DNA/α-Cyclodextrin–Rotaxane Conjugate as a New Supramolecular Material

Akinori Kuzuya,* Toshiyuki Ohnishi, and Makoto Komiyama*
RCAST, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904

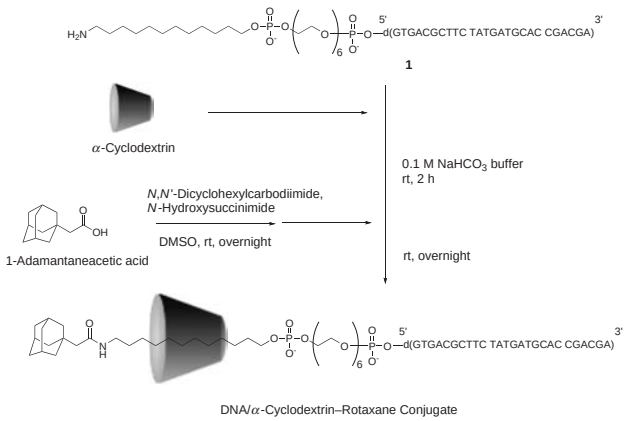
(Received June 4, 2008; CL-080567; E-mail: komiyama@mkomi.rcast.u-tokyo.ac.jp)

A hybrid molecule of cyclodextrin (CyD) and DNA, two major subjects in supramolecular chemistry, should open a new research field in nanoscience. By capping the end of a pseudorotaxane, formed between α-CyD and dodecylamine-modified DNA, with 1-adamantaneacetic acid, a DNA/α-CyD–rotaxane conjugate has been successfully synthesized and characterized.

Cyclodextrins (CyDs) are cyclic oligosaccharides composed of 5 or more α-D-glucopyranosides, and are a very popular target of supramolecular chemistry for their unique host–guest inclusion capability.[1,2] CyD-necklace, a polyrotaxane made of CyD, axle, and capping molecules, is a typical supramolecular derivative of CyD.[3–8] Various interesting applications of CyD–polyrotaxane, i.e. slide-ring gel (topological gel)[5] and insulators for conductive polymers,[6] have been proposed, and some of them have already been put into practice. Single-molecular manipulation of CyD-necklace composed of α-CyD and poly(ethylene glycol) using SPM probes has recently raised a new application of CyD-necklace as a molecular abacus.[7]

DNA is another interesting subject of supramolecular chemistry because of its accurate complementary base-pairing and its regularly formed double-helical structure. Besides, recent progress of DNA nanotechnology based on programmed assembly of branched DNA helices has widened the scope of its application toward nanomaterial science.[8,9] Various kinds of precise DNA nanostructures have been constructed so far. If such DNA nanostructure can be used as a scaffold for precise assembly of CyD-supramolecules in single-molecule manner in nanometer range, CyD-derivatives could be promoted to far more attractive and practical nanodevices. Thus, efficient preparation of DNA/CyD–rotaxane conjugate is desirable. Besides, an application for single-molecular DNA sequencing using AFM has also been proposed recently.[10,11] Despite such attractive future applications of DNA/CyD–rotaxane conjugates, there are few papers on their actual synthesis.[12] Although formation of DNA/CyD–rotaxane conjugate on a surface has been suggested,[10] as far as we know, no report on isolated DNA/CyD–rotaxane conjugate has been published to date. Here, we report an efficient synthesis and isolation of DNA/α-CyD–rotaxane conjugate in solution by using 1-adamantaneacetic acid as one of the capping molecules.

The synthetic procedure of DNA/α-CyD–rotaxane conjugate is shown in Scheme 1. First, 10 nmol of 26-mer DNA **1**, which bears a dodecylamine linker connected via hexaethyleneglycol linker at the 5′-end and was synthesized on an automated DNA synthesizer, was first mixed with 1 μmol of α-CyD in 0.1 M $NaHCO_3$ buffer (100 μL, pH 9.0) and kept at room temperature for 2 h. To the mixture was added 4 μmol of activated 1-adamantaneacetic acid in 20-μL DMSO and allowed to react overnight at 37 °C. After passing through a microspin column packed with Sephadex G-25, to the mixture was added 400 μL of water and the desired DNA/α-CyD–rotaxane conjugate was isolated by reversed-phase (RP) HPLC.



Scheme 1. The synthesis of DNA/α-CyD–rotaxane conjugate.

