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Temperature control for the expansion of artificial DNA motif

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Abstract The DNA, which forms a double helical conformation by the highly selective base-pairing rule with the width of 2 nm, is one of the polymers utilized as a template for nanoparticle assembly. Then, holiday junction structure, where two different DNA double strands intersect and replace each complement strand, is an important artificial motif for forming two-dimensional mesh-like DNA morphology. In this study, the two-dimensional sheet with the mesh-like conformation is formed and the growth of the DNA morphology is controlled by changing the temperature in the formation process. The influence of the cooling procedure on the formation process of the two-dimensional DNA sheet is observed by atomic force microscope, and the formation of double strands in the process is traced by absorption spectra. The spectroscopic results demonstrate that the two-dimensional DNA sheet is strongly dependent on the correlation between the applied cooling process and the melting temperature of double helical conformation, and that the growth of the two-dimensional DNA sheet can be manipulated by the cooling procedure. Further, we will show the size control of the DNA sheet by mixing the designed DNA which inhibits the growth of the DNA sheet.

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Introduction

The miniaturization of materials into nano level can show unique characteristic physical properties [1] and the assembly of the nanomaterials is a next step for producing an opt/electronic device with a new function [2, 3]. Recently, the macromolecules which can be self-assembled, have been utilized for the nanoparticle patterning; especially, DNA is often utilized because DNA forms various unique self-assembling structures [4], and the addressing of nanopartices on the DNA templates has been attempted by complementary DNA–DNA interaction. Double helical DNA strands usually have a right-handed spiral centering on the common axis and the nucleic acid sequences in the DNA strand form hydrogen bonds by the highly selective base-pairing rules in DNA. Mirkin et al. [5] reversibly assembled the DNA-modified gold nanoparticles by DNA–DNA interaction. And Sato et al. [6] shows the assembly of DNA-modified nanoparticles by the structural change of DNA. Further, two-dimensional assembly of gold nanoparticles by using template of DNA network have been performed [7, 8], and noble metal nanowires have been formed on a DNA template by the reducing reaction after the concentration of metal ions on DNA [9, 10]. Recently, Mao et al. [11] have formed a mesh-like two-dimensional DNA sheet utilizing holiday junction where two different DNA double strands intersect and replace each complement strand; the square DNA conformation units with the dimension of about 10 nm are built from the 8 strands of DNA single strands with the holiday junction combination in each four corners (Fig. 1a), and the square units are connected each other through the terminal DNA single strands in themselves (Fig. 1b).

In this study, we focus on the process control for the formation of the two-dimensional DNA sheet conformation

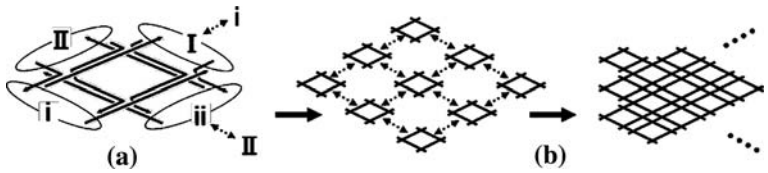

Fig. 1 Schematic representation of DNA assembly (Ref. 11). (**a**) A view of a square unit composed of 8 DNA strands. The circled single strand is designed for binding to neighboring units (**b**) a view of a mesh-like two-dimensional DNA sheet from the square units

to manipulate the expansion of the two-dimensional DNA sheet conformation. Base sequence, base length and melting temperature (T_m) are critical to the hybridization of the DNA chains; especially, temperature should be strictly controlled to hybridize long complementary DNA chains without mismatch. Considering the further application of DNA templates for nanoparticle assembly, the temperature control to manipulate the DNA template should be studied. Here we show the temperature control based on T_m to manipulate the expansion of the two-dimensional DNA sheet conformation.

Experimental method

Formation of the mesh-like two-dimensional DNA sheet

The 8 kinds of DNA single strands which were designed by Mao et al. [11] were mixed at the concentration of 100 nM in the same solution containing 10 mM HEPES (pH 7.0), 4 mM $MgCl_2$ and 1 mM EDTA. About 50 μL of the solution was heated at 90 °C for 10 min in order to anneal the DNA strands, and then the annealed DNA strands were cooled to various temperatures at the rate of 40 °C/min to form the DNA sheet. The temperature was controlled by the PCR instrument (Applied Biosystems, Japan).

