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CATALYTIC ACTIVITY OF β-CYCLODEXTRIN-HISTAMINE

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ABSTRACT. We modified cyclodextrin (CD) by a histamine group to make a model of α-chymotrypsin. Enzymatic turnover reaction was realized with CD-histamine at around neutral pH value. Compared with amino-CD, it is ascertained that this catalytic activity of CD-histamine is caused by an imidazole group. Using several substrates in the hydrolytic reactions, it shows that CD-histamine has a structural selectivity for substrates which are structurally different to each other.

1. INTRODUCTION

CD has a hydrophobic cavity which acts like a binding site of an actual enzyme. To attach a reactive functional group, we modified β-CD by a histamine group and we realized enzymatic turnover reaction in the hydrolysis of p-nitrophenyl acetate with β-CD-histamine at around neutral pH value. Catalytic rate constant of this reaction was close to an actual enzyme, α-chymotrypsin [1].

We thought that this catalytic activity was caused by an imidazole group. But β-CD-histamine has also an amino group which has a possibility to act as an active site.

This paper reports that we synthesized amino-β-CD to compare catalytic activity with β-CD-histamine and it was clear that an imidazole group acted as an active site in the hydrolysis of p-nitrophenyl acetate. And it also shows that in the hydrolytic reactions, β-CD-histamine has a structural selectivity for substrates which are structurally different to each other.

2. MATERIALS AND METHODS

C-6 mono-tosylated-β-CD and β-CD-histamine used in this work were prepared as reported previously [1]. C-6 mono-amino-β-CD was prepared as follows. A solution of C-6 mono-tosylated-β-CD (10g) and sodium azide (1g) in DMF was treated for 70 min. at 80°C. The reaction mixture was evaporated to dryness. The dried material (crude C-6 mono-azide-β-CD)

was dissolved in water and reduced by sodium borohydride (0.5g). After the reaction, the reaction mixture was evaporated to dryness to give crude mono-amino-β-CD. Mono-amino-β-CD was purified by cation exchange chromatography with a CM-Sephadex C-25 column. The purity of the product was confirmed by TLC (Rf:0.11, solvent:butanone-1M acetic acid-methanol, 2:5:3, detecting reagent:ninhydrin). Yield; 8%. Anal.Calc.for $C_{42}H_{71}O_{34}N_1 \cdot 2H_2O$: C,43.1; H,6.5; N,1.2. Found: C,42.9; H,6.3; N,1.3.

All reagents for synthesis and hydrolytic reactions were purchased from commercial suppliers and were used without further purification.

The hydrolytic reactions were followed by the appearance of p-nitrophenol spectrometrically at 400nm using a HITACHI model 220A. The reaction medium was pH 7.2 phosphate buffer and kept at 25°C.

3. RESULTS AND DISCUSSION

CD-histamine has an imidazole group and a secondary amino group (Figure 1). It is thought that the active site in the hydrolysis of p-nitrophenyl acetate is an imidazole group which has a high activity at around neutral pH value. But a secondary amino group has a possibility to act as a catalytic site.

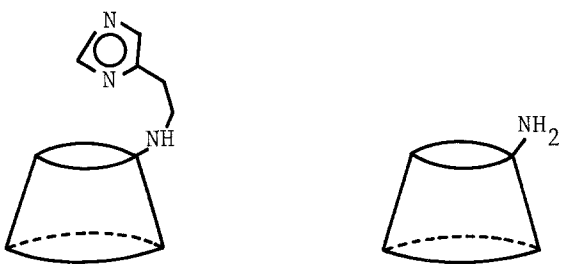
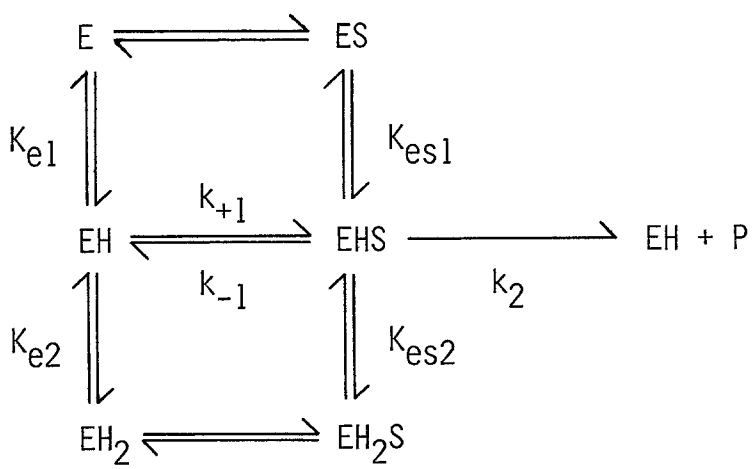


