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ORIGINAL ARTICLE

Preparation of a cyclodextrin dimer linked with a bis(picolinyl)cystine moiety and its intra- and intermolecular inclusion behavior

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Abstract A novel cyclodextrin (CD) dimer linked with a bis(picolinyl)cystine (Cys) moiety was prepared by the coupling of Boc-protected Cys with amino-modified CDs, followed by deprotection of the Boc groups and bispicolinylation. The dimer showed less affinity to an organic guest molecule compared to that of a native CD monomer. It was attributed to an intramolecular inclusion of the pyridine moiety into CD cavity. The dimer caused significant increase of its organic guest affinity by an addition of a copper ion. The included pyridine group may come out of a CD cavity to bind the copper ion and the two CDs included cooperatively and intermolecularly a guest molecule with high affinity.

Keywords Cyclodextrin dimer · Cystine · Picolinylamido group · Copper ion

Introduction

Cyclodextrin (CD) is a well-known cyclic oligosaccharide as a host molecule and its inclusion properties has been studied widely [1]. However, the inclusion by a native CD is less efficient and also less specific, when we compare it to natural systems. One of the ways to improve CD's inclusion ability is an introduction of the other CD molecule as an additional binding site. It has been demonstrated that a CD dimer that possesses suitable structure binds a guest molecule as strongly as an antibody binds its antigen [2]. As the next stage of study on CD dimers it is quite important to control its inclusion ability by a regulatory signal. On this view point Liu reported that an oligoethyleneoxide-linked CD dimer changed its guest affinity by flexibility change of the oligoethyleneoxide tether caused by its metal coordination [3]. Breslow [4] and Reinoudt [5] reported excellent photocontrol of guest inclusion by dimers respectively. In this report we will present the study of cystine (Cys) linked CD dimer. Cys possessing two pairs of carboxyl groups and amino groups is the simplest unit suitable for constructing a site for guest binding and the other for regulatory molecule binding. Two CD molecules were introduced to carboxyl groups of Cys and picolinyl (2-pyridinecarbonyl, PyCO) groups were attached to the amino moieties to prepare compound **1**. The hydrophobic picolinyl group can be intramolecularly included by a CD moiety attached to the carboxyl groups of the **1** as seen in the cases of self-inclusion reported so far [6]. Here affinity to an outer guest should be less than that of native CD. The bis(picolinylamido (PyCONH)) group coordinates a divalent metal ion such as Cu^{2+} to form a stable tetradentate 1:1 complex [7]. As a results of Cu^{2+} coordination CD cavity becomes vacant to include an outer guest. Accordingly unique switching intra- and intermolecular inclusion state of CD dimer is expected. Here preparation of dimer **1** and preliminary results of its binding behavior will be reported.

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Experimental

Genreral

β-CD was obtained from Japan Maize Products co. Ltd. Other chemicals were purchased and used as received without further purification. Thin layer chromatography (TLC) was run on a precoated silica-gel plate (Art 5554, Merck). Spot detection was carried out by an UV light and/or staining with 0.1% 1,3-naphthalenediol in ethanol–H_2O–H_2SO_4 [5:4:1 (V/V/V)] or 10% ninhydrin in acetone. A prepacked ODS column [LiChroprep RP-18, size B (25 × 310 mm), Merck] was used for a low-pressure reversed phase column chromatography. High performance liquid chromatographic analyses of products were carried out with a J'sphere ODS-M80 (4 μm, 2.0 × 150 mm, YMC Inc.) and with a YMC-Pack Diol-60 (5 μm, 8.0 × 500 mm, YMC Inc.).

