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Acceleration of Thiol Ester Hydrolysis by Cyclodextrins: Evidence from Rate and Computational Studies

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Hydrolysis of diaryl thiol esters with different substituents is studied in the presence of cyclodextrins under alkaline conditions. The presence of cyclodextrin enhances the rate of hydrolysis significantly and covalent catalysis by secondary hydroxy groups of cyclodextrin is proposed. The observed results and the proposed model find support in molecular dynamics simulations and free energy calculations.

Introduction

Cyclodextrins (CDs) consist of α-(1,4)-linked (+)-D-glucopyranose units, which exist in the form of a truncated cone-shape with a hydrophobic interior and are widely used as hosts to form inclusion complexes with small and medium sized organic molecules. Compounds with six, seven or eight glucose units are called α-, β- and γ-cyclodextrins.[1] The host-guest nature of CD-substrate complexes brings out remarkable changes in the spectroscopic and physicochemical properties as well as reactivities of the included guests.[2,3] Cyclodextrins are also shown to be good models for hydrolytic enzymes and several studies[4–19] have been carried out on the cyclodextrin-catalyzed hydrolysis of esters. For example, cycloamyloses cause a markedly stereoselective acceleration of phenol release from a variety of substituted phenyl acetates in alkaline solution.[4] In this reaction, the acyl portion of the esters is transferred to a hydroxy group of the CD forming a cycloamylose acetate, which undergoes further hydrolysis.

Cycloamylose benzoate is separated from phenol and its ester by gel filtration chromatography.[5] Hydrolysis of acetanilide is catalyzed by α-cyclodextrin and this rate enhancement is an excellent model of hydrolytic enzymes.[6] The hydrolysis of diaryl pyrophosphate in the presence of calcium ions is catalyzed by cyclodextrins and in this reaction a transfer of the phosphate residue to the cyclodextrin is observed.[7]

Tee et al. have extensively studied[8–11] the hydrolysis of substituted phenyl acetates by CDs in basic aqueous solution and also the basic hydrolysis of 1-naphthyl acetate and 2-naphthyl acetate in presence of cyclodextrin, which show that these two positional isomers differ significantly towards the cyclodextrin-catalyzed hydrolysis.[8,9] Chain length effects in the cleavage of aryl esters by cyclodextrins is studied and different transition states from *m*- and *p*- nitrophenyl alkanoates with cyclodextrin[10] are proposed. Retardation of hydrolysis of benzaldehyde dimethyl acetal is also studied and the retardation is attributed to the strong binding of the acetal inside the cyclodextrin cavity.[11]

Effect of cyclodextrin encapsulation on the hydrolysis of various anilides and esters are also studied by de Rossi et al.[12–19] In the hydrolysis of differently substituted trifluoroacetanilides studied in presence of CDs, different kinetic behavior of the substrate is observed.[12] *p*-Nitroacetanilide and *p*-trifluoroacetanilide show two types of inclusion complexes (a, 1:1 and 1:2 substrate/cyclodextrin complex) with β-CD. Both complexes react faster than the substrate and the results are justified by two different mechanisms. The pH dependent effect of β-CD on the hydrolysis rate of trifluoroacetate esters is reported.[13] These esters show pH-dependence below pH 6 and show first-order dependence on hydroxide ion concentration above pH 8. In presence of β-CD, the rate increases in a non-linear fashion at constant pH and above pH 8, but it decreases at neutral solution. Mechanistic studies[14] on the ester hydrolysis show that the reaction corresponds to general base-catalyzed addition of water and break of the leaving group with no intermediate with finite lifetime. Further studies[15] on the same system show that the less efficient acceleration by β-CD for the reactions of trifluoroacetate esters compared with acetate ester is due to smaller stabilization of the transition state for the perfluorinated esters than those of the acetates and consequently less stronger hydrolysis is observed. Effect of cyclodextrin on the intramolecular catalysis of amide hydrolysis of phthalamic acids and *N*-phenylmaleamic acid is studied and the reaction is strongly inhibited by cyclodextrin.[16] Hydrolysis of phthalic anhydride and different substituted aryl hydrogen phthalate esters are also studied.[17,18] The hydrolysis of phthalic anhydride is catalyzed by buffer

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bases and that of phthalate esters occurs with the formation of phthalic anhydride as the intermediate. Phenyl esters of perfluoroalkanoic acids (with different fluoroalkyl chains) undergo β-CD-mediated hydrolysis involving shifts in the mechanism because of different modes of inclusion of the substrate inside the CD cavity.[19] In a recent study, different substituted phenolate anions are used as bases for the hydrolysis of cyclic phthalate and maleate esters.[20]

Remarkable rate enhancement ($K_{cat}/K_{uncat} > 2000$) is achieved in glycoside hydrolysis on modifying the cyclodextrin hydroxy group with cyano group.[21] Thus it is obvious that even minor variations in the structures of the substrate as well as CD can lead to considerable variations in the kinetic behavior of CD-catalyzed reactions and in the type of complex formed.[22,23]

