Stability of 1:1 and 2:1 α-cyclodextrin–*p*-nitrophenyl acetate complexes and the effect of α-cyclodextrin on acyl transfer to peroxide anion nucleophiles

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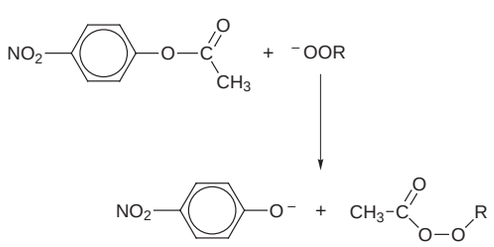
The presence of a rate maximum rather than simple saturation-type kinetics in a study of the effect of α-cyclodextrin on the hydrolysis of *p*-nitrophenyl acetate (PNPA) indicates that α-cyclodextrin forms not only 1:1 but also 2:1 complexes with PNPA. This is confirmed using a spectrophotometric method to determine binding constants directly for PNPA, giving values of 46 ± 9 and 66 ± 19 $dm^3\ mol^{-1}$ for the first and second binding steps respectively. These results contradict the majority of literature studies of this reaction in which it is assumed that only a 1:1 complex is formed. Formation of a 1:1 complex with cyclodextrin increases the reactivity of PNPA towards hydrolysis, as has been widely reported, whereas the addition of a second cyclodextrin molecule to the complex results in the PNPA taking up a less reactive configuration. The effect of α-cyclodextrin on the reaction between PNPA and the anions of hydrogen peroxide, peroxomonosulfate, peracetic acid, perbenzoic acid, 4-methylperbenzoic acid, 4-nitroperbenzoic acid, 4-sulfonatoperbenzoic acid, 3-chloroperbenzoic acid and 4-*tert*-butylperbenzoic acid is described. Linear free energy studies for transition state stabilisation of the reaction by one molecule of cyclodextrin reveal that the main pathway involves the bound PNPA reacting with free peroxide anions, although for *m*-chloroperbenzoic acid an alternative pathway may be significant. This is in contrast to the behaviour observed for the α-cyclodextrin-mediated reaction of the molecular acid form of these peroxides with a series of aryl alkyl sulfides in which the main pathway involves nucleophilic attack of the free sulfide on the cyclodextrin–peracid complex. With the exception of the *m*-chloroperbenzoic acid anion there is no evidence of transition state stabilisation of the title reaction by two molecules of cyclodextrin.

Introduction

Application of the transition state pseudo equilibrium approach[1] to the α-cyclodextrin-mediated reaction between aryl alkyl sulfides and peracids indicates a transition state in which unbound sulfide reacts with bound peracid.[2] The oxidation of iodide by the same peracids has been shown to involve an analogous transition state.[1,3] Several mechanisms could explain the enhanced reactivity of the included electrophilic peracid,[3–7] and these have recently been discussed.[2] The present work is concerned with the effect of α-cyclodextrin on reactions involving the peracid anion, which is a nucleophile. The molecule orients with the peroxide group located at the narrow end of the cavity, as does the peracid and the parent acid. The electron withdrawing carbonyl group on these molecules is likely to result in a favourable interaction with the positive dipole (narrow end) of the cavity, thus accounting for their similar orientations. This is not the case for the oppositely oriented parent anion.[8] The different effects of α-cyclodextrin on reactions involving the electrophilic peracid and the nucleophilic peracid anion may provide a useful insight into the mechanism of catalysis in these systems.

The reaction chosen for study was acyl transfer from *p*-nitrophenyl acetate (PNPA), in which nucleophilic attack by the peroxy anion takes place at the carbonyl carbon of the ester, resulting in acylation of the peroxy anion and the formation of *p*-nitrophenol,[9,10] as shown in Scheme 1. The acylated peroxide subsequently hydrolyses to the peracid and acetic acid. PNPA has been shown to form inclusion complexes with α-cyclodextrin and the preferred orientation is likely to be one in which the acetyl group is located at the wide end of the cavity, with the aryl group included within the cavity. Previous studies have looked at the effect of cyclodextrins on both the hydrolysis of



Scheme 1 R = H for hydrogen peroxide; SO_3^- for peroxomonosulfate; $C(O)CH_3$ for peracetic acid; $C(O)\text{-}C_6H_4\text{-}X$ for 3- and 4-substituted perbenzoic acids.

PNPA[11–17] and on its reaction with a range of nucleophiles.[18–20] In the present work we have used six substituted perbenzoic acid anions that are known to form inclusion complexes with α-cyclodextrin and three non-binding peroxides, allowing us to compare the relative effects of cyclodextrin on reactions of these two sets of nucleophiles with PNPA, and to make comparisons to the literature reports of the cyclodextrin-mediated reactions of other non-binding nucleophiles with PNPA.

Experimental

Materials

Details of the preparation and standardisation of peroxide solutions, and the determination of the parent acid content are given in ref. 2. Hydrogen peroxide solutions were prepared from 30 wt.% hydrogen peroxide (Aldrich). All peracid solutions contained 2×10^{-5} mol dm^{-3} EDTA. α-Cyclodextrin was obtained from Aldrich; the solutions used in kinetic studies were prepared from a 0.1 mol dm^{-3} stock solution in distilled

water. For the kinetics, an approximately 1×10^{-4} mol dm^{-3} stock solution of PNPA was prepared by adding PNPA to one litre of distilled water and allowing to stir for one hour before filtering through a 10 μm sintered-glass funnel to remove any undissolved PNPA. The PNPA solution was standardised by hydrolysing in 0.1 mol dm^{-3} sodium hydroxide solution to form *p*-nitrophenol (PNP) in the form of its anion. The PNP anion concentration, which is equivalent to the initial PNPA concentration, can then be calculated from the absorbance at 400 nm, for which the molar absorptivity is 18300 dm^3 mol^{-1} cm^{-1}.[21] For PNPA solutions used in the spectrophotometric study a reduced stirring time was employed and the pH was lowered by adding a small amount of pH 4.6 acetate buffer in order to prevent PNPA hydrolysis. This is important since PNP absorbs in the region of the PNPA spectrum,[21] and has a significant binding constant (245 dm^3 mol^{-1}).[22] From known absorptivities of PNP and its anion it was confirmed that no PNP was present in the stock solution initially or at the end of the spectrophotometric titration, which was approximately 2 hours in duration.

