Heptakis(2,3-di-*O*-carboxymethyl)-β-cyclodextrin as a pH-sensitive Host

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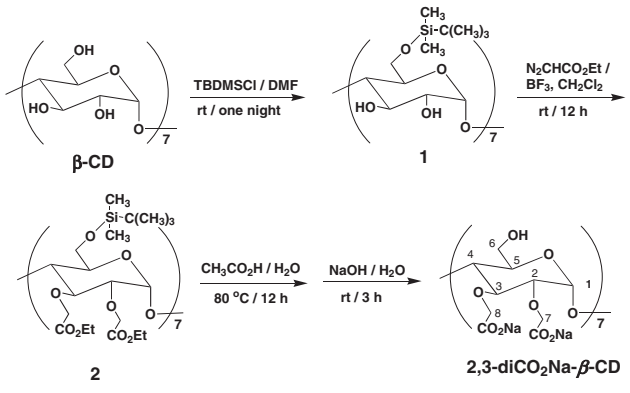
Acid-dissociation of heptakis(2,3-di-*O*-carboxymethyl)-β-cyclodextrin occurred between pD 2 and 6. At pD 2.0, 1-pyrenesulfonate was included into the host cavity with a binding constant of $2300 \pm 100\,M^{-1}$, while no complexation occurred at pD > 6.0. Much sharper pH-dependency in complexation proceeded with 1-anilino-8-naphthalenesulfonate and 2-(*p*-methylanilino)-6-naphthalenesulfonate.

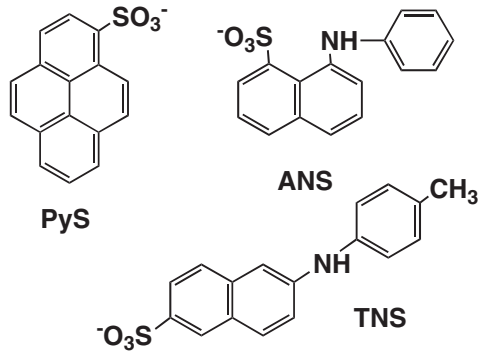
Native cyclodextrins (CDs) can act as substructures of functional host molecules. Among many modified CDs, polycationic and polyanionic CDs are very interesting because of their strong ability to form inclusion complexes assisted by Coulomb interactions with oppositely charged guest molecules. Heptakis(6-amino-6-deoxy)-β-CD in a protonated form (per-NH_3^+-β-CD) and heptakis(6-carboxymethylthio-6-deoxy)-β-CD in a dissociated form (per-CO_2^--β-CD) are well-known polycationic and polyanionic CDs,[1] respectively, which bind oppositely charged chiral guests enantioselectively.[2,3] Looking at these polyionic hosts from a different viewpoint, these hosts are expected to show pH-dependent inclusion phenomena. For example, per-CO_2H-β-CD might include an anionic guest into its cavity and pour out the guest when it dissociates to per-CO_2^--β-CD at higher pH. Unfortunately, however, neutral per-CO_2H-β-CD as well as per-NH_2-β-CD is insoluble in water. Heptakis(2,3-di-*O*-carboxymethyl)-β-CD (2,3-diCO_2H-β-CD) is one of the candidates of a pH-sensitive host which can dissolve in acidic aqueous solution. Although 2,3-diCO_2H-β-CD is a known material (CA Registry Number: 185150-02-1) and has been used as a chiral selector in capillary electrophoresis,[4] no details for synthesis have been disclosed. In this communication, we report the synthesis of 2,3-diCO_2H-β-CD and its interesting behavior as a pH-sensitive host for anionic guests.

A synthetic route is shown in Scheme 1. To a solution of heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-CD[5] (**1**, 3 mmol) in CH_2Cl_2 (25 mL) containing 10-drops of an ethereal solution of BF_3 (46%) was added ethyl diazoacetate (9.3 mmol) in CH_2Cl_2 within 3 h at $-10\,^\circ C$ and the mixture was stirred overnight at room temperature. The crude products were treated by silica gel column chromatography to isolate **2** (42% yield). TOFMS spectrum of **2** indicated that all secondary OH groups were transformed to the OCH_2CO_2Et groups. **2** (9.3 mmol) was dispersed in a mixed solution of ethanol and 70% aq acetic acid (2:3, 30 mL) and the solution was stirred overnight at $60\,^\circ C$. After the reaction, 1 M aq NaOH was added to the reaction mixture until the pH of the solution became 12.5 and the resulting solution was stirred for 3 h. After neutralization, the solvent was evaporated and the residues obtained were dissolved in water and dialyzed (Spectro/pro M.W.C.O.1000) to afford pure 2,3-di-CO_2Na-β-CD (58% yield).[6]



Scheme 1. Synthetic route of 2,3-diCO_2Na-β-CD.