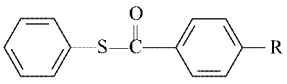
The reactivity of thiol ester and comparison of the reactivity with oxo esters have been of longstanding interest, largely because of the importance of thiol esters in enzymatic reactions of coenzyme A and cystein proteases. They are substrates in many biological transesterifications with larger negative standard free energy change and are very good acylating agents.[24] Brown et al.[25] have studied the hydrolysis of thiol esters by phosphate buffer, which catalyzes readily the decomposition of various active acyl compounds. In a recent work, alkaline hydrolysis of *S*-phenyl 4-hydroxythiobenzoate gives the product via *p*-oxoketene intermediate and an E_1CB mechanism operating through dissociation pathway is proposed.[26] CD-mediated cleavage of thiol esters like *S*-*p*-nitrophenyl thioacetate and *S*-ethyl *p*-nitrothiobenzoate have been studied and the accelerated hydrolytic cleavage of these esters proceeds via a tetrahedral intermediate, consistent with reactions catalyzed by serine protease.[27]

While recent studies on the CD-accelerated cleavage of oxo esters exhibit clearcut differences of the effect of CD dependence on the leaving group, less attention has been given to the effect of CD on the thiol ester hydrolysis particularly arenethiol esters.

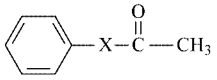
Results and Discussion

The proposed active participation of hydroxy group of CD in catalyzing organic reactions and stabilizing[28] reaction intermediates has prompted us to study the hydrolysis of different thiol esters **1**–**5** given below: substrate without substitution in both the rings (*S*-phenyl thiobenzoate, **1**), with electron-withdrawing group in one ring (*S*-phenyl *p*-nitrothiobenzoate, **2**) and with electron- releasing group in one ring (*S*-phenyl *p*-methylthiobenzoate, **3**). For comparison, hydrolysis of *S*-phenyl thioacetate (**4**) and phenyl acetate (**5**) are also studied.

The results of hydrolysis of thiol esters **1**–**5** accelerated by CD (Table 1) show that with **1**, in the absence of CD, even after one day, the conversion to hydrolyzed products is less than 5%. However in the presence of α-, β- and γ-CDs, the percentage of conversion increases very significantly. Only thiophenol and diphenyl disulfide are identified



R = H **1**
R = NO_2 **2**
R = CH_3 **3**



X = S **4**
X = O **5**

by GC and neither benzoic acid nor other aryl derived products are observed in the product mixture. As the reaction time increases, the percentage conversion also increases. Similarly an increase in the amount of CD also increases the percentage of conversion.

Table 1. Results of hydrolysis of thiol esters **1**–**3** catalyzed by cyclodextrins.[a]

Cyclodextrin	Time (h)	Conversion (%)	Products[b] (%) thiol	disulfide
S-Phenyl thiobenzoate (**1**)				
Without CD	12	4.70	–	4.70
α-CD	12	40.8	8.80	32.0
β-CD	4	20.7	5.80	14.9
β-CD	8	25.4	3.40	22.0
β-CD	12	51.8	16.9	34.9
β-CD	16	76.0	21.3	54.7
γ-CD	12	73.4	–	73.4
β-CD (2:1)[c]	12	100	–	100
S-Phenyl *p*-nitrothiobenzoate (**2**)				
Without CD	12	10.2	3.30	6.90
α-CD	12	31.7	6.5	25.2
β-CD	12	99.9	54.3	45.6
γ-CD	12	100	28.4	71.6
S-Phenyl *p*-methylthiobenzoate (**3**)				
Without CD	12	1.46	–	1.46
α-CD	12	22.0	–	22.0
β-CD	12	25.0	–	25.0
γ-CD	12	30.2	–	30.2

[a] All reactions are carried out in sodium carbonate/hydrogen carbonate buffer at pH 9.4 with 50% acetonitrile/water mixture as the solvent; in all the cases the CD:substrate molar ratio was 1:1, unless specified otherwise. [b] Analyzed by GC; error limit ± 2%. [c] Molar ratio of CD:substrate.

When the hydrolysis of thiol ester **2** is studied under identical conditions, the reaction is faster even in the absence of cyclodextrin. This is expected as the nitro group, with its very strong electron-withdrawing nature, increases the electron deficiency at the carbonyl carbon. In presence of cyclodextrins, hydrolysis becomes faster as evident from an increase in percentage of conversion. It is also interesting to note that with all three substrates **1**–**3**, an increase in cyclodextrin size (from α to γ) increases the conversion percentage.

