Cyclodextrin-Catalyzed Hydrolysis of Enantiomeric Esters Showing Stereoselectivity

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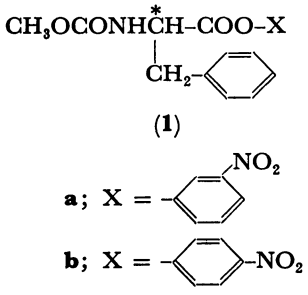
The rate constants for hydrolysis of the enantiomers of amino acid nitrophenyl esters have been determined at pH 9.00 and pH 10.0 in the presence of α- or β-cyclodextrin. α- and β-Cyclodextrins cause stereoselective acceleration in the hydrolysis of *m*-substituted enantiomers. On the other hand, less stereoselectivity is observed in the hydrolysis of *p*-substituted enantiomers. The stereoselective properties are discussed in the light of cyclodextrin-ester dissociation constants and thermodynamic parameters of the reaction.

Stereoselectivity and high catalytic reactivity are among the most interesting properties of enzyme action.[1] Several model studies on micellar,[2-9] polymer,[2,10-14] and macrocyclic[2,15-19] catalyzed reactions have been investigated in order to gain further insight into the stereoselective nature of enzyme reactions. In the course of our study on stereoselective micellar catalysis,[20,21] we found that comicelles of optically active *N*-acylhistidine or dipeptide derivatives containing histidyl residues and cationic surfactant are effective stereoselective catalysts for the hydrolysis of enantiomeric esters.

Cyclodextrin (CD) is often used as models of enzymatic reactions[22] since particular interest has attended the processes in which a substrate binds into a CD cavity and then undergoes reaction with one of CD's secondary hydroxyl groups. Stereoselectivities by CD have been reported for a number of reactions, particularly a very large selectivity was exhibited in the cleavage of chiral organophosphates such as isopropyl methylphosphonofluoridate,[16] or in the acylation of specific rigid substrates such as *p*-nitrophenyl *trans*-2-ferrocenylpropenoate.[18b] Recently, a remarkable stereoselectivity has also been reported in the hydrolysis of activated enantiomeric ester.[23]

In the present paper, the rate constants for hydrolysis of the enantiomers of amino acid *m*- and *p*-nitrophenyl esters (**1a** and **1b**) have been determined at pH 9.00 and pH 10.00 in the presence of α- or β-cyclodextrin. The substrate-binding properties, and the effect of temperature and of added surfactant on the rate constants were investigated to study the kinetic stereoselectivity.



Experimental

Materials. α- and β-Cyclodextrins were commercially available highest grade reagents (Tokyo Kasei Co. and Nakarai Chemicals Co., Japan) and were used without further purification. *m*-Nitrophenyl esters of *N*-methoxycarbonyl-D- and L-phenylalanine were prepared by standard methods.[24] Other materials have been described elsewhere.[20,21]

Kinetic Measurements. Reactions were generally monitored on a Hitachi 200 spectrophotometer or a Shimadzu 140 spectrophotometer with a thermostated cell holder. In general procedure, a solution (25 μl) of substrate in aceto nitrile was added to a buffer solution (3.00 cm³) containing the cyclodextrin or the catalyst and surfactant at desired concentrations. The formation of *m*- or *p*-nitrophenolate ion was followed spectrophotometrically at 390 or 400 nm, respectively. The pseudo-first-order rate constants were obtained from plots of log $(A_\infty - A_t)$ versus time (t) and calculated by the least-squares method. Correlation coefficients were>0.999.

Results and Discussion

Kinetics of Hydrolysis of 1 in the Presence of Cyclodextrin (CD). Kinetic studies were first examined at 25°C using pH 9.00 sodium borate buf-

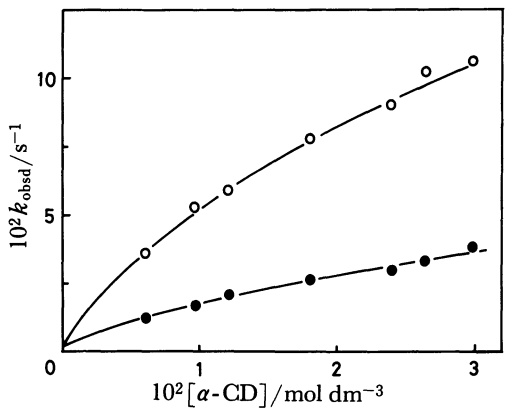


Fig. 1. Pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of **1a** as a function of the concentration of α-CD at pH 9.00, 0.02 mol dm⁻³ borate buffer (I=0.2, KCl), and 25°C; (●) D-**1a**, (○) L-**1a**, [**1a**]=1.00×10⁻⁴ mol dm⁻³.

