Notes

Pore Size Variability of DNA Nanomeshes by Polymerase Chain Reaction

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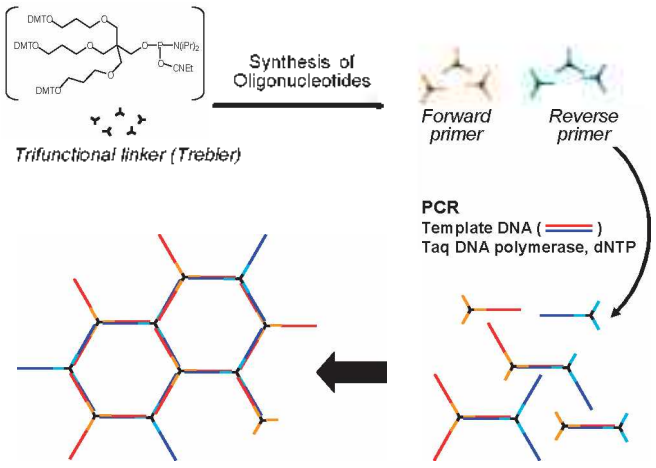
Since Seeman's pioneering work,[1-4] innumerable demonstrations have been reported in the field of structural DNA nanotechnology;[5-7] any imaginable nanostructures, in principle, could be realized by simple hydrogen bonds between complimentary bases. Recent advances in this field include the use of self-assembled DNA nanostructures as scaffolds for the construction of hierarchical, multi-component structures, which would promise more functionalizability and applicability.[6,8] Most, if not all, of the previously reported structures have been derived from the tightly controlled self-assembly of sophisticatedly but intricately designed DNA building blocks. One set of building blocks intrinsically produces one predetermined, final structure, although some attempts have been reported to tune final structures by combining several building blocks differently.[9-11] It is, however, still challengeable to develop methods for varying self-assembled DNA structures with one set of starting building blocks.



Scheme 1. Procedure for the formation of DNA nanomeshes.

In this work, we utilized the polymerase chain reaction (PCR), a method based on biological principles, to synthesize two-dimensional DNA nanomeshes; enzymes have previously been used as post-treatments after structural formation, such as rolling circle enzymatic replication,[12,13] but not been applied for directing self-assembly of DNA building blocks. Using the PCR processes, we successfully fabricated DNA nanomeshes and controlled pore sizes of the resulting nanomeshes by varying template DNA lengths. Our design principle is described in Scheme 1. Two three-armed central hubs are coupled with two different, chemically orthogonal oligonucleotides to act as forward and reverse primers during the PCR, respectively. DNA polymerases, such as *Pfu* DNA polymerase, elongate the oligonucleotide primers in the presence of the template DNAs and deoxyribonucleotide triphosphates (dNTPs), and the PCR process and hybridization spontaneously yield DNA nanomeshes by linking the PCR-amplified products through three-armed hubs. The three-armed primers were synthesized from the commercially available "Trebler" phosphoramidite, tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]ethyl-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite by a DNA synthesizer,[14-18] because organic hubs has been proved to be amenable to the generation of multiple-armed DNA structures.[19-25] Synthesis of oligonucleotides has routinely been carried out from 3' to 5' terminus, because the primary 5'-hydroxyl group is significantly more reactive than the secondary 3'-hydroxyl group, making it straightforward to protect the 5'-hydroxyl group with the DMT group and leaving the 3'-hydroxyl group available to form the phosphoramidite. In this work, we, however, used four deoxynucleotide-5'-CE phosphoramidites in order to synthesize the required primers

Table 1. Sequences of the primers used for this study.

Name	Sequence	Length
M13F	5`-Trebler-CGCCAGGGTTTTCCCAGTCACGAC-3`	24 mer
M13R	5`-Trebler-TCACACAGGAAACAGCTATGAC-3`	22 mer
ET7F	5`-Trebler-CGGAATTCTAATACGACTCACTATAGGG-3`	28 mer
ET7R	5`-Trebler-CGGAATTCGCTAGTTATTGCTCAGCGG-3`	27 mer