However, with thiol ester **3**, possessing a *p*-methyl substituent, the hydrolysis is slower than the unsubstituted thiol ester **1**, reflecting that the presence of electron-releas-

Table 2. Rate constants for the hydrolysis of thiol esters **1**–**5** in presence and absence of cyclodextrins.[a]

Thiol ester	$k_{uncat} \times 10^{-6}$ s^{-1}	$k_{cat} \times 10^{-3}$ s^{-1}			k_{cat}/k_{uncat}			K_{CDOH} (**1**/K_d) [M^{-1}]		
		α	β	γ	α	β	γ	α	β	γ
1	8.30	1.07	1.72	0.41	129	207	49.4	116(133)	323 (317)	476 (342)
2	13.3	1.58	1.18	4.47	119	88.7	336	81(115)	147 (105)	247 (188)
3	7.65	0.439	0.462	0.960	57.4	60.4	125	227(194)	337 (322)	850 (862)
4	34.3	10.9	3.30	5.05	318	96.2	147	852	146	30.14
5	14.3	1.73	–	–	121	–	–	164	–	–

[a] All reactions observed at 25 °C using KCl/NaOH buffer, pH = 11.49, I = 0.2, in 0.5% (v/v) acetonitrile/water solution, [ester] = 1×10^{-4} M. [CD] = varied from 1×10^{-4} to 5×10^{-3} M, numbers in the parentheses are the binding constants obtained from the spectrophotometric method.

ing *p*-methyl group decreases the electron deficiency of carbonyl carbon and hence the rate of reaction. Addition of all three CDs causes an increase in conversion percentage. Thus, from the results presented in Table 1, the catalytic role of cyclodextrin is evident in the hydrolysis of thiol esters **1** and **3**.

As the reaction is faster in presence of cyclodextrin and also to gain more quantitative information, the rate of hydrolysis of thiol esters **1**–**3** is monitored using a diode array spectrophotometer. For comparison, hydrolysis of acetate esters **4** and **5** are also studied. Pseudo first-order rate constants (k_{cat}) are obtained over a range of CD concentrations by monitoring the rate of product formation. The calculated pseudo-first-order rate constants for the hydrolysis of substrates **1**–**5** in aqueous KCl/NaOH buffer at pH = 11.5, in the absence and presence of CD are presented in Table 2.

With all the substrates, the reaction shows simple saturation kinetics and this kind of behavior is consistent with a reaction sequence which proceeds through a substrate/CD complexation followed by hydrolysis [see Equation (1)].

$$\text{product} \xleftarrow{k_{uncat}} \text{S} + \text{CD} \underset{K_{CDOH}}{\rightleftharpoons} [\text{SCD}] \xrightarrow{k_{cat}} \text{product} \quad (1)$$

The variation in the observed rate constant is given by Equation (2), where k_{uncat} and k_{cat} are rate constants observed in the absence and in presence of cyclodextrin, respectively. K_{CDOH} is the binding constant.

$$k_{obs} = \frac{k_{un}K_{CDOH} + k_{cat}[CD]}{K_{CDOH} + [CD]} \quad (2)$$

The extent of acceleration by CD on hydrolysis of thiol esters is reflected from the ratio of k_{cat}/k_{uncat}. Similar to the qualitative results observed from product analysis by GC, with all the three cyclodextrins substrates **1**–**3** show faster hydrolysis than the uncatalysed reaction. Among these, hydrolysis is more faster with substrate **2** than with **1** and **3**, due to the presence of electron-withdrawing nitro group present in the *p*-position. The rate acceleration is more pronounced in presence of γ-cyclodextrin (a 336-fold increase in the rate was observed).

The binding constants obtained from kinetic methods (Table 2) show that substrates **1**–**3** form stronger complexes with γ-CD. This is also evident from molecular modeling data, which show that substrates 1–3 are stabilized inside the γ-cyclodextrin cavity via hydrogen bond formation between the carbonyl oxygen of the ester with the secondary hydroxy group present at the rim of the cyclodextrin cavity (Figure 1). It is relevant to note that this type of hydrogen bonds is not observed with α- and β-CD.

Figure 2 shows the absorption spectra of substrates **1**–**3** in presence of α-CD. In all the cases, an isosbestic point is observed indicating clearly that the substrate undergoes a simple, clean unimolecular cleavage without any competing side reaction. A similar trend is also observed with β- and γ-CDs. In the case of *S*-phenyl thiobenzoate (Figure 2, a), an increase in the absorbance is observed at 263 and

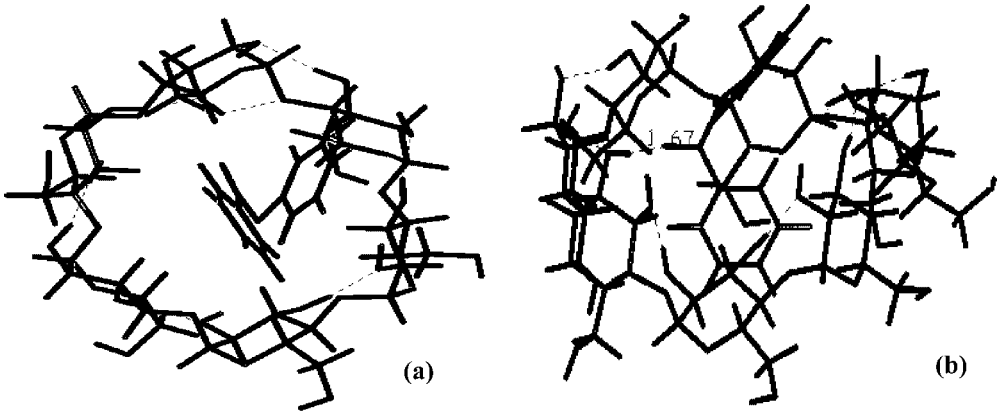


Figure 1. Structures of **1** a) α-CD and b) γ-CD cavities.

