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Nucleophilic Acceleration of the Cleavage of β-Cyclodextrin *trans*-Cinnamate by Amines

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The cleavage of β-cyclodextrin *trans*-cinnamate (**1**) was accelerated by amines such as quinuclidine and piperidine by 27- and 13-fold, respectively. The reaction involves complex formation of **1** with the amines, and proceeds via nucleophilic attack by the neutral amine, which was shown by the production of amide in the reaction of **1** with piperidine. Quinuclidine exhibited real catalysis of hydrolysis without production of amide. The present finding indicates that the rates of the rate-determining deacylation step in the cyclodextrin-accelerated hydrolyses of phenyl esters can be made larger than the rates of uncatalyzed hydrolyses by an amine such as quinuclidine, resulting in the use of cyclodextrin as a true catalyst and as a better enzyme model.

INTRODUCTION

Cyclodextrins have served as excellent models of hydrolytic enzymes (*1*, *2*). The hydrolyses of esters (*3–6*) and amides (*7–10*) proceeds via a pathway of binding, acylation, and deacylation, which is consistent with the pathway used by hydrolytic enzymes. Furthermore, the cyclodextrin-accelerated cleavage of esters and amides exhibit many of the kinetic features shown by enzymatic reactions, including saturation, stereospecificity, D,L-specificity, and competitive inhibition. However, the slow rates of the deacylation step (hydrolyses of acyl-cyclodextrins), which are smaller than the rates of spontaneous hydrolyses in the case of phenyl esters (*3–6*) and acylimidazoles (*9*), limit cyclodextrins as enzyme models and as catalysts. Thus, acceleration of the hydrolyses of acyl-cyclodextrins is very important.

In the previous paper (*11*), certain amines showed acceleration in the cleavage of cyclodextrin *trans*-cinnamate in the alkaline region, which is far from biological conditions. For example, the acceleration of cleavage of β-cyclodextrin *trans*-cinnamate by 1,4-diazabicyclo(2.2.2)octane, the best one among the catalysts examined, is sixfold at pH 12.0. Accelerations by benzimidazoles were only 1.5- to 3-fold, although they were obtained near neutrality (*12*).

In the present paper, we describe extension of the previous study leading to the finding of better catalysts which show larger accelerations near neutrality (biological conditions). It will be shown that quinuclidine (**2**) and piperidien (**3**) exhibit large acceleration of the cleavage of β-cyclodextrin *trans*-cinnamate (**1**). Acceleration by **2** is effective near neutrality, thus making cyclodextrins better enzyme models as well as better catalysts.

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EXPERIMENTAL

Materials. **1**, kindly furnished by Dr. Y. Kurono, was recrystallized before use. The purity was found to be higher than 96% by absorption spectroscopy at 273 nm (*12*). Piperidyl *trans*-cinnamide was synthesized from *trans*-cinnamoyl chloride and piperidine, and recrystallized from *n*-hexane–acetone solution; mp 120–121°C (lit (*13*) 118–119°C). Other reagents were obtained as described in the previous paper (*11*). Water used in the kinetic studies was doubly distilled.

Kinetics. The cleavage of **1** was followed at 305 nm and 20°C. The ionic strength was kept at 1.0 *M* by use of KCl. The initial concentration of **1** is about 5×10^{-5} *M*. The hydrolysis of **1** followed first-order kinetics. The specific rate constant, k_{obs}, was determined by the usual first-order equation or by the method of Guggenheim (*14*). In the D_2O experiments, pD was determined by the equation: pD = pH meter reading +0.4 (*15*).

RESULTS

Both **2** and **3** exhibited large accelerations of the cleavage of **1** as shown in Fig. 1. The k_{obs} did not increase linearly with the concentration of **2** or **3** but the increase was

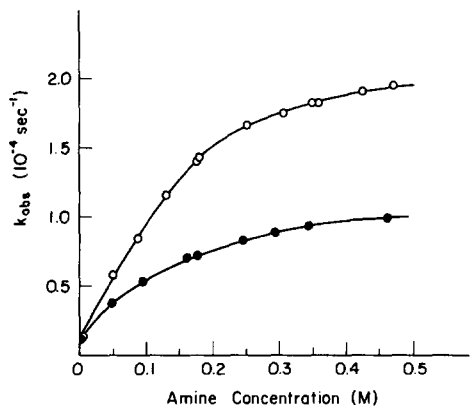

FIG. 1. Dependence of k_{obs} on the amine concentration at pH 9.85, 20°C; (○) **2**, (●) **3**.

gradual (Fig. 1), indicating that complex formation of **1** with **2** or **3** is the first step in the accelerations. Thus, the rate constant of the cleavage of **1** complexed with **2** or **3** (k_c) and the dissociation constant of the complex of **1** with **2** or **3** (K_d) was determined by use of Eq. (1) (*11*):

$$1/(k_{obs} - k_{un}) = K_d/k_c(1/[B]_0) + 1/k_c, \quad (1)$$

where $[B]_0$ is the initial concentration of **2** or **3**, and is experimentally taken as much larger than the initial concentration of **1**.

