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Thermodynamic Studies of the Cyclodextrin-Accelerated Cleavage of Phenyl Esters

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Abstract: The α-cyclodextrin-accelerated cleavage of several (*p*-methyl, *m*-methyl, *p*-nitro, *m*-nitro, and *m*-chloro) phenyl acetates was examined thermodynamically from 15 to 70 °C. The activation enthalpy and entropy for the α-cyclodextrin reactions as well as for the alkaline hydrolyses were determined. The logarithm of the magnitude of the acceleration by α-cyclodextrin over that for the alkaline hydrolyses linearly increases with decreasing difference of the activation enthalpy and entropy between the α-cyclodextrin reactions and the corresponding alkaline hydrolyses. The activation enthalpy is responsible for stereospecific acceleration by α-cyclodextrin; however, the activation entropy partly compensates the activation enthalpy. The enthalpy-controlled stereospecific acceleration by cyclodextrins is attributed to stereospecific complex formation between the cyclodextrins and the phenyl acetates. Cyclodextrins are good enzyme models, especially suited to examine the effect of enzyme–substrate complexation on enzymatic acceleration.

Introduction

Cyclodextrins (CD) have served as good models of serine proteases as reviewed recently by the present authors.[1] CDs accelerate the cleavages of phenyl esters[2,3] and acylimidazoles.[4] Furthermore, they catalyze the hydrolyses of amides such as acetanilides[5] and penicillins.[6] One of the characteristics of the CD-accelerated cleavage of esters and amides is that the reaction pathway is initiated by binding, followed by acylation of CD, and deacylation, which is identical with the pathway used by serine proteases. The CD-accelerated cleavages of phenyl acetates are highly stereospecific, with the meta-substituted compounds being cleaved much faster than the corresponding para-substituted compounds by a factor of 30–200.[2] However, it is still unknown whether activation enthalpy or entropy is responsible for the stereospecific acceleration by CD.

In this paper, thermodynamic studies on the CD-accelerated cleavages of phenyl acetates are described. The rate constants and the dissociation constants of the CD–phenyl acetate complexes were determined at various temperatures from 15 to 70 °C. The activation parameters for the cleavage of the esters and the thermodynamic parameters for complex formation are shown. Relations between the magnitude of acceleration of ester cleavage by CD and the activation parameters are described.

Experimental Section

Materials. α-Cyclodextrin (α-CD) was purified by recrystallization from water. Phenyl acetates were purchased from Eastman Kodak Co. or were synthesized by the method of Spasov.[7,8] All water used for the kinetic studies was doubly distilled.

Kinetics. The hydrolyses of phenyl esters were followed by the appearance of phenols at 300 (*p*-tolyl, *m*-tolyl, and *m*-chlorophenyl acetates) or 400 nm (*p*-nitrophenyl and *m*-nitrophenyl acetates) on a Cary Model 14 PM spectrophotometer equipped with a thermostated cell compartment. The observed rate constants of the cleavage of phenyl esters in the presence (k_{obsd}) and absence (k_{un}) of added α-CD were determined by a usual first-order equation. The values of k_c (the rate constant of the α-CD-accelerated cleavage of phenyl esters) and K_d (the dissociation constant of the α-CD–phenyl acetate complexes) were determined from the Y intercept and the slope of the plot of $(k_{obsd} - k_{un})$ vs. $(k_{obsd} - k_{un})/[\alpha\text{-CD}]$. Plots were carried out at pH 10.6 carbonate buffer, I = 0.2 M, for the cleavage of *m*- and *p*-nitrophenyl acetates or at pH 10.0 carbonate buffer, I = 0.2 M, for the cleavage of *m*-tolyl, *p*-tolyl, and *m*-chlorophenyl acetates.

The rate constant of the alkaline hydrolysis (k_{OH}) was determined from k_{un} and the ion product of water (K_w) at various temperatures.[9] The value of the limiting value of k_c ($k_{c(lim)}$), which refers to complete ionization of α-CD, was calculated by use of k_c and the values of the K_a of α-CD, where K_a of α-CD was taken as 12.1 at 25 °C[3] and it was assumed that K_a varied with the temperature in a fashion exactly parallel to the variation of K_w with the temperature.[10]

From the values of $k_{c(lim)}$ and k_{OH} at various temperatures, the activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($\Delta S^{\ddagger}$) were evaluated.[11]

The enthalpy change (ΔH_f) and entropy change (ΔS_f) of the formation of the complexes between α-CD and phenyl acetates were also determined from the kinetically determined K_d's at various temperatures.