Atomic force microscope (AFM) imaging

A 10 μL sample solution was spotted on a mica (15 × 10 mm) and then dried. To remove salts in the buffer solution, the mica plate was rinsed several times by 100 μL of distilled water. Imaging was performed at tapping AFM (SPA-400, Seiko Instruments Inc.) under air using NSC tips (MikroMacsh, Inc.).

Results and discussion

The two strands forming the double helical conformation are separated by heating the DNA solution above T_m. Here, we investigated the influence of cooling process on the formation of the mesh-like two-dimensional DNA sheet, in order to propose a cooling process which enables us to control the size of the DNA sheet. Figure 2 shows the change of the absorbance at 260 nm for the solution containing the 8 kinds of DNA strands which can form the DNA sheet, during the cooling procedure from 90 °C to various temperatures. The absorption at 260 nm decreases by hybridization of DNA single strands. The cooling to

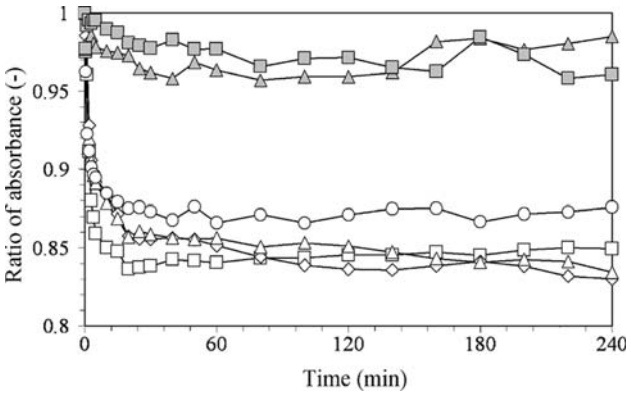

Fig. 2 The change of the absorbance at 260 nm by cooling from 90 to 80 °C (closed square), 70 °C (closed triangle), 60 °C (opened circle), 50 °C (opened triangle), 40 °C (opened rhombus), and 30 °C (closed opened square), at the cooling speed of 40 °C/min. The absorbance is normalized by the value at the start point

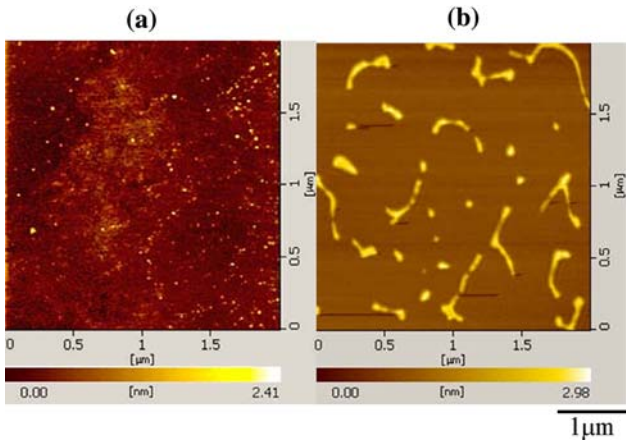

Fig. 3 AFM images of the DNA morphologies formed by the cooling from 90 to 4 °C with the keeping time of 4 h at (**a**) 70 °C and (**b**) 60 °C

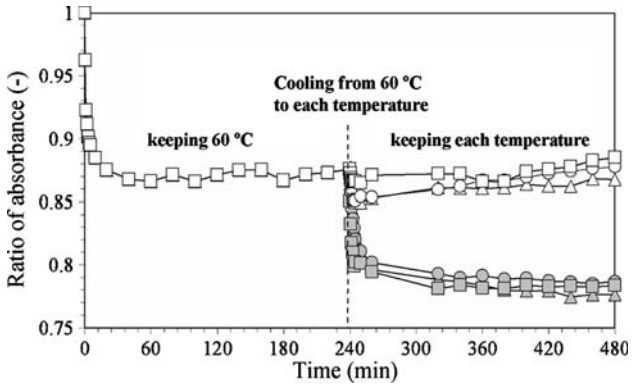

Fig. 4 The change of the absorbance at 260 nm by cooling from 90 to 50 °C (opened square), 40 °C (opened circle), 30 °C (opened triangle), 20 °C (closed square), 10 °C (closed circle), and 0 °C (closed triangle), at the cooling speed of 40 °C/min, after keeping the temperature at 60 °C for 4 h. The absorbance is normalized by the value at 90 °C