Table 1. Kinetic Parameters for Hydrolysis of **1a** and **1b** in the Presence of Cyclodextrin[a)]

Cyclodextrin	Ester	$10^3 k_{un}/s^{-1}$	$10^3 k_2/s^{-1}$	k_2/k_{un}	$10^2 K_{diss}/mol\ dm^{-3}$	$k_{2(L)}/k_{2(D)}$
α-CD	**1a** D	1.85	88.5	47.8	4.59	2.40
	L	1.93	212	110	3.12	
	1a D	(8.90)	(552)	(62.0)	(2.83)	(2.83)
	L	(8.96)	(1580)	(176)	(2.24)	
	1b D	2.92	24.6	8.45	12.0	1.16
	L	2.75	27.0	9.83	13.4	
β-CD	**1a** D	1.85	12.4	6.70	0.526	2.15
	L	1.93	26.6	13.8	0.574	
	1a D	(8.90)	(129)	(14.5)	(0.437)	(2.09)
	L	(8.96)	(269)	(30.0)	(0.486)	
	1b D	2.92	4.47	1.53	0.187	1.36
	L	2.75	6.08	2.21	0.379	
	1b D	(13.3)	(50.9)	(3.83)	(0.261)	(1.19)
	L	(13.0)	(60.5)	(4.65)	(0.428)	

a) At pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2, KCl), and 25 °C, [**1a**]=1.0×10^{-4} mol dm^{-3}, [**1b**]=1.0×10^{-5} mol dm^{-3}. The values of parentheses are at pH 10.0, 0.02 mol dm^{-3} carbonate buffer (I=0.2, KCl).

fer or pH 10.00 sodium carbonate buffer, I=0.2 in 0.83% (v/v) acetonitrile–water. Under the conditions [CD]≫[substrate], pseudo-first-order rate constants (k_{obsd}) were evaluated by monitoring the release of *m*- or *p*-nitrophenoxide ion spectrophotometrically at 390 or 400 nm. Examples of the observed pseudo-first-order rate constants, k_{obsd}, against the varying cyclodextrin concentrations are shown in Fig. 1. The data were treated by the Michaelis–Menten pathway, applicable to much of enzyme kinetics described by Scheme 1, where CD is the cyclodextrin, S is the substrate, CD·S is the CD-substrate complex, K_{diss} is the dissociation constant, and k_{un} and k_2 are the rate constants for the alkaline hydrolysis and for the reaction of the complexed ester at infinite cyclodextrin concentration, respectively. The kinetic parameters are calculated by least squared method and given in Table 1.

$$\mathrm{CD} + \mathrm{S} \underset{}{\overset{K_{diss}}{\rightleftharpoons}} \mathrm{CD{\cdot}S} \xrightarrow{k_2} \mathrm{Products}$$
$$\mathrm{CD{\cdot}S} \xrightarrow{k_{un}} \mathrm{Products}$$

Scheme 1.

In the presence of CD, the rates of hydrolysis of **1a** are accelerated (6.7—180 fold) when compared (k_2/k_{un}) to alkaline hydrolysis under the same conditions. α-CD causes much greater rate accelerations than β-CD. Similarly, α-CD shows higher stereoselectivity, $k_{2(L)}/k_{2(D)}$, than β -CD. The K_{diss} values are not very different for the enantiomeric D and L esters. Thus, the stereoselective rate accelerations are unrelated to the strength of binding. In this system, D and L esters may bind equally in the cavity of CD, but the binding of the D ester leads to the wrong orientation between the hydroxyl group of the CD and the carbonyl group of the esters.

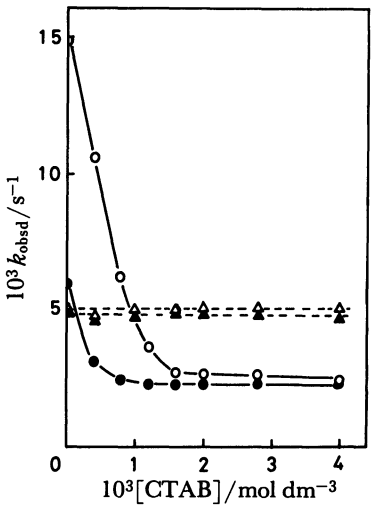


Fig. 2. Effect of CTAB on the hydrolysis of **1** catalyzed with α-CD at pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2, KCl), and 25 °C; (——) **1a** (●) D-**1a**, (○) L-**1a**, (----) **1b**, (▲) D-**1b**, (△) L-**1b**; [**1a**]=1.00×10^{-4} mol dm^{-3}, [**1b**]=1.00×10^{-5} mol dm^{-3}, [α-CD]=2.0×10^{-3} mol dm^{-3}.

