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ORIGINAL ARTICLE

Preparation of polypseudorotaxane consisting of fluorescent molecule-modified β-cyclodextrins and biotin-terminated poly(propylene glycol) with high yield

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Abstract A polypseudorotaxane was prepared using 4-(*N*,*N*-dimethylamino) benzoyl modified β-cyclodextrin (DMAB-β-CD) and biotin-terminated poly(propylene glycol) (biotin-terminated PPG) in water for 7 days. When the biotin-terminated PPG was added to a saturated solution of DMAB-β-CD at room temperature, the polypseudorotaxane was not obtained. However, with increasing temperature to 60 °C, precipitation was observed. The ^{1}H-NMR spectrum indicated that the obtained precipitate was the polypseudorotaxane. The final yield was 54%, which was higher than that previously reported for polypseudorotaxane formation using chemically modified β-CD. The polypseudorotaxane can be useful to make a polyrotaxane via avidin–biotin molecular recognition using the terminal biotin group.

Keywords Cyclodextrins · Polypseudorotaxanes · Fluorescence · Avidin–biotin

Introduction

Fluorescent molecule-modified β-cyclodextrins (β-CDs), such as 4-(*N*,*N*-dimethylamino) benzoyl β-CDs (DMAB-β-CDs) has been studied as a molecular sensor that changes fluorescent properties via forming a self-inclusion complex [1]. The DMAB moiety of DMAB-β-CD is included into the β-CD cavity in water, and DMAB-β-CD shows twisted intramolecular charge transfer (TICT) dual fluorescence [2]. When another guest molecule is included into the cavity and the DMAB moiety is exposed to outer aqueous medium, magnitude of TICT dual fluorescence decreases. However, TICT dual fluorescence of DMAB-β-CD decreases only when guest molecules can be incorporated into the cavity of β-CD, and the decrease of fluorescence reflects stimulus of guest molecules.

In recent years, CD-based polyrotaxanes as new polymeric materials have attracted much attention [3]. β-CD-based biodegradable polyrotaxanes have been focused as a new fluorescent system [4]. In this molecular design, β-CDs are threaded onto a poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (PEG-*b*-PPG-*b*-PEG) capped with α-CD via pH-cleavable hydrazone bond. All the β-CDs are released only when one or two terminal hydrazone bonds are cleaved, meaning degradation-responsive exposure of β-CD cavities. 6-(*p*-toluidinyl) naphthalene-2-sulfonate (TNS) has been used as a model guest molecule for β-CD. Inclusion complexation between β-CD and TNS in aqueous media increases the magnitude of fluorescence [5]. When TNS exists in aqueous medium, the exposed β-CD cavities can include TNS, resulting in increased fluorescent intensity. However, the fluorescent intensity is quite low because the concentration of the released β-CDs is low, and therefore, both β-CD and the fluorescent molecule are diluted to reduce the chance to form an inclusion complex. In order to enhance the

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fluorescent intensity change, TICT change of DMAB-β-CD is a good candidate for the development of β-CD-based polyrotaxane fluorescent system. In our approach, DMAB-β-CD is firstly threaded onto a biotin-terminated PPG chain and then capped with avidin–biotin interaction that has been known as non-covalent bond interactions and used for biochemical application. This interaction is the strongest [6], and the avidin–biotin complex is dissociated by the addition of large free biotin or oxidizing reagents [7]. However, most of the DMAB-β-CD molecules are expected to exist as the self-inclusion complexes in aqueous medium, which is unfavorable to the threading of the PPG chain into the β-CD cavity [8]. From these points of view, threading of DMAB-β-CD molecules to the PPG chain is a key process. In this paper, the formation of polypseudorotaxane consisting of DMAB-β-CDs and biotin-terminated PPG was examined to find suitable preparative conditions.