223 nm, which corresponds to the thiol and benzoylated cyclodextrin, respectively. The spectral changes show that with increase in time during the course of the reaction, the peak at 243 nm decreases with the simultaneous increase in the intensity of the peak at 263 nm and 223 nm with an isosbestic point at 250 nm. These absorption changes point out that the disappearance of thiol ester is accompanied by the formation of thiol and benzoylated cyclodextrins. An increase in the intensity of the peak at 223 nm (which corresponds to benzoylated cyclodextrin[4,5]) can be considered

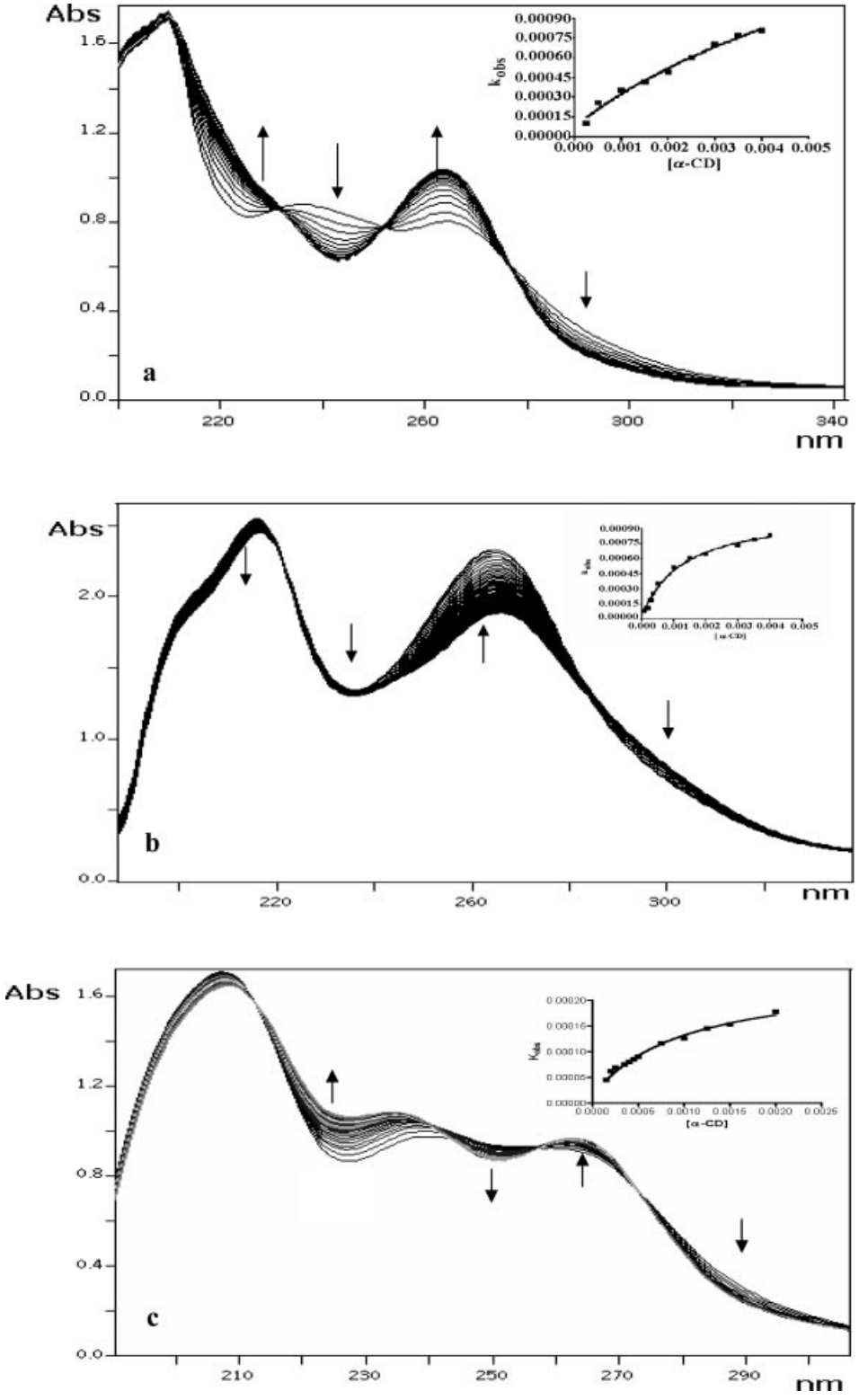


Figure 2. a) Effect of α-CD on hydrolysis of substrate **1**. b) Effect of α-CD on hydrolysis of substrate **2**. c) Effect of α-CD on hydrolysis of substrate **3**. In all the cases the [sub] = 1×10^{-4} M and [CD] = 5×10^{-3} M.

