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Inclusion Behavior of Dimer β-Cyclodextrin Bridged with Aspartic Acid Derivative

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Abstract: The β-cyclodextrin (CD) dimer bridged with aspartic acid (ASP) derivative, FITC-ASP(NH-β-CD)$_2$ (Host, FITC=fluorescein-4-isothiocyanate), was synthesized. Fluorescence polarization study showed that the novel host formed an inclusion compound, [FITC-ASP(NH-β-CD)$_2$]ATA, for which K_d was determined to be 5.0×10^{-6} mol/L by Beacon 2000 Analyzer, when ATA (Guest) = Adm-Trp-Arg-Arg-NH_2 (Adm = 1-adamantanecarboxylic acid, Trp = tryptophan, Arg = arginine), where K_d is the dissociation constant in aqueous solution at 298 K.

Keywords: β-Cyclodextrin, amino acid, peptide, inclusion compound, fluorescence polarization.

The capped cyclodextrins (CDs) are very useful macromolecular hosts in supramolecular chemistry. Previously, we demonstrated that the dimer cyclodextrin bridged with 1,2-diaminoethane causes significant enhancement of affinity to a small organic guest molecule[1]. A variety of interesting peptides have been developed as artificial enzymes. Here our designed peptide, Adm-Trp-Arg-Arg-NH_2, named ATA was synthesized by the usual solid-phase Fmoc strategy[2] with trityl resin in Shimadzu-PSSM8 peptide synthesi-zer and purified by RP-HPLC.

The novel host molecule, FITC-ASP(NH-β-CD)$_2$, was obtained according to the route outlined as **Scheme 1.**

Scheme 1

$$\text{ASP} + \text{BOC-ON} \xrightarrow{a} \text{BOC-ASP(OH)}_2 \xrightarrow{b} \text{TFA-ASP(NH-β-CD)}_2 \xrightarrow{c} \text{FITC-ASP(NH-β-CD)}_2$$

a. BOC-ON (2-t-butoxycarbonyloxyimino-2-phenylacetonitrile, 1 equiv.), NEt_3 (3 equiv.), H_2O (6ml), 15 minutes; b. DMF (15.4 ml), CH_2Cl_2 (0.4 ml), mono-6-NH_2-β-CD[3] (1 equiv.), 93 hour, TFA/H_2O (5/1, 2 ml), cooled diethyl ether (10 ml); c. NEt_3 (7 equiv.), FITC (1 equiv.), DMF (5 ml), H_2O (6 ml), 16 hour, room temp.; 21% (yield , 3 steps).

In order to obtain high purity samples for analytical purposes, the product can be subjected to column chromatography on silica gel using EtOAc/heptane as eluant. FITC-ASP(NH-β-CD)$_2$ obtained as a yellow solid was dried to constant weight and stored over P_2O_5 under vacuum. Anal. Calcd. for $C_{109}H_{154}N_4O_{74}S$: C, 47.84; H, 5.67; N,

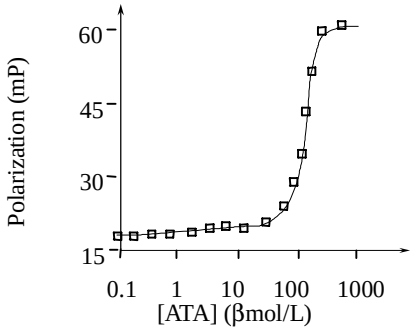
2.05; S, 1.17. Found: C, 47.93; H, 5.18; N, 1.97; S, 0.96. MALDI-TOF-MS, *m/z* 2737 for $[M+H]^+$, cacld. for $C_{109}H_{154}N_4O_{74}S$, M 2736.

ATA was diluted serially from 1000 μmol/L to 0.1 μmol/L in 16 test tubes in 190 μl of H_2O (PH = 7.5). Ten microliters of FITC-ASP-(NH-β-CD)$_2$ were added to each tube and incubated at room temperature for 10 minutes. Polarization values (mP) were measured for each sample on Beacon 2000 Analyzer. The equilibrium binding isotherm

$$\text{FITC-ASP(NH-β-CD)}_2 + \text{ATA} \underset{K_d}{\rightleftharpoons} \text{[FITC-ASP(NH-β-CD)}_2\text{]ATA}$$

(mP *vs*. [ATA]) is shown in **Figure 1**. The curve was fit by nonlinear regression using the Prism curve fitting software (Graphpad Software)[4]. The calculated dissociation constant (K_d) is equal to 5.0×10^{-6} mol/L. The binding isotherm was also analyzed by Scatchard analysis[4], which is sensitive to the presence of non-specific binding, positive or negative cooperativity, and multiple classes of binding sites. The calculated K_d from this method was 4.3×10^{-6} mol/L. The data fit best to a linear function, indicating that there is a single class of binding sites. From the measurement of ^{1}H-NMR spectra we observed the shift ($\Delta\delta$ = -0.029) of the FITC-ASP (NH-β-CD)$_2$ 3-H protons in the presence of ATA, which gave further evidence for the formation of inclusion compound of FITC-ASP(NH-β-CD)$_2$ with the peptide as a guest.

Figure 1 Equilibrium binding isotherm for FITC-ASP(NH-β-CD)$_2$/ATA interaction



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