Hydrolytic Catalysis with Complexation by Modified Cyclodextrins Having Diastereomeric Structure

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At the presence of three regiospecifically modified cyclodextrins, *i. e.*, β-CD-D-histidine ($\mathbf{I_D}$), β-CD-L-histidine ($\mathbf{I_L}$) and β-CD-histamine (**III**), the ester cleavage at neutral pH was compared concerning in enantiomeric selectivity as well as rate enhancement. Michaelis-Menten-type kinetics were observed for $\mathbf{I_D}$ and $\mathbf{I_L}$ in the phenol release of acetylalanyl (and phenylalanyl) p-nitrophenyl ester enantiomers. Second order rate constants were obtained for **III**. Stereochemical comparisons of the spatial geometry in the complexes were discussed and also the schematic scheme of the host-guest fit was discussed.

The characteristics of cyclodextrin (CD), *i. e.*, complex formation with a variety of organic compounds and the catalytic property by the hydroxyl anions of CD, have been attracting many chemists, because CD can mimic an enzymic action such as rate acceleration and stereospecific hydrolysis as a result of a 1 : 1 complex formation which was evidenced in the saturation and inhibition kinetics[1~10]. Moreover, by the reason that cyclodextrins are chiral molecules, many chiral selective complexations by use of CD have been reported. For example, the optical resolution of racemic α-bromophenylacetic acid derivatives using α-CD was reported by F. Cramer and the stereoselective inclusions of CD to the substrates were shown to be 14% in optical purity[11]. The stereospecific hydrolysis of sarin enantiomers with α-CD by C. V. Hooidonk *et al.* showed a large ratio of the dissociation constants, $K_{diss}(S)/K_{diss}(R)=6.6$ and large ratio of the rate constants=36[12]. Recently Kaiser et al. reported the hydrolyses of spin-labeled chiral esters catalyzed by α- and β-CD; the ratio of the rate constants up to be 7[13]. Kitaura and Bender prepared modified α-CD derivatives having a hydroxamic group as catalytic site and they reported that acetyl-D-phenylalanine m-nitrophenyl ester was hydrolyzed with the modified CD faster than L-isomer by 1.5 times[14]. On the other hand, modified chiral crown ethers having thiol group, were prepared by Chao and Cram. Their compounds formed complex with amino acid ester and catalyzed the hydrolysis of the enantiomeric esters with the ratio of rate constants up to 8[15].

In order to design a synthesis of enzyme model, it is essential to afford the model compounds the following two significant characters of enzymic reaction; large rate enhancement

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of a reaction and chiral recognition toward the substrates. Concerning the former object, we have already reported that α-CD introduced an imidazole group on a secondary alcohol side of CD ring, and we have showed a complex formation of Michaelis-Menten type with phenyl acetates and the large rate enhancement on hydrolysis of the substrates in the presence of the CD-imidazole catalyst[16].

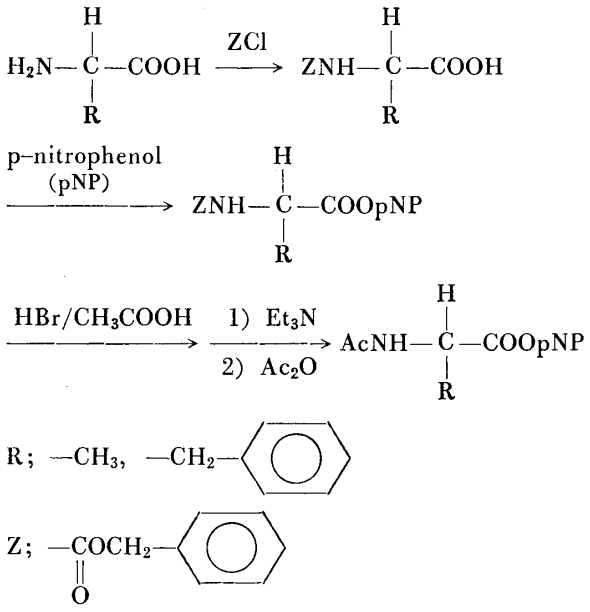
In this paper we wish to report concerning the above two objects, that is, chiral recognition on ester hydrolysis in the presence of the modified β-CD as well as rate enhancement from the stand-points of spatial geometry of the catalyst in the complex formation, were compared. For these purposes, we investigated the hyrolyses of optical active esters (acetylalanine *p*-nitrophenyl ester and acetylphenylalanine *p*-nitrophenyl ester) in the presence of modified cyclodextrins (β-CD-D-histidine ($\mathbf{I}_D$), β-CD-L-histidine, ($\mathbf{I}_L$), *n*-butylamino-β-CD (**II**) and β-CD-histamine (**III**)). **I** and **III** have imidazole group attached on the CD molecule as catalytic group, whereas **II** has no imidazole group. $\mathbf{I}_D$ and $\mathbf{I}_L$ have an opposite chirality at the catalytic site introduced on the CD molecule, and **III** has no chirality at the catalytic site introduced to the CD molecule.

