Bioconjugate Chemistry

Communication

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Cooperative Binding of Ferrocenylnaphthalene Diimide Carrying β-Cyclodextrin Converts Double-Stranded DNA to a Rod-Like Structure

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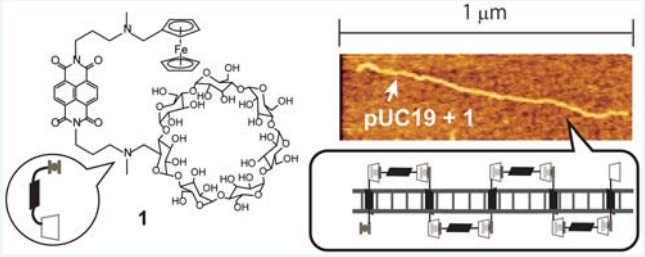
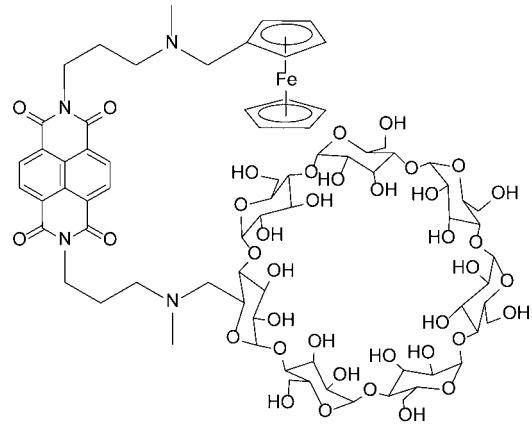
Supporting Information

ABSTRACT: Ferrocenylnaphthalene diimide carrying β-cyclodextrin (β-CD), **1**, intercalated into double-stranded DNA with a binding affinity of $K = (6.6 \pm 0.8) \times 10^4\ M^{-1}$ and a binding site size of $n = 4$, with a high positive cooperative parameter of $\omega = 14$. β-CD and ferrocene moieties of the compound contributed to the formation of the intermolecular inclusion complex on DNA. Binding of **1** resulted in conversion of the DNA duplex to a rod-like form, which was cleaved upon adamantylamine addition.



β-Cyclodextrin (β-CD) is known to form an inclusion complex with ferrocene, where the reduction and oxidation (Redox) reaction of ferrocene is inhibited by its inclusion, resulting in a positive shift of peak potential with decrease in current.[1,2] A combination of the electrochemical behavior and molecular recognition ability of β-CD as a host molecule has been utilized in several applications, such as chemical sensors.[2−5] Recently, the formation of such a complex has attracted interest because of its potential application in electrochemical DNA detection. We previously developed a DNA detection system consisting of β-CD and ferrocenylnaphthalene diimide as the DNA ligand,[6,7] with ferrocenyl-β-CD and adamantylnaphthalene diimide[8] or with a naphthalene diimide derivative having β-CD and ferrocene units.[9] Oligonucleotides carrying ferrocene or β-CD at both termini have also been studied with the aim of developing a label-free and signal-on DNA detection system.[10] Oligonucleotides carrying ferrocene or β-CD separately have also been developed in sandwich-type hybridization DNA detection systems, where inclusion complexes are formed in the assembly of two oligonucleotides through target DNA as a template.[11]

Naphthalene diimide derivative is a compound with unique properties and has applications as a semiconductor material, self-assembling nanomaterial forming nanorods or nanotubes, and DNA intercalator.[12−14,6−9] Ferrocenylnaphthalene diimide carrying β-CD (compound **1**) was designed and synthesized to facilitate the preparation of DNA nanorods. Compound **1** was connected to a naphthalene diimide with ferrocene and β-CD through a simple linker chain, similar to a previously described compound,[9] but without the triazole moiety from the linker chain. This compound **1** formed an intermolecular complex between ferrocene and the β-CD moiety upon binding to the DNA duplex, converting the DNA duplex into a rod-like form. Recent studies have demonstrated the ability of β-CD inclusion