Results

Table I shows the $k_{c(lim)}$ values of the α-CD-accelerated cleavages of phenyl acetates. The plots of $k_{c(lim)}$ and k_{OH} vs. $1/T$ (Arrhenius plots) exhibited fair straight lines, from which the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of both the α-CD-accelerated cleavages and

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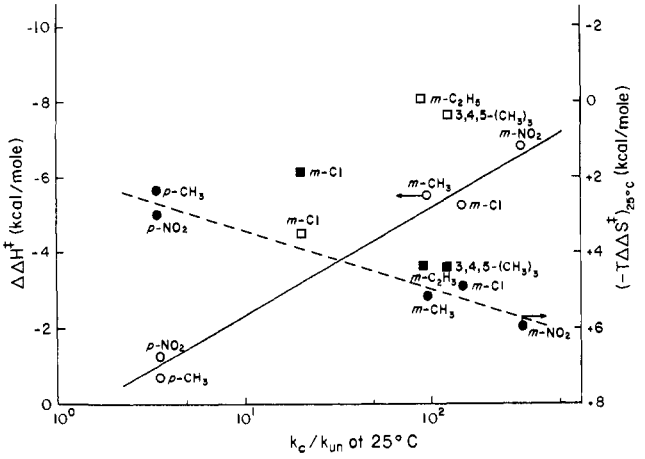

Figure 1. Relations between the acceleration by α-CD and the activation terms in the α-CD-accelerated cleavages of phenyl acetates at 25 °C; $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ are the differences between the values for the α-CD reactions and those for the corresponding alkaline hydrolyses; the round points refer to α-CD; the square points refer to β-CD (values obtained by Drs. M. L. Bender, G. A. Clowes, and R. L. VanEtten). White refers to enthalpy and black refers to entropy.

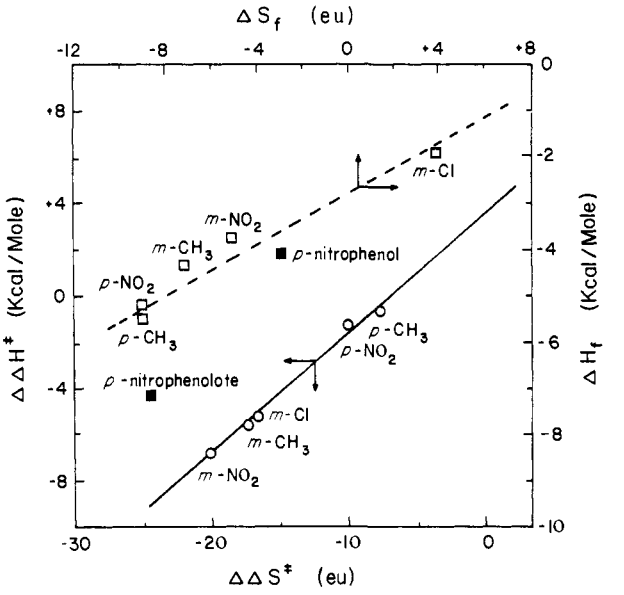

Figure 2. Plots of the difference in the activation enthalpy ($\Delta\Delta H^{\ddagger}$) vs. the difference in the activation entropy ($\Delta\Delta S^{\ddagger}$) in the α-CD-accelerated cleavages of phenyl acetates (white circles) and plots of the enthalpy change for complex formation of phenyl acetates with α-CD (ΔH_f) vs. the entropy change (ΔS_f) (squares); white squares come from the present study, and black squares are from ref 14.

Table I. Values of $k_{c(lim)}$ for the α-CD-Accelerated Cleavages of Phenyl Esters at Various Temperatures

	$k_{c(lim)}$, s^{-1}				
temp, °C	*p*-CH_3	*m*-CH_3	*p*-NO_2	*m*-NO_2	*m*-Cl
15	0.0447	1.58	0.457	42.0	5.37
25	0.0696	2.08	0.768	53.4	8.6
40	0.166	3.16	1.78	70.8	12.3
55	0.420	4.74	3.85	92.2	18.6
70			6.44	125.0	

the alkaline hydrolyses were determined as shown in Table II. In the alkaline hydrolyses, the effects of substituents show up in $\Delta S^{\ddagger}$ rather than $\Delta H^{\ddagger}$, which is consistent with the literature.[12] An electron-withdrawing substituent gives a favorable $\Delta S^{\ddagger}$. In the α-CD-accelerated cleavages, however, neither $\Delta H^{\ddagger}$ nor $\Delta S^{\ddagger}$ shows a good relationship to Hammett σ. Instead, all para compounds have larger values of both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ than all meta compounds.

