Synthesis and its DNA Detecting Ability of Fluorescent Modified β -cyclodextrins having Long Chain Connected by pyrene Unit

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Two kinds of pyrene modified β -cyclodextrins, which are different in linker parts to bind between β -cyclodextrin and pyrene unit, were synthesized. Those derivatives show different behavior for single and double strands of DNA, where short linker derivative shows no interaction for DNA, but longer linker derivative indicates there are something interaction for DNA by intercalating of pyrene unit.

Key Words : cyclodextrins, pyrene, ssDNA, dsDNA, linker, fluorescent spectra, linker

1 INTRODUCTION

Recently, a base and a particular disease sequences of DNA for human being have been studied and clarified.[1] Although, a lot of fluorescent substances are utilized to detect specific DNA sequences for particular diseases, those substances have some problem such as poor water solubility together with lack stability against heat and light.[2-5] It is well known that cyclodextrins (CyDs) work to increase water solubility and keep stability of heat and light when there is something interaction between organic molecule and CyD. Also there is another advantage because CyD has no toxicological properties for human being. Figure 1 shows the structure of β -CyD, which is a cyclic compound composed of D-glucose units connected by α -1, 4 linkages. In this study, we tried to synthesize water soluble pyrene modified CyD derivative which will be able to intercalate DNA double strand because

planer structure of pyrene unit is favorable to interact with DNA.[2] The synthetic strategy of modified CyDs is taking introduction of ethylenediamine or tetraethylenepentamine as linker which binds CyD and pyrene unit because those linkers increasing water solubility and preventing self inclusion of pyrene unit into CyD cavity. The synthetic pyrene modified β -CyDs are mono-6-(1-pyrenebutylate-pentaaminotetraethyl-aminoethyl)-amino-6-deoxy- β -CyD (**I**) and mono-6-(1-pyrenebutylate-pentaaminotetraethyl)-6-deoxy- β -CyD (**II**).

The UV-vis spectra, fluorescent spectra and circular dichroism (CD) spectra were measured when **I** and **II** interacted with single strand DNA (ssDNA, 40bp) of which sequence is ttaaaaatag gctatccctt attaatgaaa ataggaggtt and double strand DNA (dsDNA, 195bp) of which complement sequence is ctgagtcca aatgtccag ctgtttatg cttgtctct gttcccca gaccctgagt gtgtctaga gttggatga gcattgtct ctaatggtc tgaaataatt gtatttctt gcaaaaacat taagtctatt

Figure 1 Structure of β -cyclodextrin.

agaaccagc taatttcatt ttgtcatctt tatagtgtaac atattctggt gcagg in aqueous solution. This sequence shows osteoporosis.

2 EXPERIMENTAL

Preparation of Mono-6-(1-pyrenebutylate-aminoethyl)-amino-6-deoxy- β -CyD (**I**)

To solution of 1-pyrenebutyric acid (0.1378 g, 0.4779 mmol) and 1-hydroxybenzotriazole (1-HOBt, 0.1294 g, 0.9576 mmol) in a mixture of dry-DMF (50 ml) and pyridine (50 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.1712 g, 0.8931 mmol) was added at -15°C . The reaction mixture was cooled down at -25°C and stirred for 2hr and then mono-6-(2-aminoethyl)-amino-6-deoxy- β -CyD [6] (0.5053 g, 0.4293 mmol) was added. The reaction mixture was warmed up at room temperature and then stirred for 4days. The mixture was concentrated in vacuo which was treated with 300 ml of acetone. The resulting precipitates were filtered and dried up to give crude product was obtained (0.6219 g). The crude product was treated with 100 ml of water of which water soluble fraction was loaded on CM Sepahedx C-50 column chromatography (250 mm x 55 mm). After 1200 ml of water was eluted and then 0.1% aqueous ammonia (1000 ml) was eluted to obtain pure **I** fraction, which was collected and dried up in vacuo to give pure **I** (0.2868 g, 0.1981 mmol, 46.15% yield). R_f : 0.62 (propanol-water-ethyl acetate-ammonia_{aq} (28%) 5:2:3:3 by volume, TLC; silica gel 60F₂₅₄).

