Transactions of the Materials Research Society of Japan 29 [2] 439-441 (2004)

Designer DNA: Design and Synthesis of DNA Nanostructures

Rika Mizuno, Hirotaka Haruta, Takashi Morii, Takao Okada,
Kenichi Nakashi*, Takeshi Asakawa* and Hayashi Kenshi*
Research Institute of Biomolecule Metrology, 807-133 Enokido, Tsukuba, Ibaraki 305-0853, Japan
Fax: +81-29-839-4612, E-mail: mizuno@ribm.co.jp
* Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu Univ., 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

ABSTRACT: We propose a novel bottom-up approach for the fabrication of various desired nanostructures that is based on the sequence design of oligonucleotides and their self-assembly governed by the base-pair rule. Using this approach, we synthesized four types of structures, namely Y-shaped, closed Y-shaped, H-shaped and hexagonal structures, by hybridizing equimolar solutions of designed oligonucleotides. We confirmed the synthesized structures by agarose gel electrophoresis and atomic force microscope (AFM) visualization and concluded that they were consistent with our intended design and that AFM is a very useful tool for the observation of nanostructures.
Keywords: DNA, nanostructure, design, self-assembly, AFM

1. INTRODUCTION

Nanotechnology has had a strong impact on the development of new materials and devices in recent years. Research on nanodevices using DNA and biomaterials enhances the possibility of new applications for nanometer-scale electronics and medications [1-5]. The self-assembly of DNA works effectively in constructing nanostructures. In most studies, crystal-like nanostructures have been built by the repetition of basic structural units made of DNA [6-7]. We propose a novel bottom-up approach for the fabrication of various desired nanostructures that is based on the sequence design of oligonucleotides and their self-assembly governed by the base-pair rule. In this study, we made I-shaped, Y-shaped, H-shaped and hexagonal structures. The process of assembly was confirmed by agarose gel electrophoresis and atomic force microscope (AFM) visualization.

2. MATERIALS AND METHODS

2.1 Design of DNA nanostructures

Oligonucleotide sequences were carefully chosen using a newly developed, dedicated algorithm. Basically, sequences were chosen so that the Tm values between intended pairs of oligonucleotides were sufficiently high while the Tm values between all of the other possible pairings were sufficiently low. Firstly, "segment" sequences of 10 mer were selected and several of these segment sequences were connected to design the oligonucleotides. Figure 1a shows the design of the I-shaped structure, which was made with two oligonucleotides designated Ip and Iq. These oligonucleotides were designed so that the segment sequences of Ip2 and Iq3, and Ip3 and Iq2, were complementary. The Y-shaped structure consisted of three oligonucleotides: Yp, Yq, and Yr (Fig. 1b). The segment sequences of Yp2 and Yq3 were complementary. Likewise, Yq2 and Yr3, and Yr2 and Yp3, were complementary. In addition, the Y-shaped structure had adenine residues between the Yp2 and Yp3, Yq2 and Yq3, and Yr2 and Yr3 segments, serving as hinges. The segment sequences of Yp1, Yq1, and Yr1 were sticky ends that could be used for connection with other structures. The J-shaped structure consisted of one oligonucleotide, Jp, with complementary segment sequences of Jp2 and Jp3, and an adenine residue serving as a hinge between the Jp2 and Jp3 segments (Fig. 1c).

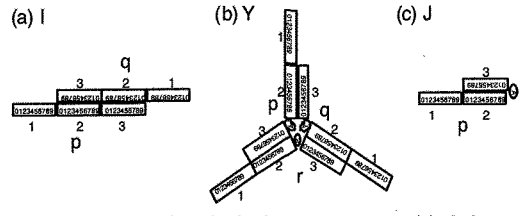

Fig. 1. Rules for designing structures: (a) I-shaped structure, (b) Y-shaped structure, and (c) J-shaped structure.

