Attachment of Monoclonal Antibody Molecules to Surface-Confined DNA Duplexes Imaged by Atomic Force Microscopy

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A detailed elucidation of the factors affecting the binding of anti-double-stranded (ds)-DNA antibody to ds-DNA immobilized on surfaces may provide insight into the pathogenicity of the systemic lupus erythematosus disease and lead to the development of surfaces for specific DNA and antibody recognition. In this work, the influence of the surface parameters on the attachment of antibody to DNA under physiologically relevant conditions was studied by magnetic alternating current mode atomic force microscopy, ellipsometry, and fluorescence spectroscopy. The DNA surface orientation (tethered vs flat lying), duplex surface density, antibody concentration, and reaction time were all found to affect the antibody/DNA conjugate formation. The mouse monoclonal antibody was specific toward ds-DNA over single-stranded DNA. The steric hindrance and reduced flexibility of surface-confined polynucleotide DNA molecules for the conjugate formation were examined by comparing the conjugates attached to calf thymus DNA preimmobilized onto a surface to those preformed in a homogeneous solution comprising DNA and antibody molecules and subsequently attached to the surface. Antibody aggregation was also found to be prevalent at surface sites where DNA/antibody conjugates existed.

1. Introduction

Anti-DNA autoantibodies are a major component of systemic lupus erythematosus (SLE). Anti-double-stranded (ds)-DNA antibodies can be detected in the sera of diseased individuals and have been shown to interact with ds-DNA to form deposits in kidneys. Thus, a detailed elucidation of the binding of anti-ds-DNA antibody to DNA may provide insight into the pathogenicity of the SLE disease. On the other hand, because the DNA duplexes could specifically bind with the anti-ds-DNA antibodies, the recognition of ds-DNA or DNA duplex formation occurring at the heterogeneous DNA biosensor surface may become possible.

The driving force for the ds-DNA/antibody conjugate formation has been postulated to be the electrostatic interaction between the negatively charged DNA phosphate groups and the positive charges associated with IgG. The secondary structure of DNA is also known to be important. It has been verified that, through the antibody deposition in the kidney, the anti-ds-DNA monoclonal antibodies initiate an inflammatory response, causing the lupus nephritis.

A number of reports have demonstrated that atomic force microscopy (AFM) is a valuable technique in revealing the details of the DNA/protein interaction at the molecular level. AFM studies on immunological...
reactions and the antibody conformations at surfaces have also been reported. Bonin et al. have employed AFM to analyze the preferential binding sites of ds-RNA for anti-ds-RNA antibodies and showed that the monoclonal anti-ds-RNA antibody J2 binds ds-RNA in an apparently sequence-nonspecific way. On the other hand, other reports have shown that somatic mutations at the periphery of the DNA binding site are particularly important in human monoclonal anti-DNA antibodies. For example, Swanson et al. and Wang et al. have shown that the binding sites on the ds-DNA can be as few as 3–4 bases, and the base sequences are important for the antibody recognition of nucleotides.

Because the antibody deposition may occur at tissue surfaces, it is possible that the surface parameters (e.g., the morphology of the surface and the coverage and orientation of nucleotides) play important roles in the antibody/nucleotide conjugation formation. For example, the surface structure and the elastic properties of the DNA have been proven to be crucial for the p53 recognition, a DNA-binding transcription factor protein that is believed to be important in tumor suppression. These factors are known to be essential for other types of biomolecular interactions. Recently, using AFM and other techniques, we have shown that these surface parameters can significantly affect the hybridization of a single-stranded (ss)-DNA probe with both polynucleotide and oligonucleotide targets in the solution. Therefore, in this work, we examined the influence of those sequence-unrelated factors, under physiologically relevant conditions (e.g., pH and ionic strength), on the extent of DNA/antibody interaction in a quantitative or semiquantitative manner. Several factors, including the DNA surface orientation (tethered vs flat lying), duplex surface density, antibody concentration used for the conjugate formation, and time duration for the antibody/DNA interaction, were investigated. We also studied the selectivity of the mouse monoclonal antibody toward ds-DNA over ss-DNA. The possible effect of steric hindrance and reduced flexibility of surface-confined DNA molecules on the extent of conjugate formation was contrasted between the conjugates formed at a calf thymus DNA-covered surface and those first formed in a homogeneous solution of calf thymus DNA and antibody and subsequently anchored to a surface. Our work demonstrates that a variety of experimental parameters may alter the binding of antibody to DNA at the surface/solution interface and suggests that a judicious choice of preparative procedures may lead to the development of surfaces capable of recognizing specific DNA/antibody binding.13