80 °C and 70 °C after the anneal at 90 °C led to no absorbance change at 260 nm (closed squares and triangles in Fig. 2, respectively), while the cooling to the lower temperature than 60 °C resulted in the observation of the rapid decrease in absorbance (60 °C; opened circles, 50 °C; opened triangles; 40 °C; opened rhombus, and 30 °C; opened squares in Fig. 2). The rapid decrease of absorbance implied the formation of double helical conformation at less than 60 °C. We estimated the T_m values from the equation of Wallance et al. [12] (T_m (°C) = 81.5 + 16.6*log[S] + 0.41*(%GC)−(500/n); S, mol of salt; n, base length (bp)); The T_m for the formation of the double strands in square unit (Fig. 1a) was 69–72 °C, and the T_m for the terminal DNA strands connecting between the square units (Fig. 1b) was 14–26 °C. The fact that the formation of the double strands in square unit means the formation of square unit itself, suggests the formation of only the square units by the cooling to less than 60 °C. Figure 3 shows the AFM images of the DNA morphologies formed by the cooling process from 90 to 20 °C with a keeping time of 4 h at 70 and 60 °C. The cooling process with the keeping time of 4 h at 70 °C led to no observation of DNA morphology (Fig. 3a), while the introduction of the holding time at 60 °C resulted in the formation of some small one-dimensional DNA morphology (Fig. 3b). This supports the suggestion that the square units are not formed at the cooling to less than 60 °C, and the formation of the one- dimensional DNA morphology by the introduction of the keeping time of 60 °C suggests that the linkages between the square units for a large two-dimensional DNA sheet should be controlled. We, further, measured the change of absorbance at 260 nm during the cooling process from 90 °C to various temperatures with a keeping time of 4 h at 60 °C (Fig. 4). The decrease in absorbance was observed only for the DNA solutions cooled to the lower temperature than 20 °C. Considering that the calculated T_m of the terminal DNA strands connecting between the square units is 14–26 °C, the cooling procedure from 60 °C to the lower temperature than 20 °C promotes the linkage between the square units. Therefore, the cooling to less than 20 °C is necessary to make the linkages between the square DNA units.

Figure 5 shows the DNA morphologies for the DNA solution cooled from 90 to 20 °C with the keeping time of

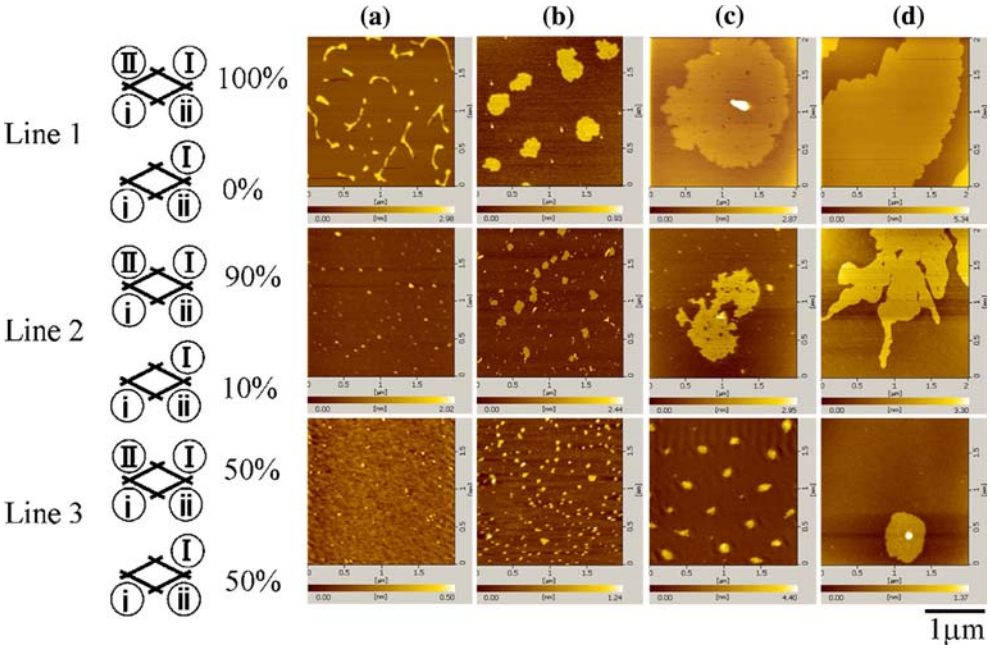

Fig. 5 AFM images of the two-dimensional DNA sheet. Each conformation was formed by cooling from 90 to 20 °C at the cooling speed of 40 °C/min, with the keeping times at 60 °C for 4 h and at 20 °C for (**a**) 0 min, (**b**) 5 min, (**c**) 10 min, and (**d**) 15 min. The designed DNA strands (Strands a–h listed in Table 1) were added into the normal DNA strands (Normal Strands a–b and Strands c–h listed in Table 1) solution. The concentration of the designed DNA strands were (line 1) 0%, (line 2) 10%, and (line 3) 50% of the total DNA strands