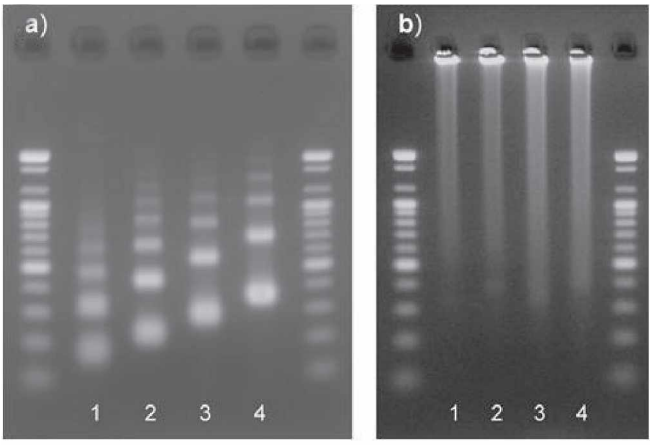


Figure 1. Agarose gel electrophoresis of PCR products. (a) After the first 40 cycles and (b) after the second 40 cycles. A set of primers and a template were used for each PCR reaction as follows: (lane 1) M13F, M13R and pUC19; (lane 2) M13F, M13R and pGEM-3zf(+); (lane 3) ET7F, ET7R and pET19b; (lane 4) ET7F, ET7R and pET22b.

from the 5'-"Trebler" phosphoramidite, which contained 3'-OH ends essential for the PCR processes. Four different primers, *i.e.* two sets of the primers, were synthesized (Table 1). M13F and M13R contained the identical sequence to the commonly used forward (F) and reverse (R) sequencing primers of the pUC/M13 vectors, respectively. The sequences of ET7F and ET7R were identical to those of T7 promoter and terminator sequencing primers, respectively.

Four different plasmid DNAs were employed as templates in the PCR process (pUC19: 135 bp; pGEM-3Zf(+): 192 bp; pET19b: 249 bp; pET22b: 325 bp). The M13 primers were used for pUC19 and pGEM-3Zf(+), and the ET7 primers for pET19b and pET22b. The length of the templates was calculated to be *ca.* 43, 62, 80, and 104 nm at their fully extended B-conformation of dsDNA, respectively. PCR was performed with *Pfu* DNA polymerase: after 40 cycles, the resulting DNAs were purified, and then another 40-cycle PCR was performed (See the Experimental Section for detail). After the first 40 cycles, we observed discrete ladder-like DNA bands that appeared as regularly spaced with the expected number of base pairs (Figure 1a). For example, when we used a combination of M13F, M13R and pUC19, the bands were separated by 135-bp (lane 1 in Figure 1a). These bands implied a gradual generation of low-molecular-weight self-assembled (or aggregated) structures formed by amplified DNAs linked by treblers; they also indicated that the intermediate structures were formed by base pairing among the three-armed DNA structures. After the second 40 cycles, the size of the resulting self-assembled structures was greatly increased and almost all DNAs remained immobile (well-bound) at the top of the 1% agarose gel (lane 1 in Figure 1b).

The PCR-amplified DNAs were purified and deposited on mica, and their morphology was characterized by atomic force microscopy (AFM). The tapping-mode AFM micrographs showed clear two-dimensional structures of DNA networks (DNA nanomeshes) (Figure 2). The height of the structures was about 3 ± 1 nm, indicative of double-stranded DNAs. The pore sizes visibly increased, as the length of the template

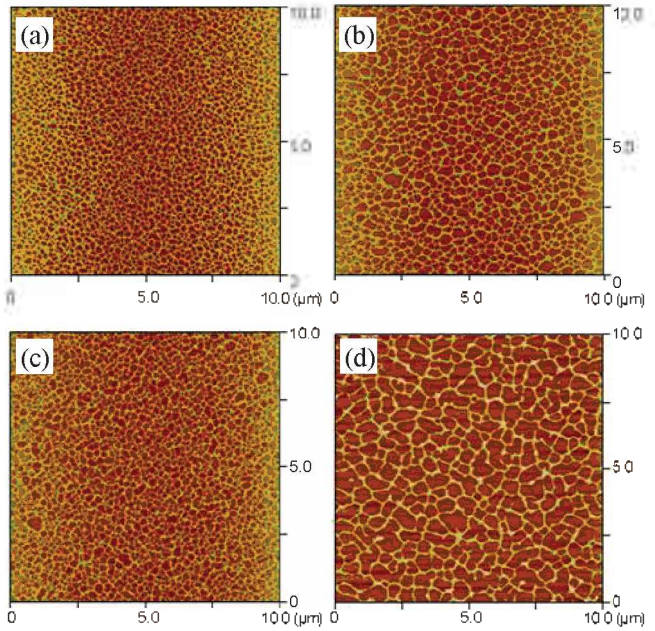


Figure 2. AFM micrographs of DNA nanomeshes on mica. (a) M13F, M13R and pUC19, (b) M13F, M13R and pGEM-3zf(+), (c) ET7F, ET7R and pET19b, and (d) ET7F, ET7R and pET22b.