Spectrophotometric determination of binding constants for PNPA

The procedure described in ref. 23 was used to determine the binding constants of the α-cyclodextrin–PNPA complexes. The determinations were made in phosphate buffer, pH 6.8, ionic strength 0.1 mol dm^{-3} at 25 °C. The α-cyclodextrin concentration ranged from 0.6×10^{-3} to 85×10^{-3} mol dm^{-3} and the PNPA concentration was 6.6×10^{-5} mol dm^{-3}.

Potentiometric determination of binding constants for HCO_3^- and CO_3^{2-}

This determination was carried out following the procedure given in ref. 8. The carbonate buffer concentration was 0.01 mol dm^{-3} and sodium nitrate was present at 0.05 mol dm^{-3}.

Kinetics

Pseudo-first order rate constants were determined from non-linear regression of the mono exponential increase in absorbance at 400 nm with time that is due to the formation of the PNP anion. The reaction was carried out at 25 °C in carbonate buffer (sodium salts), pH 10.00 and ionic strength 0.1 mol dm^{-3}. Absorbance changes were followed using an Applied Photophysics SX-17MV stopped-flow spectrophotometer. The concentration of PNPA was 1×10^{-5} mol dm^{-3} and the peroxide was in at least thirty-fold excess. The cyclodextrin concentration ranged from 0.24×10^{-3} to 38.7×10^{-3} mol dm^{-3}. One syringe contained PNPA and cyclodextrin whilst the second syringe contained peracid in double strength buffer; solutions were made up immediately prior to the runs. In these studies it was necessary to work under conditions in which the cyclodextrin concentration was in less than ten-fold excess over the peracid concentration, so it was not possible to use the approximation of $[CD] \cong [CD]_o$, where [CD] and $[CD]_o$ represent the free and total cyclodextrin concentrations respectively. The free cyclodextrin concentration was calculated using the data treatment given in ref. 2, in which only the anions of the parent acid and peracid need to be considered under these experimental conditions.

The hydrolysis of PNPA in buffer alone was followed under the same conditions as for acyl transfer to peroxides, though the much slower reaction necessitated the use of a Pharmacia Biotech Ultraspec 2000 spectrophotometer with thermostatted cell-holder.

Results

Equilibria and reactions for acyl transfer reactions

Fig. 1 shows the effect of α-cyclodextrin on acyl transfer from PNPA to water, 1(a), and to the anions of aromatic, aliphatic and inorganic peroxide anions, 1(b) to 1(i). The results are consistent with the equilibria and reactions given in eqns. (1) to (12)

Cyclodextrin complexes of peroxide anions[8]

$$P + CD \overset{K_{p11b}}{\rightleftharpoons} P,CD \quad (1)$$

$$P,CD + CD \overset{K_{p12b}}{\rightleftharpoons} P,(CD)_2 \quad (2)$$

PNPA–cyclodextrin complexes

$$S + CD \overset{K_{s11}}{\rightleftharpoons} S,CD \quad (3)$$

$$S,CD + CD \overset{K_{s12}}{\rightleftharpoons} S,(CD)_2$$

Hydrolysis

$$S \xrightarrow{k_{0(OH)}} \text{Products} \quad (4)$$

$$S,CD \xrightarrow{k_{1(OH)}} \text{Products} \quad (5)$$

$$S,CD_2 \xrightarrow{k_{2(OH)}} \text{Products} \quad (6)$$

Acyl transfer to peroxide anions

$$P + S \xrightarrow{k_0} \text{Products} \quad (7)$$

$$P,CD + S \xrightarrow{k_{1a}} \text{Products} \quad (8)$$

$$P + S,CD \xrightarrow{k_{1b}} \text{Products} \quad (9)$$

$$P,CD + S,CD \xrightarrow{k_{2a}} \text{Products} \quad (10)$$

$$P,(CD)_2 + S \xrightarrow{k_{2b}} \text{Products} \quad (11)$$

$$P + S,(CD)_2 \xrightarrow{k_{2c}} \text{Products} \quad (12)$$

that define the interactions between cyclodextrin and the reactants in this system. It was not necessary to consider reactions that involve three or more cyclodextrin molecules in the transition state. In these equations P, S and CD represent the peroxide anion, the PNPA substrate and α-cyclodextrin respectively.

Determination of PNPA binding constants

The hydrolysis data in Fig. 1(a) show a rate maximum rather than the simple saturation kinetics behaviour expected for systems in which there is only a 1:1 complex formed between substrate and cyclodextrin; a complex in which two cyclodextrins are bound to the substrate is also indicated to be present by these results. This conclusion was confirmed independently of the kinetics using a spectrophotometric technique[23] to determine values for K_{s11} and K_{s12}, the stepwise binding constants for the 1:1 and 2:1 cyclodextrin–PNPA complexes respectively. Fig. 2 shows the changes in the spectrum of PNPA with varying cyclodextrin concentration. The insets show the variation of absorbance with cyclodextrin concentration at three different

