Bioorganic & Medicinal Chemistry Letters, Vol.2, No.12, pp. 1581-1584, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

SUBSTRATE SELECTIVITY OF IMIDAZOLE-APPENDED DIMETHYL-β-CYCLODEXTRIN

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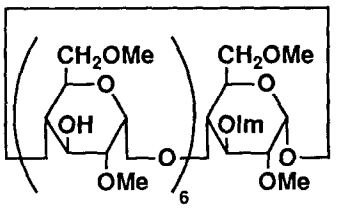
(*Received* 2 *April* 1992)

Abstract: The substrate selectivity of the hydrolysis reaction by imidazole-appended dimethyl-β-cyclodextrin (**2**) was studied using different kinds of *p*-nitrophenyl esters of amino acids. The tendency for the hydrolysis reaction of the amino acid ester by **2** reflected a difference in K_m rather than in k_{cat}. **2** had the ability to undergo a stereo-selective hydrolysis reaction.

Cyclodextrins (CDs) can form inclusion complexes with a number of molecules. For this reason, biomimetic reactions using cyclodextrins and their derivatives have been actively studied.[1] We have also prepared successful artificial enzymes by modification of cyclodextrins.[5-6] On the other hand, dimethylcyclodextrins (DMCDs) are a series of cyclic oligomers consisting of α-1,4-linked 2,6-di-O-methyl D-glucopyranose units, and have quite unique and different properties from cyclodextrins.[2-3] In a previous paper, we investigated the first successful method for modification of β-DMCD (**1**) and preparation of an artificial hydrolase, 3-[2'-(4''-imidazolyl)ethyl]dimethyl-β-cyclodextrin (**2**).[7-9] The ability of **2** to undergo a hydrolysis reaction is quite high. The k_{cat} of **2** for the hydrolysis of *p*-nitrophenyl acetate (PNPA) is 1.44 x 10^{-2} s^{-1} at pH 8. This is over twice that of the natural enzyme, α-chymotrypsin.[7] Figure 1 shows a typical time-course for the hydrolysis of PNPA under different conditions.[7] Only 1 mol% of **2** caused a 5-fold increase in the rate of hydrolysis of PNPA (10^{-3} M), compared with the condition without **2**, whereas a 5 mol equivalent of β-DMCD (**1**) caused a 60% depression in the reaction. The reaction was scarcely accelerated by imidazole with the same concentration as **2**. The binding site (β-DMCD) or active site (imidazole) alone was not effective for the acceleration of the hydrolysis reaction. The combined action of the binding site and active site of **2** caused a large acceleration of the reaction.



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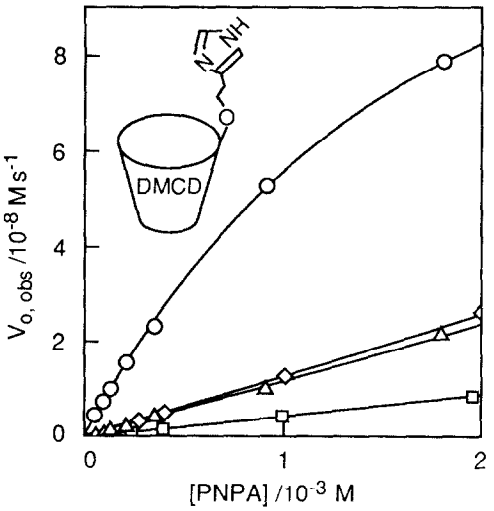

Fig. 1 Hydrolysis of p-nitrophenyl acetate in pH 7.2 phosphate buffer at 25°C;
○ in the presence of **2** (1.10×10^{-5} M),
◇ in the presence of imidazole (1.10×10^{-5} M),
□ in the presence of β-DMCD (9.67×10^{-3} M),
△ in only buffer solution.

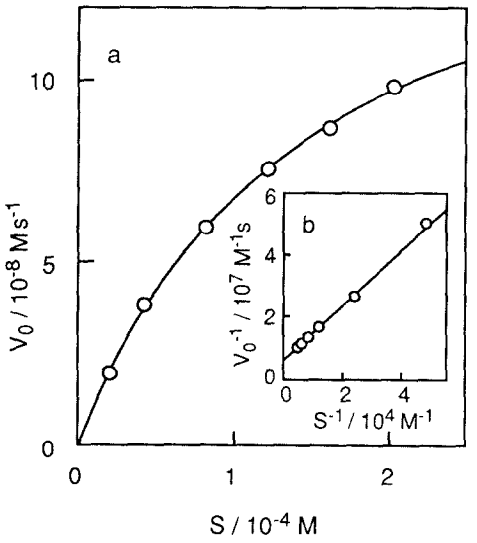

Fig. 2 Hydrolysis of *N*-Ac-L-Phe-ONp by **2** in pH 7.2 phosphate buffer at 25 °C, [**2**] = 1.53×10^{-5} M;
(a) V_0 vs [S], (b) Lineweaver-Burk plot.

This artificial enzyme had *p*-selectivity for the hydrolysis of nitrophenyl acetate isomers.[8] The detailed substrate selectivity of this compound is now of interest. In this paper, we describe the substrate selectivity of this artificial enzyme for the hydrolysis reaction of amino acid esters.

