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Cyclodextrin trimers as receptors for arranging ester and catalyst at optimized location to achieve enhancement of hydrolytic activity

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This work is dedicated to Professor Akihiko Ueno who passed away on March 23rd, 2003

Abstract—Two novel trimer receptor molecules (AC and AD trimers) consisting of two α-cyclodextrins (α-CDs) and one β-cyclodextrin (β-CD) have been designed and synthesized in order to make an optimum arrangement between a substrate and a catalyst. In the reaction systems that use trimer receptors as host molecules, the substrate and the catalyst are thought to be accommodated by two α-CDs and one β-CD, respectively. The rate for the hydrolytic reaction of a long-shaped ester was largely increased, when the trimer receptors were used as receptor molecules or molecular flasks, which provided optimum location between the substrate and the catalyst.
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Cyclodextrins (CDs) are cyclic oligosaccharides containing 6 (α-CD), 7 (β-CD), 8 (γ-CD) or more glucose units. They have a hydrophobic cavity in their structures and can accommodate various organic molecules into their cavities in aqueous solution. Because of this fascinating property, several enzyme models using CDs have widely been studied for hydrolytic reaction of ester molecules. In those cases, imidazole moieties as catalysts were fixed by covalent bonding on the edge of a CD[1–9] or on the linker of two CDs,[10] and substrates were included into the CD cavity to be arranged close to the catalyst. These modified CDs as artificial enzymes, however, accelerate the hydrolytic reaction but are not useful for a wide range of reactions because of the covalently fixed imidazole moiety as a catalyst for ester hydrolysis. If CD receptors that can arrange the catalyst and the substrate in the optimum location by non-covalent bonding could be designed, they might be useful for plural catalytic reactions and can act as reactors for various reactions. In the design of such CD-based reactor, it is very important to introduce one cavity for the catalyst and another one for the substrate to be accommodated. Based on this idea, two novel trimer receptor molecules (AC and AD trimers) have been designed and synthesized in this study (Chart 1). These two receptor molecules are geometrical isomers with two α-CDs attached at the AC and AD glucose units in β-CD. The important feature of this system is that two α-CDs can cooperatively include a long-shaped substrate and β-CD can selectively include a defined catalyst molecule (Scheme 1). It is well known that the binding constants of α-CD for various guest compounds are not so strong.[11] Therefore, two α-CDs are introduced into these trimer systems to increase the binding ability of α-CD for a particular substrate.[12] If the substrate and

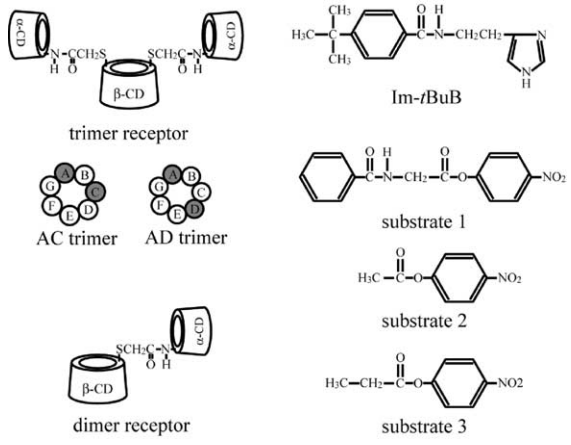

Chart 1. Structures of compounds used in this study.

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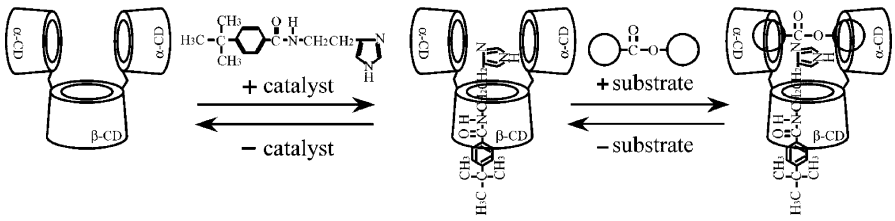

Scheme 1. Illustration of the geometrical arrangement between the catalyst and the substrate in the trimer receptor system.