^1H NMR (300 MHz, D_2O): δ = 3.3-3.8 (42H, m, $\text{C}^2\text{H}-\text{C}^6\text{H}$ of CyD), 4.8-4.9 (7H, m, C^1H of CyD), 7.3-8.3 (9H, m, aromatic-H of pyrene).

FAB-MS (m/z) 1469.

Preparation of Mono-6-(1-pyrenebutylate-pentaaminotetraethyl)-6-deoxy- β -CyD (**II**)

To a solution of 1-pyrenebutyric acid (0.2570 g, 0.8913 mmol)

and 1-HOBt (0.1314 g, 0.9724 mmol) in a mixture of dry-DMF (50 ml) and pyridine (50 ml), EDC (0.2028 g, 1.058 mmol) was added at -15°C . The reaction mixture was cooled down at -25°C and stirred for 2hr and then mono-6-(pentaaminotetraethyl)-6-deoxy- β -CyD [6] (0.5060 g, 0.3874 mmol) was added. The reaction mixture was warmed up at room temperature and then stirred for 4days. The mixture was concentrated in vacuo which was treated with 300 ml of acetone. The resulting precipitates were filtered and dried up to give crude product was obtained (0.6219 g). The crude product was treated with 100 ml of water of which water soluble fraction was loaded on CM Sepahedx C-50 column chromatography (250 mm x 55 mm). After 1200 ml of water was eluted and then 0.1% aqueous ammonia (1000 ml) was eluted to obtain pure **II** fraction, which was collected and dried up in vacuo to give pure **II** (0.1033 g, 0.06552 mmol, 16.91% yield). R_f : 0.60 (propanol-water-ethyl acetate-ammonia_{aq} (28%) 5:2:3:3 by volume, TLC; silica gel 60F₂₅₄).

^1H NMR (300 MHz, D_2O): δ = 3.3-3.7 (42H, m, $\text{C}^2\text{H}-\text{C}^6\text{H}$ of CyD), 4.7-4.9 (7H, m, C^1H of CyD), 7.4-7.8 (9H, m, aromatic-H of pyrene).

FAB-MS (m/z) 1598.

Measurement

UV-vis, Fluorescence and CD spectra were measured at 25°C using SHIMADZU-3600 Spectrophotometer, Perkin-Elmer LS 40B Fluorescence Spectrophotometer and JASCO J-720 Spectrophotometer, respectively. For the UV-vis measurement, water and phosphoric acid buffer (pH 7.0) were used as solvent for the pyrene-modified β -CyDs and DNA. When DNA solution was added to the pyrene modified β -CyD solution (1.0×10^{-5} M), the spectra change was recorded. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 340 nm and excitation and emission slits were 5 nm. Water and phosphoric acid buffer (pH 7.0) were used as solvent for the

Scheme 1 Preparation of mono-6-(1-pyrenebutylate-aminoethyl)-amino-6-deoxy- β -CyD (**I**)

Scheme 2 Preparation of mono-6-(1-pyrenebutylate-pentaaminotetraethyl)-6-deoxy- β -CyD (**II**)

β -CyDs and DNA. When DNA solution added to the pyrene modified β -CyD solution (1.0×10^{-7} M), the spectra change was recorded. For the CD measurement, water were used as solvent for the **II**. When DNA solution was added to the **II** solution (5.0×10^{-5} M), the spectra change was recorded.

3 RESULTS AND DISCUSSION

Preparations of **I** and **II**.

Pyrene modified β -CyDs (**I** and **II**), where CyD and pyrene were linked by ethylenediamine and tetraethylene pentamine, respectively, have been made in the presence of EDC and 1-HOBt. The preparation method of **I** and **II** was shown in Schemes 1 and 2.

