Construction and DNA Condensation of Cyclodextrin-Based Polypseudorotaxanes with Anthryl Grafts

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The binding of organic and biomolecules with DNA has become one of the greatest challenges for chemists and biologists in recent years because it could be applied in the design of new and efficient drugs targeted to DNA as well as in understanding how proteins could be recognized or bound to specific DNA sequences. Among these molecules, cyclodextrins (CDs) and their assemblies have attracted more and more attentions because of their high solubilization ability, low toxicity, and specific recognition ability toward many model substrates. Schneider et al. reported that the water-soluble anthryl-CD could be used as chemically switched DNA intercalators. Superior to CD monomers, CD-based rotaxanes/poly-(pseudo)rotaxanes generally have some novel chemical and physical properties, which enable their potential to act as molecular devices, molecular machines, and functional materials. Recently, the cell and DNA binding behaviors by CD-based rotaxanes/poly(pseudo)-rotaxanes have been widely investigated. Stoddart et al. reported that a self-assembled polypseudorotaxane with lactoside modified α-CDs could inhibit galectin-1-mediated T-cell agglutination. Yui and co-workers synthesized the poly(pseudo)rotaxanes with dimethylaminoethyl modified α-CD or γ-CD units, which could be applied in the endosomal escape and pDNA delivery to nucleus. Li et al. reported that the cationic polyrotaxane composed of multiple oligoethylenimine-grafted β-CDs threaded on a polymer chain could be used as efficient gene delivery vectors. Recently, we reported that the thio-CD-based polypseudorotaxanes attached on the surface of gold can cleave the pDNA under the visible-light irradiation. In the present work, we successfully prepared two new fluorescent polypseudorotaxanes (3 and 4) by threading the anthryl-modified β-CDs (1 and 2) onto the poly(propylene glycol) bis(2-aminopropylether) (PPG-NH2, MW ≈ 2000) chains (Scheme 1). Spectrophotometric and microscopic studies demonstrate that the polypseudorotaxanes 3 and 4 show good binding abilities to calf thymus DNA. Further studies by AFM show that these polypseudorotaxanes can efficiently condense the free DNA to particulate structures.

Polypseudorotaxanes 3 and 4 are obtained in satisfactory yields (49−58%) by threading the anthryl-modified β-CDs 1 and 2 on PPG-NH2 chains. From the 1H NMR spectrum of 3 in D2O, a comparison of the integral area of proton peaks indicates that the ratio between the PPG-NH2’s methyl protons (a molecule of PPG-NH2 2000 contains ca. 34 methyl protons, δ = 1.09−1.19 (d)) and the H9 proton of anthryl group in 1 (a molecule of 1 contains one H9 proton, δ = 8.40 (s)) is 10.1:1.0. In the case of 4, δ should be calculated that, for 3, one PPG-NH2 chain threads ca. 10 anthryl-modified β-CD units, and for 4, ca. 9 anthryl-modified β-CD units, which are consistent with the results of elemental analysis. Moreover, the 1H ROESY spectra of polypseudorotaxanes (see Figure S1 in the Supporting Information) show the clear NOE correlations (peak A) between the methyl protons of PPG-NH2 and interior protons of β-CD cavity, which demonstrates the threading of β-CD cavities onto the PPG-NH2 chain. XRD studies demonstrate that, although 1 and 2 are somewhat amorphous, polypseudorotaxanes 3 and 4 are crystalline, indicating that the original arrangement mode of anthryl-modified β-CDs becomes ordered after the threading of PPG-NH2. The DNA binding behaviors of 3−4 are investigated by the fluorescence titrations, where the calf thymus DNAs with different concentrations are gradually added to a solution of polypseudorotaxane. During the fluorescence titration, the fluorescence intensity of polypseudorotaxane around 413 nm gradually enhances upon the addition of varying amounts of DNA. A possible reason for the increasing fluorescence is that the anthryl groups in polypseudorotaxanes intercalate into the hydrophobic DNA grooves without the energy transfer, because the singlet energies of DNA bases are larger than that of anthryl group by at least 15 kcal/mol. By dividing the polypseudorotaxanes 3 and 4 into 10 and 9 anthryl-modified β-CD inclusion units, respectively, we can calculate the effective binding constants of every anthryl-modified β-CD inclusion unit in polypseudorotaxanes 3 and 4 with calf thymus DNA as 3.34 × 10^4 M−1 and 3.99 × 10^4 M−1 after taking into account the influence of scattering. In the control experiments, the binding constants of 1 and 2 with calf thymus DNA are also obtained as 9.44 × 10^3 M−1 and 9.68 × 10^3 M−1, by fluorescence titrations, which are 3.5 and 4.1 times lower than the corresponding values for 3 and 4, respectively. It should be noted that, because the anthryl group of 1 or 2 can be embedded into the β-CD cavity to form the self-included complex in aqueous solution, 1-adamantanol is used as a competitive reagent to exclude the self-included anthryl group from the β-CD cavity, and this conformation change is validated by fluorescence and ROESY spectra (see the Supporting Information).

Besides the fluorescence titrations, the 1H NMR and melting experiments also confirm the good DNA binding abilities of polypseudorotaxanes. By comparing the 1H NMR spectra of 1−4 before and after the addition of DNA, we find that the H9 protons of anthryl group shift downfield 0.05−0.09 ppm, whereas the H2-
In conclusion, we successfully synthesized two fluorescent polypseudorotaxanes with many anthryl grafts by threading anthryl-modified β-CDs onto a PPG-NH2 chain and found that the obtained polypseudorotaxanes could act as promising DNA concentrators, giving good binding abilities toward calf thymus DNA. Owing to these findings, these CD-based polypseudorotaxanes are expected to have many exciting applications as a sensitive analytical tool in DNA chemistry with a promising potential to control gene expression and delivery. Studies aimed to better understand the interactions between the CD-based polypseudorotaxanes and DNA as well as the design of new CD-based polypseudorotaxanes with improved DNA binding abilities is underway.

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Supporting Information Available: Experimental Details; Synthesis of 3 and 4; XRD: Fluorescence titration curves; ROESY spectra of 1/1-adamantanol and 3; 1H NMR spectra of 3 with and without DNA; plots of Alk vs temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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