2.2 Synthesis of DNA nanostructures

Oligonucleotides were synthesized by SIGMA Genosys Japan (Hokkaido, Japan). The oligonucleotides were 5'-phosphorylated with T4 polynucleotide kinase (Takara, Japan). Equimolar solutions of oligonucleotides were mixed in 10 mM $MgCl_2$-50 mM Tris-Cl (pH 8.3), heat denatured at 96°C for 10 minutes, then hybridized by cooling slowly from 96°C to 35°C using a thermal cycler (Bio-Rad, CA, USA), incubated for 20 minutes at 35°C, and cooled to room temperature. After hybridization, the oligonucleotides were 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

I-shaped, Y-shaped, and H-shaped samples were electrophoresed on 3% agarose gel, and the hexagonal sample was electrophoresed on 1% agarose gel in Tris-borate-EDTA buffer (pH 8.0). After confirmation of the bands, DNA in the bands were extracted and purified using GFX PCR and Gel Band Purification Kit

(Amersham Biosciences, NJ, USA) according to the manufactures instruction.

2.4 Sample preparation for AFM visualization

The purified samples were suspended in 1 mM $NiCl_2$-10 mM HEPES-NaOH (pH 8.0), dropped onto freshly cleaved mica, and incubated for 5 minutes. After rinsing with 5 ml of MilliQ water, the samples were dried under a stream of nitrogen gas. AFM imaging was performed in tapping mode in air with a Nanoscope IIIa instrument (Digital Instruments, Santa Barbara, CA). A silicon cantilever NCH (NanoWorld, Switzerland) was used for observation.

3. RESULTS

3.1 Designed structures

Figure 2 shows four types of nanostructures. One arrow indicates oligonucleotide segments of 18 mer. The closed Y-shaped structure (Fig. 2b) consisted of three oligonucleotides and three J-units (Fig. 1c). Each oligonucleotide was 54 bases in length. The H-shaped structure consisted of two of the Y shapes (Fig. 2a) connected by sticky ends. The hexagonal structure consisted of six Y shapes.

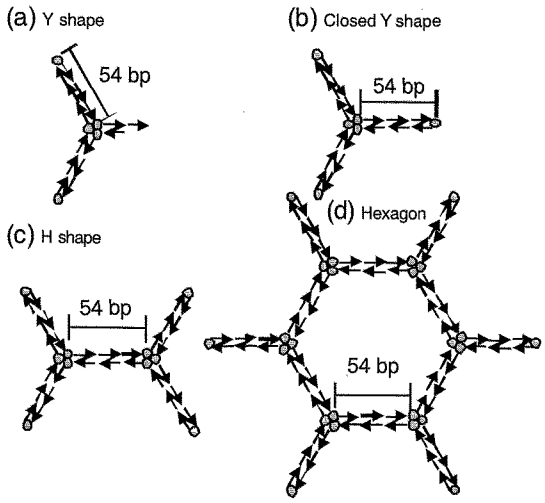

Fig. 2. Structural design of (a) Y-shaped, (b) closed Y-shaped, (c) H-shaped, and (d) hexagonal structures. An arrow indicates an oligonucleotide of 18 mer.

3.2 Electrophoretic analysis

The results of electrophoretic analysis of the structures are shown in Fig. 3. Fig. 3a shows electrophoretic analysis after the ligation of the Y-shaped, closed Y-shaped, and H-shaped structures. Lane 1 was a molecular marker of 25 base pair (bp) intervals. Lanes 2 and 3 were blank. Lanes 4 and 5 were Y-shaped structures, as shown in Fig. 2a. Lanes 6 and 7 were closed Y-shaped structures, as shown in Fig. 2b. Lane 8 was the H-shaped structure, as shown in Fig. 2c. In Fig. 3b, Lane 2 shows the result of electrophoresis after ligation of the hexagonal structure, as shown in Fig. 2d. Lane 1 consists of molecular markers of 100 bp intervals. The sample showed a mobility equal to about 800 bp of double-stranded DNA in size.