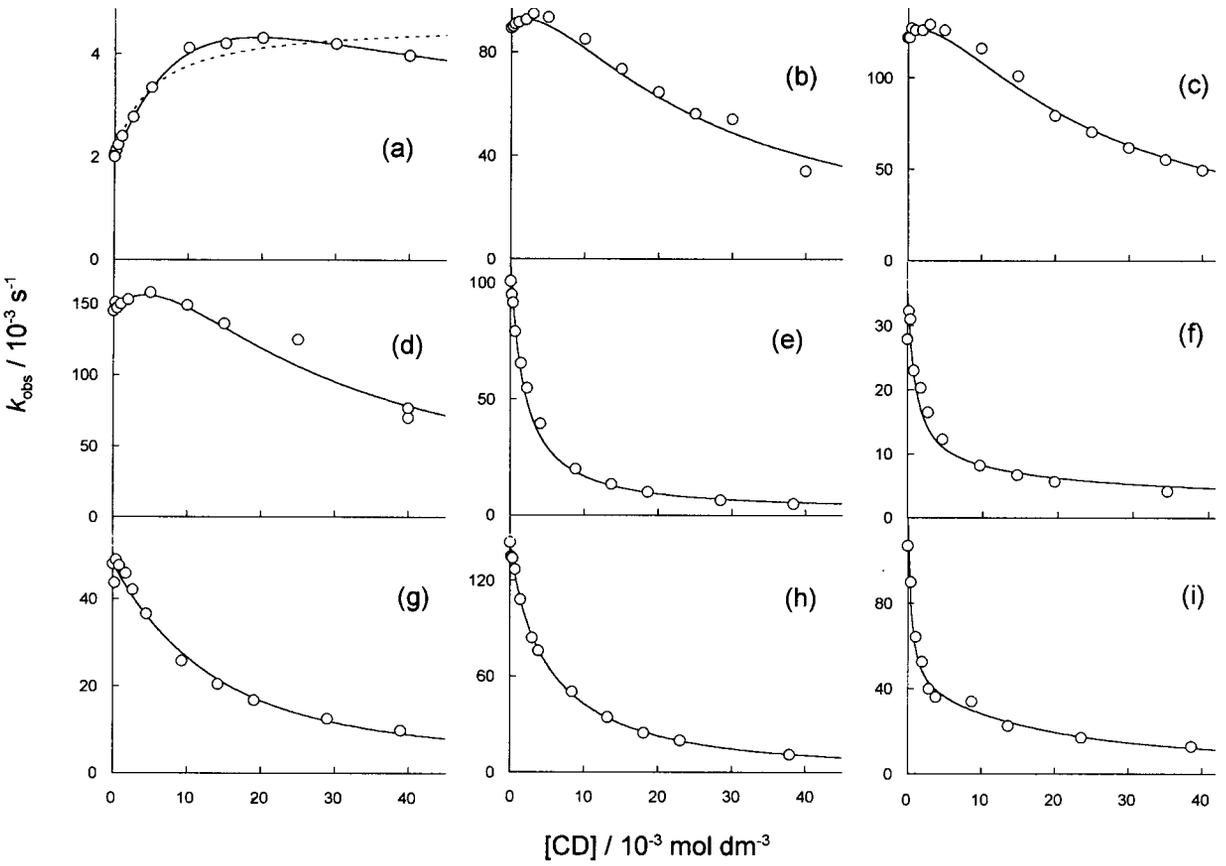


Fig. 1 Effect of α-cyclodextrin on the observed rate constant, k_{obs}, for the hydrolysis of PNPA, (a), and for the reaction between PNPA and the anions of: peracetic acid, (b); monoperoxosulfate, (c); hydrogen peroxide, (d); perbenzoic acid, (e); 4-methylperbenzoic acid, (f); 4-nitroperbenzoic acid, (g); 4-sulfonatoperbenzoic acid, (h); 3-chloroperbenzoic acid, (i). The curves show the best fits to eqn. (14) for (a) and to eqn. (17) for (b) to (i), using the stability constants and kinetic parameters given in Tables 1 and 2. Conditions in all cases were pH 10.00 carbonate buffer, ionic strength 0.1 mol dm^{-3}, 25 °C.