the catalyst are located at the optimized position without covalent bonding as shown in Scheme 1, an increase in the rate of a catalytic reaction would be observed. The hydrolytic reaction of ester compounds as a catalytic reaction was selected because the method of assay using *p*-nitrophenolate for hydrolysis of ester compounds has been extensively studied. To achieve this system, *N*-(4-imidazolylethyl)-4-*tert*-butylbenzamide (Im-*t*BuB) was synthesized as a catalytic compound (Chart 1), which can be accommodated only by β-CD (Scheme 1) since α-CD has a small sized cavity it is unfavorable for inclusion of the bulky *tert*-butyl group in Im-*t*BuB. It has been reported that the *tert*-butyl group in Im-*t*BuB is held at the secondary side of the β-CD cavity and the other end turns toward the primary side when *tert*-butyl benzene derivatives are accommodated by β-CD.[1,13,14] Thus, we can control the direction of Im-*t*BuB as the imidazole residue turns toward the part of the ester bond of the substrate. We found that the catalyst with the trimers caused an increase in the activity of the hydrolytic reaction for a relatively long-shaped substrate. Therefore, we intend to report on the selectivity of the trimers for the length of substrate and their catalytic activity for the ester hydrolysis.

Each of the trimer receptors were synthesized by a coupling reaction of 6,6′-bis(carboxymethylthio)-6,6′-dideoxy-β-CD (AC or AD isomer) and 6-amino-6-deoxy-α-CD (NH_2-α-CD).[15] The dimer receptor as a reference host compound was synthesized by a coupling reaction of 6-carboxymethylthio-6-deoxy-β-CD and NH_2-α-CD[16] (Chart 1). Histamine was coupled with *p*-*tert*-butyl-benzoic acid to give Im-*t*BuB[17] as a catalyst, and hippuric acid was reacted with *p*-nitrophenol to give *p*-nitrophenyl hippurate (**1**) as a substrate[18] (Chart 1). All these products were identified by a matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOFMS) and a ^{1}H NMR spectroscopy. The hydrolyses of all ester compounds used as substrates in this study (1 mM) with Im-*t*BuB (0.2 mM) in the presence or absence of the trimers or the dimer (0.05 mM) at 25 °C were recorded on a Shimadzu UV-3100 spectrophotometer at 400 nm (released *p*-nitrophenolate as a product) at various pH's (from pH 7.0 to pH 9.0) in 40 mM Tris–HCl buffer.

Before the effect of the trimer on rate enhancement is discussed, the structure of the inclusion complex of the trimer is examined. The binding constant of β-CD for Im-*t*BuB was obtained to be 22,000 M^{-1} by an isothermal titration calorimetry (ITC), whereas that of α-CD was too small to be estimated. This result indicates that Im-*t*BuB is accommodated only by β-CD of the trimer. Because the binding constant of β-CD for a benzene derivative having no bulky group like the substrate **1** is about 1000 M^{-1}, the affinity of β-CD for Im-*t*BuB is 20-folds larger than that for the substrate **1**. Therefore, only a few of Im-*t*BuB were excluded by the substrate **1**, and the main structure of the reactive complex of the trimer is expected to be as shown in Scheme 1, although other miner structure will exist.

The pH 8.5, which is found to be the optimum pH value, has been described in the following discussion. The combination of concentrations among the substrates, the catalyst and the receptors was made fixed at the concentration where the difference of catalytic activities for substrates in the absence and presence of receptors was maximum.

The effects of the trimers and dimer on the rate of hydrolysis of **1** at pH 8.5 are shown in Figure 1. It was observed that the presence of the AD trimer caused much higher hydrolytic activity for **1** than that of the dimer or the AC trimer in aqueous solution. From this result, we assume that the length and shape of **1** is suitable to be accommodated cooperatively by two α-CDs in the AD trimer system. The cooperative binding by two α-CDs is important to localize the substrate **1** at the optimized position for hydrolysis (Scheme 1). To confirm the validity of the concept that a substrate is accommodated cooperatively by two α-CDs in the trimer system, *p*-nitrophenyl acetate (**2**) and *p*-nitrophenyl propionate (**3**), which have the length shorter than **1**,

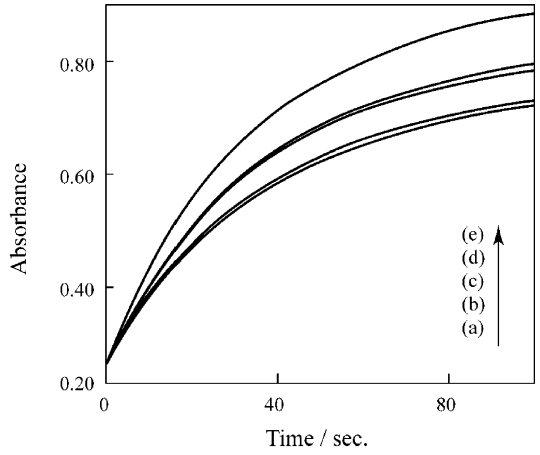

Figure 1. The changes in absorbance at 400 nm (released *p*-nitrophenolate as a product) for hydrolysis of substrate **1** in 40 mM Tris–HCl buffer at 25 °C (pH 8.5). (a) **1** alone; (b) **1** and Im-*t*BuB; (c) **1**, Im-*t*BuB and dimer; (d) **1**, Im-*t*BuB and AC trimer; (e) **1**, Im-*t*BuB and AD trimer. [**1**] = 1.0 mM, [Im-*t*BuB] = 0.2 mM, [dimer] = 0.05 mM, [AC trimer] = [AD trimer] = 0.05 mM.