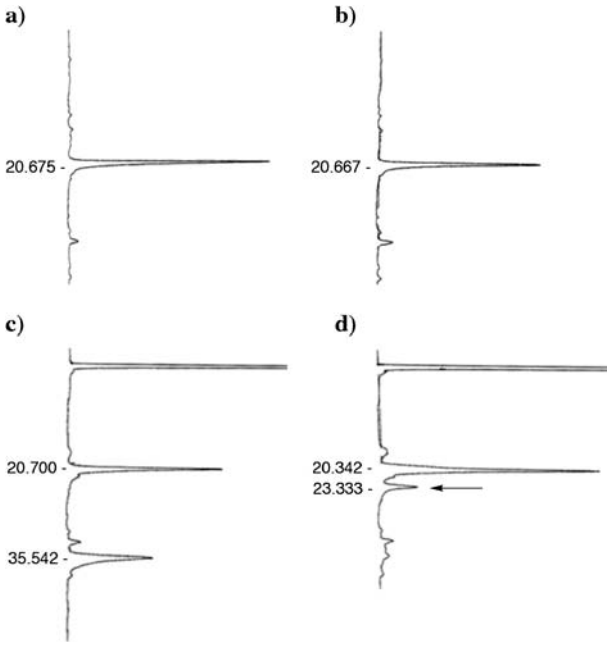


Figure 1. Reversed-phase HPLC charts of various reaction mixtures with a linear gradient of acetonitrile (0 to 50% for 50 min with 50 mM ammonium formate buffer). a) **1** alone. b) A simple mixture of **1** and α-CyD. c) **1** + activated 1-adamantaneacetic acid. d) **1** + α-CyD + activated 1-adamantaneacetic acid. The peak eluting at 23.3 min (indicated by the arrow) is the DNA/α-CyD–rotaxane conjugate. The peak at 20.7 min in c (20.3 min in d) is unreacted **1** and the peak at 35.5 min in c is **1**/adamantane conjugate without α-CyD.

Figure 1 shows the RP-HPLC charts of various reaction

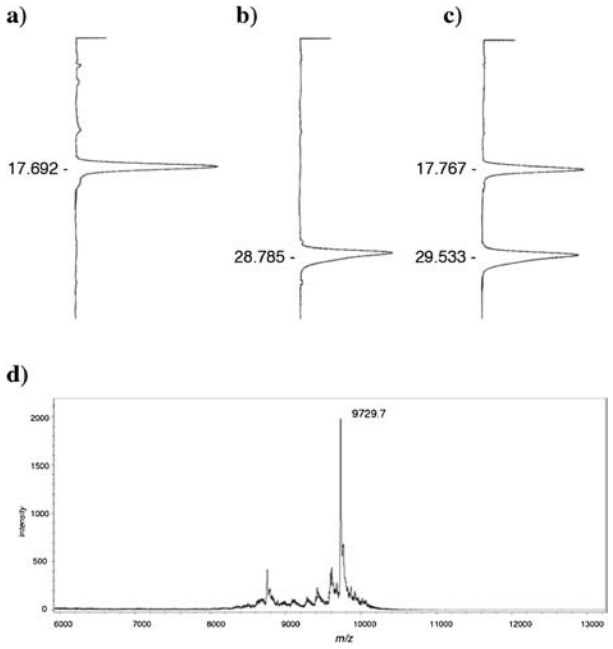


Figure 2. Characterization of the purified DNA/α-CyD–rotaxane conjugate. a) The purified DNA/α-CyD–rotaxane conjugate. b) A simple mixture of **1**/adamantane conjugate and α-CyD. c) Co-injection of the purified DNA/α-CyD–rotaxane conjugate and **1**/adamantane conjugate. d) MALDI-TOFMS of purified DNA/α-CyD–rotaxane conjugate. Calculated m/z for the desired product is 9734.5. Very small amount of **1**/adamantane conjugate (m/z 8750.5) is observed, suggesting that α-CyD slightly escapes (or is broken off) upon ionization.

mixtures. As shown in Figure 1b, a typical chart of a simple mixture of **1** and α-CyD is almost identical with a chart for **1** alone (Figure 1a). Assumedly, pseudorotaxane of **1** and α-CyD, if any, is not stable enough to be detected on RP-HPLC. When activated 1-adamantaneacetic acid was added to the solution of **1** in the absence of α-CyD, **1**/adamantane conjugate that elutes around 35 min was efficiently obtained as in Figure 1c. The coupling yield estimated from the peak area was ca. 55%. Quite interestingly, formation of **1**/adamantane conjugate was completely suppressed when **1** was incubated with α-CyD prior to the addition of the solution of activated 1-adamantaneacetic acid. Instead of **1**/adamantane conjugate, a new significant peak that elutes much earlier than the **1**/adamantane conjugate was observed (Figure 1d). The yield of this peak was ca. 14%.