The rates of hydrolysis of **1b** are slightly accelerated (1.5—12 fold) at pH 9.00 and 10.0, and also a less stereoselectivity than **1a** is observed in the presence of α and β-CD. These results clearly indicate that the large stereoselective effect accords with the large rate enhancement of the reaction. Bender et al.[18a)] showed that β-CD hydrolyzed *N*-acetyl-L-phenylalanine *m*-nitrophenyl ester 2 times faster than the D-enantiomer, while β-CD inhibited the hydrolysis of both *N*-acetyl-L- and D-phenylalanine *p*-nitrophenyl esters. The data obtained by us are different from those of theirs in the case of *p*-substituted enantiomers. This may be mainly due to differences in experimental conditions such as the pH and the structure of substrates.

Effect of Surfactant on CD Catalyzed Hydrol-

ysis. The hydrolysis of **1** in the presence of CD and hexadecyltrimethylammonium bromide (CTAB) was carried out at pH 9.00 borate buffer and 25 °C. As shown in Fig. 2, reaction rates of **1a** are decreased upon addition of CTAB to the reaction mixture. This is attributed to the complex formation between the surfactant and CD. In contrast, hydrolysis of **1b** shows no significant effect on rates in the presence of CTAB. Thus, the hydrolysis of **1b** is not inhibited under the same conditions. It should be noted here that **1b** is more reactive than **1a** with increasing concentrations of CTAB. These results indicate that the inhibitive effect of CTAB on the reaction of **1a** is attributed to the strong interactions between CD and CTAB. On the other hand, no inhibitive effect on the reaction of **1b** indicates that there are no significant differences in reactivity between CD and CTAB. The strong binding of ionic surfactants with CD was widely discussed upon the basis of conductimetric measurements.[25a)]

In the case of **1a**, the competitive inhibition constants, K_i, are determined by the method of Bender et. al,[26)] Eq. 1. By plotting the inhibitor concentration, [I], against $(k_2-k_{obsd})/(k_{obsd}-k_{un})$ an approximately straight line is obtained with a y intercept of equal to $-K_i$ and a slope of $[CD]K_i/K_{diss}$, and the results are given in Table 2. The values of K_i obtained from both enantiomeric esters are very similar with those obtained from *m*-nitrophenyl acetate.[25b)]

$$[I] = \left(\frac{k_2-k_{obsd}}{k_{obsd}-k_{un}}\right) \cdot \left(\frac{[CD]K_i}{K_{diss}}\right) - K_i \qquad (1)$$

Temperature Dependence of Reaction Rates. The effect of temperature change (5—35 °C) on the reaction rates for hydrolysis of **1a** in the presence of α- and β-cyclodextrin was examined at pH 9.00 in 0.02 mol dm^{-3} borate buffer (I=0.2 with KCl) and the kinetic parameters thus evaluated are listed in Tables 3 and 4. As discussed in previous section, stereoselective recognition is exercised in the reaction process rather than in the binding process over the whole temperature range. The stereoselectivity based on reaction rates slightly increases as the temperature is lowered. The highest stereoselectivity (2.71) is observed in the presence of α-CD at 5 °C.

In order to calculate the thermodynamic parameters accompanying the reaction process the least-squares slope was computed of the line formed by plotting $\ln(k_2/T)$ against $1/RT$. The calculated values are listed in Table 5. The ΔG^* values of α-CD are ca. 1.1—1.2 kcal mol^{-1} (1 cal=4.184 J) smaller than those of β-CD in both D and L esters. These results are compatible with those of Table 1. On the other hand, for both α- and β-CD, the ΔG^*, ΔH^*, and ΔS^* values of L substrate are ca. 0.4—0.5 kcal mol^{-1}, 1.0—1.1 kcal mol^{-1}, and 1.6—2.3 cal mol^{-1} K^{-1} smaller than those of D ester. The effect of enantiomeric selectivity is primarily to lower ΔH^* for activation. This is partly balanced by a small unfavorable change in the entropy of activation.