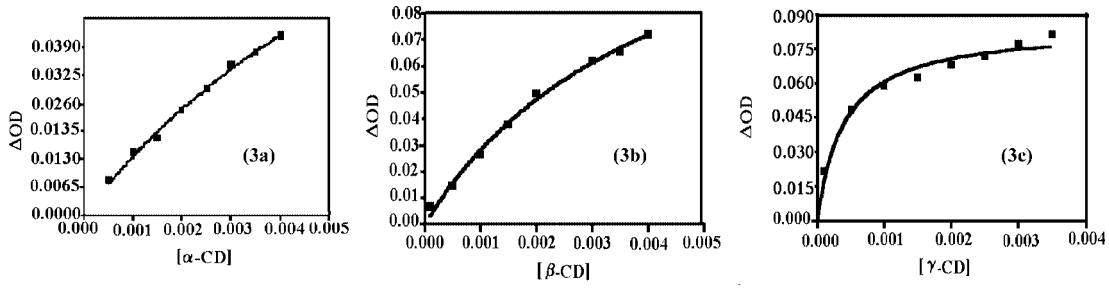


Figure 3. Change in absorbance for substrate **1** upon addition α-CD (**3a**), β-CD (**3b**) and γ-CD (**3c**), [sub] = 1×10^{-4} M and [CD] is varied from 1×10^{-4} to 6×10^{-3} M.

as a strong evidence for covalent catalysis by CD hydroxy groups. At present we are unable to isolate this product and characterize it.

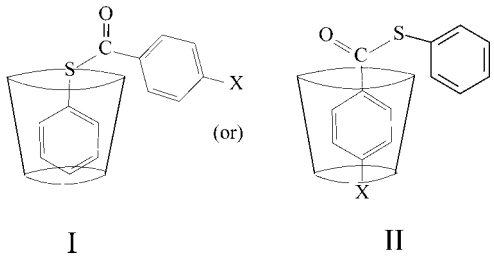
Figure 2 (b) shows the changes in the absorption spectrum of *S*-phenyl *p*-nitrothiobenzoate and here also the optical density at 263 nm increases. With substrate *S*-phenyl *p*-methylthiobenzoate (**3**), in presence of α-CD a decrease in substrate absorption and increase in thiol and benzoylated CD (233 nm) absorptions are noticed. These spectral changes are consistent with the electronic effect of the substituent groups present in the benzene ring of the ester. The insets a–c in Figure 2 were obtained from the plot of k_{obs} (rate of hydrolysis in the presence of cyclodextrin) vs. cyclodextrin concentration, which show a saturation kinetics in all the cases. The k_{cat} values were calculated from Equation (3) by a nonlinear method and the fitted values of k_{cat} and k_{uncat} are given in (Table 2).

The thiol esters **1**–**5** form 1:1 complexes with α-, β- and γ-cyclodextrins and the binding constants (K_{CDOH}) obtained from the kinetic method are close to the values estimated by a spectrophotometric method (Table 2). In this method, the change in the absorbance of substrate with CD concentration is fitted by nonlinear adjustment into the following equation Equation (3), see also Figure 3.

$$A - A_0 = \frac{[(\varepsilon_{SCD} - \varepsilon_S)K_{CDOH}[CD][S_0]}{1 + K_{CDOH}[CD]} \quad (3)$$

K_{CDOH} represents the equilibrium constant for 1:1 substrate:cyclodextrin complex. Figure 3 (see parts a–c) shows the changes in absorbance of the substrate **1** in the presence of different concentration of α-, β- and γ-CDs.

To account for the observed rate acceleration by CD on the hydrolysis of these thiol esters, it is proposed that complexation of substrate inside the CD cavity can happen (Scheme 1) with either of aryl moieties going inside the microvessel. In structure I, the phenyl group attached to the sulfur atom enters into the cavity, while the other phenyl group is outside. In structure II, the other phenyl ring bound to the carboxylate group enters into the cavity. With unsubstituted thiol ester **1**, both structures I and II are expected to be formed with equal probabilities. However, with *p*-substituted thiol esters **2** and **3**, structure II is more likely (complex formation of **1**–**3** with CD is evident from the shifts in chemical shift values of free and complexes.[31] Upfield shifts are observed in H-3 and H-5 protons in accordance with previous reports[32]).



Scheme 1. Mode of inclusion of substrate **1**–**3** into the cyclodextrin cavity.

The inclusion of aryl moiety inside the cyclodextrin cavity is also evident from calculation of the binding energies of the complexes (Table 3) obtained from energy minimization studies using AMBER[33,34] force field calculations. The computations are done in vacuo and still large negative values are obtained (indicating very high association constants). Lower binding energy values are obtained for both modes of inclusion. Interestingly, inclusion in mode II is favored more with all the three cyclodextrins.

Table 3. Binding energies[a] [kcal/mol] for **1**–**3** with various cyclodextrins.

Substrate	Cyclodextrins	Mode I	Mode II
1	α-CD	–18.63	–21.08
1	β-CD	–18.88	–22.12
1	γ-CD	–18.92	–21.30
2	α-CD	–20.81	–23.14
2	β-CD	–23.84	–24.27
2	γ-CD	–22.54	–23.46
3	α-CD	–19.42	–20.62
3	β-CD	–20.46	–22.11
3	γ-CD	–21.09	–23.04

[a] Binding energies of the substrates $\Delta E_{complex} - \Delta E_{host} - \Delta E_{guest}$; each substrate was minimized using AMBER force field, RMS derivative for each substrate is 0.0001 was achieved.