Figure 1 depicts the relationship between the acceleration by α-CD at 25 °C (k_c/k_{un}) and the difference in the activation parameters between the α-CD reactions and the alkaline hydrolyses ($\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$). Comparison of $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ instead of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ was made to normalize the values with respect to different leaving groups. Clearly, $\Delta\Delta H^{\ddagger}$ decreases (a change favorable for reaction) linearly with log (k_c/k_{un}). The meta compounds, which exhibit large accelerations by α-CD, have small values of $\Delta\Delta H^{\ddagger}$, whereas the para compounds, which exhibit small accelerations, have large values of $\Delta\Delta H^{\ddagger}$. The activation entropy term ($-T\Delta\Delta S^{\ddagger}$), however, linearly increases (unfavorable for the reaction) with log (k_c/k_{un}). Thus, whereas the activation enthalpy governs the stereospecific acceleration of the cleavage of phenyl esters by CD, the activation entropy term partly compensates the enthalpy term (Figure 2).

In Figure 1, the results on the β-cyclodextrin (β-CD)-accelerated cleavage of *m*-chlorophenyl, *m*-ethylphenyl, and 3,4,5-trimethylphenyl acetates (obtained by M. L. Bender, G. A. Clowes, and R. L. VanEtten) are also plotted. The β-CD reactions exhibit the same trends as the α-CD reactions in that $\Delta\Delta H^{\ddagger}$ decreases and ($-T\Delta\Delta S^{\ddagger}$) increases with k_c/k_{un}. However, the points of $\Delta\Delta H^{\ddagger}$ and ($-T\Delta\Delta S^{\ddagger}$) for the β-CD reactions deviate considerably in a positive direction from the straight lines seen for the α-CD reactions.

Figure 3 shows the dependences of $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ on the Hammett substituent constants. Neither $\Delta\Delta H^{\ddagger}$ nor $\Delta\Delta S^{\ddagger}$ is related to Hammett σ well, which indicates a less important role of the electronic nature of the substituents in stereospecific acceleration by α-CD. This fact also supports a predominant role of the positions of the substituents (meta vs. para).

Tables III and IV list the values of K_d, ΔH_f, and ΔS_f for the complex formation of phenyl acetates with α-CD, which were kinetically determined. As shown in Figure 2, the often observed compensation between ΔH_f and ΔS_f[13] holds here. The point for *p*-nitrophenol[14] fits the straight line for phenyl ace-

Table II. Activation Parameters for the Hydrolyses of Phenyl Esters

	α-CD-accelerated[a]		alkaline[b]			
phenyl substituents	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$ eu	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, eu	$\Delta\Delta H^{\ddagger}$,[c] kcal/mol	$\Delta\Delta S^{\ddagger}$,[d] eu
p-CH_3	9.9	−30.0	10.6	−22.4	−0.7	−7.6
m-CH_3	4.8	−40.7	10.3	−23.3	−5.5	−17.4
p-NO_2	9.0	−28.8	10.3	−18.9	−1.3	−9.9
m-NO_2	3.3	−39.5	10.1	−19.5	−6.8	−20.0
m-Cl	5.3	−36.7	10.5	−20.2	−5.2	−16.5

[a] Error analyses: $\Delta H^{\ddagger}$, ±1 kcal/mol; $\Delta S^{\ddagger}$, ±3 eu. [b] Error analyses: $\Delta H^{\ddagger}$, ±0.5 kcal/mol; $\Delta S^{\ddagger}$, ±2 eu. [c] $\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}$ (α-CD reaction) − $\Delta H^{\ddagger}$ (alkaline reaction). [d] $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}$ (α-CD reaction) − $\Delta S^{\ddagger}$ (alkaline reaction)