Spectra of **I** with ssDNA and dsDNA

The absorption and fluorescent spectra of **I** alone and with ss and dsDNA in aqueous solution or 0.1 M phosphate buffer (pH

7.0) were recorded to investigate the interaction between **I** and ss and dsDNA.

Figures 2 and 3 show the absorption and fluorescent spectra of **I** alone and with ssDNA in aqueous solution. Although, the absorption spectrum of **I** was recognized between 220 to 360 nm region, the spectrum region between 220 to 300 nm goes over the spectrum derived from DNA, the peaks between 330 to 345 nm originated from pyrene were only recorded. When ssDNA was added to the solution of **I**, the absorption and fluorescent spectra were not changed, which means that pyrene unit of **I** might not interact with ssDNA.

Figures 4 and 5 show the absorption and fluorescent spectra of **I** alone and with dsDNA in aqueous solution. When dsDNA was added to the solution of **I**, the absorption spectra were changed to strong decrease in peaks at 330 nm and 345 nm and increase in a peak at 350 nm. An isosbestic point at 347 nm was observed. But, the fluorescent spectrum was not changed, when dsDNA was

Figure 2 UV-vis spectra of **I** (1.0×10^{-5} M) at various concentration of ssDNA (1; 0; 2; 0.5×10^{-7} ; 3; 1.0×10^{-7} ; 4; 1.5×10^{-7} ; 5; 2.0×10^{-7} ; 6; 2.5×10^{-7} M) in aqueous solution.

Figure 3 Fluorescent spectra of **I** (1.0×10^{-7} M) at various concentration of ssDNA (1; 0; 2; 5.0×10^{-10} ; 3; 1.0×10^{-9} ; 4; 1.5×10^{-9} ; 5; 2.0×10^{-9} ; 6; 2.5×10^{-9} ; 7; 5.0×10^{-9} M) in aqueous solution.

Figure 4 UV-vis spectra of **I** (1.0×10^{-5} M) at various concentration of dsDNA (1; 0; 2; 2.5×10^{-8} ; 3; 5.0×10^{-8} ; 4; 7.5×10^{-8} ; 5; 1.0×10^{-7} ; 6; 1.25×10^{-7} M) in aqueous solution.

Figure 5 Fluorescent spectra of **I** (1.0×10^{-7} M) at various concentration of dsDNA (1; 0; 2; 1.0×10^{-10} ; 3; 2.0×10^{-10} ; 4; 3.0×10^{-10} ; 5; 4.0×10^{-10} ; 6; 5.0×10^{-10} ; 7; 1.0×10^{-9} M) in aqueous solution.

added to the solution of **I**. This suggested that there is something binding interaction between **I** and dsDNA.

When ssDNA or dsDNA was added to a solution of 0.1 M phosphate buffer (pH 7.0) containing **I**, the absorption and fluorescent spectra were almost not changed. It means that there is few interaction between **I** and ssDNA or dsDNA.

Spectra of **II** with ssDNA and dsDNA

The absorption and fluorescent spectra of **II** alone and with ss and dsDNA in aqueous solution or 0.1 M phosphate buffer (pH 7.0) were recorded to investigate the interaction between **II** and ss and dsDNA.

Figure 6 shows absorption spectra of **II** in aqueous solution alone and with ssDNA, which indicates dramatically change of absorbance. An isosbestic point at 347nm was observed, suggesting that there is something binding interaction between **II** and ssDNA. Also fluorescent spectra of **II** were changed in

addition of ssDNA as shown in Figure 7. When ssDNA was added to the aqueous solution of **II**, the decrease of the intensity was recognized. The almost same phenomena were observed when dsDNA was added to the aqueous solution of **II** as shown in Figures 8 and 9, respectively.