In order to compare the results obtained for diaryl thiol esters **1**–**3** with the corresponding akyl aryl systems, hydrolysis of *S*-phenyl thioacetate (**4**) is also studied in presence of CD. Interestingly, the rate of hydrolysis of this substrate is very much faster in presence of α-CD, compared to β- and γ-CDs (Table 2). The absorption spectral changes

shown in Figure 4 indicate that, as in the case of diaryl thiol esters **1**–**3**, here also the release of benzenethiol is observed, and also **4** forms a stronger complex with α-CD (Table 2). Similar result is also obtained from the binding energy calculations.

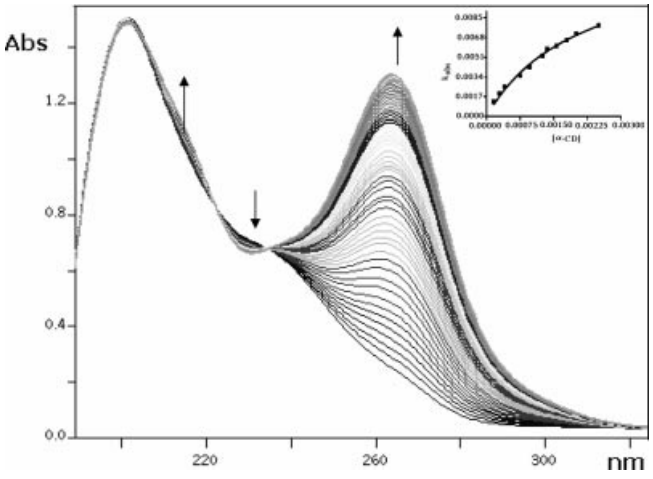


Figure 4. Absorption spectra of *S*-phenyl thioacetate **4** as a function of time in presence of α-CD.

It is proposed that the greater stabilization of substrate **4** inside α-CD may be attributed to the formation of hydrogen bonding between the secondary hydroxy groups of cyclodextrin and the carbonyl oxygen of the ester (Figure 5). As the distance between them are being closer in α-CD, compared to β- and γ-CDs, hydrogen bonding and hence stronger complexes are formed with the former.

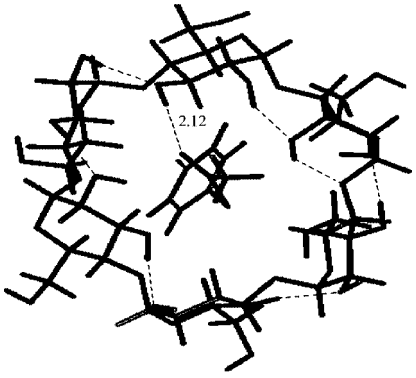
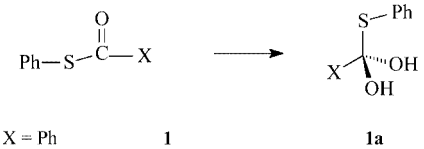


Figure 5. Energy-minimized structure of **4** in presence of α-cyclodextrin.

The kinetic behavior of thiol ester **4** is also compared with that of the oxo ester **5**, which undergoes slower hydrolysis compared to thiol esters. The greater reactivity of the thiol esters compared to the oxo esters is consistent with the greater thermodynamic stability of the latter and the greater leaving group ability of the thiolate ion relative to an alkoxide ion. The observed rate enhancement in presence of α-CD is attributed to a greater stabilization of the transition state with **4** compared to the reaction of an oxo ester **5**.

The observed results in the present study find strong support from free energy calculations. Molecular mechanics, molecular dynamics and free energy calculations are performed using Insight II/Discover program in IRIX system. First, the standard free energies of the thiol esters **1**–**4**, and the intermediates **1a**–**4a** in the absence of CD are calculated. The data, given in Table 4, indicate that, the *S*-phenyl thioacetate undergoes faster hydrolysis under alkaline condition compared to the diaryl thiol esters (the observed rate is ca. five times greater than the diaryl thiol esters) and also among the diaryl thiol esters, ester **2** undergoes faster hydrolysis compared to other diaryl thiol esters. This is also in accordance with the electronic consideration of substituent effects as outlined in the present experimental observations.



X = Ph	**1**	**1a**
= *p*-NO_2-C_6H_4	**2**	**2a**
= *p*-CH_3-C_6H_4	**3**	**3a**
= CH_3	**4**	**4a**

Table 4. Absolute Gibbs free energies [kcal/mol][a] for the thiol esters and the intermediates formed during alkaline hydrolysis.

Substrate	ΔG		$\Delta\Delta G$[b]
	thiol esters	intermediate	
1	77.73	78.28	0.55
2	86.01	87.84	1.83
3	80.54	82.25	1.71
4	48.25	38.20	–10.05

[a] Error limits in these calculations were 0.09 kcal/mol. [b] $\Delta\Delta G = \Delta G_{\text{intermediate}} - \Delta G_{\text{reactant}}$.

