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Synthesis and Reactivity of Imidazole-Appended Dimethyl-β-cyclodextrin

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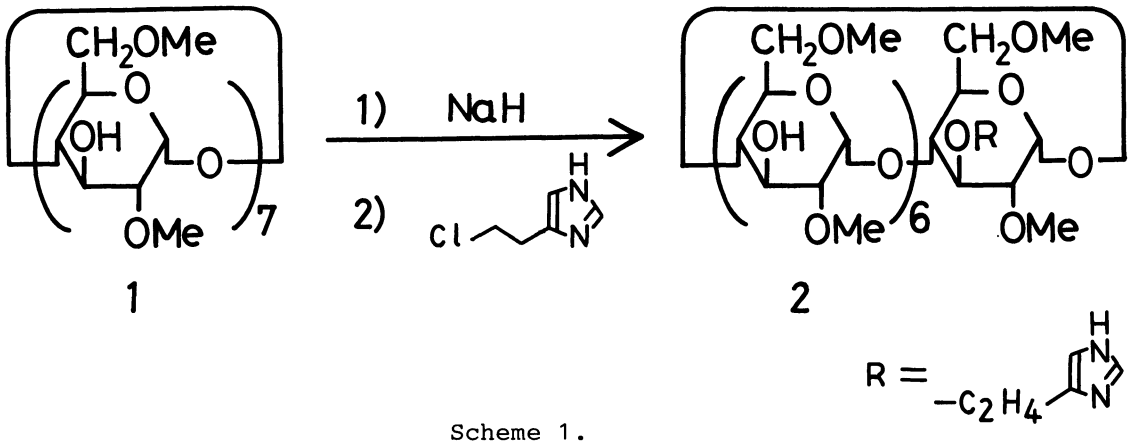
Dimethyl-β-cyclodextrin bearing imidazolylethyl group (**2**) was firstly synthesized and the catalytic activity of **2** was studied. **2** caused extensive acceleration of the hydrolysis reaction of p-nitrophenyl acetate, whereas dimethyl-β-cyclodextrin caused inhibition of the hydrolysis.

Cyclodextrins 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 shown that cyclodextrins bearing histamine or 1,4-dihydronicotinamide are effective artificial enzymes.[2,3] 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 different properties from cyclodextrins.[4] DMCDs are highly soluble in cold water and many organic solvents, whereas CDs are poorly soluble in water and insoluble in most organic solvents.[5] Their behaviors of inclusion are different. But DMCDs have never been used as enzyme models. In this communication, We firstly wish to report the preparation and characterization of β-DMCD bearing imidazolylethyl group (**2**) as an α-chymotrypsin model.

Starting material, β-DMCD (**1**) was purified by recrystallization from water and $CHCl_3$/hexane, and its purity was checked by HPLC [6] and NMR before use. β-DMCD (**1**) was treated with 3.5 equiv. of NaH at 0 - 40 °C for 4 h and 1.5 equiv.



Scheme 1.

of imidazolylethyl chloride at room temperature for 10 h in THF under argon (Scheme 1). By chromatography on silica gel ($CHCl_3/CH_3OH$), **2** was isolated as a colorless solid in 15% yield. Elemental analysis data and estimation by peak area of the ^{1}H-NMR spectrum confirmed that **2** had only one imidazolylethyl group.[7]

The rate of hydrolysis of p-nitrophenyl acetate (PNPA) was measured at 25 °C in pH 7.2 phosphate buffer in the presence of **2**, **1** or imidazole, and in the absence of them. Only 1 mol% of **2** caused 5-fold increase in the rate of hydrolysis of PNPA (10^{-3} mol dm^{-3}), comparing with the condition of absence of **2**, whereas 5 equiv. of β-DMCD (**1**) caused 60% repression. Imidazole in the same concentraion as **2** scarcely accelerated the reaction (Fig. 1). The combined action of binding site and active site of **2** made high catalytic effect for

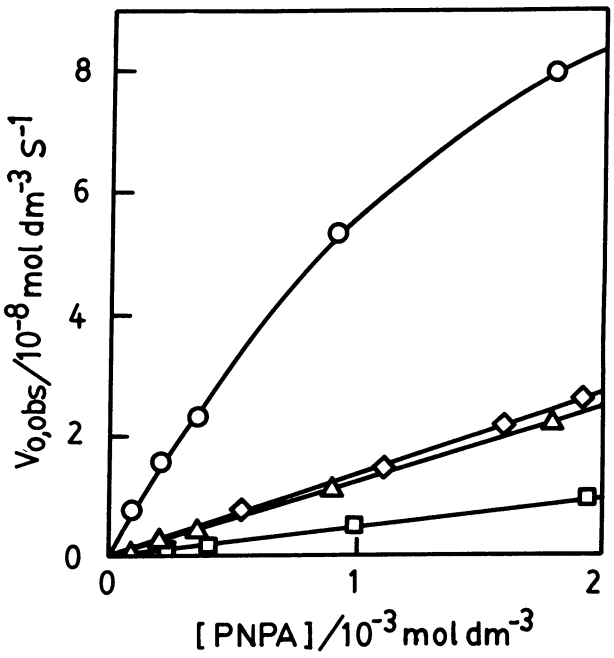


Fig. 1. Hydrolysis of p-nitrophenyl acetate in pH 7.2 phosphate buffer at 25 °C monitored by the released p-nitrophenolate ion at 400 nm,

- ○ in the presence of **2** (1.10×10^{-5} mol dm^{-3})
- ◇ in the presence of imidazole (1.10×10^{-5} mol dm^{-3})
- □ in the presence of **1** (9.67×10^{-3} mol dm^{-3})
- △ in the absence of them.