3.3 Analysis of AFM data

Figure 4 shows AFM images of the Y-shaped, H-shaped, and hexagonal structures. We were able to observe many Y-shaped structures, demonstrating that the structures were properly synthesized (Fig. 4a). Likewise, as shown in Fig. 4b, we detected many H-shaped structures as well. Fig. 4c shows an AFM image of hexagonal structures. There were a few hexagonal structures, and unclosed structures and distorted structures were also recognized. Fig. 4d is a magnified image of the hexagonal structures shown in three-dimensional representation.

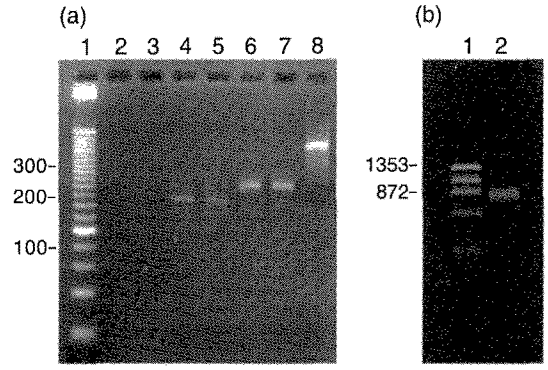

Fig. 3. Results of agarose gel electrophoresis. (a) Y-shaped and H-shaped structures separated on 3% agarose gel. Lane 1 is a 25 bp ladder of molecular size marker. (b) Hexagonal structure separated on 1% agarose gel. Lane 1 is a ϕ X174-Hae III digest molecular size marker.

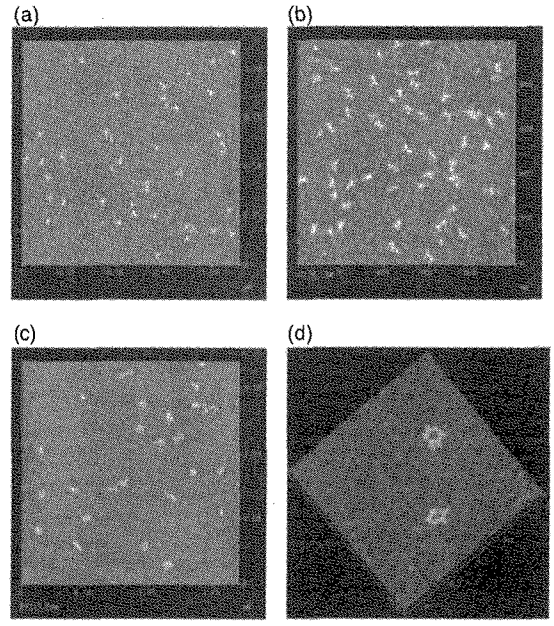

Fig. 4. AFM images. (a) Closed Y shapes. The scan size was 1.15 μm x 1.15 μm. (b) H shapes. The scan size was 995 nm x 995 nm. (C) Hexagonal shapes. The scan size was 1.7 μm x 1.7 μm. (d) Magnified image of hexagonal shapes. The scan size was 500 nm x 500 nm.

