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ORIGINAL ARTICLE

^{1}H NMR titration study of stimuli-responsive supramolecular assemblies: inclusion complexes between PEG–*b*-PEI copolymer-grafted dextran and naphthalene-appended γ-cyclodextrin via double-strand inclusion

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Abstract We have previously prepared a stimuli-responsive inclusion complex between PEG–*b*-PEI–*g*-dextran graft copolymer (PEG–PEI–dex) and γ-cyclodextrin (γ-CD) in order to investigate unique inclusion phenomena, double-axle inclusion. For further study, a γ-CD derivative, mono-6-*O*-(2-sulfonato-6-naphthyl)-γ-CD (SN-γ-CD) was additionally synthesized for ^{1}H NMR titration study, which is expected to induce the competition of pendant naphthyl group with external polymer guests. Consequently, ^{1}H NMR titration results of the inclusion complex of PEG–PEI–dex with SN-γ-CD showed stoichiometric changes, temperature-dependence, and reversibly pH-responsive properties of the inclusion complexes in terms of chemical shift variation.

Keywords Cyclodextrin · Diblock copolymer · ^{1}H NMR titration · Inclusion complex · Stimuli-responsive network · Double-strand inclusion

Introduction

After discovering the inclusion complex formation of polymers with cyclodextrins (CDs) [1, 2], supramolecular assemblies based on inclusion complexes between CDs and polymeric guests have been constantly developed in many fields of applications such as biomedical materials [3], multivalent recognitions [4], and so on. Topologies of the assemblies for such goals are mainly classified into some types containing polypseudorotaxanes, polyrotaxanes and polycatenanes. Of the supramolecular assemblies, inclusion of double chains into γ-cyclodextrin (γ-CD) retains infinite possibility applicable to complicated supramolecular architectures. For the last a few years, our group have systematically proceeded to develop polymeric guests for inclusion into γ-CD and assembling systems by their complexes, containing PEG [5] and poly(ethylenimine) (PEI) [6], and their copolymers [7]. However, characterizing the designed structures in aqueous media was always a burden to our studies because of the absence of chromogenic moieties for spectroscopic analysis.

Photo-active groups have been attached to CDs to obtain photo-responsive host molecules. Suitable size of the pendant groups on CDs makes it to be included as a guest molecule into the cavity of CDs, forming self-included hosts [8], mutual included dimers [9], or supramolecular polymers [10]. To the pendant group-appended CD derivatives, the addition of external guest molecules induces competition with the pendent groups for including into the cavity of CDs. Such positional changes of pendent groups in inclusion phenomena allow spectral changes to be detectable in aqueous media by various spectroscopic methods. This approach can provide strategic solution to understand the inclusion phenomena of polymeric guests with CDs.

In this study, we report on the results for the formation and stimuli-dependence of characteristic inclusion complexes between PEG–PEI–dex and γ-CD derivative (SN-γ-CD) in various conditions by ^{1}H NMR titration. Using SN-γ-CD, we can analyze

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threading behaviors of PEG–PEI–dex into the cavity of γ-CD by the NMR titration that shows chemical shifts of the naphthyl group on SN-γ-CD. With the NMR titration results, we discuss that the inclusion complexes are dependent on changes of temperature, pH, and composition of γ-CD with polymers.

Experimental part

Synthesis of PEG–*b*-PEI–*g*-dextran (PEG–PEI–dex)