were examined, and the rates of hydrolytic reactions for them with Im-*t*BuB were measured in the presence or absence of the trimers or the dimer. In these experiments, though the hydrolysis of **3** in the presence of the AD trimer showed rate enhancement compared with the AC trimer or the dimer, we found no rate enhancement compared with the dimer for the hydrolysis of **2** in the presence of the trimers. Figure 2 shows proportions of increase in the initial rate of hydrolytic reactions for the substrates used in this study in the presence of the dimer or the trimers. The proportion of increase in the initial rate of hydrolysis of **1** in the presence of the AD trimer showed remarkable rate enhancement, approximately 3-fold, compared with the dimer system, whereas the rate of hydrolysis of **2** was not accelerated by the AD trimer system (Fig. 2). This result implies that **1** is located comparatively at the effective position for hydrolysis in the AD trimer, as its length is suitable to be accommodated cooperatively by two α-CDs in the AD trimer system (Scheme 1). On the other hand, the length of **2** is thought not to be long enough for it to be accommodated by two α-CDs cooperatively in the trimers. That is, instead of two, one α-CD might have accommodated the substrate when Im-*t*BuB is accommodated by one β-CD in the trimers. Therefore, the ester bond region of **2** might be not located at the optimized position for the imidazole moiety of Im-*t*BuB to show high hydrolytic activity. Therefore, the phenomena of inclusion of **2** in the trimer systems might be similar to the dimer. The importance of the lengths of substrates in the trimer systems is also supported by the fact that the rate of hydrolysis of **3**, having longer length than **2**, in the presence of the AD trimer was approximately 2.4-fold higher than that in the presence of the dimer (Fig. 2). In addition, the rate of hydrolysis of **1** and **3** in the presence of the AD trimer was approximately 2.5-fold, and 1.9-fold higher respectively, than that in the presence of the AC trimer (Fig. 2). This result can be explained in terms of the geometrical arrangement between CDs and the substrate. The CPK model shows that the two α-CDs in the AD trimer face each other, whereas the two α-CDs in the AC trimer face away from each other as shown in Figure 3, and the shape of **1** or **3** is almost straight. Therefore, the geometrical positions

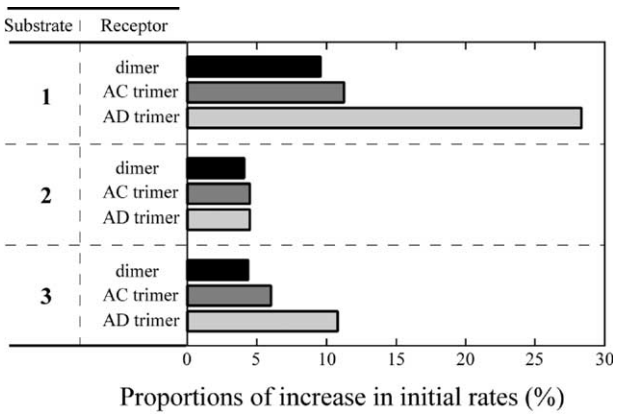

Figure 2. Proportions of increase in the initial rates of hydrolytic reactions of substrates in the presence of dimer or trimers compared with the initial rates in the absence of dimer or trimers in Tris–HCl buffer (40 mM, pH 8.5).

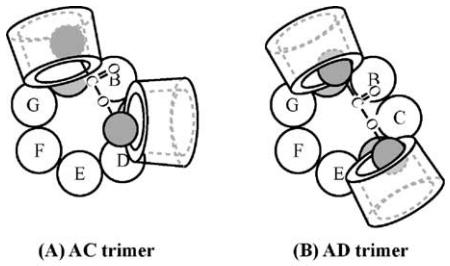

Figure 3. Orientations of α-CDs on the β-CD at AC and AD position.

of two α-CDs in the AD trimer are more favorable than that of two α-CDs in the AC trimer for the inclusion of **1** or **3**. This indicates that the shape as well as the length of the substrate plays a crucial role for the hydrolysis reaction in the presence of the CD-based receptor systems. However, the rate of hydrolysis of **1** or **3** in the presence of the AC trimer was slightly higher than those in the presence of dimer (Fig. 2). This result indicates that when the longer length esters are employed as substrates, two α-CDs play a role of increasing the binding constant for the substrate and help to enhance the rate of hydrolysis even though the geometrical positions of two α-CDs in the trimer are not favorable for the inclusion of the substrates.