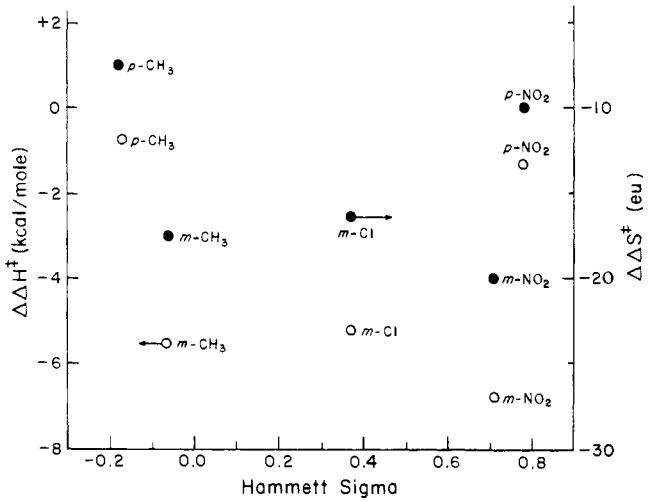

Figure 3. Plots of $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ vs. Hammett substituent constants in the α-CD-accelerated cleavages of phenyl acetates; $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ are the differences between the activation enthalpy and entropy for the α-CD reactions and those for the corresponding alkaline hydrolyses. White refers to enthalpy and black refers to entropy.

Table III. Values of the Dissociation Constants of the α-CD-Phenyl Ester Complexes (K_d) at Various Temperatures[a]

	K_d, 10^{-2} M				
temp, °C	*p*-CH_3	*m*-CH_3	*p*-NO_2	*m*-NO_2	*m*-Cl
15	0.68	1.3	1.0	1.5	0.38
25	1.1	1.7	1.2	1.9	0.42
40	1.4	2.5	2.2	2.6	0.50
55	2.1	3.3	3.2	3.4	0.58
70			4.6	4.7	

[a] Kinetically determined; each value has an accuracy of around ±10%.

Table IV. Thermodynamic Parameters for Complex Formation between α-CD and Phenyl Esters[a]

phenyl substituents	ΔH_f, kcal/mol	ΔS_f, eu
p-CH_3	−5.5	−9
m-CH_3	−4.4	−7
p-NO_2	−5.2	−9
m-NO_2	−3.8	−5
m-Cl	−2.0	+4

[a] Error analyses: ΔH_f, ±1 kcal/mol; ΔS_f, ±3 eu.

tates fairly well. However, an ionic guest, *p*-nitrophenolate,[14] showed a considerable negative deviation from the straight line, indicating a different manner (or binding force) of its complex formation than that for the other phenyl acetates and *p*-nitrophenol.

Discussion

The present study definitely shows that the stereospecificity of the cleavage of phenyl acetates by CD is attributable to the activation enthalpy term. This phenomenon can be explained sufficiently in terms of stereochemistry in the complex formation of phenyl acetates with CD.

A molecular model study in a previous paper[2] showed that the carbonyl carbon atoms of the meta-substituted phenyl acetates (which are highly accelerated by CD) are located close to the secondary hydroxyl groups of CD (nucleophile) in the inclusion complexes, whereas the carbonyl carbon atoms of the para-substituted phenyl acetates (small acceleration by CD) are at a considerable distance from the secondary hydroxyl groups of CD. Thus, the CD-accelerated cleavage of the para compounds requires large enthalpies to go from the initial complexes to the transition states. The enthalpy can be used for the perturbation of the bond lengths and bond angles in both the substrates and CD. This perturbation should be larger for the para than for the meta compounds, since the distances between the carbonyl carbon atoms and the secondary hydroxyl anions are much larger for the para than for the meta compounds. In the CD-accelerated cleavage of the meta compounds, only a small enthalpy is necessary to attack the carbonyl carbon atoms, which are originally located in the vicinity of the secondary hydroxyl anions in the initial complexes. This proximity to the anions thus results in smaller values of $\Delta\Delta H^{\ddagger}$ in the CD-accelerated cleavage of the meta compounds.

Alternatively, the meta-para specificity can be ascribed to a change of structure of the CD-substrate complex from the initial state to the transition state.[15] The aromatic portion of the para compounds could be partially pulled out of the cavity so that the carbonyl carbon atom, located far from the alkoxide ion of CD in the initial state, is shifted near the alkoxide ion in the transition state. This structural change would exhibit unfavorable enthalpy. In the CD-accelerated cleavage of meta compounds, however, this factor is small. At the present state of our knowledge, it is hard to determine which explanation is correct.