Poly(ethylene glycol) 600 monotosylate (PEGOTs) was previously synthesized with Ag_2O, followed by column separation. Using the obtained PEGOTs as a macroinitiator, PEG–*b*-POz copolymer was polymerized from 2-ethyl-2-oxazoline according to our reported method [7], which gives yellow oil (PEG–POz), after complete drying (84% yield). The resulting PEG–POz copolymer was added to HCl solution (35%, 25 mL) and stirred for 24 h at 100°C. The mixture gave PEG–PEI chain (5.2 g, 82% yield) by neutralizatng with NaOH and then lyophilizing. For the formation of a polymeric network, an activated dextran [4] (0.05 mmol) and the PEG–PEI chain (0.85 mmol) were dissolved in DMSO (50 mL) and stirred at room temperature for 48 h, followed by dialysis (MWCO = 15,000) and freeze drying to give pale yellow crystal (DS = 2.5%, 68 % yield). PEG–POz: ν_{max}(NaCl)/cm^{-1} 3444 (OH), 3333 (NH), 1644 (CO), 1541 (NH) and 1064 (OH); δ_H (300MHz, $CDCl_3$, Me_4Si) 3.58 (4H × 12, m, CH_2CH_2O), 3.40 (4H × 14, s, CH_2CH_2N), 2.40 (2H, m, $COCH_2CH_3$), 0.93 (3H, m, $COCH_2CH_3$); δ_c (75.5 MHz, $CDCl_3$) 173.8 (1, d, NCO), 71.8 (1, OCH_2CH_2OH), 70.0–69.8 (2, CH_2CH_2O), 67.9 (1, OCH_2CH_2N) 60.8 (1, CH_2OH), 44.7 (2, CH_2CH_2N), 42.5 (1, CH_2NH_2) 25.2 (1, $COCH_2CH_3$), 8.7 (1, $COCH_2CH_3$). PEG–PEI–dex: δ_H (300 MHz, DMSO-d_6) 4.81 (1H × 328, d, C_1 of dextran), 3.59–3.11 (4H × 328, C_3, C_5 and C_5 of dextran) 3.53 (4H × 12, CH_2CH_2O), 3.45 (4H × 14, CH_2CH_2N), 3.41–3.31 (2H × 328, C_2 and C_4 of dextran).

Preparation of mono-6-*O*-(2-sulfonato-6-naphthyl)-γ-CD (SN-γ-CD)

First, we modified γ-CD with bulky group to give mono-6-(*O*-2,4,6-triisopropylbenzenesulfonyl)-γ-CD according to a literature [11]. The γ-CD derivative (135 mg, 93.2 μmol) was allowed to react with the disodium salt of 2-sulfonato-naphthalene-6-ol (28.6 mg, 100 μmol) in 2 mL of DMSO at 80°C for 6 h under N_2 atmosphere. After precipitation with acetone, the salt was removed by filtration after selective dissolution of the product in DMF. After DMF was evaporated off, the product was dialyzed (MWCO = 1,000) and freeze-dried to obtain SN-γ-CD (85% yield): δ_H (300 MHz, D_2O) 8.40 (1H, H-1 of naphthyl region), 7.91–8.10 (3H, H-3, -4, and -8 of naphthyl region), 7.29–7.46 (2H, H-5 and -7 of naphthyl region), 5.18 (1H × 8, H-1 of γ-CD), 4.36 (1H, H-6 of naphthyl group-appended glucose in γ-CD), 4.23 (1H, H-5 of naphthyl group-appended glucose in γ-CD), 3.81–4.11 (3H × 22, H-2, -5, and -6 of γ-CD), 3.77–3.55 (2H × 15, H-3 and -5 of γ-CD).

^{1}H NMR titration of inclusion complexation between PEG–PEI–dex and SN-γ-CD

All NMR spectra were recorded by a 300 MHz spectrometer (Varian, Unity plus). For NMR titration, PEG–PEI–dex and SN-γ-CD was dissolved in D_2O with adjusting desired pH by using 1 wt% DCl and NaOD solution. The solution of PEG–PEI was prepared as a reference sample in the same manner. 3-Trimethylsilylpropionic acid (TPA) was added to all of solutions as an external reference. Previously, solutions of SN-γ-CD were measured to calibrate concentration-dependence of SN-γ-CD with diluting with D_2O at pH 10. For the preparation of inclusion complex, increasing concentrations of the PEG–PEI–dex solution, up to above stoichiometry for inclusion, were added to a designated solution of SN-γ-CD (13.4 mM). Final solution of the inclusion complex was titrated as increasing temperature from room to 80°C and repeatedly changing pH between 4 and 10, starting from 10. When all of solutions were changed, the mixtures were maintained with stirring for the enough time to reach to the equilibrium of the inclusion complexation.

