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DNA Morphologic Changes Induced by Spermine on a Gold Surface under DNA Crowding Conditions

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DNA immobilized on a gold surface was globule in the absence of spermine. Upon addition of spermine, the DNA underwent a structural transition that was dependent on DNA length from a globular structure to a random coil. This effect was opposite the DNA morphologic changes observed in dilute solutions.

The conformation of DNA immobilized on a surface is of relevance to research in biotechnology and nanotechnology.[1,2] There are a number of techniques for DNA immobilization. Among them, oligonucleotide immobilization on a Au surface[3,4] and on the surfaces of nanoparticles[5–7] has been widely pursued because of simplicity in preparation. Recently, DNA immobilized on a solid support system has been employed in the sequencing of genes or mRNAs.[8,9] It has already been reported that oligonucleotide length affects immobilization[10] and enzymatic reactions with DNA on a surface affected by the density and structure of the DNA. Generally, high density suppresses enzymatic activities.[11–13]

In biological systems, the density and higher-order structures of genomic DNA are important in the regulation of transcription. Genomic DNAs exist as mesoscopic-sized aggregates with histone proteins, called chromatin, and structural changes in chromatin control gene expression.[14] Recently, it was suggested that higher-order structure of chromatin is affected by its density.[15] To duplicate DNA conditions in the nucleus in vitro, the preparation of high-concentration DNA solution is needed, although it is difficult because of the high cost and low solubility of long DNA. For the investigation of DNA behavior at high density, the surface immobilization of DNA is useful, since biomolecules can be accummulated on a surface. Thus, the control of the density and higher-order structure of DNA on a surface is crucial in both artificial and biological systems.

Here we report morphologic changes in double-stranded DNAs of different lengths immobilized on a Au surface via thiol–Au bonds. Five different lengths of DNA were employed: 500, 1000, 2000, 3110, and 5000 bp. DNA length effects on the immobilization behavior and morphology of DNA were investigated under various salt conditions of the supernatant solution.

DNA immobilization to the Au surface was performed according to previously reported procedures.[3,4] Thiolated DNAs were prepared by PCR using λ-DNA as a template (see Supporting Information[31]). Thiolated DNA solution (10 nM) was allowed to stand on the Au substrate for various time periods. The substrate was washed with ultrapure water and was then immersed in 1.0 mM 11-sulfanylundecanol for 1 h. During this time, a self-assembled monolayer (SAM) formed and nonspecifically bound DNA was removed. Amounts of immobilized DNA were quantified by SYBR Gold (Invitrogen) staining with fluorescence measured using FLA 7400 (FujiFilm Co., Ltd.). Until SAM formation, no fluorescence from SYBR Gold modified DNA was observed because of quenching by Au. Treatment with 1.0 mM 11-sulfanylundecanol results in SAM formation, and fluorescence was observed as the C11 alkyl SAM film blocked the electronic interaction between SYBR Gold and Au.

First, the time dependence of DNA immobilization was investigated in 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl (Figure S1a[31]). Time courses of the immobilization of the five different lengths of DNA were similar and reached a constant value around 120 min. Maximum DNA densities were 0.58 ng mm^{-2} for all lengths of DNA. Therefore, the number of DNA molecules per unit area was inversely proportional to DNA length (Figure S1b[31]).

Amounts of immobilized DNA on the Au surface were dependent on NaCl concentration in the buffer (Figure 1a). The densities of DNAs immobilized in 1.0 M NaCl buffer were twofold higher than that in 0.1 M NaCl buffer. Higher DNA densities resulting from incubation in 1.0 M NaCl compared to the lower salt were caused by the suppression of electrostatic repulsion of DNA strands.[3] Maximum densities were similar for all strand lengths in the higher salt as they were in the low salt.

The addition of spermine altered immobilization. Figure 1b plots DNA lengths vs. DNA density in 0, 50, and 500 µM

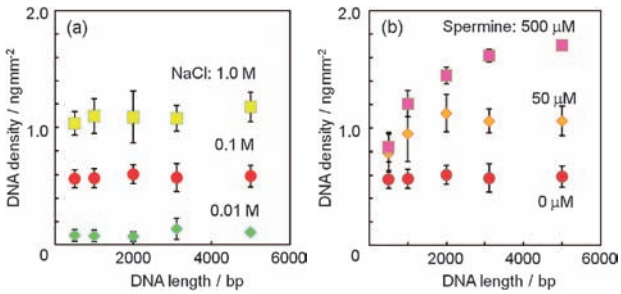

Figure 1. (a) DNA length effect on DNA immobilization in buffers containing 10 mM sodium phosphate and 0.01, 0.1, and 1.0 M NaCl at pH 7.0. DNA immobilization time was 4.0 h at room temperature. (b) DNA length effect on DNA immobilization in buffers of 10 mM sodium phosphate and 0.1 M NaCl in the presence of 0, 50, and 500 µM spermine at pH 7.0. DNA immobilization time was 4.0 h at room temperature.

