Change of Selectivity in Modified Cyclodextrin Catalyst

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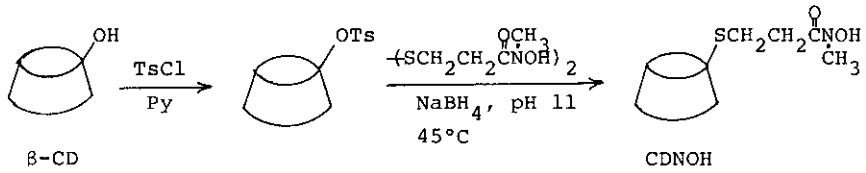
Abstract

β-Cyclodextrin having a hydroxamate functional group was newly prepared and its catalytic activity for hydrolysis of a series of mono- or dinitrophenyl acetates was estimated. Relative enhancement of catalytic hydrolysis, k_{cat}(CD-hydroxamate)/k_{cat}(CD), was very sensitive to the substrate structure, e.g., lacation of substituent nitro group(s), being 230, for p-nitro; 130, 2,3-dinitro; 66, 3,4-dinitro; and 36 for 2,5-dinitro. The reaction mechanism was discussed.

In these two decades, cyclodextrins and their derivaties have been attracting strong attentions of chemists and the basic chemistry of cyclodextrins has been much clarified.[1] Thus, unmodified α-and β-cyclodextrins are well known to catalyze the hydrolysis of phenyl acetates, for example, but this catalysis is only effective for the meta substituted substrates and for the para substituted substrates, almost no catalysis is observed.[2] This is the first example where an artificial catalyst "recognizes" the shape of substrates. The recognition of substrate shape seems to be dependent on the shape of the catalyst. This assumption was supported by the observation that cyclodextrins modified by an appropriate catalytic functional group showed the para-selectivity in a marked contrast to the unmodified cyclodextrin.[1,3] In these studies however, the origin of the rather unusual selectivity was not clear, although qualitative explanation was given. In this article, we wish to report the detailed analysis of catalyzed hydrolysis of dinitrophenyl acetates by β-cyclodexrin bearing hydroxamic acid group on the C6 position, where the substrate shape specificity is very unique.

The enzyme model, hydroxamic acid of β-cyclodextrin, (CD-NOH) was prepared by the reaction of β-cyclodextrin tosylated at 6-position (0.8 mmole) with *disulfide of β-mercapto-propanoyl-N-methylhydroxamic acid (4 mmole)* in the presence of sodium borohydride (8 mmole) at pH 11 for 3 hr. (scheme 1). After the purification by microcrystalline cellulose column chromatography (n-butanol/ethanol/H_2O=4/3/3), the product was obtained in 30% yield (Rf.0.4 on silicagel TLC, IR 1650cm^{-1}($\nu_{C=O}$)).[4]



Scheme 1

The rates of acyl transfer reaction of 3,4- 2,3- and 2,5-dinitro-phenyl acetate were measured by following dinitrophenolate ions formed in aqueous borate with the electronic spectra. In Table 1 are listed the results at pH 8.1 together with that of parent β-cyclodextrin. The observed pH-rate profile shown in Fig.1 indicates that the catlytically active species has pK_a of 9.2, strongly suggesting that the hydroxmate, $CD\text{-}NO^-$, is the active species (pK_a of unsubstituted MeCONHOH is 9.40).

Based on this assumption, catalytic rate constants for each substrates ($k_{cat}^{CDNO^-}$) were estimated and listed in Table 1.

Observed PH-rate profiles are in very good agreement with theoretical one (curved lines in Fig. 1).

Table 1. Hydrolysis of Substituted Phenyl Acetates Catalyzed by CDNOH and β-Cyclodextrin.[a)]

Substrate	k_o (sec^{-1})	k_{cat}^{app} ($sec^{-1}M^{-1}$)[b)]		Relative k_{cat}^{app} (corrected)[c)]		$\frac{k_{cat}^{app}(CDNOH)}{k_{cat}^{app}(\beta\text{-CD})}$	$k_{cat}^{CDNO^-}$ ($sec^{-1}M^{-1}$)
		β-CD	CDNOH	β-CD	CDNOH		
(3,4-DNPA)	3.6×10^{-4}	0.74	50	2.3	150	66	690
(2,3-DNPA)	5.4×10^{-4}	0.25	32	0.50	64	130	600
(2,5-DNPA)	2.0×10^{-4}	0.37	14	2.1	76	36	280
(PNPA)	2.4×10^{-5}	0.022	5.0	1.0	230	230	68

a) In aqueous borate buffer at pH 8.1, [Cat]=4×10^{-4}M, [Substrate]=2×10^{-5}M

b) $k_{cat}^{app}=(k_{obs}-k_o)/[Cat]$ [5)]

c) Relative $k_{cat}^{app}=k_{cat}^{app}(i)k_o(PNPA)/k_{cat}^{app(PNPA)}k_o(i)$

i,j-DNPA: i,j-dinitrophenyl acetate,

PNPA: p-nitrophenyl acetate

The present catalyst (CDNOH) are 36-230 times more effective than unsubstituted β-cyclodextrin. Interesting to note is the substrate shape selectivity of CDNOH, being quite different from that of β-cyclodexdrin, i.e., CDNOH catalyzes the hydrolysis of 3,4-DNPA, while β-cyclodextrin prefers 3,4- and 2,5-DNPA to 2,3-DNPA and PNPA (see relative k_{cat}^{app} in Table 1).