increased. The length of each arm in the DNA network was not strictly uniform, but the averaged ones were approximately similar to the lengths of the templates. Multiple-armed DNA structures consisting of oligonucleotides have been reported previously to generate high molecular-weight assemblies.[14-18,23,24] Their structural information has not been drawn yet in detail, but McLaughlin suggested an idealized, periodic DNA lattice with regular and defined pores.[12] We postulate that the structures were composed of many self-assembled nanomeshes with a broad range of size scales. It also should be noted that there would be a possibility of incomplete elongation of all three arms; that is, only one or two of three arms were elongated in some cases, which makes the nanomeshes disconnected. Nonetheless, the pore size variability was, however, evident. The length of the templates was translated reasonably well into the pore size by PCR. Alternatively, DNA meshes can be prepared by dropping highly concentrated DNA solutions on mica, but the control of pore sizes has not been achieved yet.[26,27]

In summary, we demonstrated that two-dimensional DNA nanomeshes could be formed, and their pore sizes were controlled simply by varying template sizes during PCR processes: as one of the most widely-used techniques in various fields, the PCR is a simple and fast method. Our work showed that the PCR method could be a powerful tool to rapidly build two-dimensional DNA nanomeshes from the DNA-template. In addition, the facile controllability of pore sizes by PCR indicated that the well-designed combination of enzymatic reactions and structural DNA nanotechnology would yield wider tools for manipulating DNA building blocks and their self-assembled structures.

Upon the preparation of this manuscript, a similar work was published by Keller *et al.*[27] Their work described the synthesis of the DNA network by PCR, which used the

identical concept of this work. However, the control of pore sizes in the nanonetwork has not been explored in their work.

Experimental Section

PCR Experiments. Trebler phosphoramidite (Glen Research, USA), deoxynucleotide-5'-CE phosphoramidites (Glen Research, USA), *Pfu* DNA polymerase (Stratagene, USA) and QIAprep spin column (Qiagen, USA) were used as received. The three-armed primers were synthesized by Genotech Co. Ltd. (Korea) on a 1-μmol scale, and then purified by reverse phase HPLC. Prior to use of each oligonucleotide, a working stock solution was prepared with 10 mM Tris buffer (pH 7.4). Two-step PCR processes were carried out to obtain DNA nanomeshes. The first PCR was performed with 2.0 μL of plasmid DNA (0.5 ng/μL), 2.0 μL of forward primer (100 pmol/μL), 2.0 μL of reverse primer (100 pmol/μL), 4.0 μL of dNTP (10 mM), 20.0 μL of 10 × reaction buffer, 4.0 μL of *Pfu* DNA polymerase (5 units/μL), and 166.0 μL of distilled water. The reaction mixture was incubated at 94 °C for 30 sec, and 40 cycles of denaturation (94 °C for 30 sec), annealing (57 °C for 1 min) and extension (72 °C for 2 min) were performed. The mixture was kept at 72 °C for 10 min and cooled down to 4 °C. After the first PCR, the PCR products were purified by a QIAprep spin column with 30.0 μL of elution buffer (10 mM tris buffer, pH 7.4). The second PCR was performed with 30.0 μL of the DNA solution from the first PCR process, 1.0 μL of dNTP (10 mM), 5.0 μL of 10 × reaction buffer, 1.0 μL of *Pfu* DNA polymerase (5 units/μL), and 14.0 μL of distilled water. The thermal cycling conditions were the same as those for the first PCR process. The PCR products were desalted by a QIAprep spin column with 30.0 μL of deionized water. The resulting DNA solutions were analyzed by electrophoresis using 2.5% agarose gel in 1 × TAE buffer as an electrophoresis buffer.

AFM Experiments. The resulting DNA structures were characterized by atomic force microscopy (AFM). The AFM imaging was performed in a tapping mode on a NanoScope IIIa multimode scanning probe microscope (Digital Instruments Veeco Metrology Group, USA) with tapping mode etched silicon probes. The resulting DNA solutions were diluted with 18.2 MΩ deionized water to concentrations of 10 ng/μL. Freshly cleaved mica (Premium Grade V-1 Muscovite Mica, Pucotech Co., Korea) was used as substrates for AFM characterizations. A 1.0 μL sample drop was spotted on a freshly cleaved mica and spread over an area of approximately 5 × 5 mm. The sample solution on the substrate was dried in air for approximately 5 min. Additional 1.0 μL of 60% ethanol/water was spotted on the same substrate, and the substrate was dried in air. AFM images were collected under ambient conditions in air. The acquired AFM images shown in this manuscript are free of modification, except for flattening to remove the background curvature of the substrate surfaces.

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