2. Experimental Section

2.1. Materials. Sodium borohydride was acquired from Metheson Coleman and Bell Manufacturing Chemists Co. (Norwood, Ohio). Sodium chloride (Fisher Scientific), (aminopropyl)trimethoxysilane (APTES, Aldrich), and sodium dodecyl sulfate (SDS, Aldrich) were used as received. An aminohexyl-modified 30-mer probe with a sequence of 5′-AGA GGA TCC CCG GCC ACC GAG CTC GAA TTC (CH2)7NH2-3′ and 47-mer targets with and without the Cy3 label with a sequence complementary to that of the 30-mer probe (5′-GAA TTC GAG CTC GGT ACC GAC CTC GCC TCT GTT GCC CTG GTT TTG AC-3′) were acquired from Integrated DNA Technologies, Inc. (Coralville, IA). A mouse monoclonal IgM (clone 11B6, ab418-1), which shows 100% reactivity to ds-DNA and 5% to ss-DNA, was obtained from Abcam (Cambridge, U.K.). Aldehyde glass substrates (TeleChem International, Inc., Sunnyvale, CA) and silicon(100) wafers (Silicon Valley Microelectronics, Inc.) were used for the DNA immobilization, hybridization, and antibody attachment. Calf thymus DNA and all other chemicals were purchased from Sigma. Water was treated by a Millipore system with a resistivity greater than 18.2 MΩ/cm.

2.2. Solution Preparation. The probe and target solution preparation followed our published procedure. The sodium borohydride solution (1.5 g of NaBH₄ dissolved in 450 mL of phosphate buffered saline (PBS), followed by the addition of 133 mL of absolute ethanol) was freshly prepared prior to each experiment.

2.3. Sample Preparations. Oligonucleotide Probe Immobilization, Duplex Formation, and Antibody Attachment. A modified version of the protocol described by Schena et al. was used to graft the aminated oligonucleotide to aldehyde-derivatized glass. The aldehyde-modified glass slides were soaked in a PBS buffer containing a 1-mM 30-mer probe. These substrates were allowed to dry for 12 h at room temperature (±25 °C), followed by rinsing twice with a 0.2% SDS solution for 2 min to remove any nonspecifically adsorbed DNA probes. After a final rinse with deionized water, the substrates were dried under a nitrogen stream. The unreacted aldehyde groups were deactivated by soaking the substrates in a sodium borohydride solution for 5 min, washing the substrates twice with a 0.2% SDS solution for 1 min, and rinsing with water for 1 min. The DNA duplex formation was achieved by immersing the duplex-covered slides in a target DNA solution for 1 h. Finally, the antibody attachment was accomplished by exposing the duplex-covered slides to phosphate buffer solution (PBS, 0.1 M) comprising various anti-DNA antibody concentrations. Different exposure times (ranging from 15 min to 14 h) were tested. For the study of the antibody attachment to polynucleotides, freshly cleaved mica sheets were first treated with 133 mL of absolute ethanol.


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Attachment of Homogeneously Formed Anti-ds-DNA Antibody/DNA Conjugates onto Mica Surfaces. ds-DNA antibody complexes were formed by mixing calf thymus DNA (final concentration = 10 \mu g/mL) with antibody (final concentration = 3 \mu g/mL) in a pH 7.0 PBS/30 mM NaCl solution for 10 min at room temperature. A total of 10 \mu L of the sample was then cast on a freshly cleaved mica surface for 1 min, followed by washing the substrate with deionized water and drying with a stream of nitrogen.

2.4. Sample Characterizations. Spectrofluorometric Measurements. A total of 100 \mu L of a 1.0 \mu M amine-modified 30-mer was spread onto the surface of an aldehyde-modified glass slide and allowed to dry at ambient temperature overnight. The resultant DNA-covered slide was rinsed with a 0.2% SDS solution and water to desorb nonspecifically adsorbed probes. The unreacted aldehyde groups were deactivated by treating the slide with a 0.3% NaBH4 solution for 5 min and subsequently rinsing with a SDS solution and water.

The probe-covered slide was incubated in 100 \mu L of a Cy3-labeled target DNA solution for 3 h. The hybridization condition was identical to that employed for the formation of unlabeled duplexes. Upon completion of the hybridization, each slide was rinsed with a washing buffer. The slide was then immersed in a 50-mL DNA-free solution at 90 °C for 5 min to denature the DNA duplex and to release the Cy3-labeled DNA from the surface. The container was then rapidly transferred to an ice water bath and allowed to stand for 20 min. Quantification of the Cy3-labeled target released was conducted by monitoring the fluorescence intensity of Cy3 at an emission wavelength of 565 nm using a Cary Eclipse spectrofluorometer (Varian Instruments).