Figure 1. β-CD-histamine amino-β-CD

[Scheme 1]



Scheme 1 shows a reaction scheme in the hydrolysis of p-nitrophenyl acetate by β-CD-histamine considering that an imidazole group acts as the active site. Ke1 and Ke2 are disociation constants for hydrogen ion of an imidazole group of β-CD-histamine. Kes1 and Kes2 are dissociation constants for hydrogen ion of complex of β-CD-histamine and substrate (p-nitrophenyl acetate).

The rate equation of this reaction scheme is shown in scheme 2. Km is the apparent Michaelis constant including k1 and k2. This scheme shows that the reaction rate vary with pH and that we can obtain dissociation constants (pKe2,pKes2) by measurement of the rate constants.[2]

[Scheme 2]

$$V = \frac{k_2[E]_0[S]}{K_m(1+[H]/K_{e1}+K_{e2}/[H])+[S](1+[H]/K_{es1}+K_{es2}/[H])}$$

Figure 2 shows the pH dependence of kcat / Km in the hydrolysis of p-nitrophenyl acetate with β-CD-histamine. In the previous report [1], pH dependence of kcat was already described. Both curved lines are in good agreement with the rate equation. From these two graphs pKe2 and pKes2 are obtained and shown in table 1.

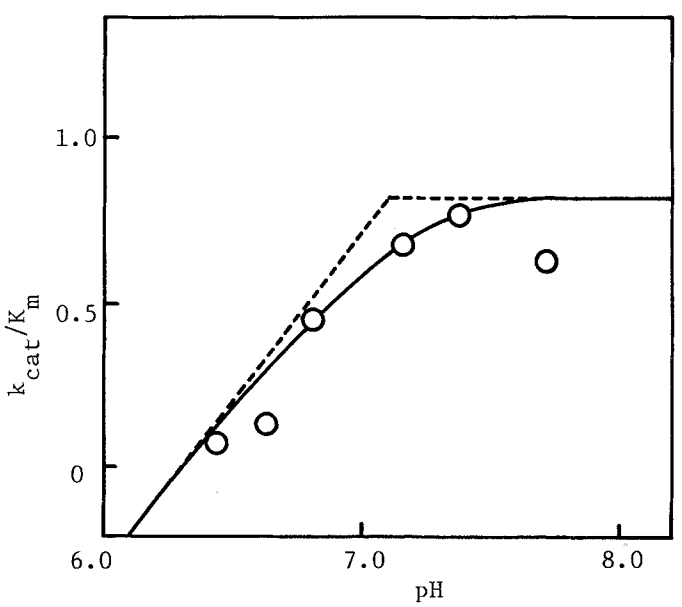


Figure 2. pH dependence of k_{cat}/K_m

Table 1 shows that pKe2, pKes2 and pKa (obtained by titration with dilute hydrochloric acid) are all at around 7. These values correspond to the dissociation constant of an imidazole group. The reason why pKa is lower than pKe2 or pKes2 is thought as follows. By forming a complex with p-nitrophenyl acetate and β-CD-histamine, intramolecular interaction with an imidazole group and CD is weakened. And then pK becomes a little bit large.

Table 1. The negative logarithms of the dissociation constant

β-CD-hiatamine	pK_{e2}	7.1
	pK_{es2}	7.2
	pK_a	6.8
Histamine	pK_a	6.14*

* Lange's handbook of chemistry

Catalytic activity of amino-β-CD was obtained by hydrolysis of p-nitrophenyl acetate in the same manner as β-CD-histamine. Figure 3 shows a Linewewver-Burk plot of this result. The data give a straight line, so this reaction is also a type of Michaelis-Menten mechanism.

