Synthesis and AFM visualization of DNA nanostructures

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Abstract

We propose a novel bottom-up approach for the fabrication of various desired nanostructures, based on self-assembly of oligonucleotides governed by Watson–Crick base pairing. Using this approach, we designed Y-shaped, closed Y-shaped, H-shaped, and hexagonal structures with oligonucleotides. These structures were autonomously fabricated simply by mixing equimolar solutions of oligonucleotides and performing hybridization. After synthesis of the nanostructures, we confirmed their validity by agarose gel electrophoresis and atomic force microscope (AFM) visualization. We detected bands of the desired molecular sizes in the gel electrophoresis and observed the desired structures by AFM analysis. We concluded that the synthesized structures were consistent with our intended design and that AFM visualization is a very useful tool for the observation of nanostructures.

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1. Introduction

Nanotechnology has had a strong impact on the development of new materials and devices in recent years. Studies on nanodevices using DNA and biomolecules will enhance the possibility of new applications for nanometer-scale electronics and medications [1–8]. The self-organization of DNA plays an effective role in constructing nanostructures [5–8]. In such studies, nanostructures called nucleo-nanocages [5], two-dimensional DNA crystals [6], barcode-patterned lattices [7], nanoribbons [8], and nanogrids [8] have been successfully constructed with DNA molecules. All of these structures were built by the repetition of structural units. In contrast to the strategy adopted in those studies, we are endeavoring to make structures with oligonucleotides whose sequences are unique from each other so that individual parts of the structure can be addressed separately. This strategy enables us to target several noteworthy applications. For example, using our structure as a framework, we will be able to align molecules such as proteins in desired complex arrangements. Furthermore, this strategy can be applied to the construction of asymmetrical structures, which cannot be constructed using a periodic design technique.

In this study, we constructed Y-shaped, closed Y-shaped, H-shaped, and hexagonal structures. The process of assembly of the structures was monitored by agarose gel electrophoresis and by atomic force microscope (AFM) visualization to evaluate whether the structures had been constructed in accordance with our design.

2. Materials and methods

2.1. Design of DNA nanostructures

Oligonucleotide sequences were selected by a specially developed computer program (Asakawa T. et al., manuscript in preparation). Basically, sequences were chosen so that the melting temperature (Tm) values between intended regions of the oligonucleotides were sufficiently high while at the same time the Tm values between all of the remaining combinations were sufficiently low. Tm is defined as the temperature at which half of the DNA double helical structure is lost [9].

Fig. 1 shows the designs of the nanostructures. Each arrow indicates an oligonucleotide of 54 mer in length. The
Y-shaped, closed Y-shaped, H-shaped, and hexagonal structures shown in the figure consisted of 5, 6, 10, and 24 oligonucleotides, respectively.

2.2. Synthesis of DNA nanostructures

Polyacrylamide gel electrophoresis-purified oligonucleotides were synthesized by SIGMA Genosys Japan (Hokkaido, Japan). The oligonucleotides were 5'-phosphorylated with T4 polynucleotide kinase (Takara, Japan). Fig. 2 shows a schematic drawing of our procedures for fabrication and confirmation of the nanostructures. Equimolar solutions of oligonucleotides were mixed in 10 mM MgCl$_2$–50 mM Tris–HCl (pH 8.3) and heat denatured at 96 °C for 10 min, then cooled slowly from 96 to 20 °C using a thermal cycler (Bio-Rad, CA, USA). The hybridized oligonucleotides were then ligated using T4 DNA ligase (Quick Ligation Kit, New England Biolabs, MA, USA) to covalently bond adjacent oligonucleotides and hold the structures stably.

2.3. Gel electrophoresis and sample purification

The fabricated structures were separated on 1% or 3% agarose gels in Tris–borate–EDTA buffer (pH 8.0). DNA was stained with SYBR-Green I (Sigma Aldrich Japan, Tokyo, Japan) and observed with Dark Reader equipment (Clare Chemical Research, CO, USA) to avoid DNA damage by UV irradiation. DNA bands of the desired molecular sizes were cut out and purified using GFX PCR and Gel Band Purification Kit (Amersham Biosciences, NJ, USA) according to the manufacturer's instruction.

2.4. Sample preparation for AFM visualization

Purified nanostructure samples were suspended in 1 mM NiCl$_2$–10 mM HEPES–NaOH (pH 8.0), dropped onto freshly cleaved mica, and incubated for 5 min at room temperature to electrostatically immobilize them onto the surface (Fig. 2). After rinsing with 5 ml of Milli Q water, the
samples were dried and observed with AFM. AFM imaging was performed using a Nanoscope IIIa (Veeco Instruments, CA, USA) in tapping mode in air (Fig. 2). A silicon cantilever NCH (NanoWorld, Switzerland) was used for observation.