Experimental

Materials

p-Nitrophenyl esters

N-Acetylalanine *p*-nitrophenyl ester enantiomers and N-acetylphenylalanine *p*-nitrophenyl ester enantiomers were prepared in the same manner described in the literatures[17,18]. The general procedure for the preparation of esters were described below.



After N-protecting of an amino acid with benzyloxycarbonylchloride, followed by the esterification of N-protected amino acid with *p*-nitrophenol and dicyclohexylcarbodiimide, the removal of the N-protecting group and acetylation produces the desired N-acetylamino acid *p*-nitrophenyl ester.

The physical properties of esters were;

Ac-D-Ala-pNP, mp. 103-103.5°,
$[\alpha]_D = +66.1°$ (c=1 in $CHCl_3$)

Ac-L-Ala-pNP, mp. 103-103.5°,
$[\alpha]_D = -68.2°$ (c=1 in $CHCl_3$)

Ac-D-Phe-pNP, mp. 137-138.5°,
$[\alpha]_D = +17.0°$ (c=2 in $CHCl_3$)

Ac-L-Phe-pNP, mp. 137-138.5°,
$[\alpha]_D = -17.8°$ (c=2 in $CHCl_3$)

All of which agreed with the values repored previously[17,18].

Modified cyclodextrins

β-CD-D-histidine ($\mathbf{I}_D$), β-CD-L-histidine ($\mathbf{I}_L$) and β-CD-histamine (**III**) were prepared and ascertified in the same manner as described in the preceeding paper[19], starting with regiospecific monotosyl CD.

Iodo-β-cyclodextrin

Mono-tosylated β-cyclodextrin (1 g) was dissolved in dry dimethylformamide (10 ml). After large excess of sodium iodide (1 g) and triethylamine (3 ml) were added to this solution, the solution was kept at 90～100°C for about 12 hrs. After reprecipitation with acetone, the reaction mixture was dried completely in vacuum overnight below 50°C. β-Cyclodextrin iodide purified by column chromatography using polystyrene gel was contaminated with tosylated β-cyclodextrin by the observation of proton nmr by *ca.* 10%. Without further purification, the conversion was estimated by iodide content according to Volhard method. More than 80% of the tosyl group was substituted to form Iodo-β-cyclodextrin. Yield; 65%, $[\alpha]_D$; 147.1° (c=0.5, water).

Histamyl-β-cyclodextrin (***III***)

β-Cyclodextrin iodide (5 g) and free histamine (2 g) were added to water (50 ml), and the solution was kept at 80～90°C for 20 hrs. The reaction mixture was condensed and after reprecipitated with acetone, dried in vacuum.

The product was purified by chromatography using porous polystyrene gel (100×5 φ cm column). After washing with water (more than 1000 ml), 30% aqueous methanol (more than 1000 ml) was made to eluate. The fractions were examined by iodine test, refractivity, optical rotation and Pauly test. The fractions which contained the product were gathered and evaporated. Ion-exchange and gel chromatography with CM Sephadex G-25 (15×2 φ cm column, solvent; water and 1 N-NH_3 solution), and Sephadex G-15(100×4 φ cm column) were used repeatedly until the purified product showed a single spot in paper chromatography (R_f; 0.23, solvent; n-BuOH: DMF: H_2O= 2 : 1 : 1, detecting reagent; I_2 vapour and Pauly reagent). The purity of the product was checked by high pressure liquid chromatography using LS-170 column (Toyo soda Co., 60×0.7 φ cm, solvent; acetonitrile-water system). Yield; 10～15%, $[\alpha]_D$=135° (c=0.5, water), ^{1}H nmr; 7.42 and 6.76 ppm due to imidazole, relative area ratio of imidazole to C_1H (4.9 ppm) of glucose showed equimolar attachment of imidazole on CD.

Anal. Calc. for $C_{47}H_{77}O_{34}N_3 \cdot 4H_2O$: C, 43.42; H, 6.59; N, 3.23. Found: C, 43.52; H, 5.99; N, 3.34. This compound was hygroscopic.

D (L)-Histidyl-β-cyclodextrin (***I***$_D$ and ***I***$_L$)

The mixture of β-cyclodextrin iodide (5 g) and free D (L)-histidine (1.5 g) in water (40 ml) was refluxed for 12 hrs., and the reaction mixture was reprecipitated in acetone. The product was purified by chromatography using porous polystyrene gel (100×5 φ cm column). After water was made to flow through the column (more than 1000 ml), aqueous methanol (30%) was made to flow (more than 1000 ml). The fractions were examined by iodine test, refractivity, optical rotation, and Pauly test.