The only difference is the magnitudes of change and decrease of absorption and fluorescent spectra, respectively. When ssDNA was used, the change and decrease magnitude was small. It suggested that the interaction between **II** and dsDNA was much stronger than that of ssDNA and **II**. It is well known the decrease and increase of the intensity of fluorescent spectra were depended on the high and lower polarities of surrounded environment for chromophore, respectively.[7-11] Both fluorescent intensities of **II** in addition of ss and dsDNA were decreased, which suggests that pyrene unit moves into the higher polarity environment than that of CyD cavity. It is just speculation of the movement of pyrene unit when ssDNA or dsDNA was added to the solution of

Figure 6 UV-vis spectra of **II** (1.0×10^{-5} M) at various concentration of ssDNA (1; 0; 2; 0.5×10^{-7} ; 3; 1.0×10^{-7} ; 4; 1.5×10^{-7} ; 5; 2.0×10^{-7} ; 6; 2.5×10^{-7} ; 7; 3.0×10^{-7} ; 8; 3.5×10^{-7} ; 9; 4.0×10^{-7} ; 10; 4.5×10^{-7} ; 11; 5.0×10^{-7} M) in aqueous solution.

Figure 7 Fluorescent spectra of **II** (1.0×10^{-7} M) at various concentration of ssDNA (1; 0; 2; 5.0×10^{-10} ; 3; 1.0×10^{-9} ; 4; 1.5×10^{-9} ; 5; 2.0×10^{-9} ; 6; 2.5×10^{-9} ; 7; 5.0×10^{-9} M) in aqueous solution.

Figure 8 UV-vis spectra of **II** (1.0×10^{-5} M) at various concentration of dsDNA (1; 0; 2; 2.5×10^{-8} ; 3; 5.0×10^{-8} ; 4; 7.5×10^{-8} ; 5; 1.0×10^{-7} ; 6; 1.25×10^{-7} M) in aqueous solution.

Figure 9 Fluorescent spectra of **II** (1.0×10^{-7} M) at various concentration of dsDNA (1; 0; 2; 1.0×10^{-10} ; 3; 2.0×10^{-10} ; 4; 3.0×10^{-10} ; 5; 4.0×10^{-10} ; 6; 5.0×10^{-10} ; 7; 1.0×10^{-9} M) in aqueous solution.

Scheme 3 Proposed an interaction between **I** and ssDNA in aqueous solution.

Scheme 4 Model of intercalation based on **II** into dsDNA in aqueous solution.

Figure 10 CD spectra of **II** (5.0×10^{-5} M) at various concentration of ssDNA (1; 0, 2; 2.5×10^{-7} , 3; 6.25×10^{-7} M) in aqueous solution.

Figure 11 CD spectra of **II** (5.0×10^{-5} M) at various concentration of dsDNA (1; 0, 2; 5.0×10^{-8} , 3; 1.25×10^{-7} M) in aqueous solution.

II. It seems that **II** can be partially included in the cavity of another **II** where the intermolecular complexation came down in addition of ssDNA as shown in Scheme 3. Because a size of ssDNA is smaller molecule than that of dsDNA, ssDNA can be included in the CyD cavity. On the other hand, dsDNA is too larger molecule to be included in CyD cavity where **II** can intercalate into dsDNA as shown in Scheme 4.

When 0.1 M phosphate buffer (pH 7.0) was used, the absorption and fluorescent spectra were almost not changed.

The CD spectra was recorded to investigate how movement of pyrene unit of **II** when ss or dsDNA was added. Figure 10 shows the CD spectra of **II** alone and with ssDNA. Like exciton coupling was observed, where the positive cotton effect at 340 nm was decreased and the negative cotton effect at 365 nm was decreased. It suggested that pyrene unit goes into the less chiral environment. On the other hand, the CD spectra of **II** alone shows positive cotton effect at 345 nm and negative cotton effect at 370 nm, which were increased upon addition of dsDNA, as shown in Figure 11. It suggested that the pyrene unit goes into more chiral environment. It is clearly contrary phenomena in addition of ssDNA and dsDNA. Original cotton effects at around 340 and 370 nm were derived from the interaction between pyrene unit and CyD cavity because of its chirality, which means that decrease of cotton effect indicate pyrene unit moves far from the CyD cavity and increase of cotton effect indicates the pyrene unit move closely the CyD cavity. In conclusion, when ssDNA was added to the solution of **II**, the pyrene unit moves far from the CyD cavity. When dsDNA was added to the solution of **II**, the pyrene unit move far from the chiral CyD cavity which resulted in a formation of intercalative binding between **II** and dsDNA.