Stereoselective Nature of CD Catalysis. Although stereoselective effects in the present catalytic systems are relatively small, it is now clear that (1) α- and β-CD cause stereoselective acceleration in the hydrolysis of **1a**, while a less stereoselectivity is observed in the hydrolysis of **1b**. (2) The hydrolysis of **1a** with CD is inhibited by added CTAB, whereas there is no significant effect for **1b** hydrolysis in the presence of CTAB. For the reaction with CD, the esters are incorporated

Table 2. Inhibition by CTAB on the Hydrolysis of **1a** Catalyzed with Cyclodextrin[a)]

Ester	α-CD	β-CD
	$10^4 K_i$/mol dm^{-3}	$10^4 K_i$/mol dm^{-3}
D-**1a**	1.8±0.4	3.3±0.5
L-**1a**	1.3±0.3	3.6±0.7

a) At pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2, KCl), and 25 °C, [α-CD]=2.00×10^{-3} mol dm^{-3}, [β-CD]=1.00×10^{-3} mol dm^{-3}, [**1a**]=1.0×10^{-4} mol dm^{-3}.

Table 3. Kinetic Parameters for the Hydrolysis of **1a** in the Presence of α-CD[a)]

	5 °C		15 °C		25 °C		35 °C	
	D	L	D	L	D	L	D	L
$10^2 k_2$/s^{-1}	0.313	0.847	1.40	3.64	8.85	21.2	21.4	46.4
$10^2 K_{diss}$/mol dm^{-3}	1.55	1.36	3.03	2.10	4.59	3.12	5.44	3.55
k_2(L)/k_2(D)	2.71		2.60		2.40		2.17	

a) At pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2 with KCl), [**1a**]=1.00×10^{-4} mol dm^{-3}.

Table 4. Kinetic Parameters for the Hydrolysis of **1a** in the Presence of β-CD[a)]

	5 °C		15 °C		25 °C		35 °C	
	D	L	D	L	D	L	D	L
$10^3 k_2$/s^{-1}	0.660	1.66	3.16	7.56	12.4	26.6	34.9	72.2
$10^3 K_{diss}$/mol dm^{-3}	1.49	2.51	2.10	2.69	5.26	5.74	6.54	7.47
k_2(L)/k_2(D)	2.52		2.39		2.15		2.07	

a) At pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2 with KCl), [**1a**]=1.00×10^{-4} mol dm^{-3}.

Table 5. Thermodynamic Parameters for Hydrolysis of **1a** in the Presence of Cyclodextrin[a)]

	α-CD		β-CD	
	D	L	D	L
$\Delta G^{\ddagger}$[b)]/kcal mol^{-1}	19.0	18.5	20.1	19.7
$\Delta H^{\ddagger}$/kcal mol^{-1}	24.9	23.9	22.0	20.9
$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1}	19.8	18.2	6.45	4.14

a) At pH 9.00, 0.02 mol dm^{-3} borate buffer (I=0.2, KCl). The parameters were calculated by plotting the data of Tables 3 and 4 in the form of $\ln(k_2/T)$ versus $1/RT$. b) For the values at 298.2°K.

into the cavity of CD and react with a hydroxyl group. Thus, *meta* is better than *para* in stereoselectivity. This is attributable to the nature of the catalyst.

The differences in reactivity between D and L enantiomers in the hydrolysis of **1a** depend on the catalytic rate constants (k_2) rather than the dissociation constant of the complex formation (K_{diss}). In the cleavage of 2,2,5,5-tetramethyl-3-(*m*-nitrophenoxycarbonyl)-1-pyrrolidinyloxyl by α-CD,[17] k_2 for the (+) enantiomer is 6.9 times larger than that for the (−) enantiomer. However, the K_{diss} values are almost equal for the two enantiomers. On the other hand, in the cleavage of chiral organophosphates,[16] the reactivity depends on both k_2 and K_{diss}. Thus, the stereoselectivity is very large in this reaction. As described by Bender and Komiyama,[22] the larger enantiomeric specificity in the cleavage of organophosphates than in the cleavage of esters is attributable to the fact that the reaction takes place directly at the asymmetric center (phosphorus atom), whereas in the cleavage of esters it occurs at the carbonyl carbon atom, which is adjacent to the asymmetric center. However, a remarkable stereoselectivity has recently been reported in the hydrolysis of activated enantiomeric esters which contain relatively-long-chain alkyl groups.[23] These results suggest that more effective stereochemical features may be obtained by different combinations of substrates than those used in the present system. In connection with this work, we have also investigated in order to gain the stereoselective nature of micellar reaction.[20,21]

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