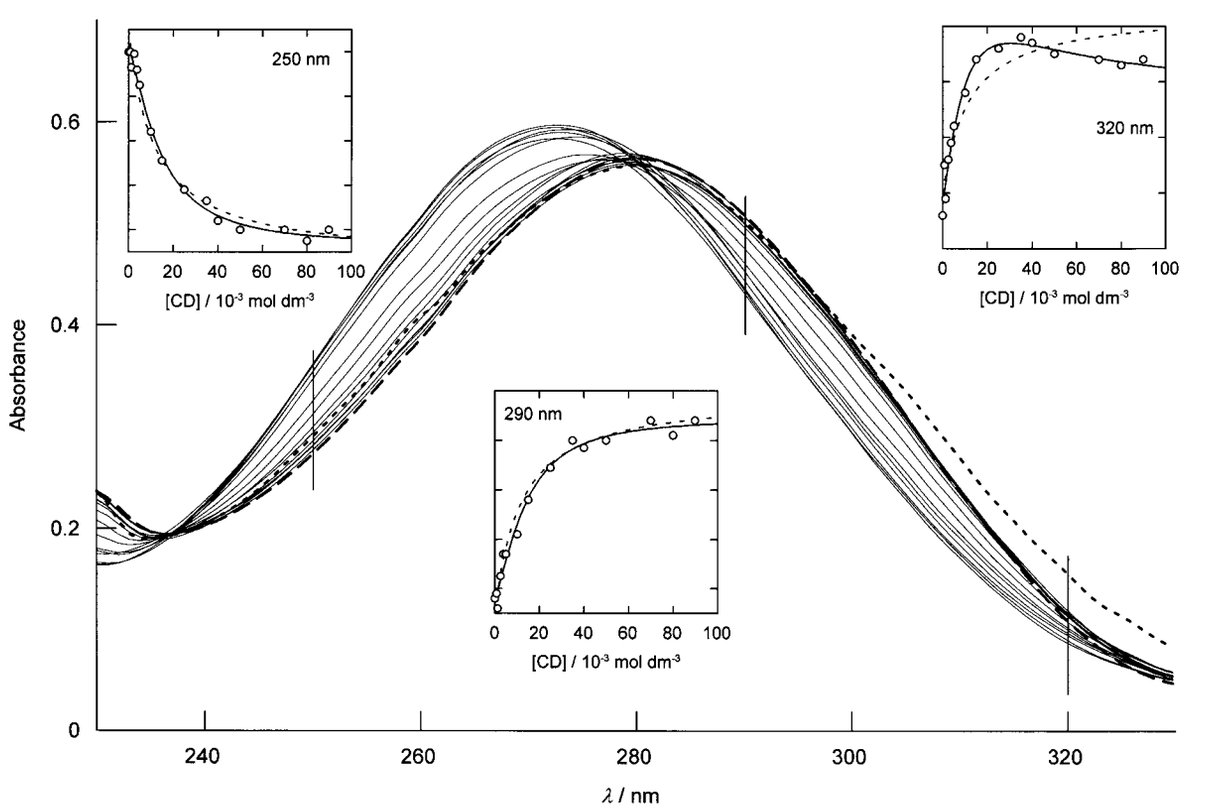


Fig. 2 Effect of α-cyclodextrin on the spectrum of 6.6×10^{-5} mol dm^{-3} PNPA in phosphate buffer, pH 4.6, ionic strength 0.1 mol dm^{-3} at 25 °C. The dotted and broken lines on the main graph are, respectively, the calculated spectra of the 1 : 1 and 2 : 1 cyclodextrin–PNPA complexes. The insets show variation in absorbance with increasing cyclodextrin concentration for 250, 290 and 320 nm; the solid line is the best fit to eqn. (13), whereas the dotted line is the best fit to eqn. (13) in which K_{s12} is set to zero.

Table 1 Comparison of literature binding constants, rate constants and transition state pseudo equilibrium constants (± standard deviation) for the hydrolysis of *p*-nitrophenyl acetate in the presence of α-cyclodextrin at 25 °C

Entry no.	Conditions	K_{s11}/dm^3 mol^{-1}	K_{s12}/dm^3 mol^{-1}	k_0/10^{-3} s^{-1}	$k_{1obs(OH)}$/dm^3 mol^{-1} s^{-1}	$k_{2obs(OH)}$/dm^6 mol^{-2} s^{-1}	K_{TS1}/dm^3 mol^{-1}	K_{TS2}/dm^3 mol^{-1}	Reference
1	pH 10.0, carbonate	46[a]	66[a]	2.00 ± 0.00	0.439 ± 0.008	6.95 ± 0.31	219.5	15.83	This work
2	pH 10.0, borate	Significant[b]	Significant[b]	2.3	—	—	—	—	13
3	pH 10.4, carbonate	95.2	—[c]	1.75	0.54[d]	—	307[e]	—	12
4	pH 11.7, phosphate	100	—	96	27	—	277	—	11
5	pH 10.6, carbonate	83	—	6.94	2.02[f]	—	291[g]	—	14

[a] Determined independently of the kinetic data using a spectrophotometric technique. [b] The authors state that formation of a 2:1 cyclodextrin–PNPA complex is likely, though they did not derive stability or kinetic parameters from the data. [c] Indicates that the parameter was not detectable. [d] Calculated from binding constant and kinetic data given in reference 12, using the relationship $k_{1obs} = k_1 K_{s11}$, where k_1 is the rate constant for the reaction catalysed by one molecule of cyclodextrin. [e] Calculated from the data given in reference 12. [f] Calculated from binding constant and kinetic data given in reference 14, using the relationship $k_{1obs} = k_1 K_{s11}$. [g] Calculated from the data given in reference 14.

wavelengths for which the solid line is the best fit to eqn. (13),

$$\frac{A(\lambda_i)}{[S]_0} = \frac{\varepsilon_0(\lambda_i) + \varepsilon_{11}(\lambda_i)K_{s11}[CD]_0 + \varepsilon_{12}(\lambda_i)K_{s11}K_{s12}[CD]_0^2}{1 + K_{s11}[CD]_0 + K_{s11}K_{s12}[CD]_0^2} \quad (13)$$

determined globally for nine different wavelengths, which describes the spectral changes upon formation of 1:1 and 2:1 cyclodextrin–guest complexes. $A(\lambda_i)$ is the absorbance at i nm and $\varepsilon_0(\lambda_i)$, $\varepsilon_{11}(\lambda_i)$ and $\varepsilon_{12}(\lambda_i)$ are the molar absorptivities for free PNPA, the 1:1 complex of PNPA and cyclodextrin and the 2:1 complex respectively. Values of 46 ± 9 and 66 ± 19 dm^3 mol^{-1} were obtained for K_{s11} and K_{s12} respectively using this technique. The dotted lines in the insets to Fig. 2 show the best fit to a simplified form of eqn. (13), in which K_{12} is set to zero.

Hydrolysis

The solid curve in Fig. 1(a) is the best fit to eqn. (14) in which

$$k_{obs(OH)} = \frac{k_{0(OH)} + k_{1obs(OH)}[CD] + k_{2obs(OH)}[CD]^2}{1 + K_{s11}[CD] + K_{s11}K_{s12}[CD]^2} \quad (14)$$

$k_{obs(OH)}$ is the observed first order rate constant and $k_{0(OH)}$, $k_{1obs(OH)}$ and $k_{2obs(OH)}$ are the zero, first and second order dependencies on cyclodextrin respectively, as defined by eqns. (15) and (16). Values for K_{s11} and K_{s12} determined by

$$k_{1obs(OH)} = k_{1(OH)}K_{s11} \quad (15)$$

$$k_{2obs(OH)} = k_{2(OH)}K_{s11}K_{s12} \quad (16)$$

spectrophotometric titration, as described above, were substituted into eqn. (14) and the best fit parameters listed under Entry 1 in Table 1 obtained by linear regression. The dotted line in Fig. 1(a), which describes the data poorly, is the best fit to a simplified form of eqn. (14) in which both K_{s12} and k_{2obs} are set to zero.

