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Pressure Effects on the Hydrolysis of Acetanilide Catalyzed by Cyclodextrins

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The rates of the hydrolysis of *p*-nitrotrifluoroacetanilide (pNFA) catalyzed by α- and β-cyclodextrins (α-, β-CD) were measured at pressures up to 2 kbar at 25 °C and at pH 6.9 in a 0.05 M[†] Tris buffer solution. The substrate binding constants, *K*, and the rate constants of the acylation process, k_{acyl}, increased monotonically up to 2 kbar. From the pressure dependence of *K*, the volume changes (ΔV) accompanying the formation of the inclusion complexes at 1 bar were $-3\pm2\,\mathrm{cm^3/mol}$ for α-CD-pNFA and $-2\pm2\,\mathrm{cm^3/mol}$ for β-CD-pNFA. The activation volumes (ΔV) for the cleavage of the anilide molecule in the acylation process were $-25\pm2\,\mathrm{cm^3/mol}$ for α-CD-pNFA and $-18\pm2\,\mathrm{cm^3/mol}$ for β-CD-pNFA. The reaction mechanisms of the cleavage of the anilide substrte by CDs were discussed on the basis of the volume parameters (ΔV and ΔV^*).

Cyclodextrins have served as a good model of a serine protease, as has been reviewed by Bender *et al.*[1] The reactions of the CD-acceleated cleavage of the phenyl ester and anilide proceed *via* the three steps of the substrate binding, the acylation of CD, and the deacylation of acyl-CD as well as by means of the chymotrypsin reaction. The first reason for the great interest in these reactions is that the rate determining step of anilide hydrolysis is acylation, whereas that of ester hydrolysis is deacylation.[2—4] Secondly, the general acid catalysis in the acylation step has been observed only in the anilide substrate.[4] Therefore, the CD-catalyzed hydrolysis of anilide can be a means to discuss the reaction mechanism of the hydrolysis of amide compounds catalyzed by hydrolytic enzymes. In order to consider such a mechanism of the acylation of CD related to general acid catalysis, the volume changes and activation volumes (ΔV and ΔV^*) given by the pressure dependence of the rate process are useful, because a proton transfer in the general-acid catalysis is accompanied by a volume decrease of 5 cm^3/mol with one hydrogen-bonding formation[5,6] Thirdly, the difference in the reaction mechanism between meta and para substrates is reflected in the stereospecificity. It is well known that meta-substituted compounds are cleaved much faster than the corresponding para-substituted ones in the ester substrates.[2] In the anilide substrates, the reverse results have been reported.[4]

In the previous reports,[7,8] the most important difference in the stereospecific cleavage of the phenyl ester catalyzed by CDs between para- and meta-substrates has been regarded as the solvation effect; the para type is solvated, while the meta type is hardly solvated at all, in the activation state from the point of view of the ΔV^* values.

In this paper, the hydrolysis rates of pNFA catalyzed by α- and β-CDs were measured at pressures up to 2 kbar at 25 °C in order to obtain the ΔV value for the formation of the inclusion complex and the ΔV^* value for the acylation process. The difference in the reaction mechanism between an ester and an anilide is discussed in terms of the ΔV and ΔV^* values for the two kinds of substrates by comparing them with the findings of previous studies.[7,8]

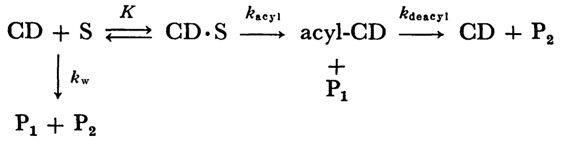
Experimental

Materials. The purification of the α- and β-CDs has been described previously.[7] The pNFA was synthesized from trifluoroacetic anhydride and *p*-nitroaniline and recrystallized 4 times from hexane-acetone; mp 149—150 °C (lit,[4] 150 °C). The ionized water was distilled. The acetonitrile was distilled over phosphorus pentaoxide before use.

Apparatus and Procedure. The method of measuring the rate of hydrolysis reactions under a high pressure has been described in a previous report.[9] The rate was measured at pH 6.9 in a 0.05 M Tris buffer solution up to 2 kbar at 25± 0.5 °C. The pressure dependence of the pH value is negligible, as the volume change of the dissociation process is -1 cm^3/mol.[10] By the use of a Hitachi 340-type spectrophotometer, the hydrolysis was monitored by means of the optical density at 390 nm attributable to the *p*-nitroaniline formed as a product. The initial concentrations of the cyclodextrins varied from 4.0×10^{-3} M to 2.0×10^{-2} M for α-CD and from 2.0×10^{-3} M to 1.0×10^{-2} M for β-CD respectively. The concentration of pNFA was 1.0×10^{-4} M.