AFM Measurements. AFM images were collected with a PicoScan SPM microscope (Molecular Imaging, Phoenix, AZ) that is equipped with the MAC mode in which the probe oscillates near its resonant frequency under an alternating magnetic field. All measurements were conducted at room temperature. This technique (MAC-AFM) has proven to be advantageous for measuring soft samples in liquid media. Probes with a 2.8 N/m spring constant and a 30-kHz resonance frequency (Molecular Imaging) were used for imaging. All images were collected upon 12 h of soaking in antibody solutions except for those related to the study on the time dependence of the duplex/antibody interaction. The imaging was carried out at a scan rate of 1 line/s with 512 data points per line and a driver current of 10 ± 5 \mu A. The amplitude change of the probe was sufficiently low, and, therefore, the imaging was essentially nondestructive to the sample. Heights ranging from 1 to 100 nm were estimated by section analysis using the PicoScan program (version 4.18) without calibration, and lateral sizes in the range of 10–1000 nm were calibrated using a calibration grid. At least three regions of each surface were examined to verify that similar structures existed throughout the sample. No filter treatment was used to modify the images. SPIP software (Image Metrology, Denmark) was used for the roughness analysis.

Ellipsometric Measurements. Ellipsometry was performed using a picometer ellipsometer (Beaglehole Instruments, Wellington, New Zealand) that utilizes phase-modulated ellipsometry for continuous real-time signal monitoring. The sensitivity is typically equivalent to an ellipticity of about 10^{-5} or to a polarization angle of 10^{-3} at a time constant of 1 s (corresponding to a film thickness of approximately 0.1 Å).

3. Results and Discussion

Figure 1 schematically depicts the procedures involved in the oligonucleotide probe/target duplex formation and the attachment of mouse monoclonal antibody at low and high antibody concentrations. Prior to the duplex formation, the amine-modified 30-mer probes were affixed onto aldehyde-modified glass slides via the well-known Schiff’s base formation. The immobilized probes were then hybridized with the target in solution to form duplexes at the surface. Mouse monoclonal antibody molecules (mAb) were attached onto the regions where ds-DNA duplexes exist. Because the anti-ds-DNA antibody used (IgM) is a pentamer, each antibody could interact with one or more ds-DNA molecules at the surface. The surface feature arising from the anti-ds-DNA antibody attachment is dependent on the antibody concentration. At higher concentrations, antibody molecules could potentially deposit onto spots where ds-DNA/antibody conjugates had formed. As will be described, the DNA surface density and orientation were also found to have a profound influence on the structure of the resultant ds-DNA/antibody conjugates.

The density of the 30-mer probe/47-mer-target duplexes at an aldehyde-modified substrate was first quantified by spectrofluorometry. In conducting this measurement, we first derivatized the 47-mer target molecules with a fluorophore (Cy3) and allowed the labeled target molecules to hybridize with the surface-bound probe on slides with an area of 0.7–1.0 cm². The surface-confined duplexes

Figure 1. Schematic representations showing the aldehyde glass slide covered with ss-DNA probe, DNA duplexes, ds-DNA/antibody conjugates produced at a lower antibody concentration, and antibody aggregates formed at a higher antibody concentration.
After the DNA hybridization, $S_a$ increased to 1.4 nm. We attribute this small change in the surface roughness to the 47-mer DNA target attachment onto the 30-mer-confined surface. Using ellipsometry, we also measured the apparent thickness of the adsorbate layer of the aldehyde-modified (2.25 ± 0.16 nm), ss-DNA-modified (2.55 ± 0.18 nm), and ds-DNA-modified (2.65 ± 0.23 nm) surfaces.