Acyl transfer to peroxides

Values of K_{s11} and K_{s12} used in the analysis of this data were those determined independently by spectrophotometric titration (Entry 1 in Table 1) and were substituted into eqn. (17).

$$k_{obs} = \frac{k_0 + k_{1obs}[CD] + k_{2obs}[CD]^2}{1 + K_{s11}[CD] + K_{s11}K_{s12}[CD]^2)(1 + K_{p11}[CD] + K_{p11}K_{p12}[CD]^2} + k_{obs(OH)} \quad (17)$$

The values of peroxide binding constants shown in Table 2, which were taken from refs. 8 and 24, were also substituted into eqn. (17). The solid curves in Fig. 1(b) to 1(i) are the best fits to eqn. (17) in which k_{obs} is the observed first order rate constant and k_0, k_{1obs} and k_{2obs} are, respectively, the zero, first and second order dependencies on cyclodextrin. In eqn. (17), k_{1obs} and k_{2obs} are defined by eqns. (18) and (19), and $k_{obs(OH)}$ is the hydrolysis

$$k_{1obs} = k_{1a}K_{p11a} + k_{1b}K_{s11} \quad (18)$$

$$k_{2obs} = k_{2a}K_{p11}K_{s11} + k_{2b}K_{p11}K_{p12} + k_{2c}K_{s11}K_{s12} \quad (19)$$

component of the reaction, as defined by eqn. (14); values for $k_{obs(OH)}$ at each cyclodextrin concentration were obtained from parameters derived from the independently determined PNPA hydrolysis data. The presence of a significant hydrolysis term necessitated that kinetic analysis of data for acyl transfer to peroxide anions was carried out on the pseudo first order rate constants. Kinetic parameters obtained from the analysis were subsequently converted to second, third and fourth order (where present) rate constants by calculating the quotient of the kinetic parameter and the peroxide anion concentration, which remained essentially constant throughout the duration of the experiment. The converted best fit parameters are listed in Table 2.

Interaction of carbonate buffer with cyclodextrin

Buffer components and inert salt components of reaction mixtures, such as sodium nitrate added to adjust ionic strength, can interact with cyclodextrin to form inclusion complexes, thus reducing the number of available binding sites for the substrates under study;[24] this has been observed for acetic acid (K_{11} = 10 dm^3 mol^{-1})[8,25] and nitrate (K_{11} = 1.4 dm^3 mol^{-1}).[26] Recent binding studies conducted in which these components were present have used the convention of expressing the free cyclodextrin concentration as the sum of the uncomplexed cyclodextrin and that complexed by the buffer or salt component.[8,24] The binding constants and transition state pseudo-equilibrium constants derived from these studies are, therefore, apparent constants and can be converted to 'actual' constants, in the absence of any buffer component, using eqn. (20);[24] K_{app} is the apparent

$$K = K_{app}(1 + K_b[B]) \quad (20)$$

binding or transition state pseudo-equilibrium constant, K is the constant in the absence of buffer components, K_b is the binding constant of the buffer component and [B] is the concentration of the specific buffer component that interacts with the cyclodextrin. With acetate buffer, for example, only the molecular acid form binds.[8]

In the present study, which was conducted in pH 10.0 carbonate buffer, it was found from a potentiometric study that CO_3^{2-} interacts with cyclodextrin to a small degree. Fig. 3 shows the variation of apparent pK_a of HCO_3^- with cyclo-

Table 2 Binding constants, rate constants (± standard deviation) and transition state pseudo equilibrium constants for the reaction of nucleophiles with *p*-nitrophenyl acetate at 25 °C. Binding constants of $K_{s11} = 46$ dm^3 mol^{-1} and $K_{s12} = 66$ dm^3 mol^{-1}, determined in the present study for PNPA, were used for the analysis of kinetic data for reactions involving peroxides

Nucleophile	K_{p11b}/dm^3 mol^{-1}	K_{p12b}/dm^3 mol^{-1}	k_0/dm^3 mol^{-1} s^{-1}	k_{1obs}/10^3 dm^6 mol^{-2} s^{-1}	K_{TS1}/dm^3 mol^{-1}
Non-binding nucleophiles					
Peracetic acid	—[a,b]	—	90 ± 3	4.6 ± 0.3	51.1
Peroxomonosulfate	—[b]	—	104 ± 2	4.9 ± 0.2	47
Hydrogen peroxide	—[c]	—	2774 ± 84	203 ± 11	73
Trifluoroethanol	—	—	13	0.813[d]	63[e]
Mercaptoethanol	—	—	13	1.429[d]	110[e]
Hydroxylamine	—	—	2.1	0.321[d]	154[e]
Imidazole	—	—	0.55	0.076[d]	139[e]
Binding nucleophiles					
Perbenzoic acid	557[b]	—	86 ± 3	3.3 ± 0.7	38
4-Methylperbenzoic acid	913[b]	—	81 ± 6	10.0 ± 2.7	123
4-Nitroperbenzoic acid	183[f]	—	53 ± 2	7.6 ± 0.4	142
4-Sulfonatoperbenzoic acid	289[f]	—	66 ± 1	5.8 ± 0.1	88
3-Chloroperbenzoic acid	2087[f]	—	92 ± 8	53 ± 6[g]	583[h]
4-*tert*-Butylperbenzoic acid	1136[i]	538[i]	86 ± 3	<8	<93

[a] Not detectable. [b] From ref. 8. [c] D. M. Davies and M. E. Deary, unpublished data. [d] Calculated from binding constant and kinetic data given in reference 19, using the relationship $k_{1obs} = k_1 K_{s11}$, where k_1 is the rate constant for the reaction catalysed by one molecule of cyclodextrin. [e] Calculated from ref. 19. [f] From ref. 24. [g] k_{2obs} also present in this case: 1.1 ± 0.43 × 10^6 dm^9 mol^{-3} s^{-1}. [h] $K_{TS2} = 20.6$ dm^3 mol^{-1}. [i] This work.