The fractions which contained the product were colleted and condensed invacuo on a rotary evaporator. Ion-exchange gel chromatography using CM Sephadex G-25 (20×3 φ cm column, solvent; 500 ml of water and then 1000 ml of 1 N-NH_3 solution) was done, and then Sephadex G-15 (150×3 φ cm column) and DEAE Sephadex G-25 (20×3 φ cm column, solvent; water, followed by 1 N-NH_3) was also used to purify the product. The purification of the product was repeated until the product showed one spot in paper chromatography (R_f; 0.18 for D-histidine compound, 0.17 for L-histidine compound, solvent; n-BuOH: DMF:

$H_2O=2:1:1$, detecting reagent; I_2 vapour and Pauly reagent). The purified product showed one peak in high pressure liquid chromatography (the same conditions mentioned above). Yield; 40～42%. These products were also hygroscopic.

For β-CD-D-histidine; $[\alpha]_D=146°$ (c=1, H_2O), mp. 164°C (dec.), ^1H nmr; 6.92, 7.90 ppm due to imidazole, 4.90 ppm due to C_1H, ^{13}C nmr; 174.4 ppm due to COOH, 118.0, 130.4, 134.9 ppm due to imidazole, 27.0 ppm due to CH_2 of the histidyl group.

Anal. Calc. for $C_{48}H_{77}O_{36}N_3$: C, 45.32; H, 6.10; N, 3.30. Found: C, 44.98; H, 5.92; N, 3.00.

For β-CD-L-histidine; $[\alpha]_D=131°$ (c=1, H_2O), mp. 163°C (dec.), ^1H nmr; 6.92, 7.92 ppm due to imidazole, 4.90 ppm due to C_1H, ^{13}C nmr; 184.7 ppm due to COOH, 124.1, 134.4, 140.9 ppm due to imidazole, 30.0 ppm due to CH_2 of the histidyl group.

Anal. Calc. for $C_{48}H_{77}O_{36}N_3\cdot 5H_2O$: C, 42.01; H, 5.79; N, 2.65. Found: C, 42.32; H, 6.44; N, 3.08.

n-Butylamino-β-cyclodextrin (***II***)

n-Butylamino-β-cyclodextrin (**II**) was prepared in the same procedure as described in the preceeding paper[19]; CD iodide which obtained through tosylation of CD, followed by iodination, was reacted with butylamine. In order to purify the product, the gel chromatography with CM-Sephadex G-15 and ion-exchange chromatography with CM-Sephadex were employed. The yield was 30%. Estimation of the butyl group introduced on the cyclodextrin was calculated to be 92% based on the ratio of the integrated nmr peak areas of the methyl protons of butyl group with C_1 protons of cyclodextrin.

Kinetics

The reaction was followed by the appearance of phenol spectrometrically using a JASCO UVIDEC Model-1 recording spectrophotometer equipped with a cell programer and thermostated cell compartment. The reaction medium was usually pH 7.21 Tris-HCl buffer solution if not mentioned. The ionic strength was kept at 0.200 with KCl. The reaction was initiated by the addition of 15 μl of a acetnitrile solution of *p*-nitrophenyl ester into 3.00 ml buffer where modified CD was dissolved at the concentration of $0.47-4.00\times10^{-3}$ M. The final ester concentration was 4×10^{-5} M for *p*-nitrophenyl ester. The hydrolysis of the substrates showed pseudo-first-order kinetics in these buffer with correlation factor more than 0.999. The infinite absorbabce A_∞ at 400 nm was observed after at least ten half-lives. Plots of $\log(A_\infty-A_t)$ against time for the reaction at the absence or in the presence of modified CD gave straight lines. The pseudo-first order rate constant at the absence of modified CD (k_{un}), and in the presence of modified CD (k_{obs}) were calculated as the slope divided by −2.303.

Results and Discussion

Determination of Dissociation Constant K_{diss} and Intracomplex Rate Constant k_2 in the Hydrolysis of N-Acetyl D, L-Amino Acid p-Nitrophenyl Esters in the Presence of β-Cyclodextrin-D, L-Histidine (***I***$_D$, ***I***$_L$)

To investigate the kinetical effect of the complex formation on ester hydrolysis in the presence of $\mathbf{I}_D$ or $\mathbf{I}_L$, the pseudo-first-order rate for the release of p-nitrophenolate from D and L Ac-Ala-pNP enantiomers at pH 7.21 in buffer solution at 25°C, in the excess concentration of (**I**) more than that of substrate

by 9～100 times as described in Experimental Section.

On the increase of the concentrations of **I**, k_{obs} showed a maximum saturation value similar to the behavior observed in enzyme kinetics. For example, the plot of k_{obs} for Ac-D-Ala-pNP *vs.* [$\mathbf{I}_L$] gave a down-curved line as shown in Fig. 1.