Application of the data in Fig. 1 to Eq. (1) gave a fair linear relationship between $1/(k_{obs} - k_{un})$ and $1/[B]_0$. Table 1 lists the values of k_c and K_d.

TABLE 1

VALUES OF k_c AND K_d FOR THE AMINE-CATALYZED HYDROLYSIS OF **1**

Amine	pH	k_c (10^{-4} sec^{-1})	k_c/k_{un}	K_d (10^{-1} M)	pK_a of amine[a]
2	9.85	3.0 ± 0.2	27	2.4 ± 0.2	10.95
	9.35	1.0 ± 0.1	28	2.3 ± 0.2	
3	9.85	1.4 ± 0.3	13	2.0 ± 0.4	11.28
triethylamine	9.85	—[b]	—	—	10.78
1,4-diazabicyclo(2.2.2)octane	7.80	—[b]	—	—	8.19

[a] Ref. (*15*).

[b] No measurable effect of amine was observed at the amine concentration of 0.175 M.

The acceleration of the cleavage of **1** by **2** was as large as 27-fold at pH 9.85. The magnitude of the acceleration by **2** as well as the K_d of the **1** · **2** complex at pH 9.35 was identical with those at pH 9.85 within experimental error. **3** also exhibited considerable acceleration of the cleavage of **1**.

Figure 2 shows the pH dependence of k_{obs} in the presence of 0.35 M **2** as well as that of k_{un} k_{obs} in the presence of **2** increases linearly with pH with a slope of unity up to pH 10. However, the slope gradually decreases with pH above pH 10, due to the ionization of **2**. Since both log k_{obs} and log k_{un} have a slope of unity below pH 10, the magnitude of acceleration by **2** is constant down to lower pH. In other words, the 27-fold acceleration by **2** is also obtained near neutrality.

The product of the reaction of **1** in the presence of **3**, a secondary amine, was analyzed by infrared and absorption spectroscopy to get information on the reaction product. The reaction conditions were [**1**] = 0.05 M, [**3**] = 0.8 M, and pD 9.85 in D_2O, where about 80% of **1** is complexed with **3** and about 1% of **3** has its neutral form. Spectroscopy was carried out after the completion of the reaction (4 days after). The

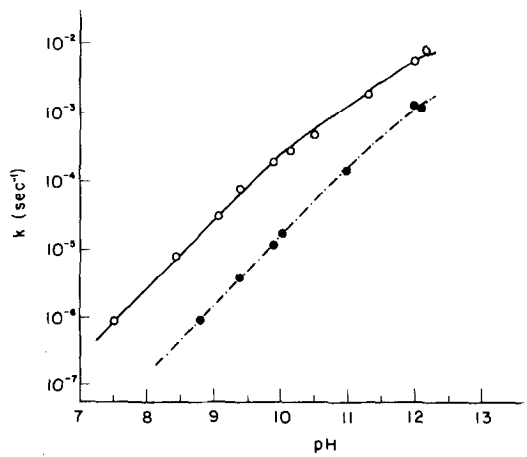

FIG. 2. pH dependence of the rate of the hydrolysis of **1** at 20°C; (–O–) in the presence of 0.35 M **2**, (–·–●–·–) in the absence of **2**.

infrared spectrum of the reaction mixture showed vibrational bands at 1640 and 1440 cm^{-1}, which are identical with those of authentic sample of piperidyl *trans*-cinnamide Thus, the major reaction between **1** and **3** is amide formation rather than hydrolysis of **1**. This assignment was supported by uv absorption spectroscopy, showing an absorption maximum at 275 nm. Piperidyl *trans*-cinnamide shows absorption maximum at 276 nm, whereas the hydrolysis product, *trans*-cinnamate ion, shows an absorption maximum at 269 nm. The reaction product of **1** with **3** at pD 12.1, where about 60% of **3** is in the neutral form, was also piperidyl *trans*-cinnamide.