Results & discussion

Synthesis of PEG–PEI–dex and SN-γ-CD

A linear bifunctional PEG–PEI was synthesized via an efficient route containing cationic ring-opening polymerization of 2-oxazoline using PEGOTs as a macroinitiator, termination with amino groups for bifunctionality, and hydrolysis for the removal of *N*-acyl groups. The prepared PEG–PEI was grafted onto dextran according to our method [12, 13]. All the

polymers were characterized by ^{1}H and ^{13}C NMR, IR, and GPC, showing clear data for chemical structure of the polymers.

Mono-6-*O*-(2-sulfonato-6-naphthyl)-γ-CD (γ-CD-NS) was prepared through a simple route reported by Ueno et al. [14]. According to the literature, they could obtain highly pure mono-6-(*O*-2,4,6-triisopropylbenzenesulfonyl)-γ-CD in good yield without column process, while common monosulfonyl γ-CDs give some byproducts such as di- and tri-adducts, which required to be separated by methods such as ion-exchange columns. The CD derivatives in synthetic process could be characterized by ^{1}H NMR. The sulfonylation for the CD derivative was confirmed by the presence of the signals from protons in triisopropyl benzene sulfonyl group and the chemical shifts of signals from the protons in 6-sulfonated glucose ring. In addition, the integration of signals from phenyl region and H-1 proton of γ-CD supports the mono-sulfonylation on γ-CD. SN-γ-CD could be obtained in high yield (85%) by using sulfonated naphthalene sodium salt.

Concentration-dependence of SN-γ-CD

To evaluate the stoichiometry of SN-γ-CD aggregates, the variation in chemical shifts as a function of concentration was investigated. Figure 1(a) shows ^{1}H NMR spectra for concentration-dependent chemical shifts of SN-γ-CD. The signals from H-5 proton of naphthyl group were downfield shifted as decreasing concentration. Coinciding with the case of β-CD [15], the concentration (C_{tot})-dependent ^{1}H NMR signal from the H-5 proton in naphthyl group of SN-γ-CD fitted well to the dimerization scheme (Equation 1). From the NMR data, it is concluded that the structure of SN-γ-CD in aqueous media is the inclusion complex that is stabilized by the mutual inclusion of the pendant groups into the γ-CD cavities of counter molecules.

$$\delta_{obs} = \delta_{mon}\frac{\sqrt{8K_D C_{tot}+1}-1}{4K_D C_{tot}} + \delta_{dimer}\frac{(4K_D C_{tot}+1)-\sqrt{8K_D C_{tot}+1}}{4K_D C_{tot}} \quad (1)$$

In addition, the concentration dependence of SN-γ-CD was investigated by another method [8] as well. The aggregation number, *n*, was obtained from the Equation 2:

$$\ln(C_{tot}\delta_{rel}) = \ln[C_{tot}(\delta_{agg}-\delta_{rel})] + \ln K + \ln(n) - (n-1)\ln\delta_{agg} \quad (2)$$

by plotting $\ln(C_{tot}\delta_{rel})$ against $\ln[C_{tot}(\delta_{agg}-\delta_{rel})]$. The chemical shift of the monomer was obtained from the intercept of plots of chemical shift versus concentration of SN-γ-CD as well as from the chemical shift of the dimer from the intercept of plots of chemical shifts as a function of the inverse concentration of SN-γ-CD. Signals gave narrow range of *n* values varying from 1.8 to 2.1, strongly suggesting a dimer structure.