Experimental

Preparation of *N*-hydroxysuccinimidyl biotin ester **1**: D-biotin (0.4 g) was dissolved in hot DMF (12 mL, 60 °C), and the solution was cooled to room temperature. Subsequently, *N*-hydroxysuccinimide (0.2 g) and dicyclohexylcarbodiimide (DCC) (0.34 g) were added into the solution. After the mixture was stirred for 2 h, the precipitated dicyclohexylurea was removed by filtration. The filtered solution was evaporated in vacuo, and diethyl ether was added into the residual solution to precipitate the product. The filtered product was washed with diethyl ether, and dried in vacuo at room temperature. Yield 0.31 g (56%); ^{1}H-NMR (DMSO-d_6) δ (ppm) 1.43–1.7 (m, 6H), 2.58 (d, 1H), 2.67 (t, 2Ht), 2.83 (m, 1H), 2.81 (s, 4H), 3.08 (m, 1H), 4.15 (m, 1H), 4.31 (m, 1H), 6.37(s, 1H), 6.44 (s, 1H); MS (ESI): m/z = 341.92 (M^+), 363.90 ($M+Na^+$), 379.87 ($M+K^+$).

Synthesis of biotin terminated poly(propylene glycol) **2**: **2** was synthesized by a modified method of Grubber et al. [9]. PPG-bisamine (0.62 g) was dissolved in DMF (6.0 mL), and then **1** (0.3 g) and Et_3N (81 μL) were added to the solution. Stirring was continued at room temperature for 24 h. Unreacted **1** was deactivated by the addition of distilled water (6.0 mL) and stirring for 2 h. The solution was evaporated in vacuo, and the residue was dissolved in 200 mmol Na_2CO_3 (7.5 mL). Undissolved residue was removed by filtration. The filtrate was saturated with NaCl, extracted with CH_2Cl_2 and dried in vacuo. Yield 20 mg (2.6%); ^{1}H-NMR (DMSO-d_6) δ (ppm) 1.01 (d, 3H) 1.30–1.75 (m, 6H), 2.13 (t, 2H), 2.58 (d, 1H), 2.82 (q, 1H), 3.09 (m, 1H), 3.30–3.50 (m, H), 4.15 (m, 1H), 4.31 (m, 1H), 6.37(s, 1H), 6.44 (s, 1H), 7.60 (s, 1H).

Preparation of polypseudorotaxane consisting of DMAB-β-CDs and biotin terminated PPG **3**: DMAB-β-CD was prepared by the method of Osa et al. [1]. The DMAB-β-CD (45 mg) was dissolved in distilled water (0.75 mL), and then **2** (5 mg) was added to the solution. The mixture was stirred for 1, 3, 7 days and room temperature, 60 °C. The mixture was stirred for 1, 3, 7 days and room temperature, 60 °C. We observed whether precipitation occurred. If precipitation was observed, the precipitated product was collected by centrifuging, and then lyophilized. We obtained the precipitated product at 60 °C and 7 days. Yield 26.9 mg (54%); ^{1}H-NMR (DMSO-d_6) δ (ppm) 1.01 (d, 3H) 1.22 (s,), 2.58 (d, 1H), 2.85 (q, 1H), 2.95 (s, 6H), 3.20–3.83 (m, H), 4.45 (s, 1H), 4.82 (d, 1H), 4.92 (d, 2H), 4.93 (d, 2H), 5.50 (d, 1H), 5.71 (s, 1H), 6.68 (d, 2H), 7.65 (d, 1H), 7.80 (d, 2H).

Results and discussion

The synthetic route of **1**, **2** and **3** was shown in Scheme 1. Biotin was activated by condensation with *N*-hydroxysuccinimide using DCC as a condensation reagent to obtain *N*-hydroxysuccinimidyl biotin ester **1** [10]. The final purification of **1** was carried out by precipitation in excess amount of diethyl ether. The results of ^{1}H-NMR and ESI-MS indicated that the obtained **1** was successfully purified. The terminal amino groups of PPG-bisamine (JEFFAMINE® D-2000 polyoxypropylenediamine M_n = 2,000) were allowed to react with **1** to obtain biotin-terminated PPG **2**. The number of biotin in one **2** molecule was ca. 2.3, which was determined by the 4-hydroxyazobenzene-2-carboxylic acid (HABA) method [11]. This result indicates that both terminal of PPG possessed each one biotin.