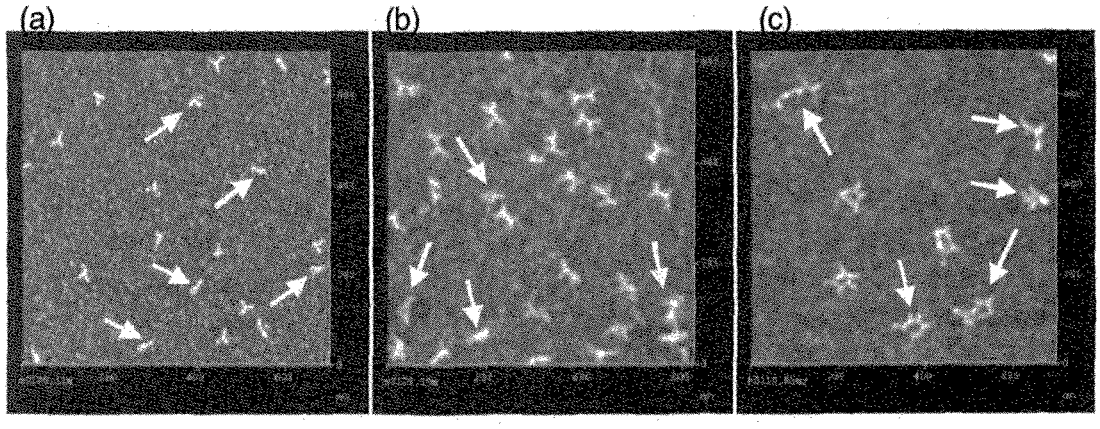

Fig. 5. Structures inconsistent with the design. (a) Closed Y shapes. The scan size was about 700 nm x 700 nm. (b) H shapes. The scan size was about 500 nm x 500 nm. (c) Hexagonal shapes. The scan size was about 700 nm x 700 nm. The white arrows indicate structures that differ from the design.

4. DISCUSSION

In this study we synthesized nanostructures and confirmed their validity by agarose gel electrophoresis and AFM visualization. AFM is becoming an increasingly useful tool in biological research and is a powerful method for investigating the structures of macromolecular assemblies [8-9]. In the electrophoretic analysis, each of the ligated structures was detected as a major band (Fig. 3). This result shows that nanostructures of the targeted size were synthesized. We also observed the structures with AFM (Fig. 4). The synthesized structures were confirmed as a single band from agarose gel electrophoresis; however, as indicated by the arrows in Fig. 5, incomplete structures were also detected. We believe that there are three reasons for these divergent results. Firstly, the structures may have been broken during the process of gel extraction and purification because of an incomplete ligation reaction in the synthesis step. Secondly, contamination from another band may have occurred when we cut out a band from the gel. Thirdly, the elastic nature of DNA may have been a factor. DNA is known to be an elastic structure influenced by its surrounding environment and length [10]. Therefore, when structures bind to the mica surface, they may shrink during sample dehydration [11]. In the next study, we need to observe AFM images of the DNA nanostructures in aqueous solution as a more natural condition.

5. SUMMARY

We have succeeded in fabricating DNA nanostructures based on the sequence design of oligonucleotides and their self-assembly governed by the base-pair rule. Agarose gel electrophoretic analysis followed by AFM visualization proved to be effective for validating the desired structures.

6. ACKNOWLEDGEMENT

This work was partly supported by the New Energy and Industrial Technology Development Organization (NEDO).

7. REFERENCES

[1] E. Braun, Y. Eichen, U. Sivan and G. B. Yoseph, Nature, 391, 775-778 (1998).
[2] T. C. Marsh, J. Vesenka and E. Henderson, Nucleic Acids Research, 23, 696-700 (1995).
[3] B. Yurke, A. J. Turberfield. A. P. Mills Jr., F. C. Simmel and J. L. Neumann, Nature, 406, 605-608 (2000).
[4] H. Yan, X. Zhang, Z. Shen and N. C. Seeman, Nature, 415, 62-65 (2002).
[5] K. Matsuura, T. Yamashita, Y. Igami and N. Kimizuka, Chem. Commun., 376-377 (2003).
[6] R. I. Ma, N. R. Kallenbach, R. D. Sheardy, M. L. Petrillo, N. C. Seeman, Nucleic Acids Research, 14, 9745-9753 (1986).
[7] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, Nature, 394, 539-544 (1998).
[8] M. Tanigawa, M. Gotoh, M. Machida, T. Okada and M. Oishi, Nucleic Acids Research, 28, (2000).
[9] H. G. Hansma, R. Golan, W. Hsieh, C. P. Lollo, P. M. Ley and D. Kwoh, Oxford University Press, 26, 2481-2487.
[10] D. Rhodes and A. Klug, Nature, 292, 378-380 (1981).
[11] H. G. Hansma, I. Revenko, K. Kim and D. E. Laney, Nucleic Acids Research, 24, 713-720 (1996).

(Received October 11, 2003; Accepted February 25, 2004)