In our dimer or trimer systems, it is difficult to estimate various kinetic values since the process of the hydrolysis reaction undergoes three constituent parts consisting of the substrate, the catalyst, and the dimer or trimers. In order to compare the catalytic activity accurately at each condition, we will find out the way to estimate various kinetic values in the dimer or trimer systems. These trimer systems, however, suggest that they could be applicable as the reactors for hydrolysis of ester compounds with the catalyst. The combinations of other kind of catalysts have the potential that might be applicable not only for hydrolysis of ester compounds but also for various reactions by controlling the geometrical arrangement between CDs, the substrate and the catalyst.

In this study, we have shown that the addition of the trimers into the solution containing ester compounds as a substrate and Im-*t*BuB as a catalyst caused enhancement in the activity of the hydrolytic reaction. In the case of using the substrate **2**, the addition of trimers caused no increase in the activity of the hydrolytic reaction. On the other hand, in the case of using the substrate **1**, the addition of the trimers showed remarkably higher hydrolytic activity. This observation suggests that the size and shape of the substrate is important for this trimer system because the substrate is required to be accommodated cooperatively by two α-CDs for achieving high hydrolytic activity. This is supported by the fact that the rates of hydrolysis of **3**, having longer length than **2**, in the presence of trimers were slightly higher than those of **2**. Again, the rate of hydrolysis of **1** or **3** in the presence of the AD trimer showed much higher hydrolytic activity than that of the AC trimer, indicating that the geometrical arrangement of the CDs in the trimer receptors is also important.

These findings lead us to improve the system by modifying the structures of trimer receptors as well as the substrates. The work on this line is now underway

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15. *AC trimer*; ^{1}H NMR (D_2O): δ = 2.70 (s, 4H, –S–CH_2–CO–), 2.87 (d, 2H, α-CD–H^6–N), 3.05 (m, 2H, α-CD–H^6–N), 3.34–3.95 (m, 116H, CDs–$H^{2\text{-}6}$, β-CD–H^6–S), 4.96–5.04 (m, 19H, CDs–H^1), MALDI-TOF MS: *m*/*z*: 3218.3 (calcd for $M + Na^+$: 3213.2) *AD trimer*; ^{1}H NMR (D_2O): δ = 2.66 (s, 4H, –S–CH_2–CO–), 2.85 (d, 2H, α-CD–H^6–N), 3.05 (m, 2H, α-CD–H^6–N), 3.30–4.00 (m, 116H, CDs–H^{2-6}, β-CD–H^6–S), 4.95–5.05 (m, 19H, CDs–H^1), MALDI-TOF MS: *m*/*z*: 3214.4 (calcd for $M + Na^+$: 3213.2).
16. *Dimer*; ^{1}H NMR (D_2O): δ = 2.66 (s, 2H, –S–CH_2–CO–), 3.05 (m, 2H, α-CD–H^6–N), 3.32–4.00 (m, 79H, CDs–H^{2-6}, β-CD-H^6-S), 4.98–5.01 (m, 13H, CDs–H^1) MALDI-TOF MS: *m*/*z*: 2186.2 (calcd for $M + Na^+$: 2185.1).
17. *Im-tBuB*; ^{1}H NMR (DMSO-d_6): δ = 1.27 (s, 9H, *t*-butyl), 2.75 (t, 2H, histamine, CH_2–CH_2), 3.46 (t, 2H, histamine, CH_2-NH), 6.83 (s, 1H, histamine, CH–N), 7.46 (d, 1H, benzene-beta), 7.58 (s, 1H, histamine, N–CH–N), 7.74 (d, 1H, benzene-alpha), MALDI-TOF MS: *m*/*z*: 271.7 (calcd for $M + H^+$: 271.4).
18. *p-Nitrophenyl hippurate* (*substrate 1*); ^{1}H NMR (DMSO-d_6): δ = 3.34 (s, 2H, –N–CH_2–C–), 7.44–7.46 (m, 1H, Ar–H^4), 7.51–7.52 (m, 2H, Ar–$H^{2,6}$), 7.56–7.57 (m, 2H, Ar–$H^{3,5}$), 7.90–7.92 (m, 2H, NO_2–Ar–$H^{3,5}$), 8.31–8.34 (m, 2H, NO_2–Ar–$H^{2,6}$).