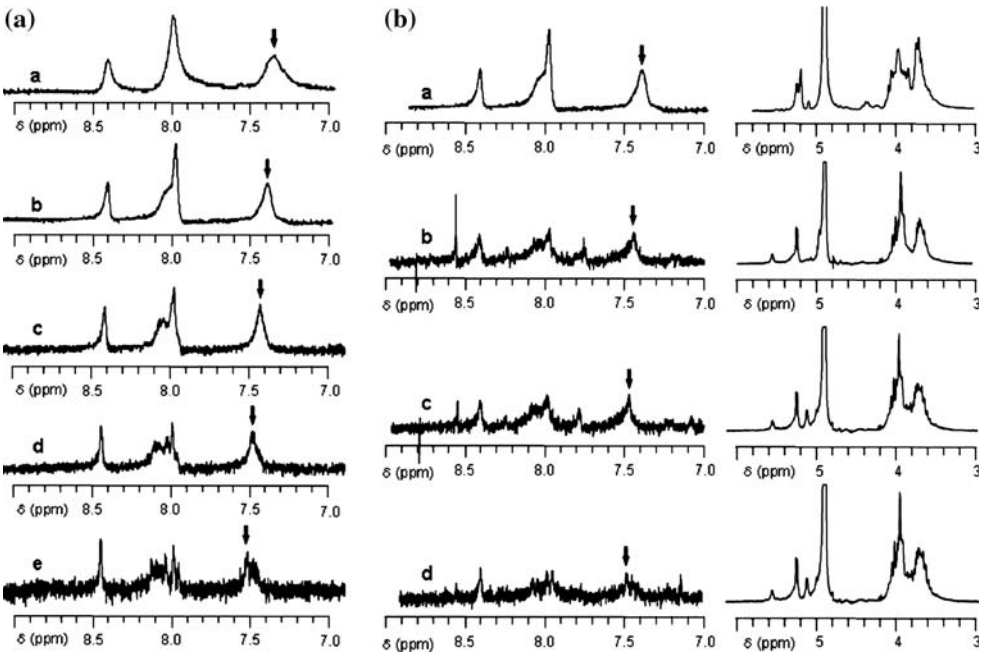

Fig. 1 ^{1}H NMR spectra of the naphthyl and aliphatic regions in SN-γ-CD and the inclusion complex with polymers. (**a**) NMR spectra for naphthalene group dependent on different concentrations of SN-γ-CD with a: 47, b: 14, c: 7.1, d: 3.6, and e: 1.8 mM in D_2O at 25°C. (**b**) NMR spectra for a: SN-γ-CD solution (13.4 mM), b: a inclusion complex of PEG–PEI and SN-γ-CD, inclusion complexes of PEG–PEI–dex copolymer and SN-γ-CD at c: 50°C and d: 80°C

^{1}H NMR titration of the inclusion complex between SN-γ-CD and PEG–PEI–dex

Although the SN-γ-CD exhibits strong fluorescence and positive dichroic peaks by fluorescence and circular dichroism spectroscopy, the signals coincide with that of the monomeric form of other similar models, in which the naphthyl group is outside of the of γ-CD cavity, which eventually is not suitable for studies on inclusion complexes. On the contrary, NMR studies can be carried out at high concentration in which SN-γ-CD prefer to the formation of a head-to-head dimer.

Figure 1(b) shows ^{1}H NMR spectra of inclusion complexes of SN-γ-CD with PEG–PEI–dex and PEG–PEI in D_2O at different temperature. In aliphatic region, changes in peaks were shown due to the presence of polymers. And, chemical shifts from H-5 proton of naphthyl group in the inclusion complexes showed downfield shifts, compared to that in only SN-γ-CD without copolymers. This indicates that naphthyl groups of SN-γ-CD were dethreaded from γ-CD cavity due to the threading of PEG–PEI chains and increase in temperature. For the inclusion complexe of SN-γ-CD, external guest molecules compete with the appended naphthyl group for inclusion into γ-CD cavity, which make the CD derivatives responsive to the guest. SN-γ-CD molecules form the head-to-head dimer that is stabilized by the mutual inclusion of the pendant groups into the γ-CD cavities of counter molecules [16]. However, the dimerization of SN-γ-CD can be disaggregated to the monomeric form by the addition of other guests because of loose fitting of the naphthyl group inside γ-CD cavity. The structure of the monomeric form has pendant naphthyl group outside of the γ-CD cavity. It can be inferred that the association constant for the inclusion between suitable external guest and γ-CD is higher than that for the dimerization of SN-γ-CD by very loose and weak mutual inclusion [15]. In our study, the addition of the PEG–PEI–dex or PEG–PEI copolymers as external guest molecules could induce the excluding of the naphthyl group from γ-CD cavity. As a result, γ-CD with wide space of the cavity includes double strands of the PEG–PEI copolymer instead of pendant naphthyl group, resulting in spectral changes in naphthyl group.