Because both AFM and ellipsometry measure the average thickness of the surface, they are subject to uncertainties associated with these surface-bound DNA molecules (e.g., nonuniformity in the molecular orientation and the elasticity of the DNA strands). The fully stretched ss-DNA probe exhibits a theoretical height of 13.6 nm and a width of 2 nm. Therefore, a 0.30-nm increase in the thickness measured by ellipsometry accompanied by the ss-DNA attachment to the aldehyde slide would correspond to a surface density of $1.2 \times 10^{-12}$ mol/cm$^2$ (or about 2.2% of a full surface coverage) of perfectly tethered DNA molecules. Similarly, when the 0.10-nm change in the film thickness brought about by the duplex formation was used, the hybridization efficiency was thus, estimated to be about 60%, which is reasonable for the DNA hybridization occurring at the solid/solution interface. The spectrofluorometric and ellipsometric results appear to be quite consistent (differing only by approximately 2 times), despite some uncertainties and several assumptions. For example, the elasticity of the DNA molecules was ignored because in the interpretation of the ellipsometric data DNA molecules were assumed to be rigid rods. Moreover, the DNA surface orientation was simplified to be uniformly tethered with respect to the substrate. Given the large experimental errors in determining the average thickness, the estimate of the surface densities of the ss-DNA and ds-DNA molecules are semiquantitative at best. Nevertheless, the comparative study based on spectrofluorometry, AFM, and ellipsometry confirms that the surface coverage of ss-DNA is not compact and not all the probes have been hybridized at a target concentration of 1 μM.

We next studied the interaction of mouse monoclonal antibody molecules with surface-confined DNA duplexes and examined the effect of antibody concentration on the surface morphology of the DNA duplex/antibody conjugates. At low antibody concentrations (0.01–0.1 μg/mL), the DNA duplex/antibody conjugates were mainly observed as globular features. The duplex/antibody conjugate surface densities (6.7 ± 1.7) × 10$^8$ complexes/cm$^2$ at an antibody concentration of 0.01 μg/mL (image not shown) and (6.9 ± 1.1) × 10$^8$ complexes/cm$^2$ at 0.1 μg/mL are both considerably lower than the aforementioned duplex density. In estimating the conjugate surface density, we only took into account the objects on the surface whose heights are equal to or greater than 10 nm. The selection of such a value partially stems from the fact that features of this height are essentially absent at the surface covered with only the DNA duplexes (Figure 3a), and the surface roughness of the slide specified by the vendor is at least 3 nm. At a lower antibody concentration (0.1 μg/}

The concentration is 1 mL (Table 1) and increases only slightly when the antibody concentration increases, the surface density of the result-

...the antibody molecules in solution can be attributed to the following.

...preformed in a solution containing both the DNA and the nucleotide duplexes are estimated to form conjugates with the antibody molecules (vide infra).

...that the tethered DNA configuration strongly disfavors the antibody attachment. This is in contrast to the situation of the interactions between DNA molecules lying on a slide covered with DNA duplex/antibody conjugates employing an antibody concentration of 1 μg/mL.

Table 1. Average Heights, Lengths, Areas, and Densities of the Conjugates or Aggregates Formed at Different Antibody Concentrations

<table>
<thead>
<tr>
<th></th>
<th>C = 0.01 μg/mL</th>
<th>C = 0.1 μg/mL</th>
<th>C = 1 μg/mL</th>
<th>C = 10 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>height, nm</td>
<td>19 ± 8</td>
<td>26 ± 6</td>
<td>28 ± 14</td>
<td>55 ± 30</td>
</tr>
<tr>
<td>length, nm</td>
<td>55 ± 23</td>
<td>55 ± 21</td>
<td>107 ± 22</td>
<td>589 ± 174</td>
</tr>
<tr>
<td>mean area of conjugates, × 10^5 nm^2</td>
<td>0.024 ± 0.012</td>
<td>0.024 ± 0.010</td>
<td>0.15 ± 0.11</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>density, × 10^5 conjugates/cm²</td>
<td>6.7 ± 1.7</td>
<td>6.9 ± 1.1</td>
<td>7.9 ± 2.3</td>
<td>0.84 ± 0.28</td>
</tr>
</tbody>
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The time of exposure of the duplex-covered slides to the antibody solutions was 12 h.

...the circular feature imaged by AFM has a mean area of (0.024 ± 0.010) × 10^5 nm^2, comparable to the literature values for the IgM molecule (0.0159 × 10^5 nm^2). At a higher antibody concentration (1 μg/mL; Figure 3c), the aggregate formed has a mean area of (0.15 ± 0.11) × 10^5 nm^2, corresponding to about 10 antibody molecules. At the highest concentration studied (10 μg/mL), the aggregates have evolved into large, oblong features with an area about (2.6 ± 1.6) × 10^5 nm^2. Such a significant increase in the conjugate size (Table 1) at the two higher antibody concentrations suggests that aggregation of antibody molecules occurs at the sites occupied by preformed ds-DNA/antibody conjugates. At 10 μg/mL, the severely aggregated antibody molecules prohibit one from clearly resolving conjugates of smaller sizes on the surface. Consequently, only the density of larger aggregates can be provided in Table 1.

