in the mixed medium with the ester (20−100 μM for pNPAlk; 100−400 μM for mNPAlk) dissolved in the same mixed medium, containing water or the CD, so that final concentrations were half these. Ester cleavage was followed by monitoring the first order production of the nitrophenolate anions at 390−410 nm, using a stopped-flow spectrophotometer with the observation cell kept at 25.0 ± 0.1 °C. Much of the work was carried out on an Aminco-Morrow Stopped-flow Accessory attached to an Aminco-DW2a spectrophotometer, interfaced to a microcomputer.[9,37] Where final absorbance values were uncertain (for dilute solutions of the longest esters) they were estimated using the Kezdy−Swinbourne method.[38] The final phases of the project were completed using an Applied-Photophysics SX17MV stopped-flow apparatus, as in other recent work.[14]

The constants K_S and k_c were obtained by non-linear least squares fitting of eq 3 to k^{obsd} values over a range of [CD] values, using in-house or commercial software based on the Marquardt Algorithm[39] and keeping k_u fixed at the observed value. As mentioned in the main text, we observed suggestions of 2:1 (CD:ester) binding for the C9 and C10 esters, at high [CD] > 8 mM. However, for consistency with the approach used for all the other nitrophenyl esters, and because we were interested in 1:1 binding and its ramifications, we kept [CD] in the range 0−10 mM, and restricted our analysis to fitting eq 3 to the data. Fitting to eq 5, which makes provision for 2:1 binding[10,17] and has one extra parameter, gave only marginally better fits and parameters with much larger uncertainties.

The binding of ACN, DMF, and DMSO to CDs in water, was probed using inhibition kinetics, as in other recent work.[15b,40] The effects of up to 0.5 M of these solvents on the cleavage of mNPA by α-CD and by β-CD in a basic phosphate buffer were measured and analyzed to estimate dissociation constants. Experiments with ACN and β-CD were inconclusive but those with DMF and DMSO gave excellent results for 1:1 binding, leading to K_d values given in Table 5.

The kinetics of cleavage of *m-tert*-butylphenyl acetate were followed by the first order appearance of the phenolate ion at 248 nm. Because this compound showed definite evidence of 2:1 binding, experiments were carried out to higher [CD] and the data were analyzed in terms of eq 5 (Figure 1), giving constants reported in Table 6.

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