Although the structures of the substrates are very much similar to each other, a relatively sharp selectivity was observed. For the best substrate,

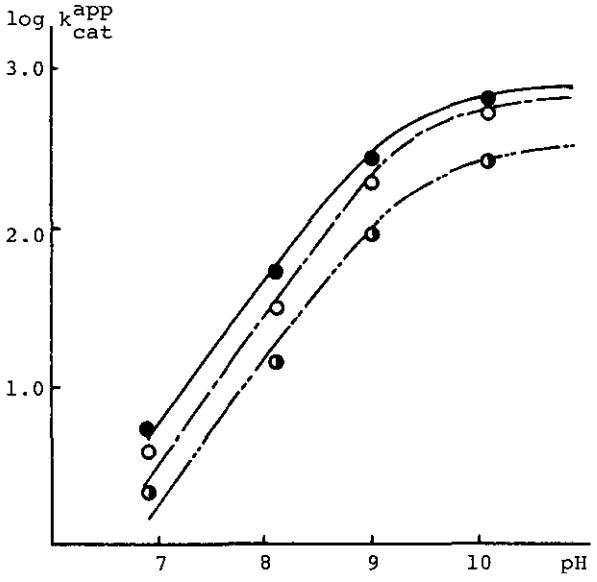


Fig.1 pH-rate profile for CDNOH
(●)3,4-DNPA, (o)2,3-DNPA, (◑)2,5-DNPA
Curved lines are drawn based on the calculation with
$k_{cat}^{app}=k_{cat}^{CDNO^-}K_a/(K_a+[H^+])$

PNPA, spatial arrangement of the ester group seems to be most appropriate for the attack by hydroxamate group and for the worst substrate, 2,5-DNPA, the spatial arrangement seems to be least appropriate since the ester group tends to point in the opposite direction to the hydroxamate (Figure 2). For the other two substrates, the spatial arrangement seems to be somewhere between. It is noteworthy that even the structures of substrates are very much similar, the present enzyme model can recognize the slight change by very simple intermolecular interaction. This interaction can be understood and estimated a

	3,4-DNPA	2,5-DNPA	2,3-DNPA	PNPA
$\frac{k_{cat}^{app}(CDNOH)}{k_{cat}^{app}(\beta\text{-CD})}$	66	36	130	230

Fig.2 Schematic Representation of Binding Modes
(●:-OAc, o:-NO_2)

priori by computation by taking every elemental interaction such as van der Waals, destruction of water structure, change in freedom of motion, formation and/or breakdown of hydrogen bonding, induced or relaxed strain etc.[6] We can be optimistic, in this sense, to expect to be able to "design" the most appropriate molecular shape of substrates toward a given enzyme (model) or the most appropriate molecular shape of enzyme models toward a given substrate.

Reference.

1) See, for example, Bender, M.L., Komiyama, M. "Cyclodextrin Chemistry" Springer Verlag, West Berlin, 1978. For more recent examples, a) Tabushi, I., Kuroda, Y., and Shimokawa, K.J. Amer. Chem. Soc., 1979, 101, 1153, b) Tabushi, I., Kuroda, Y., and Mochizuki, A. ibid., 1980, 102, 1153, c) Breslow, R., Bovy, P., and Hersh, C.L. ibid., 1980, 102, 2115.
2) Van Etten, R.L., Sebastian, J.F., Clowes, G.A., and Bender, M.L. ibid., 1967, 89, 3242.
3) Gruhn, W.B., Bender, M.L., Bioorg. Chem., 4, 219 (1975).
4) Bender, M.L., et al., reported β-cyclodextrin modified by N-methylaceto-hydroxamic acid on C2 position. Bruhn, W.B. and Bender, M.L. Bioorganic Chem., 1974, 3, 324.
5) As the preliminary results, binding constants of β-cyclodextrin for substrates were obtained by Lineweaver-Burk plot at pH 10.5, i.e., K_{bind} for 3,4-DNPA, 91 M^{-1}; for 2,3-DMPA, 53 M^{-1}; for 2,5-DMPA, 77 M^{-1} for PNPA, 160 M^{-1}.
6) Tabushi, I., Kiyosuke, Y., and Yamamura, K.J. Amer. Chem. Soc., 1978, 100, 916.

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