Results and Discussion

At 1 bar, the apparent rate constant, k_{app} increased with an increase in the concentrations of the α- and β-CDs. The hydrolysis of pNFA catalyzed by CD has been shown by VanEtten *et al.*[2] to proceed *via* the scheme of Eq. 1, where S, P, *K*, and


(1)

$$\begin{array}{ccccccc} \mathrm{CD+S} & \overset{K}{\rightleftarrows} & \mathrm{CD\cdot S} & \xrightarrow{k_{acyl}} & \text{acyl-CD} & \xrightarrow{k_{deacyl}} & \mathrm{CD+P_2} \\ \downarrow k_w & & & & + & & \\ \mathrm{P_1+P_2} & & & & \mathrm{P_1} & & \end{array} \qquad (1)$$

ks' denote the anilide, the products, the binding constant, and the rate constants of each process respectively. *K* and k_{acyl} values at various pressures and at 25 °C, as determined by means of the Lineweaver-Burk plots[7] in Fig. 1 (pNFA, α-CD), are shown in Table 1. From the linear relations of log *K* and log k_{acyl} *vs.* the pressure established for each system in Figs. 2 and 3, the ΔV values for the formation of the CD complexes and ΔV^* values for the process of the product formation at 1 bar were determined to be as shown in Table 2, along with the previous data on the α-CD- and β-CD-*p*-

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[†] 1 M=1 mol dm^{-3}.

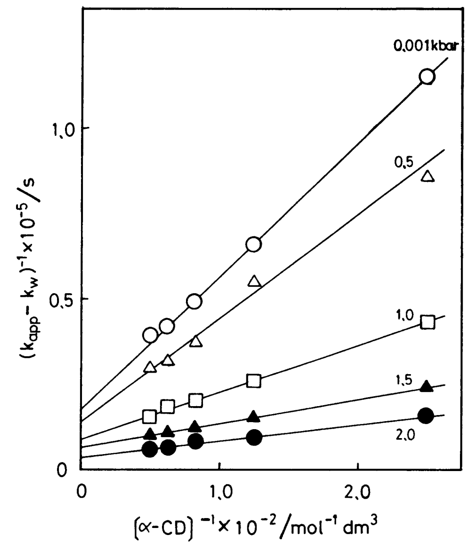

Fig. 1. Lineweaver-Burk plots for the hydrolysis of pNFA catalyzed by α-CD at each pressures, pH 6.9 (0.05 M Tris buffer) and 25 °C.

TABLE 1. RATE CONSTANTS, k_{acyl}, AND EQUILIBRIUM CONSTANTS, K, FOR THE INCLUSION-COMPLEX FORMATION OF pNFA CATALYZED BY CD AT VARIOUS PRESSURES AND AT 25 °C[a)]

P/kbar	α-CD		β-CD	
	K/M^{-1}	$10^4\times k_{acyl}/\text{s}^{-1}$	K/M^{-1}	$10^4\times k_{acyl}/\text{s}^{-1}$
0.001	55.2	0.601 (0.119)[b)]	95.9	0.235 (0.119)[b)]
0.5	57.1	0.826	97.0	0.347
1.0	62.6	1.33	103	0.538
1.5	74.8	2.31	108	0.852
2.0	70.0	3.73	87.8	1.02

a) 1% acetonitrile solutions, pH 6.9 in a 0.05 M Tris buffer. b) k_W, spontaneous hydrolysis of pNFA.

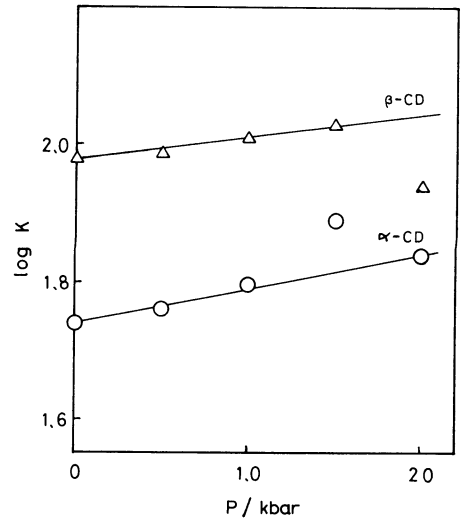

Fig. 2. Pressure dependence of log K at 25 °C.