If the DNA duplex surface density deduced by spectrofluorometry is used, only 0.04—0.05% of the oligonucleotide duplexes are estimated to form conjugates with the antibody molecules in the solution. When the antibody concentration increases, the surface density of the resultant conjugates remains relatively constant until 0.1 μg/mL (Table 1) and increases only slightly when the antibody concentration is 1 μg/mL. Such low percentages suggest that the tethered DNA configuration strongly disfavors the antibody attachment. This is in contrast to the situation of the interactions between DNA molecules lying flat on a surface and the antibody molecules in solution and even more to the case when conjugation has been preformed in a solution containing both the DNA and the antibody molecules (vide infra).

The observation that most of the DNA duplexes on the surface did not form conjugates with the antibody molecules in solution can be attributed to the following factors: (1) despite most being tethered by the heptylamine spacer, not all of the duplexes have surface orientations that favor the attachment of the antibody molecules; (2) the steric hindrance imposed by adjacent DNA molecules may affect an effective formation of DNA/antibody conjugates; and (3) at higher antibody concentrations (which should favor an extensive conjugate formation), the antibody molecules begin to aggregate at the sites where DNA/antibody conjugates have formed. Two important implications can also be drawn from these results: (1) when antibody aggregation takes place at the surface, specific interaction between the surface-bound ds-DNA molecules and the antibody molecules in solution could be further impeded and (2) the preferential deposition of antibodies over the DNA/antibody is interesting in its own right because surface-initiated aggregation and fibrillation have been noted to be important in the origination of certain neurodegenerative diseases (e.g., Huntington and Alzheimer diseases), 36,56–58

...we also found that the target concentration could alter the surface density of ds-DNA/antibody conjugates. Figure 4 shows a plot of the conjugate density versus the target concentration. When target concentrations lower than 1.0 μM were used, the density of the conjugate particles on the surface appeared to decrease proportionally. This trend is conceivable because the number of duplexes (which is related to the total number of conjugates) would decrease with the target concentration and cause a smaller number of conjugates to be formed. Note that, in generating this plot, an antibody concentration of 2 μg/mL was used for all the target concentrations, which may have led to conjugate surface densities slightly different than (within


experimental error) those based on different antibody concentrations (Table 1).

To confirm the inactivity of the antibody molecules toward ss-DNA and the absence of an extensive nonspecific adsorption, we immersed a 30-mer-covered substrate in a 10 μg/mL antibody solution for 12 h. After thoroughly washing the substrate, we found the mean particle height to be about 2.5 nm, with only about 7.8 × 10⁷ particles/cm² of heights greater than 6.9 nm (Figure 5a). The density is about an order of magnitude less than that of the ds-DNA/antibody conjugates and the height is substantially lower than the ds-DNA/antibody conjugate counterpart (19 nm). Thus, our AFM results are in reasonable agreement with the reactivity of the antibody toward ss-DNA (5%). The data also suggest that the antibody molecules do not adsorb onto the surface indiscriminately.

Although the convolution effect of the AFM tip obscures the detailed structure of the antibody molecules, it was found not to be serious enough to introduce uncertainties in the conjugate size measurements. The confirmation of the conjugate sizes was accomplished as follows. Upon casting 10 μL of a 0.01 μg/mL antibody solution onto an APTES-modified silicon surface, the resultant sample was frozen at −80 °C and promptly dried in a vacuum. This step was to reduce the elasticity and the thermal motion of the flexible antibody molecules that would affect the quality of the AFM imaging. Figure 5b displayed a representative topographic image of the resultant surface. The particles have a diameter of 48 ± 5 nm, which is in good agreement with the size of mouse monoclonal antibody molecules determined by scanning electron microscopy (30–45 nm). Therefore, we conclude that the interaction between the AFM tip and the antibody molecules is minimal and the values shown under 1 and 10 μg/mL antibody concentrations in Table 1, being greater than that of single antibody molecules, suggest that the aggregation of the antibody molecules had occurred.