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to alter the DNA structure,[10,15−17] providing insights into the regulation of DNA structure by small molecules. Therefore, in this study, we also discuss this topic with respect to our results. Compound **1** was obtained by the fractionation of its peak using reverse-phase high-performance liquid chromatography (HPLC) after the reaction of *N*-methyl naphthalene diimide derivatives with trimethylammoniummethyl ferrocene and tosylated β-CD in DMF at 60 °C. The cyclic voltammogram of compound **1** in the presence of adamantylamine showed increased peak current with negatively shifted redox potential compared to that in the absence of adamantylamine (Figure S1).

Received: November 21, 2014
Revised: February 4, 2015
Published: February 13, 2015

DOI: 10.1021/bc500535n
Bioconjugate Chem. 2015, 26, 379−382

Since β-CD is known to form a more stable inclusion complex with adamantylamine than with ferrocene,[18] this behavior can be explained on the basis of conversion from the intra- or intermolecular inclusion complex of ferrocene with β-CD to a non-inclusion complex. AFM measurement of **1** alone did not show any aggregates. Circular dichroism (CD) spectra of **1** alone showed the presence of exciton coupling in the naphthalene diimide chromophore around 300−400 nm, suggesting the stacking of two naphthalene diimide chromophores within the intermolecular inclusion complex of **1** (Figure S2). These results suggested favorable intramolecular complex formation of **1** rather than intermolecular complex formation of **1**.

Thus, compound **1** formed an intramolecular complex in an aqueous solution. Additionally, compound **1** showed an absorption maximum based on the naphthalene diimide chromophore and a hypochromic and red shift upon addition of calf thymus DNA (Figure 1A). Creation of a Scatchard plot

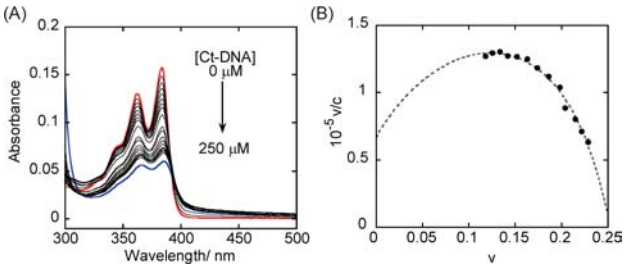

Figure 1. (A) Absorption change in compound **1** upon addition of calf thymus DNA in 10 mM MES (pH 6.25), 1.0 mM EDTA, and 0.10 M NaCl at 25 °C. (B) Scatchard plot based on its spectral change.

using the absorption change upon addition of different amounts of calf thymus DNA yielded a convex upward curve (Figure 1B). A binding constant of $K = (6.6 \pm 0.8) \times 10^4$ M^{-1} and binding site size of $n = 4$, with a positive cooperative parameter of $\omega = 14$, were obtained by fitting the data to the theoretical equation by McGhee and von Hippel, which considered the cooperative effect.[19] This finding showed that compound **1** bound to the DNA duplex with high positive cooperativity. The binding of compound **1** with double-stranded DNA was clarified by the observations of unwinding of the plasmid DNA in a topoisomerase I assay (Figure S3) and the Cotton effect as an area of induced CD around the region of the naphthalene diimide chromophore in the CD spectrum under excess amounts of calf thymus DNA (Figure S4). Association and SDS-driven dissociation of 2.5 μM compound **1** with 25 μM calf thymus DNA were completed within 45 and 70 s, respectively (Figure S5). The dissociation rate constant of compound **1** from calf thymus DNA was 0.2 s^{-1}, which was one-sixth that of the ferrocenylnaphthalene diimide derivative without β-CD (1.3 s^{-1}), without the intermolecular inclusion complex on DNA[20] (Figure S4B). Similar previously described derivatives[3] have been shown to have a very slow association process over 10 min. The rapid association of compound **1** may be explained by differences in the linker structure without triazole moieties as bulky linkers. According to these results, we propose that compound **1** intercalates double-stranded DNA every four base pairs with very high cooperativity and that this effect may be derived from the formation of an intermolecular inclusion complex between the ferrocene and β-CD moieties of compound **1**.

