Amine-catalyzed hydrolyses of cyclodextrin cinnamates
(cyclodextrin-catalyzed ester hydrolysis/acyl-cyclodextrin/acceleration of deacylation/enzyme model)

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ABSTRACT

Hydrolyses of β-cyclodextrin cinnamate (βCDC) and α-cyclodextrin cinnamate were catalyzed by amines such as 1,4-diazabicyclo(2.2.2)octane, triethylamine, quinuclidine, piperidine, diisobutylamine, and n-butylamine. The rate constant of hydrolyses of the βCDC-amine complexes follows the order: 1,4-diazabicyclo(2.2.2)octane > α-n-butylamine > quinuclidine > piperidine > triethylamine > diisobutylamine. The ratio of the catalytic rate constant for the βCDC/1,4-diazabicyclo(2.2.2)octane complex to the spontaneous rate constant for βCDC is about 8-fold and is almost independent of pH below pH 11.5; but it then drastically increases with pH above pH 11.5, up to 57-fold at pH 13.6 which is much higher than previous attempts. The pH-rate constant profile and isotope effect with deuterium oxide solvent indicate that 1,4-diazabicyclo(2.2.2)octane, included in βCDC, assists the catalytic nucleophilic attack by hydroxide ion toward the carbonyl carbon of βCDC. Acceleration of deacylation of acyl-cyclodextrins, by amines, has made the cyclodextrin-catalyzed hydrolyses of esters an even better model of hydrolytic enzyme reactions than those developed previously.

It is well known that cyclodextrins catalyze the hydrolyses of phenyl esters (1). The cyclodextrin-catalyzed hydrolysis of esters can be used as a model of serine esterase-catalyzed hydrolyses, because it proceeds through the pathway of binding, acylation, and deacylation steps which is characteristic of enzymatic reactions. Besides, cyclodextrin reactions exhibit many kinds of kinetic features shown by enzyme reactions, including stereospecificity, competitive inhibition, saturation, D-L specificity, etc. (2). However, a much smaller rate constant for the deacylation of acyl-cyclodextrins than that of the corresponding acylation resulted in the ineffective use of cyclodextrins as catalysts (2, 3). Thus, acceleration of deacylation of acyl-cyclodextrins is quite important to make cyclodextrins an even better enzyme model. Recently, Kurono et al. (4) showed that the hydrolyses of α-cyclodextrin cinnamate and β-cyclodextrin cinnamate are accelerated by 3- and 2-fold, respectively, when 5-nitrobenzimidazole is included in the cyclodextrin cinnamates. This acceleration, however, is not sufficient for cyclodextrin to be a true enzyme model.

In this report, accelerations of hydrolyses of α-cyclodextrin cinnamate (αCDC) and β-cyclodextrin cinnamate (βCDC) by other amines, which come closer to a true enzyme model, are reported. The amines studied include 1,4-diazabicyclo(2.2.2)octane (DABCO; AzagbicOct), triethylamine (Et3N), quinuclidine (QCD), piperidine (Pip), diisobutylamine (iBu2NH), and n-butylamine (BnNH2). The pH-rate constant profile and solvent isotope effect with deuterium oxide for AzagbicOct on the hydrolysis of βCDC are shown. Furthermore, a mechanism for the amine-catalyzed hydrolysis of αCDC or βCDC is proposed.

EXPERIMENTAL

Materials. βCDC and αCDC were kindly furnished by Y. Kurono. They were recrystallized before use. The purity of βCDC and αCDC was found to be higher than 96% and 98%, respectively, by absorption spectroscopy at 273 nm, which is the isosbestic point between trans-cinnamic acid and βCDC or αCDC. AzagbicOct was purified by recrystallization and had a melting point of 158° (159–160° reported, see ref. 5). Other reagents were purchased from the Aldrich Chemical Co.