In order to determine pK_a of 2,3-diCO_2H-β-CD, ^{1}H NMR spectral changes of this CD in D_2O containing 0.1 M NaCl were measured as a function of pD at $25\,^\circ C$. A sigmoid pD-titration curve measured by following the chemical shifts of the methylene protons at the 7-position (see Figure 1) of 2,3-diCO_2H-β-CD indicated that the dissociation of 2,3-diCO_2H-β-CD was initiated at pD 2 and completed at pD 6. The apparent pK_a value was ca. 5.



Since 2,3-diCO_2H-β-CD has fourteen hydrophilic CO_2H groups at a wider rim of β-CD, this CD can dissolve even in acidic aqueous solution. In acidic D_2O solution, 2,3-diCO_2H-β-CD included 1-pyrenesulfonate (PyS) into its cavity. Figure 1 shows ^{1}H NMR spectra (TSP as an external standard) of 2,3-diCO_2H-β-CD in D_2O at pD 2.0 (DCl) in the absence and the presence of PyS. In the absence of PyS, the methylene protons at the 7- (4.360 ppm, $J = 33$ and 17 Hz) and 8-positions (4.719 ppm, $J = 77$ and 16 Hz) of 2,3-diCO_2H-β-CD showed the AB patterns. Such a novel coupling pattern having extremely large coupling constants was also observed with the methylene protons of heptakis(3-*O*-ethoxycarbonylmethyl)-β-CD in CD_3OD.[1b] Upon complexation with PyS, the signals due to the methylene protons at the 7- and 8-positions shifted to 4.268 ($J = 39$ and 17 Hz) and 4.352 ppm (144 and 16 Hz), respective-

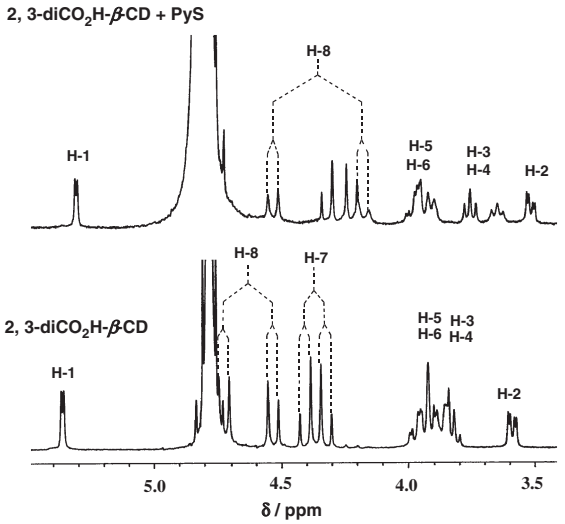


Figure 1. ^{1}H NMR spectra of 2,3-diCO_2H-β-CD (5×10^{-4} M) in D_2O containing 0.1 M NaCl in the absence and the presence of PyS (5×10^{-4} M) at pD 2.0 (DCl).

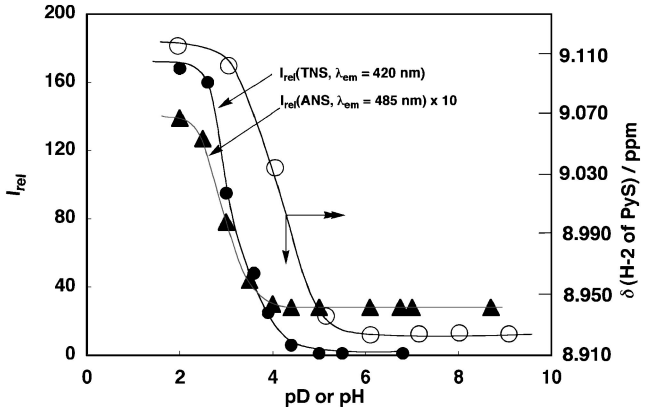


Figure 2. Changes in the chemical shift of the proton at the 2-position of PyS (5×10^{-4} M) in D_2O at pD 2.0 and in the fluorescence intensities of ANS (2×10^{-5} M) and TNS (5×10^{-6} M) in H_2O at pH 2.0 in the presence of 2,3-diCO_2H-β-CD and/or 2,3-diCO_2^--β-CD as a function of pD or pH at 25 °C. The initial concentrations of 2,3-diCO_2Na-β-CD were 2.5×10^{-3}, 5×10^{-4}, and 1×10^{-4} M for PyS, ANS, and TNS, respectively. ANS and TNS were excited at 400 and 335 nm, respectively.