Polypseudorotaxane formation between DMAB-β-CD and **2** was carried out in water. Since the driving force of polypseudorotaxane formation is mainly hydrogen bonding between CDs, the formation is often accompanied with precipitation because of column-like crystalline structure of the polypseudorotaxane [12]. However, in the case of chemically modified CDs, the precipitation would not be caused by the polypseudorotaxane formation [8], which is dependent upon the types of chemical modification and combination of linear polymer and CDs. When **2** was mixed with saturated aqueous solution of DMAB-β-CD at room temperature, the

precipitation was not observed even after 7 days. The 2D-ROESY NMR spectra of this reaction mixture showed that no cross-peaks between H(3, 5) protons of β-CD and methyl groups of PPG were observed, indicating that the threading of biotin-terminated PPG into the cavity of DMAB-β-CD did not occur (data not shown). On the other hand, precipitation was observed when the temperature of the reaction mixture was 60 °C after 7 days. This result suggests that an increase in the solution temperature and long reaction time contributes to enhancing the inclusion complexation between DMAB-β-CDs and **2**, followed by crystallization of polypseudorotaxane consisting of DMAB-β-CDs and biotin terminated PPG **3**. Figure 1 shows ^{1}H-NMR spectrum of **3**. All the peaks attributed to DMAB-β-CDs, PPG, and biotins were confirmed. The number of DMAB-β-CD units in **3** was calculated from the ^{1}H-NMR spectrum. The threading number of DMAB-β-CDs was calculated to be ca. 8 (stoichiometric number for the PPG segment: 17). The yield of **3** was 54%, which was extremely higher than the previous report of polypseudorotaxane formation between chemically modified β-CD and PPG [12]. Liu et al reported that the polypseudorotaxane formation between L-tryptophan-modified β-CD (Try-β-CD) and amino-terminated PPG chains [13]. However, the yield of the polypseudorotaxane was very low (6%). It is known that the Try-β-CD, as well as DMAB-β-CD, forms self-inclusion complexes in aqueous solution [14]. The authors indicated that this result might be the conformational equilibrium between the free and self-included conformer of Try-β-CD in the aqueous solution. With the addition of **2** at 60 °C, the DMAB moiety was exposed to outer aqueous medium via forming a complex with **2** to give **3**. Due to the self-inclusion ability of DMAB-β-CD, one can imagine that most of the DMAB-β-CDs exist as a self-included conformer which is unfavorable to the threading of PPG into the β-CD cavity. The result of precipitation with a longer reaction time at higher temperature suggests that the biotin-terminated PPG can be competitively included in the cavity of DMAB-β-CD, and increasing the threading number of DMAB-β-CD leads to crystallization of the polypseudorotaxane.