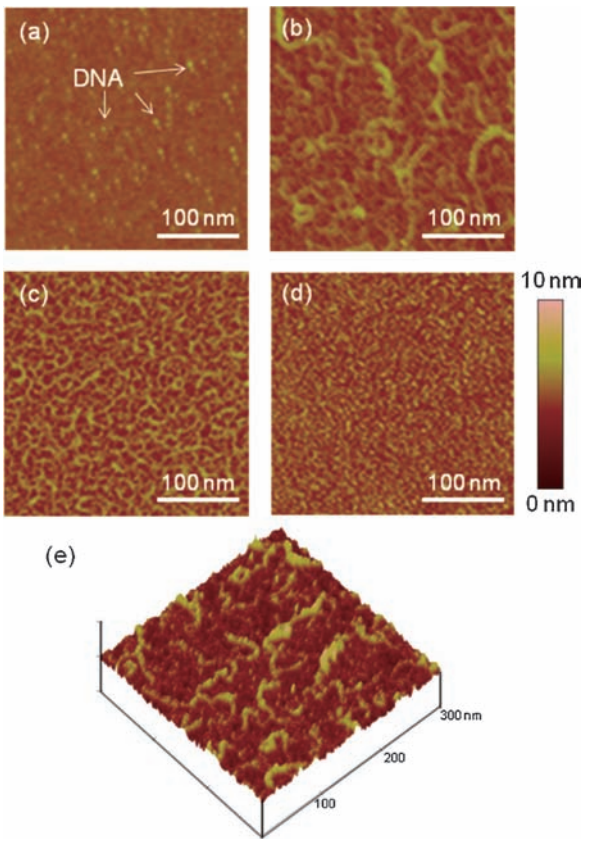

Figure 2. AFM images of immobilized 5000-bp DNA on the Au surface under various conditions: (a) DNA immobilized in 10 mM sodium phosphate buffer (pH 7.0) containing 1.0 M NaCl. (b) DNA immobilized in 10 mM sodium phosphate buffer (pH 7.0) in the presence of 500 μM spermine and 0.1 M NaCl. (c) DNA of image (a) after treatment with 500 μM spermine and 0.1 M NaCl containing buffer for 12 h at rt. (d) DNA of image (b) after treatment with 1.0 M NaCl containing buffer for 12 h at rt. (e) 3D view of AFM image of (b).

spermine. Without spermine, in 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl, DNA densities on the surface did not depend on length. With the addition of 50 μM spermine to the same buffer, density increased with strand length and was saturated at 2000 bp. In the case of 500 μM spermine, DNA densities increased as length increased for all chain lengths.

In order to investigate DNA morphologies in the absence and presence of spermine, atomic force microscopy (AFM) was performed on surfaces. DNA (5000 bp)-immobilized substrates were washed with ultrapure water and dried prior to AFM observation. When 10 mM sodium phosphate buffer (pH 7.0) containing 1.0 M NaCl was used as the immobilization buffer, particles were observed on the surfaces. These particles on the surface were not observed when surfaces were not treated with 11-sulfanylundecanol (Figure S2a[31]), suggesting that DNAs formed globule structures on the Au surface in this condition which were observed as particles (Figure 2a). When 5000-bp DNA was immobilized in 0.1 mM NaCl and 500 μM spermine, fibrous forms were observed (Figures 2b and 2e). We assume that random coil DNAs became entangled on the surface which were observed as fibrous structure by AFM. Formation of random coil structures should allow higher accumulation of DNAs on the surface than would formation of globule forms, since entangled form of randam coil DNAs increased in thickness and roughness of DNA layer (Figures S2b[31] and S2c[31]); this mechanism may result in the higher densities of DNA observed in the presence of spermine.

Interestingly, the particle structures could be converted to random coil upon treatment of the surface with buffer containing spermine and vice versa. Figure 2c is an image of immobilized DNA after the treatment of Figure 2a surface with a buffer containing 500 μM spermine overnight. In contrast, the treatment of Figure 2b surface prepared in the presence of spermine with a buffer containing 1.0 M NaCl resulted in the disappearance of the fibrous structures on the surface (Figure 2d).

