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Letter

A novel cyclodextrin homodimer with dual-mode substrate binding and esterase activity

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Abstract

A novel cyclodextrin homodimer with two β-cyclodextrin units tethered from the primary side by a tris(2-aminoethyl)amine linker was found to be hydrolytically active. The hydrolysis of a ditopic activated carbonate ester was accelerated 150-fold and a Lineweaver–Burk plot showed a biphasic curve indicative of two different binding modes for the activated carbonate ester.

Keywords: Cyclodextrins; Artificial enzymes; Enzyme mimics; Enzyme models

1. Introduction

Cyclodextrins have proved to be very useful in the design of artificial enzymes [1,2]. The hydrophobic cavities of cyclodextrins are highly receptive to a wide variety of aromatic and aliphatic guest molecules. Typical dissociation constants of the cyclodextrin inclusion complexes in water vary [3] in the range of 1×10^{-3}–1×10^{-4} M. The strength of binding can be improved by the utilization of two covalently linked cyclodextrin units per guest molecule (cyclodextrin dimers) [4–9]. This approach yielded [9] K_d's as low as 10^{-9} M, dissociation constants comparable to that of a medium-affinity antibody–antigen complex.

As a part of our research effort [10,11] in the design and synthesis of functional models of enzymes, we have targeted β-cyclodextrin homodimer **3**. The design included tripodal tris(2-aminoethyl)amine (tren) as linker, considering the fact that tren would exist primarily as a dication at pH's near neutrality. Thus, protonated and neutral amino groups will coexist, opening the way to an effective general acid/general base catalysis. This mode of catalysis is widely utilized in a number of hydrolytic enzymes, including ribonuclease A.

2. Results and discussion

The homodimer **3** is synthesized in 3 steps from β-cyclodextrin (Scheme 1). Recently reported [12] tosylation procedure worked well in our hands, leading to an analytically pure 6-(p-

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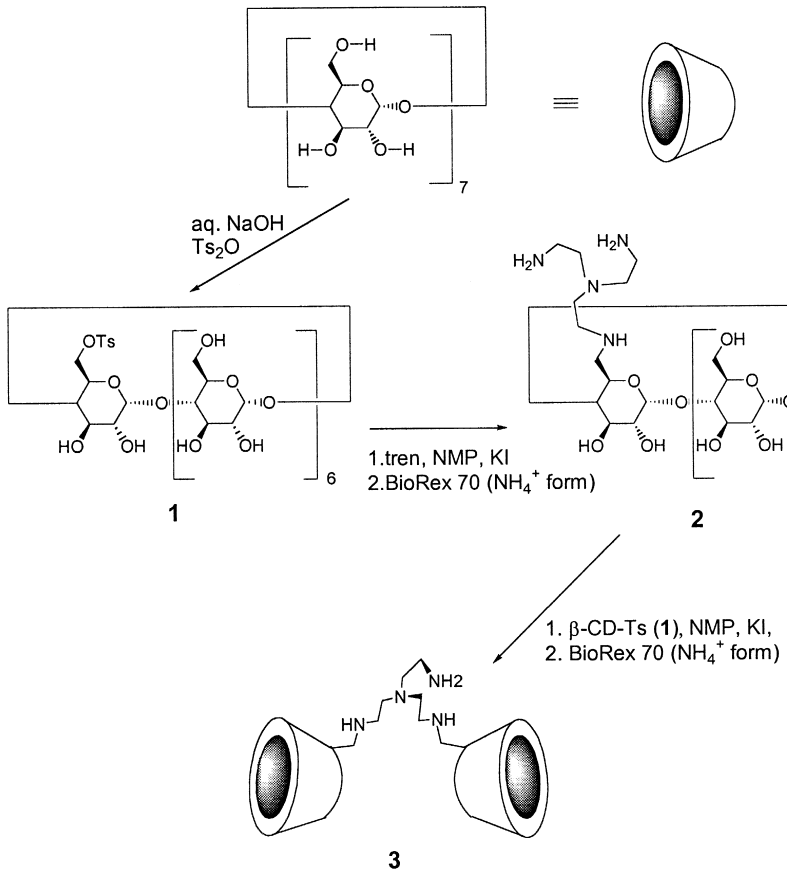

Scheme 1. Synthesis of the cyclodextrin homodimer **3**.