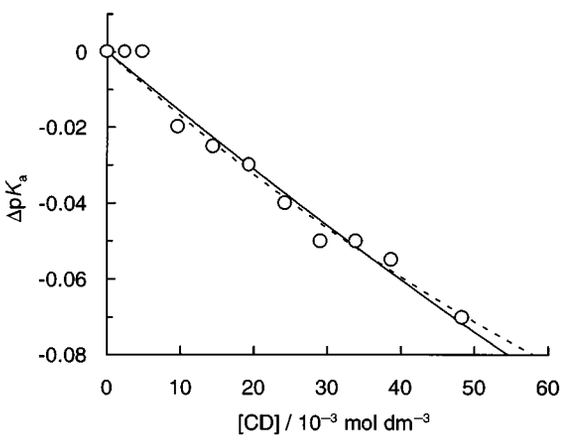


Fig. 3 Effect of α-cyclodextrin on the pK_a of 10 × 10^{-3} mol dm^{-3} HCO_3^- at 25 °C in 0.05 mol dm^{-3} sodium nitrate. The dotted line is the best fit to eqn. (21), whereas the solid line is the best fit to eqn. (21) in which it is assumed that HCO_3^- does not bind (K_{11a} is set to zero).

dextrin concentration. The dotted line is the best fit to eqn. (21),

$$\Delta pK_a = \log \frac{1 + K_{11a}[CD]}{1 + K_{11b}[CD]} \qquad (21)$$

which yields the meaningless values of 3.4 ± 4.0 and 7.8 ± 4.4 dm^3 mol^{-1} for K_{11a} and K_{11b} respectively, whereas the solid line is the best fit to eqn. (21), in which it is assumed that HCO_3^- does not bind (K_{11a} is set to zero), yielding a K_{11b} value of 4.0 ± 0.1 dm^3 mol^{-1}; the latter value was used. All binding and pseudo-equilibrium constants in this paper are expressed as apparent constants in the presence of 0.0231 mol dm^{-3} CO_3^{2-}. A correction factor of 1.092 can be used to convert to constants in the absence of carbonate buffer.

4-*tert*--Butyl perbenzoate

With the exception of 4-*tert*-butylperbenzoic acid, peracid anion binding constants used in the analysis of kinetic data were taken from the potentiometric titration studies described in refs. 8 and 24. The binding constants listed for the 4-*tert*-butylperbenzoic acid anion have, however, been redetermined in the present paper using global analysis of kinetic data for the reaction of the 4-*tert*-butylperbenzoic acid with 4-nitrophenyl methyl sulfide;[1,8] potentiometric titration data;[8] and the data from the present study for the reaction of the anion. This was done because 4-*tert*-butylperbenzoic acid is a complex case, with the molecular acid form and its anion each forming both 1:1 and 2:1 cyclodextrin–guest complexes.[8] Consequently, analysis of the original potentiometric data was not possible without a combined analysis involving kinetic data for the reaction of the molecular acid form with 4-nitrophenyl methyl sulfide.[8] The values arising from that analysis are likely to have been biased towards K_{11a} and K_{12a}, since the molecular acids were the species involved in sulfide oxidation. It is important, therefore, to redetermine the binding constants using the additional set of data for acyl transfer from PNPA. Fig. 4 shows the best fit to each of the sets of data using the global analysis. Table 3 compares the original binding and kinetic data with the re-determined values. The kinetic and potentiometric titration data are described well by the results of the global analysis, whilst the binding and kinetic parameters are not significantly altered. It should be noted that the differences in competitive binding for acetate, nitrate and carbonate (present study) were accounted for in the global analysis.

Table 3 Comparison of the original[1,8] and re-determined values of binding and kinetic parameters for 4-*tert*-butylperbenzoic acid and its anion

Parameter	Original value	Re-determined value
K_{p11a}/dm^3 mol^{-1}	524 ± 68	806 ± 83
K_{p12a}/dm^3 mol^{-1}	369 ± 75	446 ± 76
K_{p11b}/dm^3 mol^{-1}	839 ± 18	1136 ± 80
K_{p12b}/dm^3 mol^{-1}	398 ± 75	538 ± 105
k_0 (sulfide oxidation)/dm^3 mol^{-1} s^{-1}	24.4	24.5
k_{1obs} (sulfide oxidation)/10^3 dm^6 mol^{-2} s^{-1}	144 ± 3	157 ± 8
k_{2obs} (sulfide oxidation)/10^6 dm^9 mol^{-3} s^{-1}	37 ± 1.4	76 ± 17

Discussion

Binding constants for PNPA

The evidence produced in this study for the existence of both 1:1 and 2:1 α-cyclodextrin–PNPA complexes supports the observation of Easton *et al.* that α-cyclodextrin-mediated hydrolysis of PNPA at higher cyclodextrin concentrations is inconsistent with Michaelis–Menton kinetics, probably due to the formation of a less reactive 2:1 cyclodextrin–PNPA com-