Figure 2 shows atomic force microscopic (AFM) images of linearized pUC19 DNA (2686 bp) mixed with different

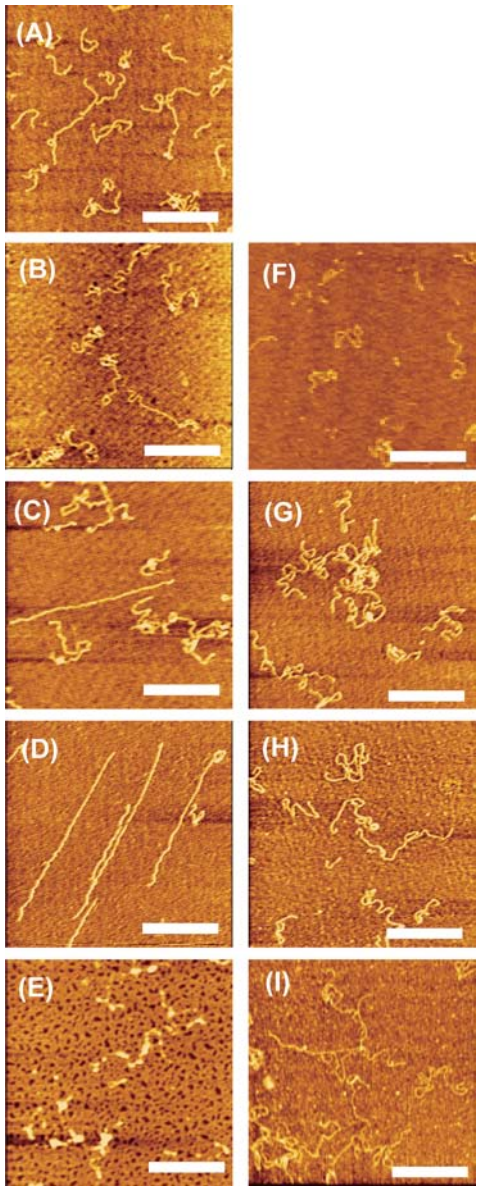

Figure 2. AFM images of 1.5 μM linearized pUC19 in the absence (A) or presence (B−I) of compound **1**. (B) 1.5 μM pUC19 + 0.30 μM compound **1**, (C) 1.5 μM pUC19 + 0.75 μM compound **1**, (D) 1.5 μM pUC19 + 1.5 μM compound **1**, (E) 1.5 μM pUC19 + 3.0 μM compound **1**, (F) 1.5 μM pUC19 + 0.30 μM compound **1** + 60 μM adamantylamine, (G) 1.5 μM pUC19 + 0.75 μM compound **1** + 150 μM adamantylamine, (H) 1.5 μM pUC19 + 1.5 μM compound **1** + 300 μM adamantylamine, and (I) 1.5 μM pUC19 + 3.0 μM compound **1** + 60 μM adamantylamine. The white bar scale indicates a length of 500 nm.

amounts of compound **1**. A randomly curving, corded, linear DNA duplex was observed in the case of DNA alone (Figure 2A). In contrast, a rod-like form of the DNA sample was observed when compound **1** was mixed in a 1:1 ratio with DNA (Figure 2D). Such a rod-like structure was not observed for β-CD alone (Figure S6). Similar previously described derivatives[3] were also shown to exhibit a rod-like DNA form without repeatability. Compound **1** repeatedly yielded rod-like DNA by reaching a state of equilibrium quickly through rapid

DOI: 10.1021/bc500535n
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association and dissociation. The average DNA lengths were estimated as 398 and 935 nm before and after addition of compound **1**, respectively, by image analysis using *ImageJ* software (Figure S7). However, the DNA lengths obtained were not precisely measured because the DNA length observed by AFM is known to be shorter than the theoretical length when the length is over 50 nm.[21] In our experiment, pUC19 DNA fragments with a theoretical length of 913 nm were observed as strings with an average length of only 398 nm (range, 275−650 nm). The average DNA length after binding to compound **1** increased approximately 2-fold to 935 nm (range, 650−1400 nm). A previous report also showed that DNA lengths were elongated by 50% and 33% after binding to intercalators with $n = 2$ or $n = 3$, respectively.[22] According to the extrapolation of data presented in this paper, the DNA length in the case of $n = 4$ was expected to be elongated by 25% to approximately 1140 nm. This result seems to be in good agreement with the obtained result.