These facts show that the reaction of **1** with **3** at any pH proceeds through nucleophilic attack by **3**, resulting in the formation of a stable amide. This result is consistent with the formation of dichloroacetamide in the reaction of ethyl dichloroacetate with ammonia (*17*).

On the other hand, the reaction mixture of **1** and **2** showed a product with an absorption maximum at 269 nm, which is *trans*-cinnamate. Considering the result of the **3**-accelerated cleavage of **1**, this catalysis proceeds through nucleophilic attack by **2** rather than general base attack. **2** does not form a stable amide with **1**, since **2** is a tertiary amine, and thus forms *trans*-cinnamate ion.

D_2O solvent isotope effects on the cleavage of **1** catalyzed by **2** were examined at pH 9.35 and 9.85. The observed D_2O solvent isotope effect is 1.2 ± 0.2 irrespective of pH, which is consistent with nucleophilic attack by **2**.

In spite of the large acceleration of the cleavage of **1** by **2** and **3**, triethylamine, which has a similar pK_a to that of **2** or **3**, showed no measureable acceleration. This fact indicates an important role of the stereochemistry of binding of amines with **1** for acceleration. 1,4-Diazabicyclo(2.2.2)octane [pK_a 8.19 (*15*)] showed no acceleration at pH 7.8.

DISCUSSION

The present results show that the amines such as **2** and **3** markedly accelerate the cleavage of acyl-cyclodextrins. Acceleration by **2** is real catalysis, although that by **3** is attributable to amide formation. The slow rate of the deacylation step has been a big shortcoming of cyclodextrins both as catalysts and as enzyme models (*1*, *2*). For example, the rate constants of the deacylation step (hydrolyses of α-cyclodextrin benzoates) in the α-cyclodextrin-accelerated cleavage of *m*-nitrophenyl benzoate, *m*-chlorophenyl benzoate, and *m*-chlorophenyl *p*-nitrobenzoate are 4.6×10^{-4}, 4.6×10^{-4}, and 7.5×10^{-3} sec^{-1}, respectively, at pH 10.6, 25°C, and they are smaller than the rate constants of the alkaline hydrolyses of these substrates (1.54×10^{-3}, 5.5×10^{-4}, and 1.63×10^{-2} sec^{-1}, respectively). Thus, α-cyclodextrin can not be called a true catalyst, though the rate constants of the cleavage of these substrates in the presence of 0.01 *M* α-cyclodextrin (the first step of the α-cyclodextrin-accelerated hydrolyses) are more than 10-fold larger than the rate constants of the alkaline hydrolyses (*4*). Cyclodextrins in the absence of amines should be called as accelerating reagents of ester cleavage rather than as catalysts of hydrolysis.

However, when the deacylation step of α-cyclodextrin-accelerated cleavages of these substrates is catalyzed by **2**, their rate constants are 1.24×10^{-2}, 1.24×10^{-2}, and 2.03

$\times 10^{-1}$ sec^{-1}, respectively, which are 8.1-, 23-, and 12-fold larger than those of the corresponding alkaline hydrolyses. Here, the magnitude of the acceleration of the hydrolyses of α-cyclodextrin benzoates by **2** was taken as identical with that of the hydrolysis of **1** by **2**. Thus, with the aid of the amine such as **2**, cyclodextrins can be true catalysts.

However, it should be noted that simple addition of amines to cyclodextrin-accelerated cleavage of phenyl esters retards the cleavage of phenyl esters through competitive binding of the amine and the phenyl ester to the cyclodextrin cavity. Thus, cyclodextrins covalently attached to amines should be good catalysts and enzyme models; hopefully this will be reported in the near future.

This finding also allows cyclodextrins to be better enzyme models, since a large acceleration of the hydrolyses of acyl-cyclodextrins by **2** is also obtained near neutrality. 4-, and 6-Nitrobenzimidazoles could show acceleration of hydrolyses of acyl-cyclodextrins near neutrality (*12*). However, the magnitude of the acceleration by them is only 1.5- to 3-fold, which is much smaller than those by **2**.

In conclusion, the cleavage of **1** was accelerated by **2** and **3**. The reaction proceeds through nucleophilic attack by the amines. Thus, **2** shows real catalysis of hydrolysis, although the reaction of **1** with **3** results in amide formation. With the aid of an amine such as **2**, the rates of the rate-determining deacylation step in the cyclodextrin-accelerated hydrolyses of phenyl esters can be made larger than the rates of uncatalyzed hydrolyses, which make cyclodextrin a true catalyst and a better enzyme model.

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