

CYCLODEXTRIN HOMO- AND HETERO-DIMERS AS ENZYME MODELS

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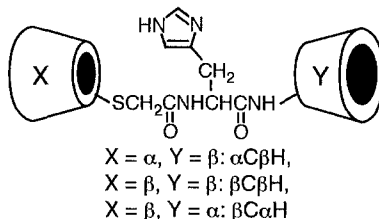
ABSTRACT

Three kinds of dimers (one homo-dimer of β -CD and two kinds of hetero-dimers of α -CD and β -CD) were synthesized as artificial hydrolases. The dimers were prepared by the condensation of 6-deoxy-6-(L-histidylamino)-cyclodextrin and 6-(carboxymethylthio)-6-deoxycyclodextrin with dicyclohexylcarbodiimide. The enzyme-like activities were studied by measuring the rates for the cleavage reaction of some kinds of nitrophenyl alkanoates. They showed large acceleration ability and substrate specificity for the acyl chain length of the substrate. As the acyl chain length of the substrate was longer, the transition state was more stabilized by the dimers. The only homo-dimer showed allosteric behavior with 1.8 Hill constant, when *p*-nitrophenyl methoxyethoxyacetate was used for the substrate.

1. INTRODUCTION

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. We have been studied imidazole-modified cyclodextrins, which show maximum activity for ester cleavage reactions in neutral pH region and the turnover property. The most successful enzyme model in our studies is imidazole-appended 2,6-dimethyl- β -cyclodextrin [1]. This model caused about 1000-fold acceleration of the hydrolysis of *p*-nitrophenyl acetate, and k_{cat} for this reaction of this model is $2.67 \times 10^{-2} \text{ s}^{-1}$, 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, the 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 the catalytic activity of the former after making inclusion complex is larger than that of the latter. So, if the



binding ability of an enzyme model can be improved, total catalytic acceleration of the model will be larger than that of the natural enzyme. Recently O. S. Tee reported that 2 : 1 (CD/substrate) complex played an important role for the cleavage of aryl alkanooates. Some CD dimers have very strong binding ability. So, imidazole-appended CD dimers would be expected to be better artificial hydrolases. In this symposium, we present the syntheses of CD homo- and hetero-dimers and these enzyme-like activities.

2. MATERIALS AND METHODS

2.1 Materials

Imidazole-appended cyclodextrin dimers were prepared by the condensation of 6-deoxy-6-(L-histidylamino)-cyclodextrin and 6-(carboxymethylthio)-6-deoxy-cyclodextrin with dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). 6-Deoxy-6-(L-histidylamino)-cyclodextrin was obtained by the reaction between 6-amino-6-deoxy-cyclodextrin and N^{α} -*tert*-butyloxycarbonyl- N^{im} -tosyl-L-histidine in the presence of DCC and HOBt followed by deprotection. 6-(Carboxymethylthio)-6-deoxy-cyclodextrin was synthesized from 6-deoxy-6-iodo-cyclodextrin and methyl sodium sulfidoacetate followed by demethylation. These compounds were identified by elemental analysis, some kinds of NMR spectra including 2D NMR, and mass spectra.

2.2 Methods

Ester cleavage reaction was followed by monitoring the appearance of nitrophenolate ion using a Simadzu UV-3100 spectrophotometer. The reaction was conducted in a quartz cell in the water-jacketed cell holder of the UV-3100. The temperature was maintained at 25 °C by a HAAKE F3 circulating water bath. The reaction was initiated by adding a stock solution of the ester in acetonitrile or DMSO to a buffer solution in the quartz cell. The rates used in the calculation of rate constants were averages of at least three determinations which agreed within 3 %.