3. Results

3.1. Confirmation of nanostructures by agarose gel electrophoresis

The constructed nanostructures were assayed with agarose gel electrophoresis (Fig. 3). The Y-shaped, closed Y-shaped, H-shaped, and hexagonal structures showed mobilities equal to about 180, 220, 370, and 800 base pairs (bp), respectively, of double-stranded DNA fragments (Fig. 3a and b, lane 7). The respective structures were built with 270, 324, 540, and 1296 nucleotides, having molecular sizes equal to 135, 162, 270, and 649 bp of double-stranded DNA fragments. Therefore, each of the nanostructures showed a mobility about 1.2–1.4 times lower than that of double-stranded DNA fragments of equal molecular size. We consider this result to be reasonable because movement of the structures in the agarose gel matrices may have been hindered by their complex shapes.

A hexagonal structure consists of six kinds of Y-shaped structures. One, two, three, four, five, and six of these Y-shaped structures were mixed, hybridized, and applied to agarose gel electrophoresis (Fig. 3b, Lanes 2–7). Increasingly large molecular sizes were observed, further supporting the fact that hexagonal structures had been successfully constructed. An increasing incidence of smear bands around major bands was also shown, indicating the existence of incomplete structures (Fig. 3b). The percentage of complete structures was estimated to be about 25% by densitometric analysis of the gel image.

As a result of the electrophoresis analysis, it was found that Y-shaped, closed Y-shaped, H-shaped, and hexagonal structures seemed to have been successfully constructed.

3.2. Confirmation of nanostructures by AFM observation

Closed Y-shaped, H-shaped, and hexagonal structures were visualized with AFM (Fig. 4). As shown in Fig. 4a, almost all of the structures were Y-shaped. The Y-shaped structure was designed with 54 bp of double-stranded DNA on a side, equivalent to about 18 nm on the assumption that the length of one base pair is 0.34 nm, which is the theoretical length when the DNA fragment is in B-form, a typical conformation of DNA double helical structure in which the helical structure repeats after ten residues on each chain. The bar shown in the Fig. 4a is 100 nm in length, showing that the sizes of the observed Y-shaped structures were in good agreement with our design. Likewise, the sizes of the observed H-shaped structures shown in Fig. 4b were also reasonable. Images of the hexagonal structures are shown in Fig. 4c. Most of the structures seemed to be hexagonal in shape and reasonable in size, although the construction was incomplete in a majority of cases. Cross-sectional analysis of the AFM data showed that the height of these DNA structures was 0.5–0.7 nm, which is a typical value for double-stranded DNA molecules when observed with AFM in air [10].

As a result of the AFM observations, it was visually confirmed that closed Y-shaped, H-shaped, and hexagonal structures had been successfully constructed, although the construction efficiency was rather low in the case of hexagonal structures. It can also be noted that AFM visualization was thereby shown to be an efficient means of evaluating nanostructures.

4. Discussion

In this study, we synthesized nanostructures with DNA and confirmed by agarose gel electrophoresis and AFM imaging that Y-shaped, H-shaped, and hexagonal structures had been successfully constructed, although the construction efficiency was rather low in the case of the hexagonal structures. The reason for this low construction efficiency may be low ligation efficiency caused by steric hindrance, degradation during the process of extraction from the agarose gel, or a structural problem in our design. Studies of three- and four-arm DNA double-helical junctions have revealed that the angles flanking their branch points are flexible [11,12]. One of the reasons why our hexagonal structures were not constructed efficiently may be this type of flexibility. Winfree et al. [6] applied an antiparallel double-crossover structure to overcome this flexibility and
succeeded in constructing two-dimensional DNA crystals. Yan et al. [7] exploited double-crossover molecules and four four-arm junctions [8] as rather rigid structures and successfully constructed barcode-patterned lattices [7] and nanogrids [8] with DNA. According to their results, it may be possible to accomplish both addressability and stability with a design like the double-crossover structure and our strategy to use oligonucleotides whose sequences are different from each other in an entire structure.

Agarose gel electrophoresis of hexagonal samples showed a single band, whereas AFM images of the hexagonal structures contained many unclosed and distorted structures. This information cannot be obtained from electrophoresis analysis. AFM data can also be used to measure the length of nanostructures of nanometer order. In this way, the combination of gel band detection and AFM imaging of purified samples from the gel band is an effective technique for ascertainment of nanostructures.

5. Conclusions

We were able to synthesize four types of nanostructures with DNA molecules in good agreement with our intended designs. It was also demonstrated that AFM observation with agarose gel electrophoresis is a powerful methodology for confirming DNA nanostructures.

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References


Fig. 4. AFM images of (a) closed Y-shaped, (b) H-shaped, and (c) hexagonal DNA nanostructures. Scan sizes are shown below each image. The color bar shows the height from 0 to 2 nm.