NMR titration study was performed to monitor the formation and stimuli-responsive property of the inclusion complexes of SN-γ-CD with PEG–PEI–dex or PEG–PEI in D_2O under various conditions. Figure 2 shows changes in chemical shifts from H-5 proton in naphthyl group as the feed of polymers and temperature. In Fig. 2(a), the variations in chemical shifts increased as adding the copolymers. This result indicates that the added copolymers act as external guests to induce the exclusion of naphthyl group to the outside of γ-CD cavity, resulting in downfield shifts of the signals. Also, the addition of PEG–PEI gave bigger changes in chemical shifts than that of PEG–PEI–dex. The differences can be easily inferred by considering their structures in inclusion complexation. In the case of PEG–PEI–dex, the number of SN-γ-CD for inclusion was fewer than in the case of PEG–PEI because of the presence of dextran backbone. Figure 2(b) shows variations of the chemical shifts as temperature. As increasing temperature, signals from naphthyl groups were downfield shifted, indicating all guest molecules threaded into γ-CD cavity were excluded to the outside of the cavity. This also means that dimers of SN-γ-CD were remained after maximum formation of the inclusion complexes.

The inclusion complexes of SN-γ-CD and copolymers were reversibly dependent on pH variation. The pH-dependence for the homodimerization of SN-γ-CD was excluded for discussing on pH-reversible phenomena of competitive inclusion between external polymer guest and pendant guest because 2-sulfonato-6-naphthyl group has little effect on the inclusion to the γ-CD cavity. Figure 3 shows the reversible profile of

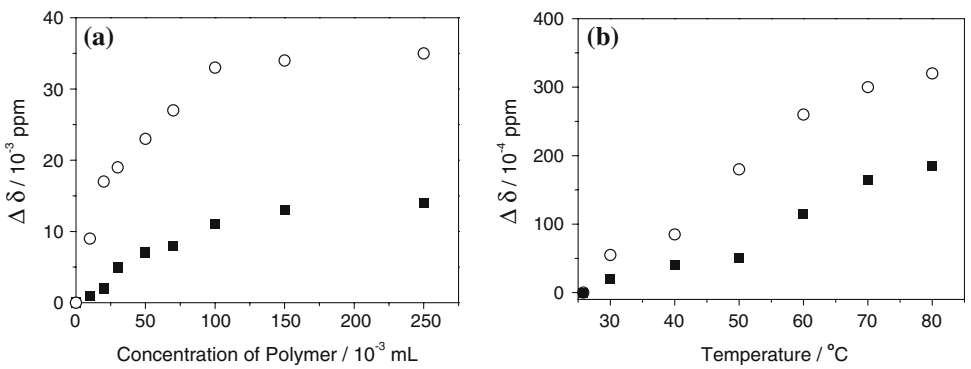

Fig. 2 ^{1}H NMR titration of the inclusion complexation between PEG–b-PEI–g-dex and SN-γ-CD. (**a**) as increasing PEG–b-PEI–g-dex and (**b**) as increasing temperature. Opened circle: PEG–PEI and closed square: PEG–PEI–dex

