CHEMISTRY LETTERS, pp. 1089-1092, 1994.

Coupled Cyclodextrin Appending Imidazole as an Enzyme Model

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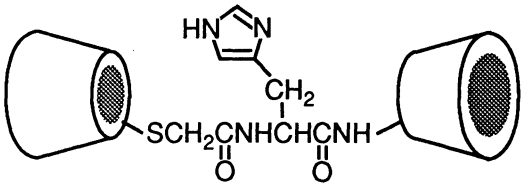
Novel imidazole-appended coupled β-cyclodextrin (**6**) was prepared by the condensation of 6-deoxy-6-(L-histidylamino)-β-cyclodextrin and 6-(carboxymethylthio)-6-deoxy-β-cyclodextrin with dicyclohexylcarbodiimide. The enzyme-like activities of **6** were studied by measuring the rates for the hydrolysis reaction of some kinds of p-nitrophenyl alkanoates (C2, C3, C6, and C12). **6** showed large acceleration ability and substrate specificity for only p-nitrophenyl C6 ester.

Cyclodextrins (CD's) have been extensively studied as enzyme models and as molecular receptors due to their abilities to bind various hydrophobic compounds into their hydrophobic cavities.[1] CD-catalyzed hydrolyses of esters proceed quite similarly to enzymatic hydrolyses. However there are some differences between CD-catalyzed hydrolyses and enzyme-catalyzed hydrolyses: a) The maximum rate constant for CD-catalyzed reaction is obtained at pH 12, while that for a natural enzyme, α-chymotrypsin, is obtained at pH 7. b) CD does not undergo turnover in the hydrolysis reaction and only stoichiometric acylation of CD proceeds. For the purpose of solving these problems and achieving an enzyme model which has more of the features of a real enzyme, we have been studied imidazole-modified



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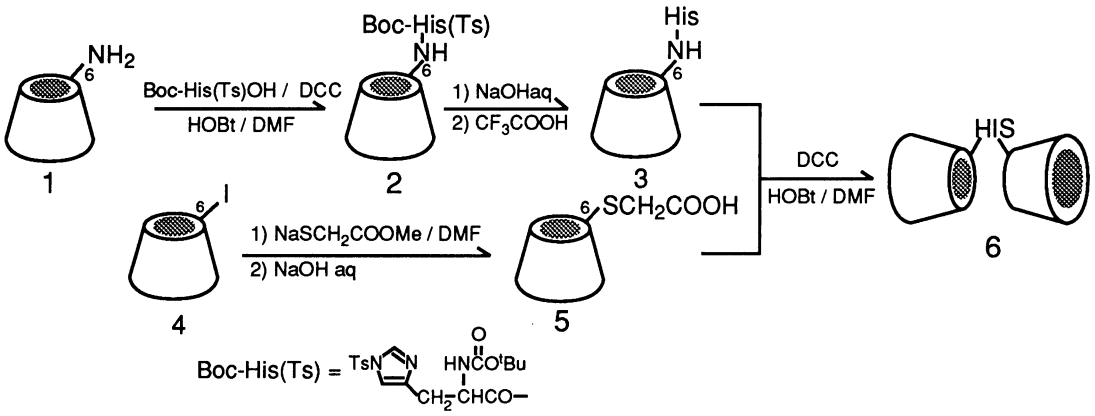
cyclodextrins, which show maximum activity for a hydrolysis reaction in neutral pH region and the turnover property.[2] The most successful enzyme model in our studies is imidazole-appended 2,6-dimethyl-β-cyclodextrin.[2c] This model caused about 1000-fold acceleration of the hydrolysis of p-nitrophenyl acetate, and k_{cat} for this reaction of this model is 2.67 x 10^{-2} s^{-1}, which is over twice as much as that of α-chymotrypsin. However K_m of the former is much bigger than that of the latter. Therefore, total reaction activity, k_{cat} / K_m of the former is still smaller than that of the latter. This means that the binding ability of the enzyme model is much smaller than that of the natural enzyme, whereas catalitic activity of the former is larger than that of the latter after making inclusion complex. So, if the binding ability of an enzyme model can be improved, total catalitic acceleration of the model will be larger than that of the natural enzyme.

To increase the binding ability of CD, a number of coupled CDs have been synthesized.[3] The binding constant of this derivative to some guest went up to over 10^8 M. So, imidazole-appended coupled CD is expected to be a better enzyme model for α-chymotrypsin. In this paper, we report the synthesis of imidazole-appended coupled CD and its enzyme-like activity.

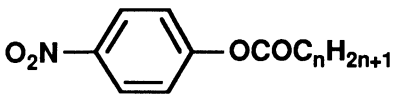
Imidazole-appended coupled cyclodextrin (**6**) was prepared by the condensation of 6-deoxy-6-(L-histidylamino)-β-cyclodextrin (**3**) and 6-(carboxymethylthio)-6-deoxy-β-cyclodextrin (**5**) with dicyclohexylcarbodiimide (DCC) as shown in scheme 1. **3** was obtained by the reaction between 6-amino-6-deoxy-β-cyclodextrin (**1**) and N^{α}-*tert*-butyloxycarbonyl-N^{im}-tosyl-L-histidine in the presence of DCC and 1-hydroxybenzotriazole (HOBt) followed by deprotection. **5** was synthesized from 6-deoxy-6-iodo-β-cyclodextrin (**4**) and methyl sodium sulfidoacetate followed by de-methylation. These compounds were identified by elemental analysis, some kinds of NMR spectra including 2D NMR, and mass spectrum.[4]



Scheme 1.