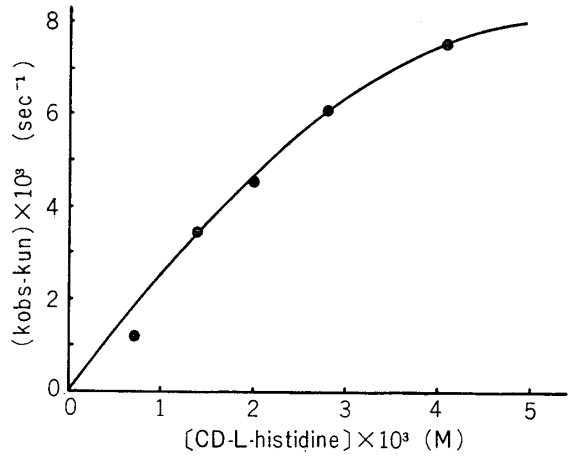


Fig. 1 A Plot of the Liberation of *p*-Nitrophenyl from Acetyl-L-alanine *p*-Nitrophenyl Ester (k_{obs}) as a Function of [*β*-CD-L-histidine]; Conditions Indicated in Table 1.

The data were treated by a variant of Michaelis-Menten kinetics previously employed for investigation of CD catalysis involving complex formation[6]. Eadie-type plots of these experimental data in Fig. 1. gave a straight line as shown in Fig. 2. By plotting k_{obs}-k_{un} against (k_{obs}-k_{un})/[modified-CD], the values of -K_{diss} and k_2 were obtained as the slope and y-intercept of the line. Also the case of ($\mathbf{I}_D$) was shown in Fig. 3.

Dissociation constants K_{diss} and intracomplex rate constants k_2 obtained in this manner are summarized in Table 1. It is of importance to compare the values of K_{diss} and k_2 with those in non-modified CD-catalysis. Modified CD (**I**) showed the values of K_{diss} ranged from 1.2 to 19×10^{-3} M and k_2 ranged from 0.17 to

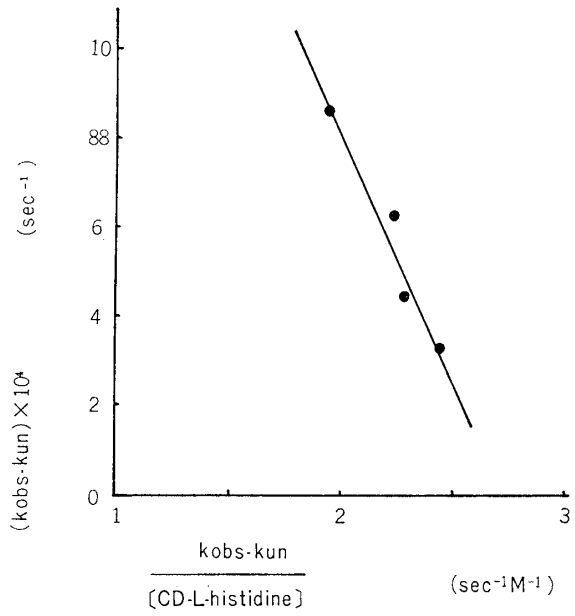


Fig. 2 Eadie Plot of the Liberation of *p*-Nitro-Phenol from Acetyl-L-alanine*p*-Nitrophenyl Ester in the Presence of *β*-CD-L-histidine; Conditions Indicated in Table 1.

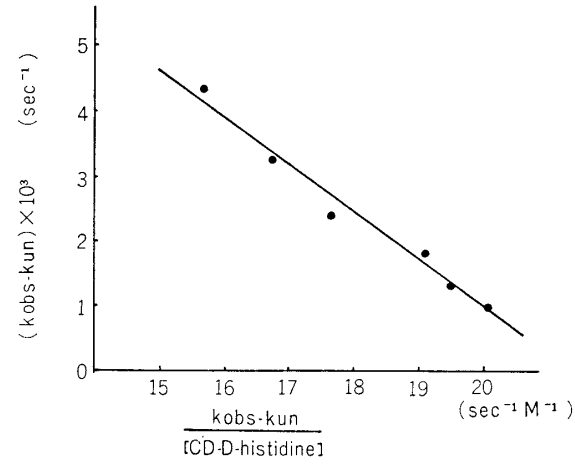


Fig. 3 Eadie Plot of the Liberation of *p*-Nitrophenol from Acetyl-D-phenylalanine *p*-Nitrophenyl Ester in the Presence of *β*-CD-D-histidine; Conditions Indicated in Table 1.