The comparison between the activation parameters of the CD reactions and those of the corresponding alkaline hydrolyses is more complex than the comparison between the values of the CD-accelerated cleavages of para and meta compounds described above. In the former case, the pK_a values of the nucleophiles are different (the secondary hydroxyl anion of CD, pK_a = 12.1,[2] and hydroxide ion, pK_a = 15.74[16] at 25 °C), whereas in the latter case they are the same. However, the change of nucleophile from the CD anion to hydroxide ion is not likely to be the cause of the extremely large change of the activation parameters seen, since the reactivity of strongly basic oxygen anions toward phenyl esters is remarkably insensitive to the basicity of the anion. For example, the rate constant of the hydrolysis of *p*-nitrophenyl acetate catalyzed by 2,2,2-trichloroethanol (pK_a = 12.24) is only 2.2 times larger than that catalyzed by hydroxide ion (pK_a = 15.74).[17]

The smaller value of $\Delta H^{\ddagger}$ for the CD-accelerated cleavage (both of the meta and para compounds) than the alkaline hydrolyses is attributable to complex formation between CD and the substrates preceding reaction, which is consistent with enzymatic reactions. The reactions lose translational and rotational entropy on complex formation, resulting in a much larger rate acceleration than that in intermolecular reactions where an entropy loss should directly increase the free energy of activation. This theory was initially proposed by Page and Jencks.[18] A later study by Larsen[19] indicated that the effect shows up in $\Delta H^{\ddagger}$ rather than in $\Delta S^{\ddagger}$, since the entropy loss described above is largely canceled by an entropy gain due to re-formation of water structure around the reactants.

In the alkaline hydrolyses of phenyl acetates, translational and rotational entropy is lost in the formation of the transition state. However, most (or part) of this entropy loss is compensated by entropy gain due to the destruction of the water cage around the reactants. Thus, a loss in activation enthalpy results from decreased hydrogen bonding in water, leading to an unfavorable activation enthalpy in alkaline hydrolyses. On the other hand, these factors are not operative in the CD-accelerated cleavage of phenyl acetates, since the reactants experience these factors in prior complex formation. Consequently, CD-accelerated cleavages have smaller activation enthalpies than corresponding alkaline hydrolyses.

Compensation between the activation entropy and the activation enthalpy has been often observed for reactions in aqueous solution including enzymatic reactions.[20] Structural changes in reactants and in the surrounding water are probably taking place in a parallel way.

The loss of the translational entropy on complex formation cannot be the origin of the meta-para specificity, which can be definitely ascribed to the stereochemistry of the complex as described above. Meta and para compounds should exhibit a similar loss of entropy when they form complexes with CD. They can differ only if the stereochemistry of the complex is appropriate for reaction or not.

It was not possible to compare the enthalpy-controlled CD reactions with enzymatic reactions (although we would like to do so) because of the tremendously complex features in enzymatic reactions.[20c] The deacylation steps of the α-chymotrypsin-catalyzed hydrolysis of a large number of substrates are entropy controlled, $\Delta H^{\ddagger}$ being constant for these substrates.[20a,21] In the deacylation of the α-chymotrypsin-catalyzed hydrolyses of other substrates, however, compensation between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ was found, with either $\Delta H^{\ddagger}$ or $\Delta S^{\ddagger}$ controlling the reaction.[20] Furthermore, in the acylation step of the α-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl acetate, the Arrhenius plot was not linear with a sharp kinetic anomaly at 20.9 °C, indicating a conformational change of the enzyme.[22] Thus, it is very hard to ascertain what thermodynamic factor is most important in enzymatic acceleration.

Complexation of CDs with guest compounds itself is a complicated reaction. X-Ray crystallography showed that it is accompanied by a conformational change of CDs.[23] The binding force of CD-guest complexes is still controversial.[1,23] Thus, the analogy between CD reactions and enzymatic reactions might have limitations.

In conclusion, it was found that the CD-accelerated cleavage of phenyl esters is enthalpy controlled. The theoretically predicted acceleration due to a loss of translational and rotational entropy in complex formation[18,19] was experimentally shown in this oversimplified enzyme model. It has been a subject of controversy whether enthalpy or entropy governs enzymatic reactions. The present result sheds some light on this problem, although it does not solve the problem.

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