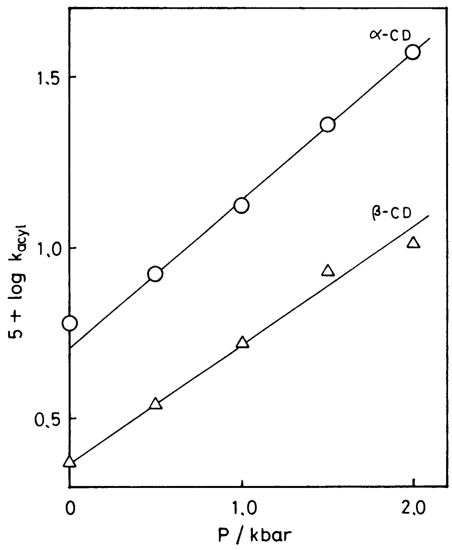

Fig. 3. Pressure dependence of log k_{acyl} at 25 °C.

TABLE 2. VOLUME CHANGES FOR INCLUSION-COMPLEX FORMATION AND ACTIVATION VOLUMES IN THE ACYLATION STEP AT 25 °C AND AT 1 bar

	pNFA		pNPA[a)]	
	α-CD	β-CD	α-CD	β-CD
$\Delta V^{b)}/\text{cm}^3\,\text{mol}^{-1}$	−3	−2	0	0
$\Delta V^{*b)}/\text{cm}^3\,\text{mol}^{-1}$	−25	−18	−16	−13

a) From Ref. 7. b) ±2 cm^3/mol.

nitrophenyl acetate (pNPA) systems.[7)]

Substrate Binding. The ΔV values in the anilide substrate, as shown in Table 2, are −3 cm^3/mol for α-CD and −2 cm^3/mol for β-CD; those values are slightly more negative than those of the ester substrate (0 cm^3/mol for α- and β-CDs).[7)] This volume difference is probably attributable to the differences in the inclusion mechanism between the NH group of anilide and the ether oxygen atom of the ester.

The X-ray analysis[11)] of the β-CD-inclusion complex of *p*-nitroaniline (pNA) as a model for the β-CD·pNFA complex has shown that the pNA molecule is wholly included in the cavity of β-CD. If the *p*-nitrophenol (pNP) part of pNPA is wholly included in the cavity of CD, the ΔV value of pNFA for the inclusion process decreases to be 2.5 cm^3/mol larger than that of pNPA, because the value of 2.5 cm^3/mol is the difference in the molar volumes between the NH_2 group (10.54 cm^3/mol) and the OH group (8.04 cm^3/mol).[12)] The estimated value is in good agreement with the experimental value of the volume difference of 2 cm^3/mol.

From the X-ray analysis of the α-CD-inclusion complexes of *p*-iodoaniline (pIA)[13)] and pNP[14)] as models for the pNFA- and pNPA-α-CD complexes, both the NH_2 group of pIA and the OH group of pNP molecules are seen to stick out from the cavity of α-CD. Since both groups exist in the aqueous media before and after the complexation, the volume differences between the NH_2 and OH groups must be cancel each other out. The difference in the electron-donating power in the NH_2 and OH groups related to the hydrogen-bonding formation may be estimated from the bond-dissociation energy of the charge-transfer (CT) complex between I_2 and an electron-donor molecule. The energy of I_2-diethyl ether as the model for the ether oxygen of pNPA is 2.1 kcal/mol,[††] and that

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[††] 1 cal=4.184 J.

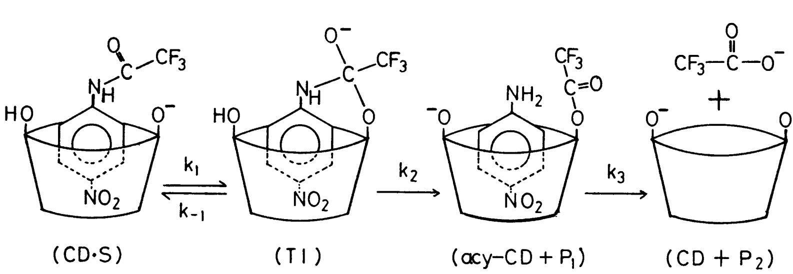

Fig. 4. Schematic drawing of the proposed reaction scheme of α-CD-catalyzed hydrolysis of pNFA after the complexation.

of I_2-*N*,*N*-dimethylaniline for the NH group of pNFA is 8.2 kcal/mol.[16] The amino nitrogen atom of the NH_2 group is more than 0.4 nm apart from any atom in α-CD.[13] After the complexation with pNFA, a hydrogen-bonding bridge among the NH group of pNFA, a water molecule, and the OH group of α-CD may be formed by the strong electron-donating power of the NH group. As the O···O distance between the water molecule and its second neighboring water molecue in an aqueous solution is approximately 0.4—0.5 nm,[15] the hydrogen-bonding system can form between the NH group of pNFA and the OH group of the α-CD through the bridge of a water molecule. Such a hydrogen-bonding system is supported by the X-ray analysis of the α-CD·pIA complex.[13] On the other hand, the ether oxygen atom of pNPA probably can not form such a hydrogn bond because of the lower power of its hydrogen-bonding formation than that of the NH group of pNFA. The volume change for a hydrogen-bonding formation is —5 cm^3/mol.[5,6] This value is in agreement with the value of —3 cm^3/mol for the volume difference between the ester and anilide substrates.