$17\times10^{-2}\,sec^{-1}$. These figures are comparable with the values observed by van Etten *et al*[6]. in the complexation and catalytic reaction between *β*-CD and *p*-nitrophenylacetate at pH 10.60 to be 6.1×10^{-3} M for K_{diss} and $6.34\times10^{-2}\,sec^{-1}$ for k_2. Essentially similar mode of reaction accompanying complex formation oc-

Table 1 Intracomplex Rate k_2 and Dissociation Constant K_{diss} for Enantiomeric Ester Cleavage with β-CD-D, L-Histidine ($\mathbf{I}_D$, $\mathbf{I}_L$)

Substrate	In the presence of $\mathbf{I}_D$				In the presence of $\mathbf{I}_L$			
	k_2 $\times 10^2 sec^{-1}$	K_{diss} $\times 10^3 M$	k_2/K_{diss} $sec^{-1}M^{-1}$	k_2/k_{un}	k_2 $\times 10^2 sec^{-1}$	K_{diss} $\times 10^3 M$	k_2/K_{diss} $sec^{-1}M^{-1}$	k_2/k_{un}
Ac-Ala-pNP D	7.3±0.5	19±1	3.8	49	0.25±0.05	8.9±2.1	0.28	1.7
L	5.4±0.6	13±2	4.2	36	0.17±0.05	5.2±0.8	0.33	1.1
D/L	1.4	1.5	0.90	—	1.5	1.7	0.85	—
Ac-Phe-pNP D	17±1	7.8±0.1	22	130	0.52±0.6	1.4±0.4	3.7	4.0
L	14±1	4.7±0.1	30	110	0.40±0.1	1.2±0.1	3.4	3.1
D/L	1.2	1.7	0.73	—	1.3	1.2	1.1	—

All determinations were made at 25.0±0.1° using pH 7.21±0.1 Tris-HCl buffer, I=0.2, 0.5% acetonitrile. Error limits for k_2 and K_{diss} are standard deviations. [Substrate] $=4.00\times10^{-5}$ M, [$\mathbf{I}_D$, $\mathbf{I}_L$] $=0.47\sim4.00\times10^{-3}$ M (5～7 points).

cured for the reaction of these esters in the presence of **I**, though the introduced histidine residue might occupy some space at the secondary alcoholic side of CD cavity and might exclude the guest molecule to some extent. Although Kitaura and Bender[14] reported that in the presence of thier modified CD the concentration dependence of the catalytic action was observed merely to be a linear, the present observations for complexation were strikingly a direct kinetical evidence for the modified CD catalysis and gave an expect to design an effective geometric specificity for a better enzyme model by chemical modification of CD molecules.

*Determination of Second Order Rate Constant in the Hydrolysis of the esters in the Presence of β-CD, Butylamino-β-CD (**II**), L-Histidine and β-CD-Histamine (**III**)*

Ac-Ala-pNP and Ac-Phe-pNP enantiomers were hydrolyzed in the presence of **III**, at pH 7.21 in Tris-HCl buffer at 25°C. For example, the plot of the pseudo-first order constants for release of phenol from Ac-L-Ala-pNP against [**III**] gave a straight line as shown in Fig. 4.

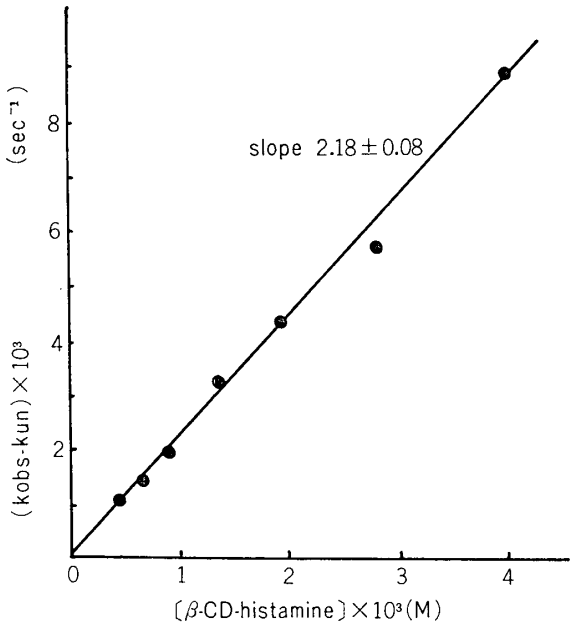


Fig. 4 Rate of the phenol liberation of *p*-nitrophenol as a function of β-CD-histamine concentration at pH 7.21, Tris-HCl buffer, ionic strength=0.200, at 25.0±0, 1°C, with 0.5 vol% acetonitrile, [ester] $=4.00\times10^{-5}$ M.

The slope of this line gives second order rate constant for this reaction. The other second order rate constants for **III** and L-histidine were calculated in the same way and summarized in Table 3. **III** showed a modest L-selectivity (L/D=1.3) in the second order rate constant for the hydrolysis of D and L-Ac-Ala-

pNP.

From the data obtained, K_{diss} of the **III** and the esters could not be calculated directly. However, on the assumption of the complexation between **III** and the ester, K_{diss} should be more than 2×10^{-2} M from the kinetical evidence. The reason why **III** did not show a saturation kinetics on plotting k_{obs} *vs.* [**III**] in the same reaction condition as $\mathbf{I}_D$ and $\mathbf{I}_L$, could be considered as follows. The flexibility of the ethylene group between imidazole and CD ring might disturb the complexation to some extent and increase the value of K_{diss} too large to observe in the present kinetics. Although this common phenomena were reported for the case of α-CD-histamine[16] and α-CD-derivatives[14,20,21], inhibition effect with the addition of cyclohexanol indicated the complex formation between these CD derivatives and substrates[16,20] or spectrometric measurement of K_{diss} implyed the complexation[14,21]. All of these CD-derivatives provided some distance and flexbility between CD and catalytic site introduced, which might give a increase for K_{diss} values.

