Preparation and characterization of plasmid DNA network via both triple helix formation and photo-crosslinking

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Abstract

New self-assembled network of plasmid DNA was prepared via both photo-crosslinking and triple helix formations, and evaluated the conformation of DNA network by electrophoresis. Two kinds of TFO sequences were designed to form the triple helix with pUC19 at different positions. In addition to the triple helix formation, a photo-crosslinking and lectin-molecular recognition were applied to form pUC19 network. Psoralen, photoreagent, and biotin were attached to 5'- and 3'-ends of the TFO, respectively. The biotin–TFO–psoralen conjugate, pUC19, and SA were mixed in a buffer and stand at 4 °C. The results of electrophoresis study indicated that both triple helix formation and photo-crosslinking with pUC19 were necessary for the network formation. Therefore, this method can provide stable plasmid DNA network that may be useful to manipulate nanostructure using typical plasmid DNA conformational changes: supercoiled circular and open circular forms.

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1. Introduction

It is known that nanoscience and nanotechnology are frontier areas. A particularly intriguing feature of the nanoscale is the scale on which biological systems build their structural components, such as microtubules and microfilaments. A key property of biological nanostructures is molecular recognition, leading to self-assembly and the templating of molecular structures. For example, it is well known that three complementary strands of DNA form a triple helix. In recent years, triplex DNA has attracted considerable interest because of its possible biological functions in vivo and its wide variety of potential applications, such as regulation of gene expression, site-specific cleavage of duplex DNA, mapping of genomic DNA, and gene-targeted mutagenesis. Homopyrimidine oligonucleotides bind to the major groove of DNA at homopurine–homopyrimidine sequences where they form a triple helix [1–3]. Thymine and protonated cytosine form two hydrogen bonds with A·T and G·C base pairs (bp), respectively. The triple helix formation with complementary oligo nucleotides (TFO) can prevent sequence-specific proteins from binding to their recognition sequence. Therefore, they might elicit specific biological responses. In this way, the triplex formation occurs largely due to their ability to recognize specific sequences within double-strand DNA, which may lead to form supramolecular architectures in a self-assembled manner.

Plasmid DNA appears in different forms: circular with different degree of supercoiling, partially cleaved or linear, or multimeric as concatamers or catenates [4,5]. As for a monomeric plasmid, its prominent forms are the supercoiled or covalently closed circular form (SC) and the open circular form (OC) [4,5]. The superhelix density is affected by a change in the environment such as the concentration of an intercalator [6], temperature [7], topoisomerase [8], and so on. In this way, plasmid DNA responds to the external stimuli to change the degree of supercoiling. Regarding such an unique topological change as stimuli-responsive properties, plasmid DNA is considered to be used as new functional materials. Such the plasmid DNA may be promising materials for creating nanoscale systems [9–11]. The goal of molecular nanotechnology is a self-replicating, programmable architecture of molecular manipulation in both biological and non-biological manner [12]. Considering the feed of nanotechnology, the self-assembly of small molecular building-blocks (plasmid
DNA) is one of the most powerful strategies to maintain the stimuli responsive properties of the plasmid DNA in the self-assembled and nanometer-scaled devices.

In previous work [13], we reported on novel supramolecular plasmid DNA network prepared through two kinds of intermolecular interactions: triple helix formation between plasmid DNA and biotinated-DNA, and self-assembly of the biotinated-DNA and streptavidin (SA). A plasmid DNA molecule provides two binding sites for the TFO sequence, the 5' -terminal of which is biotinated. Further, the biotinated-TFO could bind with SA to form a plasmid DNA network. From the results of AFM observation, OC and SC networks showed a catenane-like and a rod-like structure, respectively. Our results suggest that the change from OC to SC in the network is promising to manipulate the nanostructure. However, stability of the network was not enough in aqueous conditions. In the present study, we report on the plasmid DNA network via site-specific photocrosslinks using TFO–psoralen conjugates. Psoralens are bifunctional photoreagents that form covalent bonds with pyrimidine base of nucleic acids. When intercalated at 5'-TpA-3' steps in double helical DNA, they form crosslinks between the two strands after UV light irradiation [14]. TFO–psoralen conjugates could recognize specific sequence in plasmid DNA, since one has already reported that an intercalating agent attached to the 5' end of the oligonucleotide strongly stabilized triple helix formation as a result of intercalation at the triplex–duplex junction [15,16]. Therefore, attaching the psoralen to the 5'-end of the TFO may stabilize the DNA network. TFO–psoralen conjugates can chemically crosslink with plasmid DNA, which may strongly stabilize the network structure.