3-[2'-(4''-imidazolyl)ethyl]dimethyl-β-cyclodextrin (**2**) was prepared from β-DMCD[4] by a method previously reported.[7] *p*-Nitrophenyl esters of the amino acids were prepared by a common method.[10] The rate of the hydrolysis reaction of the *p*-nitrophenyl ester of the amino acid was measured under the conditions of a large excess of substrate at 25 °C in a pH 7.2 phosphate buffer solution. The hydrolysis reaction was followed by monitoring the appearance of *p*-nitrophenolate at 400 nm using a HITACHI 220A spectrophotometer. The reaction was conducted in a quartz cell in the water-jacketed cell holder of 220A. Temperature of the reaction mixture was maintained at 25 °C using a HAAKE F3 circulating water bath. The reaction was initiated by adding a stock solution of ester in acetonitrile to a buffer solution in the quartz cell. The rates used in the calculation of the kinetic parameters were averages of at least three determinations which agreed within 3 %.

Substrate			k_{cat}	K_m	k_{cat} / K_m	k_{un}	k_{cat} / k_{un}
	R_1	R_2	10^{-2} s^{-1}	10^{-4} M	M^{-1} s^{-1}	10^{-4} s^{-1}	-
a	Ac	Ala	3.71 ± 0.10	13.5 ± 0.5	27.5	14.4 ± 0.3	26
b	*t*-Boc	Ala	3.25 ± 0.13	3.34 ± 0.24	97.2	0.724 ± 0.032	449
c	Ac	Gly	5.19 ± 0.54	14.7 ± 0.5	35.4	5.62 ± 0.12	92
d	*t*-Boc	Gly	3.12 ± 0.10	3.87 ± 0.15	80.5	1.29 ± 0.01	242
e	*t*-Boc	Leu	1.88 ± 0.05	2.19 ± 0.13	85.8	0.303 ± 0.001	621
f	Ac	L-Phe	1.10 ± 0.01	1.48 ± 0.02	73.9	8.61 ± 0.23	13
g	Ac	D-Phe	2.38 ± 0.01	4.78 ± 0.05	49.7	8.61 ± 0.26	28

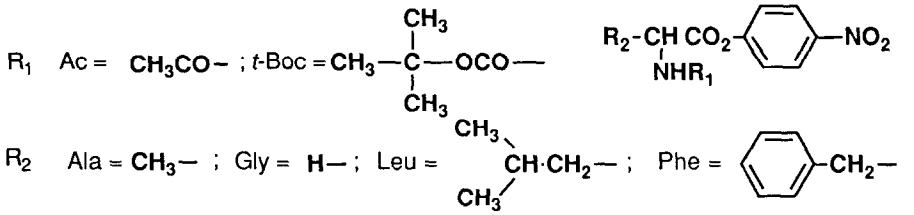

Table 1. Kinetic parameters for the hydrolysis reactions of p-nitrophenyl esters of amino acids at 25 °C in a phosphate buffer by **2**.

By plotting kinetic data in the form of $1 / (V_{0,obs} - V_{un})$ vs. $1 / [ester]$ (Lineweaver-Burk plot), a straight line was obtained (Fig. 2). This suggests that the reaction by **2** proceeds by the Michaelis-Menten mechanism in a manner similar for a nitrophenyl acetate.[7] The kinetic parameters, k_{cat} and K_m, were calculated by the nonlinear-least-squares fitting of kinetic data to the Michaelis-Menten equation (Table 1).

The selectivity of the hydrolysis reaction by **2** was found in the difference of K_m rather than k_{cat}. The value of K_m depends on the liability to be bound into the DMCD's cavity or depends on the number of bound sites of the substrate. K_m for the substrate, which has multiple bound sites, is smaller than that for the substrate which has only one bound site, i.e., the *p*-nitrophenyl group of *N*-Ac-Ala-ONp (**a**) and *N*-Ac-Gly-ONp (**c**). The protecting group, *t*-Boc, and a side chain could be also bound by **2**. Therefore, K_m for *N*-Ac-Ala-ONp (**a**) and *N*-Ac-Gly-ONp (**c**) are significantly larger than that for other esters. The value of k_{cat} depends on the reactivity of the substrate with the artificial enzyme after making an inclusion complex or depends on the structure of the

inclusion complex. In the case of the substrates used in this study, the difference in k_{cat} is not large. This means that the difference in the reactivity of substrates in the cavity of **2** is not large. In only buffer solution, the rates for the hydrolysis reaction (k_{un}) of **b** and **d** were smaller than those of **a** and **c**. Although *t*-Boc is bulky and has a blocking effect for the attack of water or hydroxyl ion to a carbonyl group of the ester in aqueous solution and *t*-Boc is usually used as a protective group, *t*-Boc is ineffective for the protection of the ester group in the reaction with **2** . This suggests a difference between the general catalytic reaction and host-guest reaction, whereas it is not clear why k_{cat} is almost the same.

Optical isomers, *N*-Ac-L-Phe-ONp (**f**) and *N*-Ac-D-Phe-ONp (**g**), were used as a substrate. k_{cat} of **g** is twice as large as that of **f**, but K_m of **g** is three times larger than that of **f**. Therefore, k_{cat} / K_m for **f** is 1.5 times larger than that for **g**.

The reactivities of **2** with some kinds of *p*-nitrophenyl esters of amino acids were studied. **2** had the ability of substrate selectivity and this selectivity was caused by the difference in K_m rather than k_{cat}. **2** has the ability to undergo a stereo-selective hydrolysis reaction but this ability is not high. This may be due to the relatively symmetrical structure of **2**.

Acknowledgment: This work was supported by a Grant for International Joint Research Project from the NEDO Japan.

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