Comparison between the Diastereomeric Catalysts $\mathbf{I}_D$ and $\mathbf{I}_L$

Kinetical comparisons between the diastereomeric modified CD in ester cleavage were summarized in Table 2. The difference in the geometry of the inside of CD cavity should be reflected in these ratios $\mathbf{I}_D/\mathbf{I}_L$ for k_2, K_{diss} and k_2/K_{diss}.

As far as the rate constant of the intracomplex ester cleavage k_2, $\mathbf{I}_D$ reacted with Ac-Ala-pNP and Ac-Phe-pNP more powerfully than $\mathbf{I}_L$ by a factor of 29～35. These rate enhancement may be caused by the difference between the diastereomeric complexes of $\mathbf{I}_D$ and $\mathbf{I}_L$ with substrates. The spatial structure of $\mathbf{I}_D$ favours so much for the catalytic reactivity of $\mathbf{I}_D$ in ester cleavage.

On the other hand, K_{diss} of $\mathbf{I}_D$ increased 2～6 times greater than that of $\mathbf{I}_L$. These results suggest that the host molecule $\mathbf{I}_D$ binded weakly with guest ester, though it catalyzed more powerfully than $\mathbf{I}_L$. On the contrary, catalyst $\mathbf{I}_L$ binded more strongly with the substrates though it catalyzed the hydrolysis more weakly.

The values k_2 divided by K_{diss} may show apparent second order rate constant including complexation and intracomplex catalytic reaction. The overall phenol release of Ac-Ala-pNP k_2/K_{diss} with $\mathbf{I}_D$ was faster than those with $\mathbf{I}_L$ by 13～14 times. Also alanyl ester was catalyzed by $\mathbf{I}_D$ faster than $\mathbf{I}_L$ by 6～9 times.

These all results could be attributed to the difference in the conformational geometry of the host-guest complexes. $\mathbf{I}_D$ and $\mathbf{I}_L$ are a kind of diastereomer each other. Moreover, the complexes of **I** with an optical active substrate

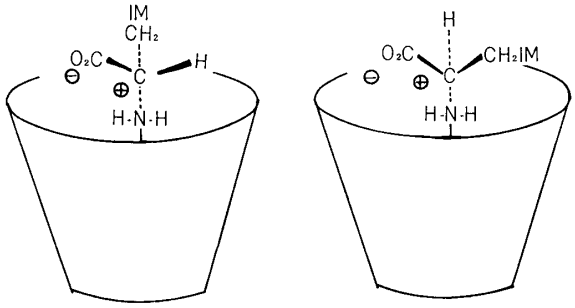
Table 2 Kinetical Comparisons with the Modified CD ($\mathbf{I}_D$ and $\mathbf{I}_L$) in Ester Claevage

Modified CD	k_2 $\times10^2$ sec^{-1}				K_{diss} $\times10^3$M				k_2/K_{diss} sec^{-1}M^{-1}			
	Ac-Ala-pNP		Ac-Phe-pNP		Ac-Ala-pNP		Ac-Phe-pNP		Ac-Ala-pNP		Ac-Phe-pNP	
	D	L	D	L	D	L	D	L	D	D	D	L
$\mathbf{I}_D$	7.3	5.4	17	14	19	13	7.8	4.7	3.8	4.2	22	30
$\mathbf{I}_L$	0.25	0.17	0.52	0.40	8.9	5.2	1.4	1.2	0.28	0.33	3.7	3.4
$\mathbf{I}_D/\mathbf{I}_L$	29	32	33	35	2.1	2.5	5.6	4.1	14	13	5.9	8.5

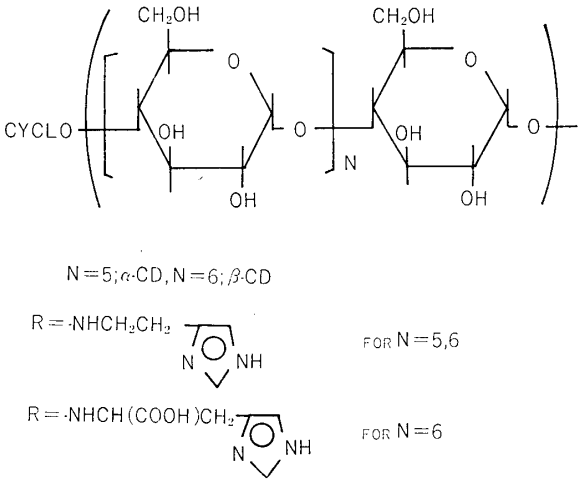
Experimental conditions are given in Table 1.

should also be called a kind of diastereomeric complexes. Therefore it is reasonably considered that the binding power or the stability between the diastereomeric complexes as well as the catalytic mode between the guest and host molecules should be different. The difference of D-histidine and L-histidine attached on the cyclodextrin molecule cause a difference of the geometrical fit of the functional groups (imidazole and carbonyl carbon) between guest and host, a steric hindrance on complexation, and so on.