The peak at 23.3 min in Figure 1d was isolated and further analyzed by RP-HPLC and MALDI-TOFMS (Figure 2).[13] As in Figure 2a, the isolated product gave a sharp single peak on RP-HPLC even after the whole work-up procedure (drying, desalting, concentration determination, etc.) that lasts at least a few days. Another possible explanation of this product is that α-CyD is not threaded by the dodecyl chain but forms an extremely stable inclusion complex with the adamantane at the end of **1**/adamantane conjugate from the outside of the conjugate. This is less probable because the size of adamantane is much larger than the cavity of α-CyD, and rather fits β-CyD. Consistently, the retention time of **1**/adamantane conjugate did not change at all even when it is mixed with α-CyD (Figure 2b) or β-CyD (data not shown) as long as 2 days before HPLC analyses. However, it is certain that the change of retention time is drastic, and the isolated product elutes significantly early for a molecule bearing a highly hydrophobic core like adamantane (Figure 2c). It is possible that some kind of interaction between α-CyD and adamantane is enhanced by an intramolecular effect in the conjugate so as to compensate the hydrophobic nature of the adamantane.[14]

MALDI-TOFMS analysis of the isolated product has clearly shown that the product is indeed DNA/α-CyD–rotaxane conjugate and contains only one α-CyD (Figure 2d). The m/z number of the main peak in the spectrum is 9729.7, which is in quite good accordance with the calculated number of 9734.5 for DNA/α-CyD–rotaxane conjugate. Besides the desired main product, very small amount of **1**/adamantane conjugate (m/z 8750.5) can be seen in the spectrum. Considering that no peak corresponding to **1**/adamantane conjugate was detected around 30 min in Figure 2a, α-CyD may slightly escape from the complex upon laser irradiation or within ionization.

In conclusion, we have successfully synthesized and isolated DNA/α-CyD–rotaxane conjugate. Preparation of DNA-nanostructure/CyD–rotaxane composite is now under way in our laboratory.

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by the Global COE Program for Chemistry Innovation.

References and Notes

1 A. Harada, *Adv. Polym. Sci.* **1997**, *133*, 141.
2 A. Harada, *Acc. Chem. Res.* **2001**, *34*, 456.
3 A. Harada, M. Kamachi, *Macromolecules* **1990**, *23*, 2821.
4 A. Harada, J. Li, M. Kamachi, *Nature* **1992**, *356*, 325.
5 Y. Okumura, K. Ito, *Adv. Mater.* **2001**, *13*, 485.
6 M. J. Frampton, H. L. Anderson, *Angew. Chem., Int. Ed.* **2007**, *46*, 1028.
7 H. Shigekawa, K. Miyake, J. Sumaoka, A. Harada, M. Komiyama, *J. Am. Chem. Soc.* **2000**, *122*, 5411.
8 N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237.
9 T. H. LaBean, H. Li, *Nano Today* **2007**, *2*, 26.
10 R. Benison, U.S. Patent Appl. 2004 0214177, **2004**.
11 S. Qamar, P. M. Williams, S. Lindsay, *Biophys. J.* **2007**, *94*, 1233.
12 Even simple DNA/CyD conjugate has not been reported so much: a) I. L. Medintz, G. P. Anderson, M. E. Lassman, E. R. Goldman, L. A. Bettencourt, J. M. Mauro, *Anal. Chem.* **2004**, *76*, 5620. b) K. Fujimoto, Y. Muto, M. Inouye, *Bioconjugate Chem.* **2008**, *19*, 1132.
13 The eluent for HPLC was re-prepared between the experiments in Figure 1 and Figure 2. The different elution time is presumably because of slight differences of the buffer (ammonium formate) concentration.
14 J. E. H. Buston, J. R. Young, H. L. Anderson, *Chem. Commun.* **2000**, 905.

Published on the web (Advance View) August 27, 2008; doi:10.1246/cl.2008.996