Kinetics. The hydrolysis of βCDC or αCDC was assayed by absorption spectroscopy at 305 nm and 20°. The ion strength was maintained at 1.0 M (KCl) unless otherwise noted. The rate constants were determined by first-order kinetics, with at least two half-lives. The change of pH in the sample solution, before and after the reaction was carried out, did not exceed ±0.02 pH units.

When amine (B) forms a complex with βCDC or αCDC (S), the observed rate constant (kobs) of the hydrolysis of βCDC or αCDC in the presence of B is expressed by Eq. 1:

kobs = k1[S] + k2[B] + k3(SB) [1]

where k1 and k3 are the first-order rate constants for S and SB, respectively; k2 is the second-order rate constant for the hydrolysis catalyzed by B, which is not included in S. For the hydrolyses in the present paper, the second term in the right-hand side of Eq. 1 is much smaller than the first and third terms, because a plot of kobs versus [B] showed saturation at large values of [B]. Thus, rearrangement of Eq. 1 using Eq. 2, under the condition that [B] > > [S], gives Eq. 3 or Eq. 4:

Kd = ([B]o - [BS])/([S]o - [SB])/[SB] [2]

1/(kobs - k2) = Kd/k2(1/[B]o + 1/k2) [3]

k2 = (kobs - k3)Kd/[B]o + 1) [4]

where Kd is the dissociation constant of BS and the subscript o refers to the initial concentration.

The values of k2 and Kd were determined by plotting 1/(kobs - k2) versus 1/[B]o in Eq. 3. When the value of Kd was determined by this procedure, k2 could then be determined by the use of Eq. 4.

For D2O experiments, pH was determined by the equation: pD = pH meter reading + 0.4 (6).

RESULTS

The hydrolysis of βCDC or αCDC was accelerated by AzagbicOct, Et3N, QCD, Pip, and BnNH2, where the rate acceleration approached a maximum at high values of [B]o. On the other hand, isobutylamine hardly affected the rate of hydrolysis of βCDC.

Abbreviations: αCDC, α-cyclodextrin cinnamate; βCDC, β-cyclodextrin cinnamate; AzagbicOct, 1,4-diazabicyclo(2.2.2)octane; Et3N, triethylamine; QCD, quinuclidine; Pip, piperidine; iBu2NH, diisobutylamine; BnNH2, n-butylamine.
Fig. 1 shows the plot of $1/(k_{obs} - k_d)$ versus $1/[B_0]$ for the hydrolysis of $\beta$CDC at 20° and pH 12.0; O, Aza2bicOct; •, Et$_3$N.

Table 1 lists the values of $k_{BS}$ and $K_d$ as well as the ratio of $k_{BS}$ to $k_d$. The dissociation constant, $K_d$, of the $\beta$CDC–Aza2bicOct complex obtained at pH 12.0 is equal to that obtained at pH 10.0, within experimental error. The $K_d$ value for the αCDC–Aza2bicOct complex is about three times that for the $\beta$CDC–Aza2bicOct complex. This result is consistent with the finding by Kurono et al. (4) that the $K_d$ value for the complex between αCDC and 5-nitrobenzimidazole is about 1.5 times that for the complex between βCDC and 5-nitrobenzimidazole. Besides, the ratio of $k_{BS}$ to $k_d$ in catalysis by Aza2bicOct is 1.7-fold larger for βCDC than for αCDC.

In the hydrolysis of βCDC, as for the $k_{BS}$ values: Aza2bicOct $>$ BtNH$_2$ $>$ QCD $>$ Pip $>$ Et$_3$N $>$ iBt$_2$NH, whereas for $K_d$: BtNH$_2$ $>$ Et$_3$N $>$ Aza2bicOct $>$ Pip $>$ QCD. Thus, no relationship was observed between catalytic activity of the base and its complex formation constant. Besides, the catalytic activity is not linear with the pK$_a$ value of the bases. The geometry of the complex between βCDC and the amine should govern the magnitude of $k_{BS}$ in the same manner as the geometry of the complexes between cyclodextrins and phenyl esters governs their hydrolyses (1). The high catalytic activity of Aza2bicOct can be also ascribed to the presence of two basic centers, which increases the frequency factor for catalysis. This interpretation is supported by the fact that $k_{BS}$ for QCD is about 6% that for Aza2bicOct, though $K_d$ for QCD is smaller than that for Aza2bicOct. QCD has a similar structure to Aza2bicOct, except that one of two nitrogen atoms in Aza2bicOct is replaced by a carbon atom.