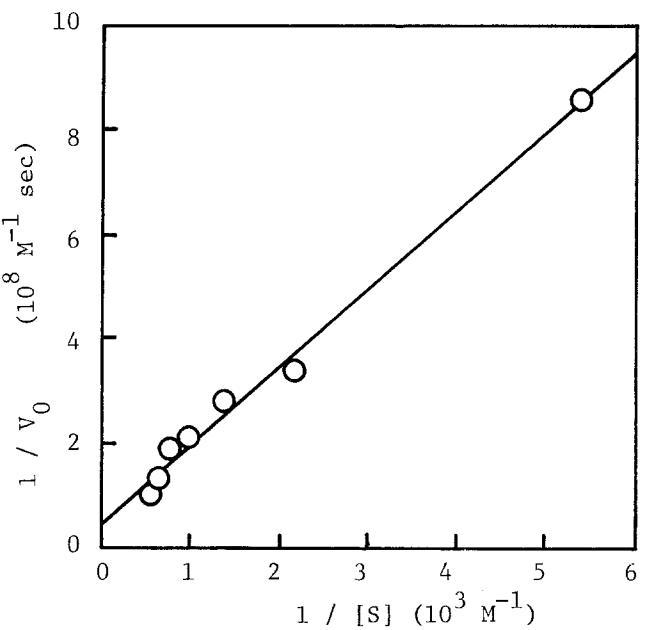


Figure 3. Lineweaver-Burk plot of hydrolysis of p-nitrophenyl acetate with amino-β-CD

The pKa value of amino-β-CD obtained by titration with dilute hydrochloric acid and back titration with dilute sodium hydroxide solution was 6.3. It is thought that this lower pKa value for the amino compound is due to the effect of the hydrophobic cavity and hydroxyl groups of β-CD. Table 2 shows catalytic rate constants in the hydrolysis of p-nitrophenyl acetate. Compared with β-CD-histamine, the value of kcat/Km of amino-β-CD is about 1/20. The catalytic activity of amino-β-CD is too low to explain that the catalytic activity of β-CD-histamine depends on its amino group.

These results suggest that it is an imidazole group, not an amino group, acts as the active site of β-CD-histamine in the hydrolysis of p-nitrophenyl acetate.

Table 2. Catalytic rate constants in the hydrolysis of p-nitrophenyl acetate.

Catalyst	$k_{cat}(10^{-4}\ sec^{-1})$	$K_m(10^{-3}\ M)$	$k_{cat}/K_m(M^{-1}\ sec^{-1})$
amino-β-CD	1.29	3.60	0.04
β-CD	≈ 0	-	-
β-CD-histamine	21.9	2.84	0.77

To confirm a selectivity of β-CD-histamine for substrates, experiments of the hydrolysis with β-CD-histamine were made using several kinds of substrates as follows; N-tert-butoxycarbonyl-L-glutamine-p-nitrophenyl ester (Boc-Gln-ONP), Boc-L-asparagine-p-nitrophenyl ester (Boc-Asn-ONP), Boc-Glycine-p-nitrophenyl ester (Boc-Gly-ONP), Boc-L-Leucine-p-nitrophenyl ester (Boc-Leu-ONP). In these cases, all hydrolytic reactions with β-CD-histamine were detected as a reaction type of Michaelis-Menten mechanism. Catalytic rate constants in the hydrolysis with β-CD-histamine are shown in Table 3. Compared with Boc-Gln-ONP and Boc-Asn-ONP, the structural difference is only one carbon in the side chain of amino acid. But reactivity (kcat/Km) is different about three times. In these four substrates, in the case of Boc-Asn-ONP, the re-

Table 3. Catalytic rate constants in the hydrolysis with β-CD-histamine

Substrates	$k_{cat}(10^{-2}\ sec^{-1})$	$K_m(10^{-3}\ M)$	$k_{cat}/K_m(M^{-1}\ sec^{-1})$
Boc-Gln-ONP	0.323	0.47	6.96
Boc-Asn-ONP	1.607	0.82	19.54
Boc-Gly-ONP	0.227	0.24	8.94
Boc-Leu-ONP	0.037	1.14	0.33

activity is the highest and in the case of Boc-Lue-ONP, it is the lowest. It is thought that these differences of activities depend on the fittness to form a complex and also the structural configuration between the substrate and the active site of β-CD-histamine when the complex is formed. At present, only the data by spectroscopic analysis are taken, but more information about the structural selectivity of β-CD-histamine for substrates will be available from X-ray analysis and NMR analysis. Such experiments are now in progress. These studies will be applied to design new artificial enzymes.

4. ACKNOWLEDGMENT

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