Figure 2B,C shows AFM images in the cases of 1:0.2 and 1:0.5 ratios, respectively, of DNA to compound **1**. Interestingly, rod-like and random coil DNA can be seen together in this AFM image. In particular, for samples with a DNA to compound **1** ratio of 1:0.5, all DNA fragments in the AFM imaging areas showed rod-like structures, and this result was reproducible. This result also showed that compound **1** bound to double-stranded DNA with high positive cooperativity; binding of **1** to double-stranded DNA promoted its binding to the neighboring site of DNA as well. For DNA to compound **1** ratios of over 1:2, DNA aggregates were observed (Figure 2E). This was expected to have resulted from the formation of an intermolecular bridge between DNA strands. In all cases, the DNA complex recovered to the initial random coil structure of DNA upon addition of excess adamantylamine (Figure 2F−I). This may have resulted from the replacement of ferrocene with adamantylamine in β-CD and suggested that formation of the rod-like DNA and DNA aggregates was derived from the β-CD and ferrocene moieties of compound **1**. This finding is also proof that such major structural changes can be attributed to the formation of an inclusion complex between β-CD and ferrocene or adamantylamine. This inclusion behavior was also observed in electrochemical experiments. The cyclic voltammogram of compound **1** was studied in the absence and presence of the linearized pUC19 DNA (Figure 3). Redox peaks based on the inclusion complex of compound **1** with β-CD were observed for the sample with compound **1** alone (Figure 3a), and a decreasing peak current with a slightly negative shift of redox potential was observed upon addition of DNA (Figure 3b). An increased current peak with a negative shift of the peak was observed upon further addition of adamantylamine to the complex of compound **1** with DNA (Figure 3c). This result also showed that formation of the inclusion complex of ferrocene with β-CD could also be observed, even when the compound was bound to the DNA duplex. Although the peak current of compound **1** alone was larger than that upon addition of adamantylamine to the complex of compound **1** with DNA, the inclusion complex of compound **1** was formed, even when the compound was bound to the DNA. We expected that compound **1** alone formed an intramolecular inclusion complex with its ferrocene and β-CD moieties and that compound **1** was concentrated on the DNA duplex by cooperative intercalation through the inclusion of ferrocene with β-CD, resulting in the rod-like structure shown in Figures 4B and 5. This complex collapsed upon addition of adamantylamine.

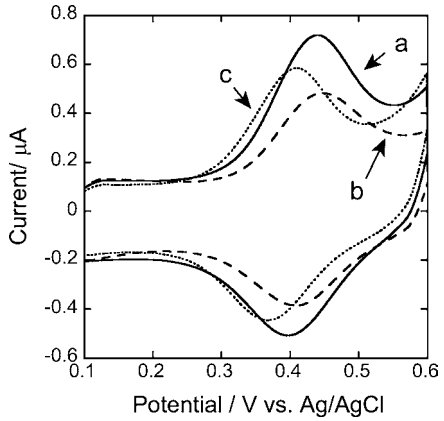

Figure 3. Cyclic voltammogram of 10 μM compound **1** in the absence (a) or presence of 10 μM linearized pUC19 (b). (c) Cyclic voltammogram of 10 μM **1** in the presence of 10 μM linearized pUC19 and 2.5 mM adamantylamine.