4 CONCLUSION

We succeeded in a synthesis of **I** and **II** in a high yield. Although these compounds in a structure are only different in linker parts, they showed different interactions for ss and ds DNA. We are now investigating in a development of detectable reagent using of CyDs and pyrene system with sensitivity and selectivity.

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References

- [1] K. M. Applebaum, M. R. Karagas, D. J. Hunter, P. J. Catalano, S. H. Byler, S. Morris and H. H. Nelson, "Polymorphisms in Nucleotide Excision Repair Genes, Arsenic Exposure, and Non-Melanoma Skin Cancer in New Hampshire", *Environ. Health. Perspect.*, **115**, 8, (2007).
- [2] L. Hernandez-Folgado, C. Schmuck, S. Tomic and I. Piantanida, "A novel pyrene-guanidiniocarbonyl-pyrrole cation efficiently differentiates between ds-DNA and ds-RNA by two independent, sensitive spectroscopic methods", *Bioorg. Med. Chem. Lett.*, **18**, 2977-2981, (2008).
- [3] M. Asakawa, K. Endo, K. Kobayashi, H. Toi and Y. Aoyama, "Enantioselectivity in the Interaction of Carf Thymus DNA with Acridine Derivatives Having an Amino Ester or Amino Alcohol Substituent", *Bull. Chem. Soc. Jpn.*, **65**, 2050-2055, (1992).
- [4] L. Perez-Flores, A. J. Ruiz-Chica, J. G. Delcros, F. Sanchez-Jimenez and F. J. Ramirez, "Intercalation and groove binding of an acridine – spermine conjugate on DNA sequences: an FT – Raman and UV – visible absorption study", *J. Mol. Struct.* **744-747**, 699-704, (2005).
- [5] X. Jiang, L. Shang, Z. Wang and S. Dong, "Spectrometric and voltammetric investigation of interaction of neutral red with calf thymus DNA: pH effect", *Biophys. Chemist.* **118**, 42-50, (2005).
- [6] H.-J. Schneider and F. Xiao, "Binding and catalysis with a metal-induced ternary complex of an ethylenediamine-substituted cyclodextrin", *J. Chem. Soc., Perkin Trans. 2*, 387-391, (1992).
- [7] R. Ostaszewski, E. Wilczynska and M. Wolszczak, "The Synthesis of a New Type of Anthracene DNA Intercalator", *Bioorg. Med. Chem. Lett.*, **8** 2995-2996 (1998).
- [8] M. Narita, J. Itoh, T. Kikuchi and F. Hamada, "A High Sensitivity Fluorescent Chemo-sensory System Based on β -Cyclodextrin Dimer Modified with Dansyl Moieties", *J. Incl. Phenom, Macrocyclic Chem.*, **42**, 107-114, (2002).
- [9] F. Hamada, K. Ishikawa and Y. Higuchi, "Strong Binding Between Acidic Guests and Fluorescein Modified γ -Cyclodextrin via Hydrogen Bonding" *J. Incl. Phenom, Mol. Recognit. Chem.*, **25**, 283-294, (1996).
- [10] M. Narita, S. Mima, N. Ogawa and F. Hamada, "Selective Fluorescent Molecular Sensing by Bis Dansyl-Modified γ -Cyclodextrin Dimer", *Anal. Sci.*, **16**, 865, (2000).
- [11] M. Narita, N. Ogawa and F. Hamada, "Fluorescence Molecular Sensing for Endocrine-disrupting Chemicals and Their Analogues Based on Dansyl or Anthranilate Modified β - and γ - Cyclodextrins", *Anal. Sci.*, **16**, 37, (2000)