In the pH region investigated were the values: Aza2bicOct $>$ Et$_3$N; BtNH$_2$ $>$ QCD $>$ Pip $>$ Et$_3$N $>$ iBt$_2$NH, whereas for $K_d$: BtNH$_2$ $>$ Et$_3$N $>$ Aza2bicOct $>$ Pip $>$ QCD. The ratio of $k_{BS}$ to $k_d$ for the complexes between γCDC and 5-nitrobenzimidazole and between βCDC and 5-nitrobenzimidazole is about 1.5 times that for the complex between αCDC and 5-nitrobenzimidazole (4). Besides, the catalytic activity is not linear with the pK$_a$ value of the bases. The geometry of the complex between βCDC and the amine should govern the magnitude of $k_{BS}$ in the same manner as the geometry of the complexes between cyclodextrins and phenyl esters governs their hydrolyses (1). The high catalytic activity of Aza2bicOct can be also ascribed to the presence of two basic centers, which increases the frequency factor for catalysis. This interpretation is supported by the fact that $k_{BS}$ for QCD is about 6% that for Aza2bicOct, though $K_d$ for QCD is smaller than that for Aza2bicOct. QCD has a similar structure to Aza2bicOct, except that one of two nitrogen atoms in Aza2bicOct is replaced by a carbon atom.

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The rate constant ($k_{BS}$), based on the concentration of hydroxide ion, for the hydrolysis of the βCDC–Aza2bicOct complex in D$_2$O was found to be 1.41 ± 0.02 times that of H$_2$O at pH 11.0, 11.0, or 12.0; the ratio of the autoprotolysis constant of H$_2$O and D$_2$O was taken as 6.5 (9). This result indicates nucleophilic attack by hydroxyl anion toward βCDC, because the nucleophilicity of deuteroxide anion is 20–40% higher than hydroxide anion (10–15). Nucleophilic attack by Aza2bicOct toward βCDC is ruled out by the D$_2$O effect (16). The rather
poor leaving group, $\beta$-cyclodextrin, in $\beta$CDC, does not favor nucleophilic catalysis by Aza$_2$biOct (17).

**DISCUSSION**

The present study showed that the deacylation of acyl-cyclodextrins is much enhanced by amines such as Aza$_2$biOct, BtNH$_2$, QCD, Pip, and Et$_2$N. Small rate constants for the deacylation of acyl-cyclodextrins are one of the defects of the cyclodextrin-catalyzed hydrolysis of esters as a model of serine esterases, though cyclodextrin reactions exhibit many enzyme-like features (2). Thus, the present finding of acceleration of the deacylation step by amines provides further potentiality for cyclodextrins to be an even better enzyme model. For example, the observed rate constants for the acylation and the deacylation in the $\beta$-cyclodextrin-catalyzed hydrolysis of m-chlorophenyl benzoate are $2.2 \times 10^{-3}$ sec$^{-1}$ and $2.7 \times 10^{-4}$ sec$^{-1}$, respectively, under the conditions that $[\beta$-cyclodextrin]$_0 = 10^{-2}$ M at pH = 10.6, 25$^\circ$, and $I = 0.2$ M (3). The observed rate constant for the acylation is four times ($5.5 \times 10^{-4}$ sec$^{-1}$) that for the alkaline hydrolysis of m-chlorophenyl benzoate. However, $\beta$-cyclodextrin cannot be called a true catalyst here, because the rate constant for the deacylation of $\beta$-cyclodextrin benzoate is smaller than that for the alkaline hydrolysis of m-chlorophenyl benzoate. However, the rate constant for the deacylation of $\beta$-cyclodextrin benzoate, catalyzed by complexed Aza$_2$biOct, can be $1.8 \times 10^{-3}$ sec$^{-1}$ (which is 3.3-fold larger than that for the alkaline hydrolysis of m-chlorophenyl benzoate), when the accelerated deacylation by complexed Aza$_2$biOct is tentatively assumed to be the same (6.7 fold) as that for the hydrolysis of $\beta$CDC. Thus, $\beta$-cyclodextrin in the presence of Aza$_2$biOct can be called a true catalyst for ester hydrolysis, and it is much better enzyme model than $\beta$-cyclodextrin in the absence of Aza$_2$biOct.