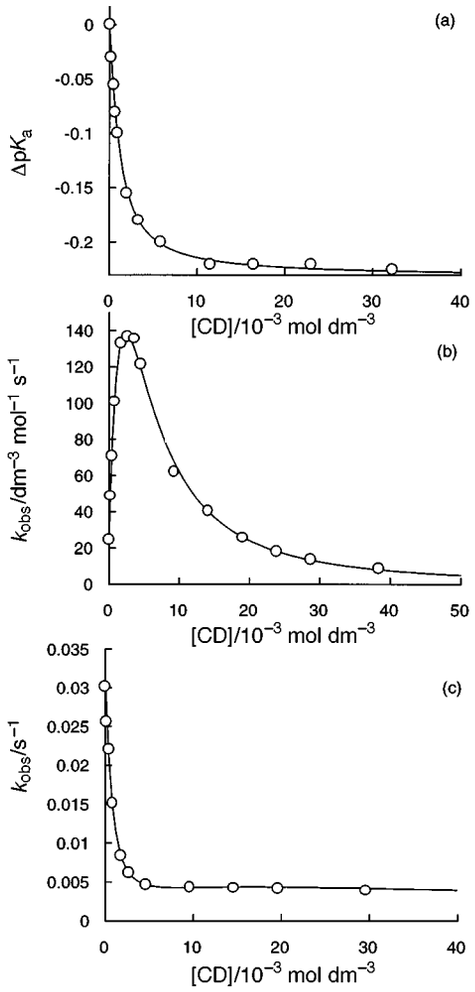
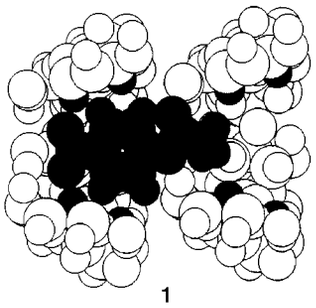


Fig. 4 Effect of α-cyclodextrin on: (a) ΔpK_a for 4-*tert*-butylperbenzoic acid; (b) the observed second order rate constant, k_{obs}, for the reaction between 4-*tert*-butylperbenzoic acid and methyl 4-nitrophenyl sulfide; and (c) the observed first order rate constant, k_{obs}, for the reaction between the 4-*tert*-butylperbenzoic acid anion and PNPA. The curves are the best fits obtained simultaneously using a global non-linear regression analysis of all three sets of data in which the binding constants were common parameters to the three regression equations used [eqn. (5) in reference 8 for (a); eqn. (6) in reference 8 for (b); and eqn. (17) in the present paper for (c)]. The parameters are listed in Tables 2 and 3. Conditions for (a) and (b) are given in reference 8; conditions for (c) were pH 10.0 carbonate buffer, ionic strength 0.1 mol dm^{-3}, 25 °C.

plex.[13] These results contradict previous literature studies on this reaction, in which only a 1:1 complex was considered, with a binding constant of *ca.* 100 dm^3 mol^{-1}. The literature binding constants are listed in entries 3–5 in Table 1 and were derived entirely from kinetic data for the hydrolysis of PNPA in the presence of cyclodextrin.[11,12,14] However, the cyclodextrin concentrations used in these studies (typically 0 to 10 mmol dm^{-3}) were not sufficient for the effect of the 2:1 cyclodextrin–PNPA complex to manifest, a fact that can be appreciated from a consideration of Fig. 1(a). It is preferable, where technically feasible, to determine reliable binding constants independently of the kinetics, since the possibility of kinetic data being described by several, very different, combinations of binding and kinetic parameters, is very real.[27] Spectrophotometric titration methods, as used in the present case, provide a reliable method, although it is important that the data analysis encompasses the entire spectrum of the substrate in order to prevent ambiguity over the number of binding steps involved. The spectrophotometric data for PNPA shown in Fig. 2, for example, can be described by both a 1:1 and 2:1 binding equation at 250 and 290 nm, whereas at 320 nm it is evident that only the 2:1 binding equation is adequate.



1

Structure **1** shows a possible conformation of the 2:1 complex that has the alkyl group of the ester included in the narrow end of the second cyclodextrin. A similar conformation is likely for *p*-nitrophenyl alkanoates of increasing alkyl chain length, though only *p*-nitrophenyl propionate has been reported to form a 2:1 complex.[11]

A small degree of co-operativity is observed for the α-cyclodextrin–PNPA complex, *i.e.* $K_{s12} > K_{s11}$. This has also been observed for aryl alkyl sulfides,[23] substituted benzoic acids and their anions, and alkyl peracids.[8] The effect has been ascribed to an enhanced interaction between opposite dipoles of the primary and secondary hydroxy rims that occur as a result of the conformational and electrostatic changes brought about by substrate inclusion.[23]

Effect of α-cyclodextrin on acyl transfer

The presence of a rate maximum in Fig. 1(a) for the PNPA hydrolysis reaction indicates that a 2:1 cyclodextrin–PNPA complex is formed that results in the PNPA taking up a less reactive configuration with respect to attack by the secondary alcohol moieties of the cyclodextrin. The different effects of α-cyclodextrin on acyl transfer from PNPA to peracid anions are shown in Fig. 1(b) to 1(i); the corresponding data for 4-*tert*-butylperbenzoic acid are shown in Fig. 4(c). For the non-binding peroxide anions, hydrogen peroxide, peracetic acid and peroxomonosulfate, Fig. 1(b)–(d), there is a slight increase in k_{obs} at low cyclodextrin concentrations, followed by a decline. This observation clearly shows that there is catalysis by cyclodextrin for these reactions to some small degree. For peroxides that form inclusion complexes with α-cyclodextrin, there is only a decline in k_{obs} with increasing cyclodextrin concentrations, with the extent of this decline dependent on the relative magnitudes of: (a) transition state stabilisation by cyclodextrin and (b) the strength of inclusion complex formation and the number of binding steps involved. Values of k_{obs} for the anion of 4-*tert*-butylperbenzoic acid, for example, rapidly fall to the hydrolysis baseline, reflecting the strong 1:1 and 2:1 cyclodextrin–guest complexes formed by this peracid anion and the relatively weak transition state stabilisation observed for this reaction. This is very different behaviour to that observed for the reaction of the molecular acid forms of these peroxides, where significant increases in k_{obs} are observed at low cyclodextrin concentrations, as shown in Fig. 4(b) for the reaction of 4-*tert*-butylperbenzoic acid with methyl 4-nitrophenyl sulfide.[1,8]