A CPK sacle molecular model may suggest the difference of the two catalysts, $\mathbf{I}_D$ and $\mathbf{I}_L$, assuming; (i) that the initial catalytic action of the imidazole in the ester cleavage is nucleophilic attack to the carbonyl carbon atom of the ester, (ii) that the carboxylic group and imino group in the molecules form an ion pair each other which may face to the outside of the CD cavity as much as possible on account of its hydrophilicity, and (iii) that guest molecules (esters) may require to form complexes as approximately same orientation and position in complexes with either $\mathbf{I}_D$ or $\mathbf{I}_L$. These two directions of bond from α-carbon of histidine attached on a C-3 carbon of CD molecule, restricted and differed distinctly between $\mathbf{I}_D$ and $\mathbf{I}_L$ as is shown in Scheme 1. In the case of $\mathbf{I}_D$ molecule, the axis of C_α–H bond directed tangently on the above surface of the CD molecule, whereas C_α-methyleneimidazole bond stands almost perpendicularly to the open side of CD cavity. On the contrary, in the case of $\mathbf{I}_L$ molecules, stereochemical restriction which mainly comes from the assumption (ii), requires contrary position of H and methyleneimidazole on α-carbon in the histidine residue. Catalytic active site of $\mathbf{I}_L$ (imidazole) positioned



Scheme 1 Schematic Scheme of Specific Geometry of the Diastereomeric Catalysts $\mathbf{I}_D$ (left) and $\mathbf{I}_L$ (right).



Scheme 2 Structures of Imidazole-Containing Cyclodextrins.

in the direction to the outside of the cavity of CD, which is disadvantage for the catalytic action to the guest molecules included in the CD cavity, although it may favor for the complexation on the accunt of less steric distubarance at the entrance of the CD cavity.

Comparison between the Substrates of Alanyl and Phenylalanyl Esters

In the case of Ac-Ala-pNP as a substrate, it was clear that a 1 : 1 complex formation may occur in a single mode of conformation at the reaction of $\mathbf{I}_D$ and $\mathbf{I}_L$ with Ac-Ala-pNP. However the molecule Ac-Phe-pNP have two possible binding sites namely, the p-nitrophe-

nyl group and the phenyl group of phenylalanine moiety. By this reason, the hydrolyses of Ac-Phe-pNP enantiomers catalyzed by the modified CD (**I**) should be considered to be a double possible modes of reaction scheme. This speculation may be assisted by the kinetical aspects: the dissociation constants of the complexes of Ac-Phe-pNP with **I** was rather small by a factor of 2-6 in comparison with those of Ac-Ala-pNP; and that the decreased D/L factors of the complexation between **I** and phenylalanyl ester compared with those of **I** and alanyl esters. In summary, phenylalanyl esters may participate in complexation either in two binding sites. The presence of phenyl moiety in phenylalanyl esters may cause generally more tight complex formation and gave a larger acceleration in k_2/k_{un}.

Chiral Selectivity toward Enantiomeric Esters

Chiral selectivity for D-, L-substrates of Ac-Ala-pNP and Ac-Phe-pNP with diastereomeric catalysts $\mathbf{I}_D$ and $\mathbf{I}_L$ both in the binding step and in the hydrolytic step was observed and calculated the ratio of D ester toward L isomer in K_{diss} and k_2 as in Table 1. In all four combinations between two substrates and two catalysts, the enantiomeric ratios k_2 (D)/k_2 (L) were 1.2-1.5. This showed D-isomer selective in the hydrolytic step. On the contrast, the ratios of K_{diss}(D)/K_{diss}(L) were 1.2-1.9, showing L-isomer selective in binding step by the complexation. These results indicated that intracomplex rate constant k_2 and dissociation constant K_{diss} cancelled each other in overall enantiomeric selectivity. In other words, asymmetric selectivity of binding step and that of hydrolytic step cancelled each other and weakened the overall selectivity. Therefore the apparent second order rate (k_2/K_{diss}) showed relatively small chiral recognition; the enantiomeric ratio D/L of this value ranged 0.7 ($\mathbf{I}_D$−Ac-Phe-pNP) to 1.1 ($\mathbf{I}_L$−Ac-Phe-pNP). It was previously reported hat α-CD-N-(N, N′-dimethylaminoethylacetohydroxamic acid) reacted Ac-L-Phe-pNP faster than D-isomer by a factor of 1.2 in second order rate constant[14]. This value can be compalable with the present result of the k_2/K_{diss} ratios which were relatively poor optical selectivity attributed to the situation above mentioned.