(Boc-Cys-NHCD) **2**

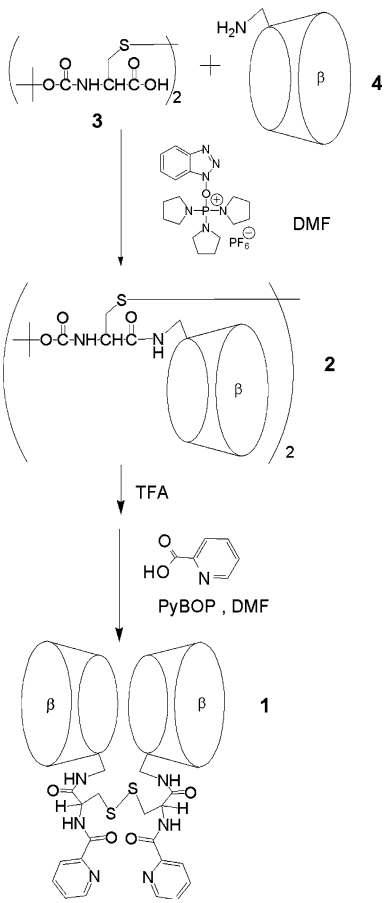
N,N′-Bis(Boc)-L-Cys {$(Boc\text{-}Cys)_2$} **3** (199 mg, 4.51×10^{-4} mol) and 6-amino-6-deoxy-β-CDx **4** [8] (1.20 g, 1.06×10^{-3} mol) were dissolved in anhydrous dimethylformamide (DMF) (6 cm^3). The reaction mixture was neutralized with *N,N*-diisopropylamine, followed by the addition of (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) (549 mg, 1.05×10^{-3} mol) under ice cooling. After stirring the reaction mixture at room temperature for 2 days, the solvent was evaporated off in vacuo and the residue was dissolved in water (500 cm^3). The solution was applied to a low-pressure reversed phase chromatography. After an elution with water (700 cm^3), a gradient elution from water (1500 cm^3) to 25% aq. CH_3OH (1500 cm^3) gave the desired compound **2** (892 mg, 73.8%); *Rf* value on TLC 0.07 [1-propanol/ethyl acetate/water/28% aq. NH_3 (3/3/2/1) (v/v/v/v)] ; t_R [column, J'sphere ODS-M80; gradient, 0–30% aq. MeCN (30 min); flow rate, 0.2 cm^3/min] 11.2 min, [column, Diol60; eluent, water; flow rate, 1.0 cm^3/min] 9.9 min; δ H (400 MHz, D_2O), 1.43 (18 H, s, CH_3), 4.60–4.95 [14H, C(1)H of CD], 7.39 (2H, brs, urethane NH], 7.60 (2H, brs, amidoNH); *m/z* (LSIMS) 2670.6 (M^+); Found C 40.95, H 6.28, N 1.90, S 2.54, Calcd. for $C_{100}H_{166}O_{74}N_4S_2 \cdot 13H_2O$ C41.32, H 6.18, N 1.90, S 2.54.

(PyCO-Cys-NHCD) **1**

The Boc-protected compound **2** (257 mg, 9.62×10^{-5} mol) was treated with trifuloroacetic acid (TFA) (2.0 cm^3) under ice cooling for 1 h. After evaporation off of TFA in vacuo, the residue was dissolved in dry DMF (2.0 cm^3) and the excess of TFA was neutralized by addition of *N,N*-diisopropylamine. Picolinic acid (30.0 mg, 2.43×10^{-4} mol) and PyBOP (120 mg, 2.30×10^{-4} mol) was added and pH was adjusted to 8.5 by addition of *N,N*-diisopropylamine under ice cooling. The mixture was stirred for 1 h and it was poured into acetone-ether (1/1 v/v) (50 cm^3). The precipitate was collected by centrifugation (3000 rpm, 10 min) and it was dissolved in water (100 cm^3). The solution was applied to a low-pressure reversed phase chromatography. After an elution with water (300 cm^3), a gradient elution from water (300 cm^3) to 40% aq. CH_3OH (300 cm^3) gave the desired compound **1** (180 mg, 69.9%); *Rf* value on TLC 0.36 [1-propanol/ethyl acetate/water/28% aq. NH_3 (1/1/1/1) (v/v/v/v)] ; t_R [column, J'sphere ODS-M80; gradient, 0–30% aq. MeCN (30 min); flow rate, 0.2 cm^3/min] 24.0 min, [column, Diol60; eluent, water; flow rate, 1.0 cm^3/min] 13.0 min; δ H (200 MHz, $[^2H_6]Me_2SO$), 4.70–4.90 [14H, C(1)H of glucose], 7.52–7.66 [2H, C(5)H of pyridine], 7.88 (2H, brd, urethane NH), 7.93–8.03 [4H, C(3)H and C(4)H of pyridine], 8.61 [2H, brd, C(6)H of pyridine], 8.91 (2H, brd, amido NH); *m/z* (FABMS) 2681.6 ($M+H^+$); Found C 42.99, H 6.35, N 3.02, S 2.78, Calcd. for $C_{102}H_{156}O_{72}N_6S_2 \cdot 9H_2O$ C43.07, H 6.17, N 2.95, S 2.25.