Acylation Process. The mechanism of the α-CD-catalyzed hydrolysis of pNFA after the complexation, as proposed by Komiyama and Bender,[4] is shown in Fig. 4 (Scheme 1). Here, TI is the tetrahedral intermediate produced as a result of the nucleophillic attack by the oxide ion from the secondary hydroxyl group of CD on the carbonyl carbon atom of pNFA. Then, the rate constant of the acylation step is given by Eq. 2, where $K_1 = k_1/k_{-1}$:

$$k_{\text{acyl}} = K_1 \cdot k_2. \tag{2}$$

The activation volume in the acylation step is given by Eq. 3:

$$\Delta V^* = \Delta V_1 + \Delta V_2^{\ddagger}. \tag{3}$$

Since there is no difference in the volume change on the K_1 process between the ester and the anilide, the ΔV_1 values were estimated to be —16 cm^3/mol for α-CD and —13 cm^3/mol for β-CD in a previous report.[7] From these values and Eq. 3, we can determine that the $\Delta V_2^{\ddagger}$ values in the process of k_2 are —9 (=—25—(—16)) cm^3/mol for α-CD and —5(=—18—(—13)) cm^3/mol for β-CD.

From the isotope effects in the acylation step, Komiyama and Bender reported[4] that, in the breakdown of the TI (k_2 process), an un-ionized secondary hydroxyl (OH) group functions as an intracomplex general acid catalyst, either directly or *via* water molecules. From the discussion of the substrate binding, the formation of the hydrogen-bonding bridge $[(\alpha\text{-CD})\text{-OH}\cdots\underset{\text{H}}{\text{O}}\text{-H}\cdots\text{NH-(pNFA)}]$ in the TI for α-CD has already been expected, but for β-CD, the NH group of pNFA located in the hydrophobic cavity of β-CD has already been dehydrated. If the proposed general acid catalyst functions *via* water molecules except for the hydrated water molecules, the formation of a hydrogen bond between the hydrated water molecule and the secondary water molecule can be expected to be related to the general acid catalysis on the basis of the value of —5 cm^3/mol.[5,6] The value of $\Delta V_2^{\ddagger}$=—9 cm^3/mol for α-CD suggests the formation of two hydrogen bonds, while the value of —5 cm^3/mol for β-CD suggests one hydrogen bond. Following the discussion of the substrate-binding and acylation process, we can propose the reaction mechanism shown in Fig. 5 (Scheme 2). That is, an OH group of α-CD in the activation state is expected to function *via* a free (not hydrated) water molecule through the oxygen atom of a water molecule in the hydrogen-bonding system in TI. Accordingly, it is considered that two water molecules may exist between the OH group of α-CD and the NH group of pNFA. On the other hand, the OH group of β-CD functions directly as a intracomplex general acid catalyst.

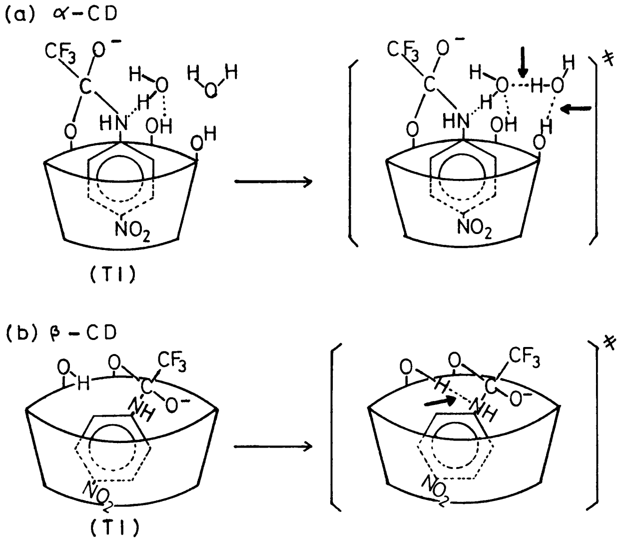

Fig. 5. Schematic drawing of the reaction mechanism of CD on the process of TI cleavage.

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