2. Experimental

2.1. Materials

TFO1 [thiophosphate-5'-TCTTTTTTTCCT-3'-biotin] and TFO2 [thiophosphate-5'-TTTTCTCCTT-3'-biotin] were purchased from QIAGEN Co. (Hilden, Germany). pUC19 was purchased from TaKaRa Co. (Tokyo, Japan). 4,5',8-trimethylpsoralen were purchased from Tokyo Kasei Co. (Tokyo, Japan). All other chemicals used were of reagent grade.

2.2. Preparation of 4'-iodomethyl-4,5',8-trimethylpsoralen (I)

The 4'-iodomethyl-4,5',8-trimethylpsoralen was prepared according to the reports [17,18]. The 4,5',8-trimethylpsoralen (230 mg, 1 mmol) was dissolved in 50 ml of acetic acid at 60 °C and 850 μl of iodomethylmethyllethr was added to the solution. The mixture was stirred in the dark at room temperature for 24 h.
The precipitate was collected and dried in vacuo to obtain a yellow powder.

\[ ^1\text{H NMR (CDCl}_3, \text{ppm}): 8 7.59 (\text{C5 H}), 6.26 (\text{C3 H}), 4.22 (\text{C4' CH2}), 2.50–2.60 (\text{C4, C5', C8 CH3}). \]

2.3. Preparation of TFO–psolaren conjugates (Scheme 1)

TFO1 [thiophosphate-5'-TCTTTTTTTCCT-3'-biotin] (0.2 \( \mu \text{mol}) or TFO2 [thiophosphate-5'-TTTTCTCCTT-3'-biotin] (0.2 \( \mu \text{mol}), 18\text{-crown-6 (25 mg), 4'}\text{-iodomethyl-4',5',8-trimethylpsoralen (2.4 \( \mu \text{mol}), dimethylformamide (100 \( \mu \text{l}), and H}_2\text{O (100 \( \mu \text{l}) were incubated with stirring at 40}^\circ\text{C for 24 h. The psoralen-substituted oligonucleotide was precipitated in isopropanol for 12 h at }-20^\circ\text{C and recovered by centrifugation at 12,000g for 40 min.}

2.4. Preparation and confirmation of plasmid DNA networks (Scheme 2)

Self-assembled plasmid DNA networks were prepared by mixing the pUC19, TFO1–psolaren, TFO2–psolaren and SA. Firstly, SA (0.3 \( \mu \text{g), TFO1–psolaren (0.04 \( \mu \text{g}) and TFO2–psolaren (0.04 \( \mu \text{g}) were mixed and then pUC19 (20 \( \mu \text{g) was added in 10 \( \mu \text{l of Tris–acetate buffer containing 30 mM of MgCl}_2 (\text{pH 5.0}). The molar ratio of pUC19, SA, TFO1–psolaren and TFO2–psolaren is 2:1:1:1. The mixture was incubated at 4\text{oC for over night. The plasmid DNA network was precipitated in isopropanol for 12 h at }-20^\circ\text{C and recovered by centrifugation at 12,000g for 40 min. The precipitated plasmid DNA network was dissolved in Tris–EDTA Buffer (pH 7.4). The solution was irradiated for 10 min using 500 W ultrahigh pressure Hg lamp equipped with a high-pass filter. And then the mixture was analyzed by electrophoresis on agarose gel.}

3. Results and discussion

Fig. 1 shows the electrophoretic migration profiles of reaction mixture, pUC19 only, and the negative controls. After UV irradiation in the mixture of pUC19, SA, TFO1 and TFO2, 3 bands were observed as seen in lane 2. Two of them are the same molecular weight of pUC19, but the band at higher molecular weight position was not seen in pUC19 (lane 1). Such the new band was not observed before the UV irradiation (lane 3). These results indicate that the plasmid DNA network was formed after photo-crosslinking with UV irradiation. Before UV irradiation, it is possible to form plasmid DNA network via triple helix. As shown in Fig. 2, TFO1 [5'-TCTTTTTTTCCT-3'] and TFO2 [5'-TTTTCTCCTT-3'] form triple helix with 12mer sequence of pUC19 at position 1437 and with 10mer sequence at position 225, respectively. However, triple helix has been already known to be unstable in neutral conditions. So, it is considered that the network is dissociated in that condition. The sample of lane 4 was prepared using pSTV28 instead of pUC19. The pSTV28 that have no complementary sequence for TFO1 and 2 is negative control. So, it is easily imagined that pSTV28 cannot form a triple helix with TFO1 and 2. As expected, there were no bands of the high molecular weight such as the lane 2. This result suggests that both the photocrosslinking and the triple helix formation are necessary for the DNA network formation.

4. Conclusion

Biotin–TFO–psolaren conjugates were used for preparing a plasmid DNA network in combination with avidin–biotin interaction. From electrophoretic migration profiles, plasmid DNA network was prepared by using both TFO recognition and psolaren photo-crosslinking with plasmid DNA. A study of change in the network structure between OC and SC by external stimuli is now in progress.

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