Table 1 DNA sequences used for the size control of the mesh-like two-dimensional DNA sheet

Strand a (88mer)
5′-GTATGCTGATAGGACAATGAGTAGCTATTGGTGATCAACGTTAAGATACCAGTGGACGAATCGTTTTCGATTCGTGGCTGTCAGTGAG-3′
*Normal Strand a (63mer)
5′-GTATGCTGATAGGACAATGAGTAGCTATTGGTGATCAACGTTAAGATACCAGTGGACGAATCG-3′
Strand b (58mer)
5′-CAGTATGGACGTAGATACTGTGCTAACGATATTCGAACTAGCGTCATCGGACGATCAG-3′
*Normal Strand b (63mer)
5′-CAGTATGGACGTAGATACTGTGCTAACGATATTCGAACTAGCGTCATCGGACGATCAGAGACG-3′
Strand c (63mer)
5′-CATTGGTAGTGCCTGTAATAATGTTGACTGCGGTTACCGTACTAATTGCTGTACCTGAGTGAG -3′
Strand d (63mer)
5′-TGACAGCCTGTCGAGTAGATCGTATGAATAGATGGCATCGCTGTAAATCCTGTGTCACCTCAC -3′
Strand e (100mer)
5′-GTGACACACCGATGACGCTAGTTCGAATATCGTTAGCACAGTATCTACGTGGTACAGCAATTAGTACGGTAACCGCAGTCAACATTATTACACCTATCAG-3′
Strand f (100mer)
5′-CTGATCGTGGATTTACAGCGATGCCATCTATTCATACGATCTACTCGACACCACTGGTATCTTAACGTTGATCACCAATAGCTACTCATTGTGGCACTAC-3′
Strand g (26mer)
5′-CAATGCTCACTCACCATACTGCGTCT-3′
Strand h (26mer)
5′-CATACCGATTCGTGGCTGTCAGTGAG-3′

* The combination of Strands a–h forms the DNA lattice DNA with the part of connection to three-way, and the combination of Normal Strands a–b and Strands c–h forms the normal DNA lattice with the part of connection to four-way

4 h at 60 °C. When the DNA sample was immediately dried after the cooling to 20 °C, only one-dimensional DNA morphologies were observed (Fig. 5a). Whereas, the introduction of a keeping time at 20 °C resulted in the formation of the two-dimensional DNA sheet, and the area of the DNA sheet was expanded as the keeping time at 20 °C was increased (line 1 in Fig. 5b–d). The construction of the networks between the square units is progressed during the keeping time at 20 °C in the cooling procedure. Therefore, it is important to control the formation of the square conformation units and the linkages between the square units, and the DNA morphologies can be controlled by the setting of the keeping time at each T_m.

In addition, we attempted to inhibit the expansion for the two- dimensional DNA sheet by adding other DNA conformation. We added the DNA sequences which forms the square units with the terminal DNA strands only in three directions; the illustration in lines 2 and 3 of Fig. 5 represents that the growth in the II direction is inhibited. The addition of the designed DNA strands listed in Table 1 into the normal DNA strands solution influenced the DNA morphologies; the increase of the amount of the designed DNA strands critically decreased the size of the two dimensional DNA sheet (line 2 and 3 in Fig. 5). Therefore, the formation of the two dimensional DNA sheet is critically influenced by the design of DNA sequence, and it is possible to control the size of the DNA sheet by mixing the DNA strands which inhibit the growth of the DNA morphology.

In conclusion, we could manipulate the size of the two dimensional DNA sheet by controlling the cooling process utilizing T_m values, and we also indicated the possibility of the control of size by mixing the DNA strands which inhibit the growth of the DNA sheet. The application of the controlled DNA sheet to the assembly of nanoparticles is in progress.

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