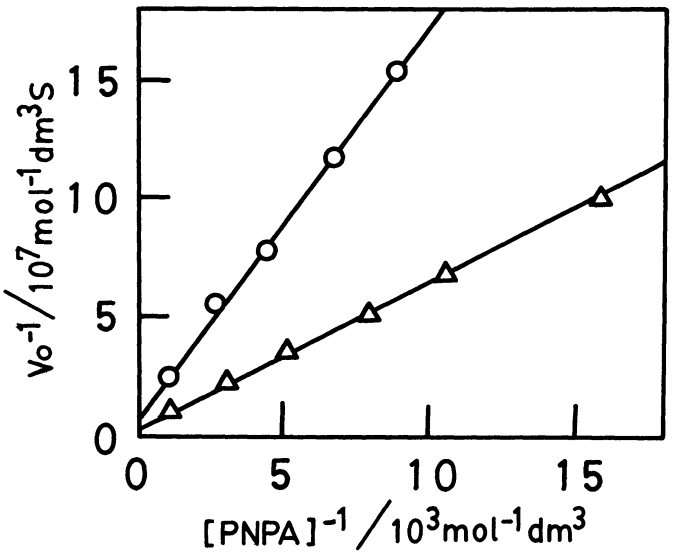


Fig. 2. Lineweaver-Burk plot for hydrolysis of p-nitrophenyl acetate with **2**,
○ at pH 7.2
△ at pH 8.2.

the hydrolysis. By the plotting kinetic data in the form of $1/(V_{obs} - V_{un})$ vs. 1/[PNPA] (Lineweaver-Burk plot), a straight line was obtained (Fig. 2). It suggests that this reaction proceeds by a Michaelis-Menten mechanism as same as cyclodextrins and their derivatives. From this plot, k_{cat} and K_m were obtained (Table 1). At pH 7.2, k_{cat} and k_{cat}/K_m of **2** are nine times larger than those of β-CD bearing a histaminyl group at the C-6 of the glucose unit (β-CD-His).[2] And also, around optimum pH of α-chymotrypsin or pH 8, the rate of the reaction was measured. k_{cat} of **2** is over twice as much as that of α-chymotrypsin.

The first successful method for modification of β-DMCD was demonstrated as an α-chymotrypsin model and the new model compound (**2**) extensively enhanced the rate of hydrolysis reaction. Further work will be needed to make clear the nature of **2**. However these results indicate that **2** is an excellent artificial enzyme and modification of β-DMCD is effective for making artificial enzymes.

Table 1. Kinetic parameters for hydrolysis of p-nitrophenyl acetate

	pH	k_{cat} / 10^{-2} s^{-1}	K_m / 10^{-3} mol dm^{-3}	k_{cat}/K_m / s^{-1} mol^{-1} dm^3
2	7.2	1.44	2.60	5.54
	8.2	2.67	2.90	9.21
β-CD-His	7.2	0.16	2.63	0.63
α-chymotrypsin[a)]	8.0	1.1	0.04	275

a) Ref. 8.

References

1) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag (1978).

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3) C-J. Yoon, H. Ikeda, R. Kojin, T. Ikeda, and F. Toda, J. Chem. Soc., Chem. Commun., 1986, 1080.

4) J. Szejtli, J. Inclusion Phenom., 1, 135 (1983).

5) Solubility in 100 ml water at 20 °C: β-DMCD 55 g, β-CD 1.8 g.

6) K. Koizumi, Y. Kubota, T. Utamura, and S. Horiyama, J. Chromatogr., 368, 329 (1986).

7) Found: C, 48.58; H, 7.00; N, 1.75%,
Calcd for $C_{61}H_{104}O_{35}N_2CHCl_3$: C, 48.20; H, 6.85; N, 1.80%;
^{1}H NMR (C_6D_6) δ=7.69 (1H, s, imidazole), 7.08 (1H, s, imidazole), 6.59 (1H, s, $CHCl_3$), 5.41 (6H, bs, C_1-H), 5.26 (1H, bs, $C_{1'}$-H), 4.99 (6H, bs, C_3-OH), 4.43 (6H, bs, C_3-H), 4.32 (1H, bs, $C_{3'}$-H);
$CHCl_3$ might be derived from the solvent of chromatography.

8) V. T. D'Souza, K. Hanabusa, T. O'Leary, R. C. Gadwood, and M. L. Bender, Biochem. Biophys. Res. Commun., 129, 727 (1985).

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