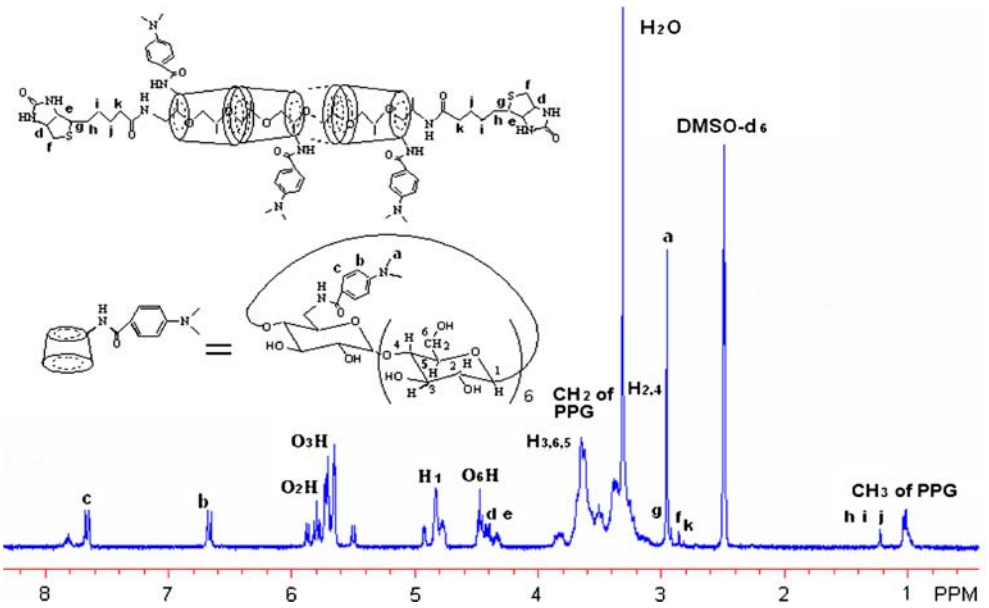
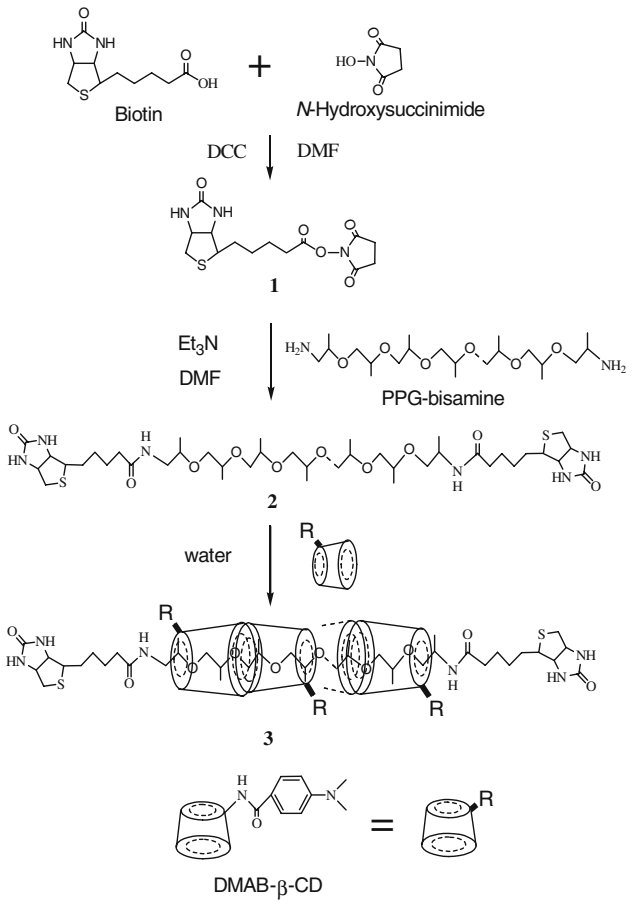


Fig. 1 ^{1}H-NMR spectrum of polypseudorotaxane consisting of DMAB-β-CDs and biotin terminated PPG **3** in DMSO-d_6



Scheme 1

Conclusion

A polypseudorotaxane consisting of DMAB-β-CD and biotin-terminated PPG was prepared in water at various conditions. Relatively high temperature and longer reaction time was needed to obtain the polypseudorotaxane with high yield. The preparation of a polyrotaxane using the polypseudorotaxane mixing with avidin will be reported our force coming paper (A. Ito et al., in preparation).