The ratio of the rate constant for the complexed Aza$_2$biOct-catalyzed deacylation of $\beta$-cyclodextrin benzoate to that for the alkaline hydrolysis of m-chlorophenyl benzoate is 3.3 and is almost independent of pH because both rate constants increase proportionally with pH. In the absence of Aza$_2$biOct, however, the ratio of the rate constant for the alkaline hydrolysis of m-chlorophenyl benzoate to that for the spontaneous deacylation of $\beta$-cyclodextrin benzoate should increase with pH, and attain the value of 16 at pH 13.6.

Neither Aza$_2$biOct, Et$_2$N, nor QCD can form a stable amide from $\beta$CDC or $\alpha$CDC, since they are tertiary amines. On the other hand, some part of the observed change of absorbance at 305 nm in the presence of Pip, Ib$_2$NH, or BtNH$_2$, may be due to the formation of amides as in ammonia-catalyzed ester cleavage (17). However, lack of acceleration of the hydrolysis by Ib$_2$NH implies that the important reaction in the presence of Pip, Ib$_2$NH, or BtNH$_2$ is hydrolysis of $\beta$CDC rather than the amide formation.

The value ($1.6 \times 10^{-5}$ sec$^{-1}$ at pH 10.0, 20$^\circ$, $I = 1.0$ M) of the rate constant, $k_s$, for the spontaneous hydrolysis of $\beta$CDC in the present paper is close to that ($1.8 \times 10^{-5}$ sec$^{-1}$ at pH 10.0, 20$^\circ$, $I = 0.15$ M) obtained by Kurono et al. (4). The latter value was calculated from the value of $k_s$ ($3.47 \times 10^{-4}$ hr$^{-1}$) at pH 7.35, 25$^\circ$, using the activation energy of the reaction (31 kcal/mol). The slightly smaller value in the present paper is due to the larger ionic strength.

The pH-rate constant profile in Fig. 2 and the D$_2$O effect show that the hydrolysis of $\beta$CDC by complexed Aza$_2$biOct is associated with attack by hydroxide anion. The alkaline hydrolysis of esters proceeds via the formation of a tetrahedral addition intermediate and the rate-determining step is nucleophilic attack by the hydroxide anion (18, 19). Aza$_2$biOct, which is in the neighborhood of the attacking hydroxide anion as the result of inclusion in $\beta$CDC, can assist attack by the hydroxide anion toward $\beta$CDC. The assistance by Aza$_2$biOct probably takes place through hydrogen bonding between the hydrogen atom of the hydroxide anion and the nitrogen atom of Aza$_2$biOct. The hydrogen bonding can stabilize the transition state more than the ground state (14) and enhance the nucleophilicity of the hydroxide anion in the ground state. Both effects result in the rate enhancement. The catalysis by amines other than Aza$_2$biOct probably proceeds in the same way as that by Aza$_2$biOct.

In summary, we find that the deacylation of acyl-cyclodextrins is much enhanced by certain amines. This implies that cyclodextrins can be even better model enzymes in the presence of these amines.

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