© The Chemical Society of Japan   1985

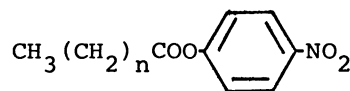# CYCLODEXTRIN-CATALYZED HYDROLYSIS OF ESTERS BEARING
A HYDROPHOBIC TAIL

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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 (cycloocta-
amylose, 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.

$$CH_3(CH_2)_n COO\!\!-\!\!\langle\!\!\bigcirc\!\!\rangle\!\!-\!\!NO_2$$

Substrates:
1, n=0
2, n=2
3, n=5
4, n=8

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

Table 1. Kinetic parameters for ester hydrolysis [a]

Substrate	Host	$\dfrac{k_{un} \times 10^3}{s^{-1}}$ [b]	$\dfrac{k_c \times 10^2}{s^{-1}}$ [c]	$\dfrac{K_m \times 10^3}{M}$ [d]	k_c/k_{un}
1	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
3	γ-CD	1.47 ± 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
1	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
3	β-CD	1.47 ± 0.16	2.08 ± 0.23	4.1 ± 0.7	12.5
4	β-CD	0.77 ± 0.07	2.19 ± 0.28	2.2 ± 0.5	25.5

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 4 is 2.6-fold larger than that for 1. This effect of the long alkyl chain on the rate probably arises from the folded structure of 4 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

Type A   Type B
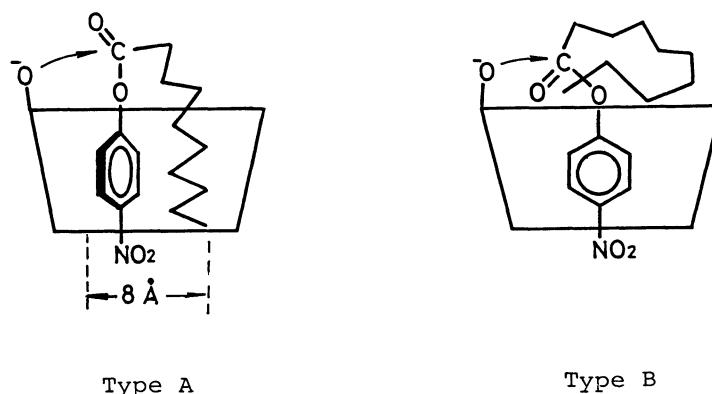
by the CPK model shows that the thickness of the folded structure of $\underline{4}$ is about

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 $\underline{4}$. 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 $\underline{4}$, 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 $\underline{4}$. It should be noted that the long alkyl chain of $\underline{4}$ forms an

effective hydrophobic cap in the complex B as shown by the strongest binding among

the four substrates.

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.

References

1) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag, New York (1977).

2) A. Ueno, Y. Tomita, and T. Osa, J. Chem. Soc., Chem. Commun., 1983, 976; Chem. Lett., 1983, 1635; Tetrahedron Lett., 1983, 5245; A. Ueno, F. Moriwaki, Y. Hino, and T. Osa, J. Chem. Soc., Perkin Trans. 2, in press.

3) Data for four p-nitrophenyl esters (1 - 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., 104, 1789 (1982).