DNA condensation has been studied in the presence of various multivalent cations[16–21] and polymer crowding agents.[22–24] In the absence of multivalent cation, long DNA aggregates are not very tight and take a rope-like sturucture based on a random coil. In the presence of multivalent cations, long size DNA condenses to form a globule structure through an entropically favored process with binding of multivalent cations and release of Na^+ ions. Surface behavior is clearly different from the behavior in a dilute solution. On the surface, globule structures were observed in the absence of spermine, while fibrous structures were in the presence of spermine. In this case, DNA compaction might be driven by an excluded volume effect caused by Coulombic repulsion of anionic backbones of DNA molecules to form globule structures. The addition of spermine to the solution above the surface resulted in decondensation to form fibrous structures on the basis of the entangle of randam coil DNA. The difference in DNA behavior might result from the difference in effective DNA concentration. The concentration of DNA in nucleotides in the dilute solution was 60–100 μM, whereas that on the surface of our system was calculated to be 15 mM assuming that the DNA layer was 50 nm thick. Such a high concentration system is close those in vitro calculated to occur in the presence of molecular crowding agents that mimic the high concentration of biomolecules inside cells.[25,26] We have reported that molecular crowding affects secondary and tertiary structures and the activities of nucleic acids.[27–30] The present results strongly suggest that the mesoscopic structures of larger DNAs are also affected by DNA concentration.

To investigate the effect of DNA length on morphology, AFM was performed on immobilized 500-bp DNA. In 10 mM sodium phosphate (pH 7.0), 1.0 M NaCl without spermine, globule structures were observed (Figure 3a). In 10 mM sodium phosphate (pH 7.0) and 0.1 M NaCl with 500 μM spermine, DNAs were random coils (Figure 3b). The 500-bp DNAs formed smaller structures than did the 5000 bp in the presence of spermine. Unlike the 5000-bp DNA, when globule structures of 500-bp DNA were incubated with spermine containing buffer, the particle structure was not completely lost (Figure 3c). Treatment of 500-bp DNA immobilized in the presence of spermine with spermine-free buffer did result in the morphologic change to globule structures (Figure 3d) as was observed for 5000-bp DNA. The mechanism that results in the length-dependent differences observed in reversibility of structures formed on the surface are unclear.

In summary, the higher-order structures formed by duplex DNA upon immobilization on a gold surface were different in

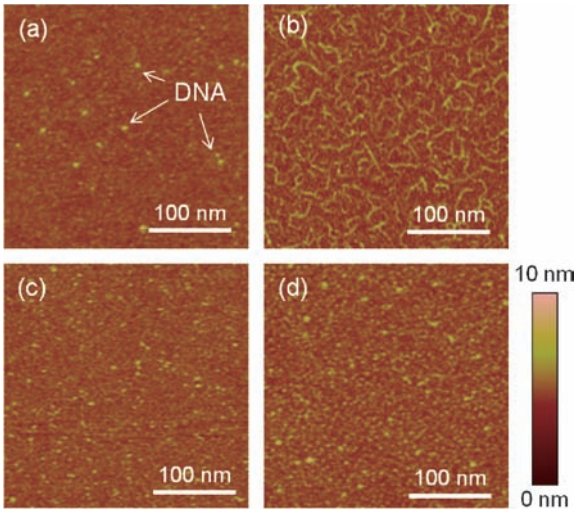

Figure 3. AFM images of immobilized 500-bp DNA on the Au surface in various conditions. (a) DNA immobilized in 10 mM sodium phosphate buffer (pH 7.0) containing 1.0 M NaCl. (b) DNA immobilized in 10 mM sodium phosphate buffer (pH 7.0) in the presence of 500 μM spermine and 0.1 M NaCl. (c) DNA of image (a) after treatment with 500 μM spermine and 0.1 M NaCl containing buffer for 12 h at rt. (d) DNA of image (b) after treatment with 1.0 M NaCl containing buffer for 12 h at rt.

the absence and presence of spermine. Independently of length from 500 to 5000 bp, globule structures were observed in the absence of spermine, and extended and entangled structures were formed in the presence of spermine. These results were opposite those observed in the dilute solutions and may be caused by the high effective DNA concentration present on the surface. These results indicated that DNA duplexes in the range of hundreds of base pairs immobilized on a surface behave in a manner thought to mimic the structures and compaction in crowding conditions that exist in cells.

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