CYCLODEXTRIN-CATALYZED HYDROLYSIS OF ESTERS BEARING A HYDROPHOBIC TAIL

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 γ -Cyclodextrin-catalyzed hydrolysis of p-nitrophenyl esters bearing a long alkyl chain proceeded with larger rate acceleration than those with a short alkyl chain. Such rate acceleration behavior was not observed with β -cyclodextrin. The results suggest that the esters with a long alkyl chain take a folded structure (Type A) in the γ -cyclodextrin cavity.

Hydrolysis of esters by cyclodextrin (CD) hydroxyls through inclusion complexes has intensively been studied as a model reaction of esterase.¹⁾ Many substrates have been checked with α -cyclodextrin (cyclohexaamylose, α -CD) and β -cyclodextrin (cycloheptaamylose, β -CD) to elucidate structural factors which determine the rate of the reaction. The cavity size of γ -cyclodextrin (cyclooctaamylose, γ -CD) is too large to bind usual substrates tightly, but we have shown that its binding and catalytic abilities can be improved by appending a spacer moiety which narrows the large γ -CD cavity.²⁾ Another approach to attain accelerated hydrolysis by γ -CD is designing good substrates. Here we report the enhanced rate acceleration observed for

p-nitrophenyl esters which bear an alkyl chain (tail) as a spacer.³⁾ To our knowledge this is the first report for the reaction in which substrates undergo a structural change to fit the cavity of the catalyst, CD.

The reaction buffer was prepared by adding five volumes of 0.02 M (lM = 1 mol

 $CH_3(CH_2)_n COO$ NO2

Substrates:

<u>1</u>, n=0

- 2, n=2
- 3, n=5
- 4, n=8

Substrate	Host	$\frac{k_{un} \times 10^3}{s^{-1}}^{b)}$	$\frac{k_{c} \times 10^{2}}{s^{-1}}$	$\frac{\kappa_{m} \times 10^{3}}{M}$	k _c /k _{un}
<u>1</u>	γ - CD	3.67 ± 0.08	6.41 ± 0.71	6.7 ± 0.2	17.4
2	γ-CD	1.87 ± 0.13	2.27 ± 0.21	4.6 ± 0.5	14.5
<u>3</u>	γ−CD	1.47 <u>+</u> 0.16	4.73 ± 0.27	6.7 ± 0.5	32.2
4	γ-CD	0.77 ± 0.07	3.54 ± 0.31	5.8 ± 0.6	45.8
<u>1</u>	β-CD	3.67 ± 0.08	9.85 ± 1.48	12.5 ± 1.0	24.5
2	β-CD	1.87 ± 0.13	2.54 ± 0.34	7.1 ± 0.9	14.9
<u>3</u>	β-CD	1.47 ± 0.16	2.08 <u>+</u> 0.23	4.1 ± 0.7	12.5
<u>4</u>	β-CD	0.77 <u>+</u> 0.07	2.19 ± 0.28	2.2 <u>+</u> 0.5	25.5

Table 1. Kinetic parameters for ester hydrolysis a)

a) In 50% dimethyl sulfoxide - 50% water (pH 12.84); temperature, 25 °C.

b) First-order rate constant for hydrolysis of the substrate in the absence of CD.c) Maximum catalytic rate constant.

d) Dissociation constant of the substrate-CD complex.

dm⁻³) carbonate buffer (pH 9.54) to five volumes of dimethyl sulfoxide, the resultant solution having pH of 12.84. Kinetic runs were initiated by injecting 10-µl acetonitrile solution of substrate (1.5 mM) into CD solutions (3 ml) which were equilibrated to 25 °C in the spectrophotometer chamber. The maximum catalytic rate constants (k_c) and dissociation constants (K_m) were obtained from Lineweaver-Burk plots (Table 1).

The k_{un} value, which is the rate constant in the absence of CD, decreases with increasing length of the alkyl chain due to the electronic properties of the substrates and the steric hindrance by the alkyl chains caused upon the attacking of OH⁻ to the carbonyl of the substrates. The rate acceleration therefore has been estimated by using k_c/k_{un} values. The data obtained with γ -CD show that the k_c/k_{un} value increases with increasing length of the tail after once diminished at 2. The attained k_c/k_{un} value of 46 for <u>4</u> is 2.6-fold larger than that for <u>1</u>. This effect of the long alkyl chain on the rate probably arises from the folded structure of <u>4</u> in the γ -CD complex (Type A). On the other hand, the K_m value is hardly influenced by the length of the tail, ranging around 6 mM. Examination



by the CPK model shows that the thickness of the folded structure of $\underline{4}$ is about 8 Å which is the same as the diameter of γ -CD cavity. The cavity of γ -CD is therefore rather narrow and leaves no vacant space in the complex. As a result, $\underline{4}$ is likely to be favorably involved in the γ -CD cavity, so as to hold its carbonyl group near the correct position to which an alkoxide anion of the secondary hydroxyl of γ -CD can easily attack. Despite these arguments, possibility of mixing of another type of complexation such as Type B still remains.⁴

β-CD has a cavity with a diameter of 6.5 Å which is too narrow to form the Type A complexes, so the structure of the β-CD complexes should be Type B. As expected from the different complexation types, we observed different hydrolysis behavior; both K_m and k_c/k_{un} values diminish with increasing length of the tail except for the rate parameter of <u>4</u>. The stronger binding of the substrates with longer tails may arise from the fact that the alkyl chains increase the hydrophobic nature of the environment around the substrate, acting like hydrophobic caps for CD cavity. The trend of decreasing in the k_c/k_{un} value might be related to the unfavorable involvement of the substrates in the β-CD cavity, which makes the ester carbonyl apart from the correct position. In the case of <u>4</u>, the long tail is hard to allow deep involvement because of the steric hindrance against the rim of β-CD. The resultant shallow binding may be the reason for the exceptional rate behavior of <u>4</u>. It should be noted that the long alkyl chain of <u>4</u> forms an effective hydrophobic cap in the complex B as shown by the strongest binding among the four substrates.

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In conclusion, the accelerated hydrolysis by γ -CD observed for the esters bearing a long alkyl chain can be explained on the basis of a folded structure of the substrates in the γ -CD complexes.

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- 3) Data for four p-nitrophenyl esters $(\underline{1} \underline{4})$ are presented here. We attempted to obtain data for esters with longer alkyl chains, but measurements were not performed because of their poor solubility in the buffer solution used in this work.
- 4) The coexistence of two complexes similar to the types A and B was proposed by Turro et al. in explaining photoluminescence data of a detergent in the presence of γ-CD N. J. Turro, T. Okubo, and C. -J. Chung, J. Am. Chem. Soc., <u>104</u>, 1789 (1982).

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