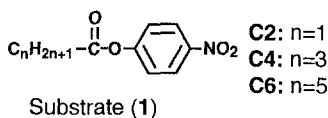
3. RESULTS AND DISCUSSION

3.1 Cleavage Reaction of *p*-Nitrophenyl Alkanoates

The enzyme-like activities of three kinds of dimers were studied by measuring the rates for the cleavage reaction of some kinds of nitrophenyl alkanoates in a 16.1 % DMSO aqueous phosphate buffer solution of pH 7.8 at 25 ° C. The reaction proceeded by a Michaelis-Menten mechanism, showing saturation behavior with increasing substrate concentration. Table 1 shows the dependence of kinetic parameters on the acyl length

TABLE 1 Kinetic Parameters for the Cleavage of *p*-Nitrophenyl Alkanoates by Cyclodextrin Homo- and Hetero-dimers

catalysis	substrate	k_{cat} / 10^{-4} s^{-1}	K_m / 10^{-4} M	k_{cat}/K_m / $\text{M}^{-1}\text{s}^{-1}$	K_{TS} / 10^{-5} M	k_{cat}/k_{un} / -
$\alpha\text{C}\beta\text{H}$	C2	2.54	9.14	0.278	4.89	18.7
	C4	1.13	3.31	0.341	1.77	18.7
	C6	1.73	1.70	1.02	0.726	23.4
$\beta\text{C}\beta\text{H}$	C2	8.04	38.7	0.208	6.98	55.4
	C4	2.32	5.53	0.420	1.54	35.9
	C6	4.76	5.78	0.824	0.939	61.6
$\beta\text{C}\alpha\text{H}$	C2	1.47	8.32	0.177	8.21	10.1
	C4	0.995	1.83	0.544	1.19	15.4
	C6	0.815	1.19	0.685	1.13	10.5



of the esters.

The transition state stabilization (K_{TS}) of the cleavage reaction was evaluated using the Kurz approach as presented by O. S. Tee. As the acyl chain length of the substrate was longer, the transition state was more stabilized by the dimers.

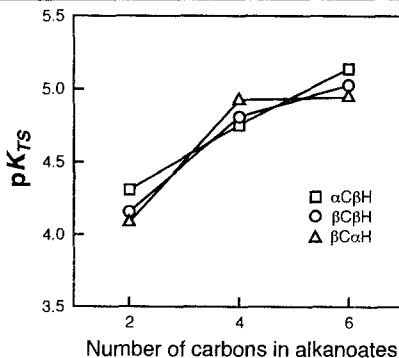


Fig. 1 Dependence of pK_{TS} on acyl length of the substrates for the cleavage of *p*-nitrophenyl alkanoates by the cyclodextrin dimers.

3.2 Allostery of Homo-Dimer

The presence of some cooperativity between two CD cavity of the CD dimer is suggested by the result of dependency on the acyl chain length of the substrates in the reaction, but it could not confirmed by the reaction with the alkanoate esters, because of

low solubility of the ester in water. So, we synthesized a new ester that can be more soluble in water and the cleavage ability of three kinds of dimers were studied in high substrate concentration condition. The only homo dimer showed sigmoid behavior with increasing substrate concentration as shown in Fig. 2, whereas two kinds of hetero-dimers showed simple saturation behavior. This sigmoidal behavior was fitted to the Hill equation and the Hill constant was 1.8. This means that high cooperativity exists between two β -CD cavity of the homo dimer but the hetero dimers have no allostery. The MWC model can explain the allosteric interactions of natural allosteric proteins. The most important assumption of the MWC model is symmetry of the structure of the protein. So, our result, that the only homo-dimer showed allostery, would be supported by the MWC model.

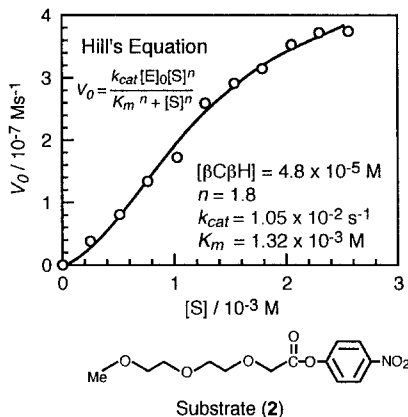


Fig. 2 Dependence of the initial rate on the substrate concentration for the cleavage of the substrate (2) by β CDH in pH 7.4 phosphate buffer (0.1M) / acetonitrile (15 : 1) at 25 °C.

4. CONCLUSION

Three kinds of CD dimers were synthesized as artificial hydrolases. As the acyl chain length of the substrates were longer, the transition state was more stabilized by the dimers. We constructed a new artificial allosteric system with 1.8 Hill constant by the use of CD homo-dimer and *p*-nitrophenyl methoxyethoxyethoxyacetate as the artificial enzyme and the substrate, respectively.

Acknowledgments

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Reference

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