tolylsulfonyl)-β-cyclodextrin. The tren derivative of β-cyclodextrin has been reported earlier [13,14], we have adapted the procedure in which NMP is substituted for DMF as the solvent for the nucleophilic substitution in order to minimize formylation. Thus, tren-derivative of cyclodextrin was obtained in multigram quantities. We reacted this compound with the excess 6-tosyl-β-cyclodextrin in NMP at 70°C, in the presence of catalytic amounts of NaI. The crude reaction mixture was purified first by precipitating the CD-compounds with acetone. The resulting material was further purified by cation exchange chromatography; an aqueous solution of the precipitated product was applied to Biorex-70 column (NH_4^+ form) and eluted first with water to removed uncharged species and then with 1.0 M aqueous NH_4OH. Repeated evaporations of added water under reduced pressure resulted in the desired compound **3** in analytically pure state: ^{1}H NMR, (D_2O, 400 MHz) δ 2.6–3.1 (m, 12H), 3.6–4.1 (m, 84H), 5.1–5.2 (s, 14H); ^{13}C NMR, (D_2O, 100 MHz) δ 38.1, 38.3, 38.5, 38.7, 60.8, 72.5, 72.6, 73.7, 81.7, 102.4; MS (FAB) 2380 (M^+); Elemental analysis: Found: C, 45.15; H, 6.73; N, 2.31. $C_{90}H_{154}N_4O_{68}$ requires C, 45.42; H, 6.52; N, 2.35.

Hydrolytic activity of the tren-homodimer was studied using ditopic substrate bis-*p*-nitrophenylcarbonate. The hydrolysis reaction was monitored spectrophotometrically at 20°C; *p*-nitrophenolate ion absorbance at 400 nm followed. First, the hydrolytic activity as a function of pH was studied. As expected, the maximum rate acceleration was observed at pH 7.5.

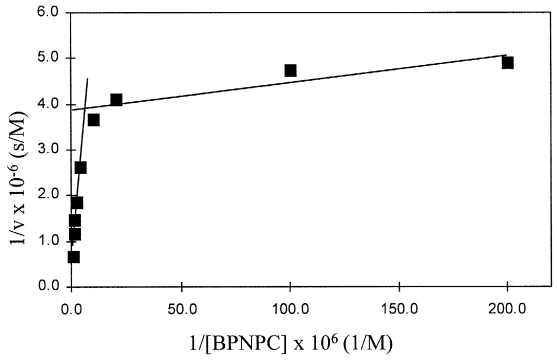

Fig. 1. Lineweaver–Burk plot of bis-*p*-(nitrophenyl)carbonate hydrolysis catalyzed by the homodimer **3** (2.5 mM).

This corresponds to a pH where two of the tren nitrogens are protonated. The decrease in the hydrolysis rate due to an increase from pH 7.5 to 8.5 is a clear indication that nucleophilic catalysis is not involved. The rate enhancement for the hydrolysis in the presence of 2.5-mM cyclodextrin dimer was 150-fold, a remarkable rate acceleration considering the fact that it is due to general acid/general base catalysis.

Kinetic saturation was seen with higher substrate/catalyst ratios. Lineweaver–Burk double reciprocal plot appeared to be biphasic, resulting two different K_m values (Fig. 1). Thus, two different binding modes, one with $K_m = 8.0 \times 10^{-5}$ M and one with $K_m = 1.5 \times 10^{-7}$ M is in effect. It appears at low concentrations of the substrate where both cyclodextrin cavities are involved in accommodating the substrate, whereas near saturation, a weaker binding mode with one aromatic ring of the substrate included inside the homodimer is in effect. A control experiment with tren-β-CD (**2**) was also carried out for the hydrolysis reaction. Under the same set of conditions, this compound resulted only a 39-fold rate increase. The saturation kinetics and Lineweaver–Burk plot did not have any unexpected features ($K_m = 1.1 \times 10^{-4}$ M).

3. Conclusion

The additional rate acceleration with the dimer demonstrates that the immobilization of the substrate by the cooperative action of the two cyclodextrin units is in effect. The rate acceleration, although modest, is still remarkable considering that it is solely due to general acid/general base catalysis. Also remarkable is the biphasic LB-plot, clearly indicating the two different binding modes for bis-*p*-(nitrophenyl)carbonate. The utilization of CD-dimers is likely to yield more enzyme-like artificial enzymes, with large catalytic turnovers. Our work along these lines is in progress.

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