These results also imply that the specificity for chiral selectivity may be determined by the delicate balance between the asymmetric binding and the asymmetric reactivity.

Rate Acceleration

As is shown in Table 1, rate accelerations k_2/k_{un} in the presence of $\mathbf{I}_L$ were less than 4.0. There was merely a modest acceleration for the case. However, in the presence of $\mathbf{I}_D$ catalyst, the rate acceleration was enhanced by a factor up to 130. The same extent of acceleration in the neutral pH was reported in the reaction of p-nitrophenyl acetate with α-CD-histamine previously; the rate acceleration k_{obs}/k_{un} was approximately 120 at pH 8.02[16]. As far as the rate acceleration, the present $\mathbf{I}_D$ catalyst showed compalable rate enhancement as that of α-CD-histamine compound. Thus the geometric specificity in the conformation of complex might suggest a close analogy of these two modified CD system such as in the length or direction between the active site of the catalyst and the reaction site of the ester.

Another aspects for rate acceleration was determined through the (apparent) second order rate constants. These results were sum-

Table 3 Kinetical Comparisons with Various Catalysts in Enantiomeric Ester Cleavage

Catalyst	Second Order Rate Constant ($sec^{-1}M^{-1}$)		D/L	Second Order Rate Constant ($sec^{-1}M^{-1}$)		D/L
	Ac-D-Ala-pNP	Ac-L-Ala-pNP		Ac-D-Phe-pNP	Ac-L-Phe-pNP	
β-CD	inhibition	inhibition	0.99*	inhibition	inhibition	0.94*
II	—	—		inhibition	inhibition	1.1*
L-histidine	0.14	0.13	1.1	—	—	
III	1.7	2.2	0.78	13	12	1.1
$\mathbf{I}_D$**	3.8	4.2	0.90	22	30	0.73
$\mathbf{I}_L$**	0.28	0.33	0.85	3.7	3.4	1.1

Rate constants were determined spectrometrically at 400 nm in Tris-HCl buffer solution at pH 7.21, I=0.200, at 25.0°C, [catalyst] $=4.00\times10^{-3}$ M for pseudo-first order rates k_{obs} and $0.47\sim4.00\times10^{-3}$ M (7 points) for second order rate, [substrate] $=4.00\times10^{-5}$ M, with 0.50% acetonitrile. (*) showes k^D_{obs}/k^L_{obs}. (**) k_2/K_{diss} values in Table 1 were used for second order rate constant.

marized in Table 3. For the catalysts of β-CD and **II**, apparent second order rate constant showed negative values on plotting of pseudo-first-order rate constant *vs.* catalyst concentration. The rate of ester hydrolysis at the presense of β-CD and **II** was inhibited, probably by the protection of the reaction site of the ester on complexation. Second order rate constants for **III** may contain some attributions by intermolecular reaction with substrates beside intracomplex catalysis within CD cavity. In the combination of α-CD-N-methylacetohydroxamic acid and p-nitrophenyl acetate, Gruhn and Bender reported that intermolecular attribution should be less than 5% and that predominant pathway involved the formation of the complex[20]. Hence, assuming the predominant pathway through complexation for the present reaction between **III** and the esters, it can be compared meaningfully with the k_2/K_{diss} values which have been obtained for the case in the presence of $\mathbf{I}_D$ and $\mathbf{I}_L$ in Table 1.

Among three modified catalysts, the order of the rate constant was $\mathbf{I}_D>\mathbf{III}>\mathbf{I}_L$ in the proportion of 14 : 7 : 1-2 approximately. Again assuming the more fixed structure of $\mathbf{I}_D$ and $\mathbf{I}_L$ which was based on the internal ionic interaction between carboxylic acid and imino group of histidine residue as shown in Scheme 1, both the enhancement of k_2/K_{diss} in the presence of $\mathbf{I}_D$ and the decrease of k_2/K_{diss} in the presence of $\mathbf{I}_L$ compared with the second order rate constant in the presence of **III** can be explained as follows. The fixation of the catalyst conformation such as in $\mathbf{I}_D$ does favour the specific catalysis when it affords an appropriate spatial fit between active site in catalyst and reaction site in substrate. However, when fixation does not give a spatial fit, it merely retards the reaction rate as in the case of $\mathbf{I}_L$. The catalyst having more free rotation of the ethylene segment between active site and binding site such as in **III** may catalyze the reaction, but the true flexibility might decrease the complexation with the substrates. From the data obtained, the value of K_{diss} should be in the order of $\mathbf{III}>\mathbf{I}_D>\mathbf{I}_L$. In the comparison between **III** and $\mathbf{I}_D$, although kinetical analogy in rate enhancement invokes the similarity of spatial geometry of the functional group, better complexation power of $\mathbf{I}_D$ as well as the favorable fixation of the conformation, may afford the superiority of $\mathbf{I}_D$ to **III** in the overall rate

constants.

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