ly. Upfield shifts were observed with the protons at the wider rim of 2,3-diCO_2H-β-CD and slight downfield shifts were measured with the protons at the narrower rim. These results definitely indicate that the PyS molecule is bound to the OCH_2CO_2H group side of 2,3-diCO_2H-β-CD. Job's plot for ^{1}H NMR signals of 2,3-diCO_2H-β-CD revealed the formation of a 1:1 complex. The binding constant (K) for complexation of PyS with 2,3-diCO_2H-β-CD was determined from ^{1}H NMR titration to be $2300 \pm 100\ M^{-1}$ in D_2O containing 0.1 M NaCl at pD 2.0 (DCl) and 25 °C. 2,3-diCO_2H-β-CD poured out PyS as increasing pD as shown in Figure 2, where changes in the chemical shift of the proton at the 2-position of PyS are shown as a function of pD. The chemical shift at pD > 6.0 was the same as that of PyS in the absence of the CD, indicating that PyS was not bound to 2,3-diCO_2^--β-CD at all at pD > 6.0.

In order to generalize pH-dependent inclusion of 2,3-diCO_2H-β-CD, 1-anilino-8-naphthalenesulfonate (ANS) and 2-(*p*-methylanilino)-6-naphthalenesulfonate (TNS) were used as the guests. Both fluorophores are well known as hydrophobic fluorescent probes. Figure 2 shows the relative fluorescence intensity changes of ANS and TNS in aqueous solutions containing 2,3-diCO_2H-β-CD and/or 2,3-diCO_2^--β-CD as a function of pH. In acidic solution at pH 2, the fluorescence intensity of TNS was much larger than that of ANS. This is reasonably interpreted in terms of the difference in K values. The size of TNS is suitable to be included into the CD cavity.[7] The K values of ANS and TNS for complexation with 2,3-diCO_2H-β-CD, which were determined in D_2O at pD 2.0 (DCl) containing 0.1 M NaCl from the ^{1}H NMR titration, were 71 ± 4 and $6300 \pm 400\ M^{-1}$, respectively. In both cases, sharp pH-dependency was observed. The acid-dissociation of 2,3-diCO_2H-β-CD causes electrostatic repulsion between the CO_2^- groups of the host and the anionic host and guest leading to ejection of the guest from the CD cavity. The pH-dependency curves shown in Figure 2 do not necessarily correspond to the pH-titration curve for determining pK_a of 2,3-diCO_2H-β-CD. The pH-sensitivity in ejection of a guest from the CD cavity should depend on the nature of the guest. PyS has a larger hydrophobic part as compared with ANS and TNS. Therefore, the PyS molecule might tend to resist the ejection from the cavity of partially dissociated 2,3-diCO_2H-β-CD.

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References and Notes

1 a) K. Tsujihara, H. Kurita, and M. Kawazu, *Bull. Chem. Soc. Jpn.*, **50**, 1567 (1977). b) F. Guillo, B. Hamelin, L. Jullien, J. Canceill, J.-M. Lehn, L. De Robertis, and H. Driguez, *Bull. Soc. Chim. Fr.*, **132**, 857 (1995). c) A. Gadelle and J. Defaye, *Angew. Chem., Int. Ed. Engl.*, **30**, 78 (1991).

2 T. Kitae, T. Nakayama, and K. Kano, *J. Chem. Soc., Perkin Trans. 2*, **1998**, 207; K. Kano, S. Arimoto, and T. Ishimura, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 1661.

3 a) K. Kano and H. Hasegawa, *J. Am. Chem. Soc.*, **123**, 10616 (2001). b) K. Kano, H. Hasegawa, and M. Miyamura, *Chirality*, **13**, 474 (2002).

4 For example; a) H. Jin, F. Li, J. Gu, R. Fu, Y. Liu, and R. Dai, *Fenxi Huaxue*, **24**, 1387 (1996); CAN 126:162355. b) H. Wang, J. L. Gu, F. H. Fang, J. D. Ji, H. D. Tian, and R. N. Fu, *Anal. Chim. Acta*, **359**, 39 (1998).

5 K. Takeo, H. Mitoh, and K. Uemura, *Carbohydr. Res.*, **187**, 203 (1989).

6 Analytical data for 2,3-diCO_2Na-β-CD: IR (KBr): 3418, 2932, 1607, 1427, 1331, 1030 cm^{-1}. ^{1}H NMR (400 MHz, D_2O, TSP) δ 3.586 (dd, 1H, $J = 9.0$ and 3.0 Hz), 3.794–3.979 (m, 4H), 4.360 (dd, 2H, $J = 33$ and 17 Hz), 4.719 (dd, 2H, $J = 77$ and 16 Hz). Anal. Calcd. for $C_{70}H_{77}O_{63}Na_{14} \cdot 18H_2O$: C, 32.59; H, 4.69%. Found: C, 32.38; H, 4.48%.

7 M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, **98**, 1875 (1998).

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