Results and discussions

Cys was modified to prepare **1** as shown in Scheme 1. *N,N′*-Bis(Boc)-L-Cys **3** was coupled with 6-amino-6-deoxy-β-CD **4** [8]. Firstly on a reaction by use of dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) those have been the most popular in situ condensing reagents, TLC analysis of reaction mixture showed appearance of a product with *Rf* 0.36 followed by the desired product with *Rf* 0.07. In order to complete the reaction, four days reaction by use of excess amount of coupling reagents (8.7 times as much as **3**) was needed. Chromatographic purification using a low pressure reversed phase column and also gel permeation column gave the amide **2** (29.6%). The low isolated yield was due to by-products which may be esters from the reaction of hydroxyl group of CD and carboxyl groups of **3**. In place of DCC-HOBt reagents we adopted (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) [9]. The reagent is more reactive and is often used for a coupling reaction of amino acids possessing bulky side chains. The reaction by use of PyBOP equimolar to Bis(Boc)-Cys **3** was completed sooner (2 h) and also it



Scheme 1

gave almost no by-products. Accordingly the desired **2** was easily purified by reversed chromatography in yield of 73.8%.

The purified compound **2** was treated with trifluoroacetic acid (TFA) for deprotection of the two Boc groups and reacted with picolinyl chloride. TLC analysis of the reaction mixture showed more than three products. Purification with a gel permeation chromatography gave the desired dimer **1** but its yield was low (37.8%). It suggested that in the reaction not only amino groups but also hydroxyl groups of CD molecule of Boc-deprotedcted **2** reacted with the corresponding acid chloride. In place of the acid chloride esterification, coupling of picolinic acid with the deprotedcted **2** by use of PyBOP was adopted. The reaction selectively gave the desired CD dimer **1** in isolated yield of 69.9%. The structures of all these products were determined with ^{1}H-NMR, mass spectrometric analyses and also elemental analyses.

On a ROESY spectrum of dimer **1**, NOEs between pyridine protons and CD H3 and H5 were observed (Fig. 1). It suggested that the CD cavity of the **1** was occupied by its pyridine moiety to form an intramo-

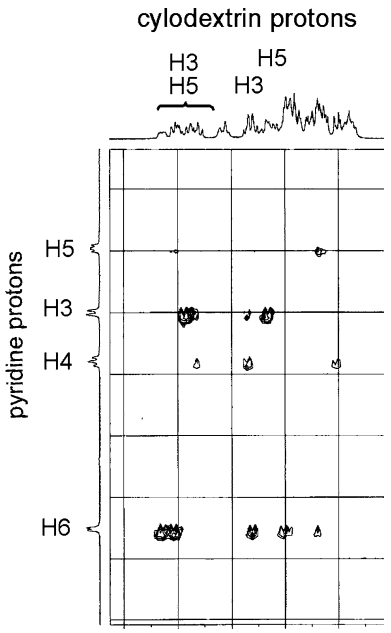


Fig. 1 ROESY spectrum (600 MHz, D_2O, mixing time 0.2s) of dimer **1**

lecular complex as we expected. We performed preliminary guest binding experiments of **1** by use of UV-Vis spectral titration. The results demonstrated that an association constant of methyl orange with dimer **1** was 500 M^{-1} while a native β-CD was 2790 M^{-1}. The observed lower affinity to the outer guest than that of native CD supported the self-inclusion state of **1** to inhibit competitively an outer guest inclusion. Addition of a copper ion to the **1** increased significantly its affinity to methyl orange. The association constant became 7420 M^{-1} that was 15 times larger than that of the self-included **1**. It suggested that the included pyridine group came out of a CD cavity to bind the copper ion and the two CDs included cooperatively and intermolecularly the guest molecule. Further binding experiments and complex structure analyses using NMR and circular dichlorism spectral analyses are underway in order to study inclusion behavior of CD dimer **1** possessing pyridine moieties for self-inclusion and also metal coordination.

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