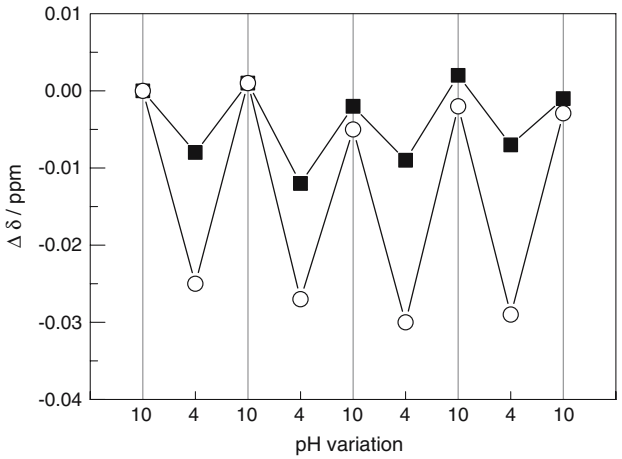

Fig. 3 Reversible properties of the inclusion complex between PEG–PEI–dex and SN-γ-CD on pH, which was shown by the variation of chemical shifts versus repeated changes in pH from 10 to 4. Closed squares: PEG–PEI–dex and SN-γ-CD, opened circles: PEG–PEI and SN-γ-CD

the inclusion complex under repeated pH changes. In similar manner, larger variations in chemical shift for PEG–PEI was shown, compared with those for PEG–PEI–dex. The values of the variation for PEG–PEI and PEG–PEI–dex were roughly consistent with the maximum value under the changes of polymer concentration and temperature, although the values were lower. This result demonstrates that the number of SN-γ-CD molecules dethreaded by the protonation of PEI under lowering pH to 4 is fewer than that by increasing temperature. Also, the reversible changes of the values showed a little deviation, confirming the presence of PEG chain. In the network structure by inclusion complexes of PEG–PEI–dex, SN-γ-CD can be still threaded on neutral PEG chain even under pH 4. Therefore, in higher pH (~10), SN-γ-CD can be threaded anywhere onto double strands of both blocks of neutral PEG–PEI copolymer, which gives relatively tight network, while the network is looser at lower pH (~4). This result suggests that supramolecular networks between PEG–PEI–dex and SN-γ-CD alter their structures by movement of SN-γ-CDs under pH variation, which such phenomena can be inferred in the case of γ-CD as well (see Chart 1 for better understanding).

Conclusions

The inclusion complexes between SN-γ-CD and PEG–PEI–dex were formed and investigated by ^{1}H NMR titration. Although the naphthyl group appended to SN-γ-CD can compete with external PEG–PEI chains for inclusion into γ-CD cavity, the dimer of SN-γ-CD was disaggregated by threading of external guests. Accordingly, the formation of inclusion complexes could be observed as the amounts of polymer, temperature, and pH changes. Also, the inclusion complexes showed reversible changes in chemical shifts under repeated pH variations, causing deformation in the network structure. After this, it is expected that this NMR titration method and the use of SN-γ-CD can be utilized as a useful tool to monitor a great variety of inclusion complexes with aliphatic chains.

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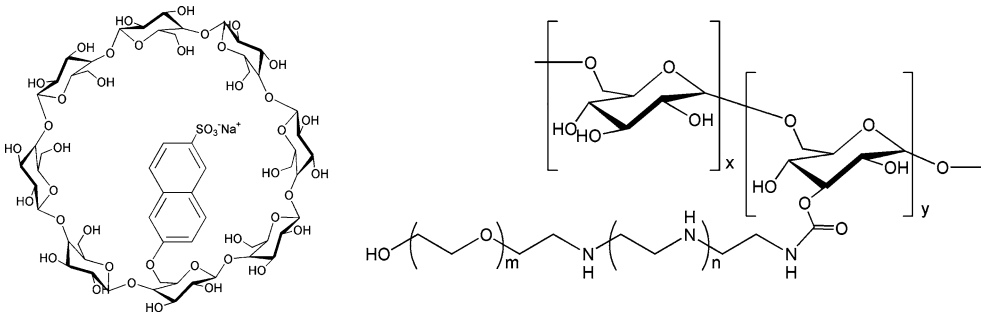
Chart 1 Chemical structures of SN-γ-CD and PEG–PEI–dex



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