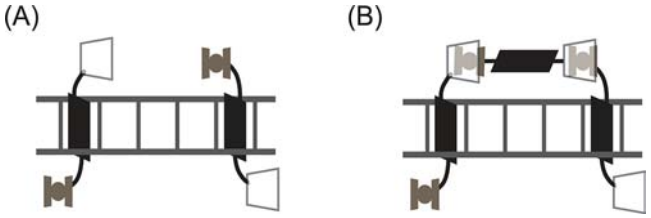

Figure 4. Schematic illustration of the intermolecular inclusion complex of compound **1** on the DNA duplex.

Compound **1** bound to double-stranded DNA every 4 base pairs. However, this length did not seem to be sufficient for

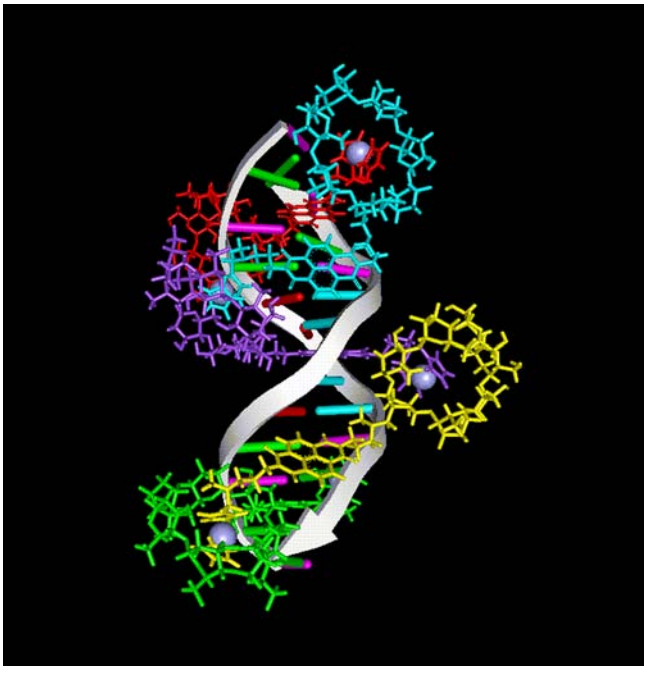

Figure 5. Molecular modeling of the intermolecular inclusion complex of compound **1** on the DNA duplex.

DOI: 10.1021/bc500535n
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complex formation between ferrocene and β-cyclodextrin moieties on double-stranded DNA, as shown in Figure 4A. When compound **1** contributed as a cross-linking molecule, as shown in Figure 4B, binding of compound **1** every 4 base pairs and complex formation on double-stranded DNA could be realized. Although spectral changes for **1** upon addition of double-stranded DNA occurred due to the intercalated molecules rather than cross-linking, binding of compound **1** every 4 base pairs was reasonable according to the results of Scatchard analysis. Under the condition of a 1:1 ratio of compound **1** and DNA base pair, **1** can bind to the DNA duplex every 4 base pairs in light of its binding affinity, where an excess amount of **1** might contribute cross-linkage between ferrocene and β-CD of **1** on DNA duplex and the linearization seen at a DNA to compound **1** ratio of 1:1, where the ratio of compound **1** to DNA-bp was 1:2. Figure 5 shows the molecular modeling of the complex containing the intercalated compound **1** bound to the DNA duplex and the cross-linked compound **1**. However, the cross-linked compound **1** did not appear to bind to the groove of the DNA duplex because of the absence of the positive Cotton effect under the naphthalene diimide chromophore (Figure S4). The rod-like structure of the DNA duplex induced by compound **1** was maintained in aqueous solution, and the random coil structure was observed by high-speed AFM upon addition of adamantylamine (Figure S8, Movie S1).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Theoretical methods and additional results for synthesis of **1**, cyclic voltammogram, topoisomerase I assay, circular dichroism spectra, stopped flow analysis, distribution of DNA length of linearized pUC19 observed AFM, and high-speed AFM (Figures S1−S15; Movie S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Young Scientific Research (B) to S. S. (No. 24750071) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT).

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DOI: 10.1021/bc500535n
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