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

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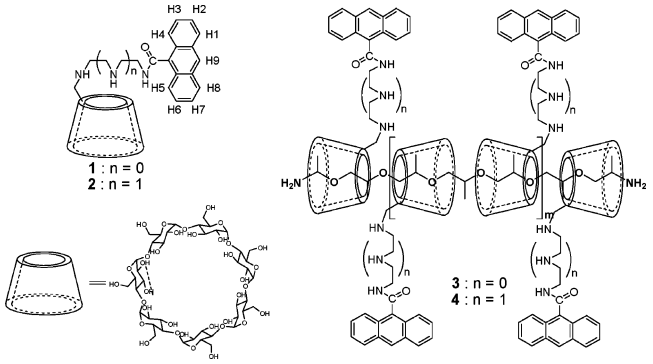
The binding of organic and biomolecules with DNA has become one of the greatest challenges for chemists and biologists in recent years because it could be applied in the design of new and efficient drugs targeted to DNA as well as in understanding how proteins could be recognized or bound to specific DNA sequences.[1] 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.[2] 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.[3] Recently, the cell and DNA binding behaviors by CD-based rotaxanes/poly(pseudo)-rotaxanes have been widely investigated.[4] Stoddart et al. reported that a self-assembled polypseudorotaxane with lactoside modified α-CDs could inhibit galectin-1-mediated T-cell agglutination.[4a] 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.[4b,c] 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.[4d] Recently, we reported that the thioCD-based polypseudorotaxanes attached on the surface of gold can cleave the pDNA under the visible-light irradiation.[4e] In the present work, we successfully prepared two new fluorescent polypseudorotaxanes (**3** and **4**) by threading the anthryl-modified β-CDs (**1** and **2**)[5] onto the poly(propylene glycol) bis(2-aminopropylether) (PPG-NH_2, 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-NH_2 chains. From the ^{1}H NMR spectrum of **3** in D_2O, a comparison of the integral area of proton peaks indicates that the ratio between the PPG-NH_2's methyl protons (a molecule of PPG-NH_2 2000 contains ca. 34 methyl protons, $\delta = 1.09\sim1.19$ (d)) and the H9 proton of anthryl group in **1** (a molecule of **1** contains one H9 proton, $\delta = 8.40$ (s)) is 10.1:1.0. In the case of **4**, this ratio changes to 10.9:1.0. Therefore, we can calculate that, for **3**, one PPG-NH_2 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 ^{1}H ROESY spectra of polypseudorotaxanes (see Figure S1 in the Supporting Information) show the clear NOE correlations (peak A) between the methyl protons of PPG-NH_2 and interior protons of β-CD cavity, which

Scheme 1



demonstrates the threading of β-CD cavities onto the PPG-NH_2 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-NH_2. 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.[6] 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,[5] 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 ^{1}H NMR and melting experiments also confirm the good DNA binding abilities of polypseudorotaxanes. By comparing the ^{1}H 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-

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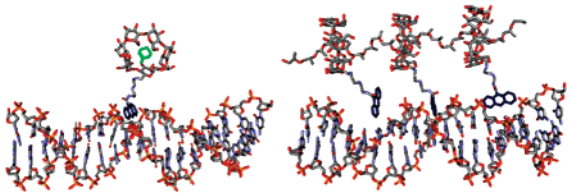

Figure 1. Energy-minimized structures of (left) **2** (with 1-adamantanol) and (right) **4** with DNA. Hydrogen atoms were omitted and structures were colored by atom type: gray, carbon atoms; red, oxygen atoms; pale blue, nitrogen atoms in the β-CD and DNA moieties; green, 1-adamantanol; dark blue, anthryl group.

(7) and H3(6) protons of anthryl group shift upfield 0.04∼0.08 ppm after the addition of DNA, which indicates that the anthryl groups of **1**−**4** intercalate into the DNA grooves.[2] Moreover, no dethreading of anthryl-modified β-CDs are observed during the DNA binding. On the other hand, the intercalation of probe molecules into the DNA double helix usually increases the helix melting temperature, i.e., the temperature at which the double helix denatures to the single stranded DNA.[7] Herein, the melting temperatures of calf thymus DNA in the presences of **1**−**4** are determined by monitoring the absorption of DNA bases at 260 nm as a function of temperature. The results show that the melting temperature of free DNA (74 °C) increases 3, 4, 6, and 6 °C, respectively, in the presence of **1** (with 1-adamantanol), **2** (with 1-adamantanol), **3**, and **4**, respectively. These enhancements of helix melting temperatures clearly indicate the increased stability of double helix induced by anthryl-modified β-CDs and polypseudorotaxanes, especially by polypseudorotaxanes **3** and **4**. Moreover, the higher melting temperatures induced by polypseudorotaxanes also demonstrate their stronger intercalation abilities into the double helical DNA.

To explore the possible DNA binding mechanism of anthryl-modified β-CDs and polypseudorotaxanes, the molecular modeling studies are performed by using the Insight II program to give the computational minimum-energy structures between anthryl-modified β-CDs or polypseudorotaxanes and DNA. (Figure 1) The results shows that the anthryl group in **1** or **2** may prefer intercalating in the minor DNA groove, but the ones in **3** or **4** can intercalate in both the minor and major DNA grooves. The minimum-energy structures of **1** or **3** with DNA are calculated to be similar to that of **2** or **4**, respectively.

To get the visible information about the interactions between polypseudorotaxane and DNA, atomic force microscopic (AFM) studies were performed. Figure 2 shows the typical AFM images of calf thymus DNA in the absence and presence of polypseudorotaxane **3**. Without the polypseudorotaxane, the free DNA exists as loose clews (Figure 2a). After the addition of **3** (w/w = 1:1), the originally loose DNA clews turn to the solid particles with an average diameter of ca. 100 nm (Figure 2b). This phenomenon clearly demonstrates the good DNA condensation ability of polypseudorotaxane **3**, and the driving force of the DNA condensation induced by **3** may be not only the electrostatic interactions between the protonated amino groups in polypseudorotaxane and the negatively charged phosphates in DNA, but also the intercalation of multiple anthryl groups of **3** into the DNA grooves as verified by molecular modeling studies. In the control experiments, the calf thymus DNA shows the similar condensation behavior in the presence of polypseudorotaxane **4**, but exhibits no appreciable condensation in the presence of **1** (with 1-adamantanol) or **2** (with 1-adamantanol).

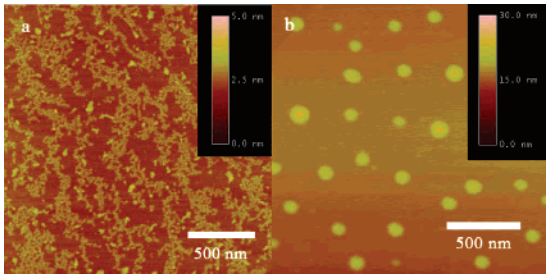

Figure 2. AFM images of (a) calf thymus DNA and (b) its condensate induced by polypseudorotaxane **3** on mica in tapping mode. (a) DNA (2 ng/μL), (b) DNA (2 ng/μL) with **3** (2 ng/μL).

In conclusion, we successfully synthesized two fluorescent polypseudorotaxanes with many anthryl grafts by threading anthryl-modified β-CDs onto a PPG-NH_2 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**; ^{1}H NMR spectra of **3** with and without DNA; plots of A/A_0 vs temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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