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References

1. Hamasaki, K., Ikeda, H., Nakamura, A., Ueno, A., Toda, F., Suzuki, I., Osa, T.: Fluorescent sensors of molecular recognition. Modified cyclodextrins capable of exhibiting guest-responsive twisted intramolecular charge transfer fluorescence. J. Am. Chem. Soc. **115**, 5035–5040 (1993)
2. Grabowski, Z.R., Rotkiewicz, K., Rettig, W.: Structural changes accompanying intramolecular electron transfer: focus on twisted intramolecular charge-transfer states and structures. Chem. Rev. **103**, 3899–4031 (2003)
3. (a) Cacialli, F., Wilson, J.S., Michels, J.J., Daniel, C., Silva, C., Friend, R.H., Severin, N., Samorì, P., Rabe, J.P., O'Connell, M.J., Taylor, P.N., Anderson, H.L.: Cyclodextrin-threaded conjugated polyrotaxanes as insulated molecular wires with reduced interstrand interactions. Nat. Mater. **1**, 160–164 (2002); (b) Wilson, J.S., Frampton, M.J., Michels, J.J., Sardone, L., Marletta, G., Friend, R.H., Samorì, P., Anderson, H.L., Cacialli, F.: Supramolecular complexes of conjugated polyelectrolytes with poly(ethylene oxide): multifunctional luminescent semiconductors exhibiting electronic and ionic transport. Adv. Mater. **17**, 2659–2663 (2005); (c) Choi, H.S., Lee, S.C., Yamamoto, K., Yui, N.: Block-selective movement of γ-cyclodextrins in polyrotaxanes of PEI-*b*-PEG-*b*-PEI copolymer. Macromolecules **38**, 9878–9881 (2005) ; (d) Ooya, T., Choi, H.S., Yamashita, A., Yui, N., Sugaya, Y., Kano, A., Maruyama, A., Akita, H., Ito, R., Kogure, K., Harashima, H.: Biocleavable polyrotaxane-plasmid DNA polyplex for enhanced gene delivery. J. Am. Chem. Soc. **128**, 3852–3853 (2006)
4. Ooya, T., Ito, A., Yui, N.: Preparation of α-cyclodextrin-terminated polyrotaxane consisting of β-cyclodextrins and pluronic as a building block of a biodegradable network. Macromol. Biosci. **5**, 379–383 (2005)
5. Alvarez-Parrilla, E., Al-Soufi, W., Cabrer, P.R., Novo, M., Tato, J.V.: Resolution of the association equilibria of 2-(*p*-Toluidinyl)-naphthalene-6-sulfonate (TNS) with β-cyclodextrin and a charged derivative. J. Phys. Chem. B **105**, 5994–6003 (2001)
6. (a) Green, N.M.: Adv. Protein Chem. **29**, 85 (1975); (b) Wilchek, M., Bayer, E.A.: The avidin-biotin complex in bioanalytical applications. Anal. Biochem. **171**, 1–32 (1988); (c) Green, N. M.: Avidin and streptavidin. Methods Enzymol. **184**, 51–67 (1990)
7. Green, N.M.: Avidin. 3. The nature of the biotin-binding site. Biochem. J. **89**, 599–609 (1963); (b) Green, N.M.: Spectroscopic evidence for the participation of tryptophan residues in the binding of biotin by avidin. Biochim. Biophys. Acta **59**, 244–246 (1962)
8. Nelson, A., Stoddart, J.F.: Dynamic multivalent lactosides displayed on cyclodextrin beads dangling from polymer strings. Org. Lett. **5**, 3783–3786 (2003)
9. Kaiser, K., Marek, M., Haselgrübler, T., Schindler H., Gruber, H.J.: Basic studies on heterobifunctional biotin-PEG conjugates with a 3-(4-Pyridyldithio)propionyl marker on the second terminus. Bioconjugate Chem. **8**, 545–551 (1997)
10. Um, S.H., Lee, G.S., Lee, Y.J., Koo, K.K., Lee, C., Yoon, K.B.: Self-assembly of avidin and D-biotin-tethering zeolite microcrystals into fibrous aggregates. Langmuir **18**, 4455–4459 (2002)
11. Green, N.M.: Spectrophotometric determination of avidin and biotin. Methods Enzymol. **18A**, 418–424 (1968)
12. (a) Harada, A., Kamachi, M.: Complex formation between poly(ethylene glycol) and a-cyclodextrin. Macromolecules **23**, 2821–2823 (1990); (b) Harada, A., Okada, M., Li, J., Kamachi, M.: Preparation and characterization of inclusion complexes of poly(propy1ene glycol) with cyclodextrins. Macromolecules **28**, 8406–8411 (1995)
13. Liu, Y., Wang, H., Chen, Y., Ke, C.F., Liu, M.: Supramolecular aggregates constructed from gold nanoparticles and L-Try-CD polypseudorotaxanes as captors for fullerenes. J. Am. Chem. Soc. **127**, 657–666 (2005)
14. Wang, H., Cao, R., Ke, C.F., Liu, Y., Wada, T., Inoue, N.: Diastereomeric molecular recognition and binding behavior of bile acids by L/D-Tryptophan-modified β-cyclodextrins. J. Org. Chem. **70**, 8703–8711 (2005)