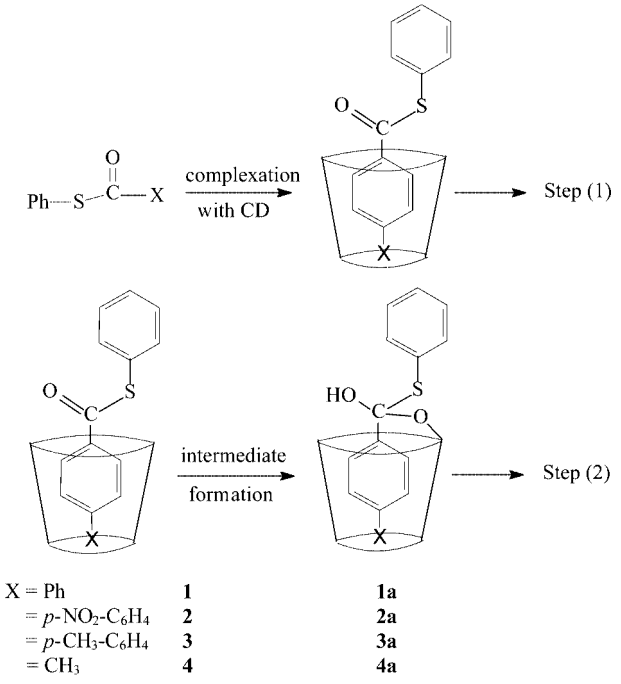
In order to study the role of cyclodextrin, free energy calculations on the CD-substrate complex and the corresponding intermediate, have been performed. The free energy change for the reactants (CD and substrate) is higher than its complex ($-\Delta\Delta G$) i.e. complexation free energy for the substrate is very low (Table 5). The more negative free energy change for the *S*-phenyl thioacetate in presence of α-CD explains readily the dramatic rate increase and high binding constant values in presence of the α-CD (852 M^{-1}). As, the CD-mediated hydrolysis proceeds through the complexation (step 1), followed by the formation of intermediate (step 2), the overall free energy change for the reaction can be calculated by addition of $\Delta\Delta G$ values for steps 1 and 2 (Scheme 2). The sum given in Table 5 indicate that the CD-mediated hydrolysis is faster than the uncatalysed reaction. With all the three substrates **1**–**3** and a dramatic increase in rate is predicted for **4** in presence of α-CD.

The rate enhancement observed in thiol esters **1** and **3** upon inclusion inside the cyclodextrins may be explained as follows (Scheme 3). Ionization of CD hydroxy groups in a basic medium results in hydroxide anions, which acting as a nucleophile attacks more readily the nearby carbonyl carbon of the thiol ester. A similar catalytic role is reported earlier with a wide range of esters. The initially formed thi-

Table 5. Absolute Gibbs free energies (kcal/mol)[a] for complex, and intermediate formed during the hydrolysis in presence of CD and the overall free energy change for the cyclodextrin mediated reaction.

Substrate	ΔG for the substrate + β-CD	ΔG for the complex	ΔG for the intermediate	$\Delta\Delta G_1$[b]	$\Delta\Delta G_2$[c]	$\Delta\Delta G_3$[d] $\Delta\Delta G_1 + \Delta\Delta G_2$
1/β-CD	420.1	397.1	415.9	–23.0	18.8	–4.20
2/β-CD	428.6	403.9	424.9	–24.7	21.0	–3.70
3/β-CD	422.2	402.2	413.8	–20.0	11.6	–8.40
4/α-CD	385.3	313.1	309.1	–72.2	–4.00	–76.2

[a] Error Limit in these calculations are 0.21 kcal/mol. [b] $\Delta\Delta G_1 = \Delta G$ complex – ΔG substrate + CD. [c] $\Delta\Delta G_2 = \Delta G$ intermediate – ΔG complex. [d] $\Delta\Delta G_3$ is the free energy change for the overall reaction in presence of cyclodextrin.



Scheme 2. Hydrolysis pathway for substrates **1**–**3** in presence of cyclodextrin.

ols can undergo an air oxidation to give disulfide. Thus the present study on the hydrolysis of thiol esters and the rate enhancement by CD illustrate versatile role of CD in effecting covalent catalysis.