The enzyme-like activities of **3** and **6** were studied by measuring the rates for the hydrolysis reactions of p-nitrophenyl alkanoates in an aqueous phosphate buffer solution of pH 7.8 at 25 °C at conditions that made no 1 : 2 complex. The reaction proceeded by a Michaelis-Menten mechanism, showing saturation behavior with increasing substrate concentration. Table 1 shows the kinetic parameters of **3** and **6** for the hydrolysis reactions of some kinds of p-nitrophenyl alkanoates (C2, C3, C6, and C12). **6** showed strong substrate specificity for only C6 ester. k_{cat} / K_m of **6** for C6 is 40 times larger than that for C2, whereas k_{cat} / K_m of **3** for C6 is as much as that for C2. This specificity is due to the difference of K_m rather than k_{cat}. **6** has a 550 times larger binding ability (1/ K_m) for C6 than C2, whereas the binding ability of **3** for these substrates is almost the same and K_m of **3** is as much as a dissociation constant of non-modified CD or usual modified CD.

Recently Tee and co-workers studied the dependence of alkyl chain length for the cleavage of the p-nitrophenyl alkanoates (C2 - C8) by α- or β-cyclodextrin in an aqueous phosphate buffer of pH 11.7.[7] They found the cleavage process involved two molecules of CD for long-chain alkanoates. The long alkyl chain of an ester was included in a CD and the aromatic group was also included in another CD. In the case of coupled cyclodextrin (**6**), it is thought that one of CD moieties in **6** includes the alkyl chain of an ester, and the other CD moiety includes the p-nitrophenyl group. k_{cat} of **6** for C6



n =1 **C2**
2 **C3**
5 **C6**
11 **C12**

Table 1. Kinetic Parameters for hydrolyses of p-Nitrophenyl Alkanoates by 3 and 6 [a]

Catalyst	Substrate	k_{cat} / 10^{-3} s^{-1}	K_m / 10^{-3} M	k_{cat} / K_m / M^{-1} s^{-1}	k_{cat} / k_{un} / -
	C2	4.70 ± 0.21	6.73 ± 0.39	0.698	234
3	C3	4.22 ± 0.12	5.08 ± 0.21	0.830	285
	C6	1.63 ± 0.45	1.97 ± 0.62	0.827	91.6
	C2	2.69 ± 0.15	3.70 ± 0.32	0.726	134
	C3	2.83 ± 0.51	3.17 ± 0.86	0.893	191
6	C6	0.191 ± 0.011	0.00673±0.00100	28.4	10.7
	C12	0.0862±0.0162	0.0125 ±0.0013	6.90	12.2

a) at 25 °C in an aqueous phosphate buffer of pH 7.8 (1/15 M),
for C2 and C3, [Catalyst] = 3.5 x 10^{-5}, [Substrate] = 2 x 10^{-4} - 1 x 10^{-3},
for C6 and C12, [Catalyst] = 3.6 x 10^{-6}, [Substrate] = 5 x 10^{-6} - 7 x 10^{-5}.

and C12 is much smaller than that for C2 and C3. In the case of **3**, there is not much different among these substrates. k_{cat} seems to be very sensitive to the relative position of the imidazole group with respect to the carbonyl group of the substrate.

In conclusion, we have observed strong substrate specificity for only C6 ester in the hydrolysis reactions of p-nitrophenyl alkanoates. If imidazole is placed in the best position in a coupled cyclodextrin, this model has a larger acceleration ability than does a real enzyme.

We are grateful to Nihon Shokuhin Kako Co. LTD. for providing us for β-cyclodextrin.

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4) **3**: FD-MS 1271 (M+1); Anal. Found: C, 41.16; H, 6.63; N, 4.01%. Calcd for $C_{48}H_{78}N_4O_{35} + 7H_2O$: C, 41.25; H, 6.64; N, 4.00%. ^{1}H NMR (D_2O) δ 2.74-2.82 (2H, m, CH_2-Im), 3.10 (1H, t, J = 9.52 Hz, H_4), 3.23 (1H, dd, $J_{6a,6b}$ = 14.65 Hz, $J_{5,6b}$ = 6.84 Hz, H_{6b}), 6.77 and 7.57 (2H, s, Im). **6**: Anal. Found: C, 40.36; H, 6.43; N, 2.01; S, 1.26%. Calcd for $C_{92}H_{148}N_4O_{70}S + 15H_2O$: C, 40.43; H, 6.57; N, 2.05; S, 1.17%. ^{1}H NMR (D_2O) δ 2.76 (1H, dd, $J_{6a,6b}$ = 14 Hz, $J_{5,6b}$ = 7.5 Hz, $CH_{6a}\underline{H_{6b}}S$), 2.94-3.01 (2H, m, $\underline{CH_{6a}}H_{6b}S$ and $\underline{CH_a}H_b$-Im), 3.08 (1H, dd, J = 6 Hz and 14 Hz, $CH_a\underline{H_b}$-Im), 3.24 (1H, t, J = 9.5 Hz, H_4 (histidine side)), 3.23-3.44 (2H, m, SCH_2CO), 6.96 and 7.70 (2H, s, Im).

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(Received February 22, 1994)