Transition state pseudo equilibrium constants

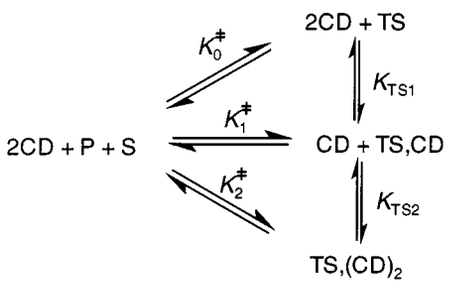
The transition state pseudo equilibrium constant approach has proved to be a very useful tool in the elucidation of multiple pathway reaction systems.[1–3] The transition state pseudo equilibrium constants K_{TS1} and K_{TS2} are calculated from eqns. (22)

$$K_{TS1} = \frac{K_1^{\ddagger}}{K_0^{\ddagger}} = \frac{k_{1obs}}{k_0} \qquad (22)$$

and (23), which are derived from the thermodynamic cycle

$$K_{TS2} = \frac{K_2^{\ddagger}}{K_1^{\ddagger}} = \frac{k_{2obs}}{k_{1obs}} \quad (23)$$

shown in Scheme 2. These quantities reflect the stabilisation



Scheme 2

imparted to the transition state as a result of its association with one and two molecules of cyclodextrin respectively.

Table 1 compares the K_{TS1} value obtained for PNPA hydrolysis in the present study with those calculated from the literature data for similar studies. The values are similar, though the literature values, which are derived from kinetic parameters calculated using an assumption of only 1:1 complexation, are slightly higher than those obtained in the present study. The involvement of a second cyclodextrin molecule, as reported in the present study, gives rise to a small K_{TS2} of 16 dm^3 mol^{-1}, which is about a third of the value of the second binding constant, K_{s12}, for PNPA.

Table 2 lists K_{TS1} and K_{TS2} values, where present, for acyl transfer to peracid anions and for reactions between PNPA and various nucleophiles studied by Tee and Gadosy.[19] The table is divided into those nucleophiles known to form complexes with α-cyclodextrin, and those that do not, including all those studied by Tee and Gadosy. The K_{TS1} values of between 47 and 154 dm^3 mol^{-1} for the non-binding nucleophiles (47 to 73 dm^3 mol^{-1} for non-binding peroxide anions) indicate a small degree of transition state stabilisation as a result of PNPA being bound in the α-cyclodextrin cavity. The lowest values are approximately equal to K_{s11} for PNPA (46 dm^3 mol^{-1}), indicating that the cyclodextrin is not making the PNPA more reactive as a result of inclusion. This 'neutral binding' behaviour has also been observed for 2:1 inclusion complexes of alkyl peracids, where the second cyclodextrin does not participate in the reaction, unlike the first.[2] The variation in values for the parameters in Table 2 may reflect some difference in the ability of these nucleophiles to approach the included PNPA, possibly as a result of steric or electrostatic interactions.

For those nucleophiles that do bind to cyclodextrin, with the exception of the *m*-chloroperbenzoic acid anion, the K_{TS1} values are similar in range to the non-binding nucleophiles. This indicates a transition state in which the free peracid anion reacts with the bound PNPA molecule [eqn. (9)]. These conclusions are further supported when logarithms of K_{TS1} values obtained for this reaction, which are proportional to the Gibbs free energy of stabilisation of the transition state by one molecule of cyclodextrin, are compared to similar quantities for other reactions. A strong correlation would imply a similar mechanism of catalysis for the two types of reaction, as in Fig. 5, which shows a previously published correlation between log K_{TS1} values for the oxidation of iodide and methyl 4-nitrophenyl sulfide by the molecular acid form of the peracids (filled circles).[2] The slope of 0.95 indicates a common catalytic mechanism for these two substrates, which we have shown from other free energy studies to be due to the activation of bound peracid by cyclodextrin.[2] By contrast, when a similar comparison is made in Fig. 5 for the reaction of the peracid anions with PNPA (open circles), a much lower slope is

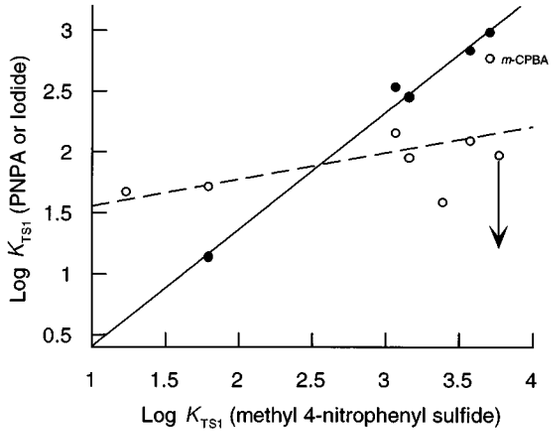


Fig. 5 Linear free energy relationships. Filled circles: correlation between log K_{TS1} values for the α-cyclodextrin-mediated oxidation of methyl 4-nitrophenyl sulfide and iodide by peracids. Open circles: correlation between log K_{TS1} values for the α-cyclodextrin-mediated oxidation of methyl 4-nitrophenyl sulfide by peracids and log K_{TS1} values for the reaction between PNPA and peracid anions.

obtained and there is considerable scatter about the dotted line that is the best fit to all the points; clearly, the transition state stabilisation observed for this reaction is very different from that for peracid oxidations of iodide and methyl 4-nitrophenyl sulfide substrates and is consistent with bound PNPA reacting with free peracid anion [eqn. (9)], for which a relatively invariant relationship would be expected. The scatter in this plot may reflect steric and electrostatic interactions during the approach of the nucleophile to the relatively conformationally free acetyl group at the wide end of the cyclodextrin cavity. The *m*-chloroperbenzoic acid anion shows a significant deviation from the other peracid anions in Fig. 5, and also shows a second order dependence on cyclodextrin concentration in its reaction with PNPA (Table 2). The reaction mediated by two cyclodextrin molecules most probably involves a transition state in which both the peracid anion and the PNPA are bound [eqn. (10)].

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