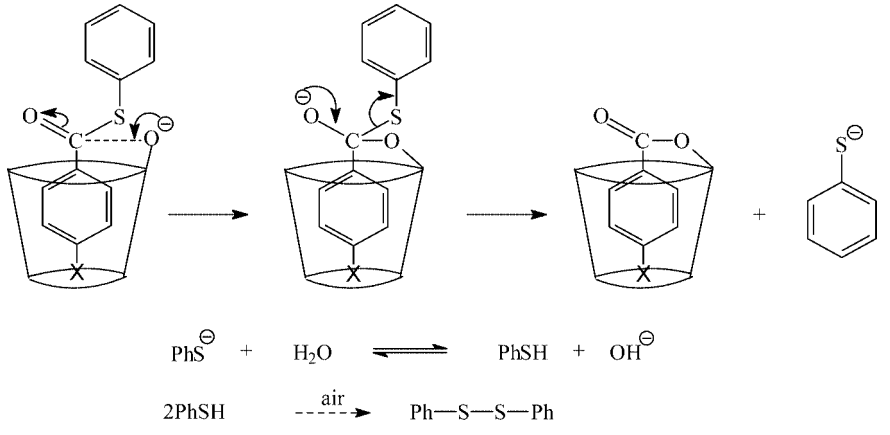
Conclusion

The present study highlights the role of CDs in achieving rate enhancement in hydrolysis of thiol esters, which play a major role in many biochemical processes such as that of acetyl co-enzyme A, which is widely acclaimed as the universal carrier of acyl group in biological systems. The study also establishes another interesting facet of CD chemistry, namely covalent catalysis and thus gives more insight into the use of CDs as useful enzyme models.

Experimental Section

General Methods: Cyclodextrins (α and γ from American Maize Products, Indiana, β from Aldrich) were used as received. Thiol esters **1**–**5** were prepared using a reported procedure[29] and APTES-K10 montmorillonite clay composite was used as the catalyst. Their structures and purity are confirmed by ^{1}H NMR and melting point data. CD complexes of substrates **1**–**5** were prepared according to a reported procedure.[30] Hydrolysis of thiol esters were carried out by taking 250 mg of cyclodextrin complexes in 50:50 acetonitrile/buffer solution at pH- 9.99 (carbonate/hydrogen carbonate buffer) and stirred for the required time reported in the Table 1. Products were extracted with dichloromethane and were analyzed by capillary GC (SHIMADZU GC-17A, SE 30–10% capillary column, high purity nitrogen as the carrier gas with FID detector).

In addition to GC analysis the hydrolysis of the thiol esters was also monitored by kinetics studies. Kinetics of ester hydrolysis was followed using a diode array spectrophotometer (Analytik Jena specord S 100). All reactions were monitored at 25 °C at constant



Scheme 3. Cyclodextrin-mediated covalent catalysis of diaryl thiol ester.

ionic strength of 0.02 μ. Pseudo-first order kinetics was followed by varying the concentration of CD from 1×10^{-4} M to 5×10^{-3} M at pH 11.9 (KCl/NaOH buffer). The absorption spectra were recorded for the reaction mixture at a time interval of 10 s and in all the cases the rate constants were measured by following the appearance of thiol at 263 nm.

Non-linear regression analysis were carried out using prism software (trial version) in an IBM compatible personal computer with a Microsoft Windows 98® operating system. Correlations were fitted by "F" test and the significance of the parameters were analysed by using the Student t-test. In both the cases the significance was measured by an α value of 0.05.

Geometries of the complexes are obtained using Molecular Mechanics/Molecular Dynamics Calculations by Insight II/Discover program. The initial structures of host and guest molecules were constructed by Insight II/Discover on Silicon Graphics IRIS workstation. We had adapted AMBER force field to express the MM energies of cyclodextrin hosts, thiol esters **1**–**4** and complexes. In the MD simulation, the initial structure was subjected to a conformational search in which 300 K constant temperature MD were carried out for 5 ns. The conformation data were saved every 80 ps, during the 5-ns snapshot, and the energies of these conformers were minimized to 0.001 kcal/mol gradient.

The technique of absolute Gibbs free energy calculation is general and can be applied in a transparent manner to systems in vacuo or in solution, under any conditions of volume and/or temperature. This approach is a special case of thermodynamic integration (TI) approach to free energy calculations, which is a general method for computing the changes in free energy upon going from one thermodynamic state to another. Absolute free energy constrains one of these imply states to be a model system for which the absolute free energy is known analytically. By integrating from a known, model state to the final real state, the absolute free energy becomes the sum of the numerically computed thermodynamic integration step and the analytical absolute free energy of the model state. These calculations have been carried out on guests, hosts, intermediate of the complex and substrate and the complexes of CDs with guest. All free energy simulations in this work were carried out with the default settings: $\Delta\lambda = 0.005$ (the spacing between windows); six windows were used to go from the initial to the final state, quadrature points: 6 (the number of Gauss–Legendre quadrature points), sampling: 10 (the frequency at which $e^{-\Delta H/kT}$ is sampled). In each window equilibration was carried out for 200000 steps (200 ps) followed by data collection for 80000 steps. The simulations and discussions given above are based primarily on the works cited in ref.[33d,33e].

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[31] ^{1}H-NMR chemical shifts exhibit upfield shift for H-3, H-5 protons of all thiol esters for example the chemical shift (in Hz) for uncomplexed β-CD H1 - 1462, H2 - 1014, H3 - 1105, H4 - 1033, H5 - 1096, H6 - 1125 and for β-CD (**1**) complex H1 - 1462, H2 - 1014, H3 - 1104, H4 - 1033, H5 - 1092, H6 - 1125 such change in chemical shift values are known to be the consequence of diamagnetic anisotropic effect of the aromatic ring residing inside the cavity and are considered to be evidence for the formation of inclusion complex in aqueous CD solution.

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