While the previously described experiment shows that antibody molecules preferentially bind to ds-oligonucleotides, it is not clear whether the binding occurs exclusively at the solution ends of the tethered molecules and how the antibody molecules would interact with polynucleotides. We, therefore, placed calf thymus DNA molecules on a mica surface. Attachment of antibody molecules was found to take place both at the ends of and along the calf thymus strands (Figure 6a,b). It is apparent that the formation of DNA/antibody conjugates is not unique to ds-oligonucleotide in the tethered configuration. The detection of antibody molecules along the strand and at the strand ends is consistent with other studies. One of the driving forces for the observed conjugate formation is electrostatic attraction. It is possible that the orientation of the DNA molecules affects the accessibility of the phosphate groups to the incoming positively charged antibody molecules. As implied by Figure 1, we speculate...
that the specific interactions can also occur through the variable regions of the antibody molecules. The driving force behind such an interaction could be arising from several factors, including electrostatic interaction and the unique motif associated with the ds-DNA molecules (e.g., binding to the DNA grooves).

To investigate whether the confinement of the calf thymus DNA at the surface affects its binding to the antibody in solution, we compared the above two images to that of a surface covered with DNA/antibody conjugates preformed in a homogeneous solution (Figure 6c). The number of antibody molecules per unit length of DNA strand appeared to have increased from 5 to 9 molecules/µm, suggesting that the extent of DNA/antibody interaction is higher in solution than that at the surface/solution interface. Conceivably, the flexibility of the immobilized DNA molecules is much less than those in solution, and, consequently, the reduced flexibility should impede the formation of a large number of conjugates. Compared to Figure 6a,b, the size of the conjugates in Figure 6c is about 30% greater. Because the sizes of the conjugates in all three images are still comparable to single antibody molecules (within the experimental errors), the small size discrepancy may suggest that the DNA strands with antibody molecules pre-attached might be less firmly attached to the surface, which could enhance the interaction between the tip and the surface-confined species. In any case, the variation of the imaged conjugate size by 30% does not unambiguously indicate a serious aggregation of antibody molecules, suggesting that the aggregation shown in Figure 3 might be triggered or promoted by the tethered DNA molecules, which experience a much greater surface congestion and have a shorter strand for the attachment of antibody molecules.

To probe the time dependence of the conjugate formation, ds-DNA-covered slides were immersed in a 10 µg/mL antibody solution for different periods of time. Figure 7a,b depicted two AFM images after 15-min and 2-h immersions, respectively. Globular particles with a width of 48 ± 13 nm and a density of 5 × 10⁸ conjugates/cm² were the only species in the former image, and the density increased only slightly (7 × 10⁸ conjugates/cm²) in the latter. After a 10-h soaking, more conjugates, with widths within 40–80 nm, were found to deposit onto the surface (Figure 7c). Larger aggregates with an average width of 138 ± 94 nm began to appear after soaking the surface in an antibody solution for 12 h or longer (Figure 7d). Therefore, it is also clear that both the antibody concentration and the time for the DNA/antibody interaction can influence the conjugate formation and antibody aggregation.

4. Conclusions

The attachment of mouse monoclonal anti-ds-DNA antibody molecules to surface-confined oligonucleotides and polynucleotides has been studied by MAC-AFM. With the use of spectrofluorometry and ellipsometry, the surface densities of the ss-DNA probe and DNA duplexes have also been measured. These measurements enabled us to gauge the extent of antibody attachment to surface-tethered DNA duplexes. For the oligonucleotide duplexes...
studied, only a small fraction of the DNA molecules (~0.04–0.05%) was found to be involved in the DNA/antibody conjugate formation. Several factors (tethered DNA orientation, the steric hindrance to the antibody attachment imposed by neighboring DNA molecules, and the tendency of antibody to form aggregates at sites where DNA/antibody conjugates have formed) may have inhibited an extensive formation of the DNA/antibody conjugates. Our data clearly illustrates that antibody binds to ds-DNA much more strongly than to ss-DNA, suggesting that these antibody molecules may be used to distinguish surface-confined DNA duplexes from ss-DNA molecules. When a polynucleotide, such as calf thymus DNA, was immobilized with its strand lying flat on the surface, more antibody molecules can be attached per DNA strand (Figure 6). This observation supports that the tethered DNA configuration disfavors the formation of DNA/antibody conjugates.

For the DNA duplex hybridized between a tethered oligonucleotide probe and a target in the solution, the surface density of the DNA/antibody conjugate is also highly dependent on the target concentration used for the hybridization and the antibody concentration employed for the DNA/antibody conjugate formation. However, the effect of antibody concentration on the conjugate formation is more complex. At high antibody concentrations (1 µg/mL or higher), antibody molecules tend to agglomerate on top of the DNA/antibody conjugates. While the exact reason is yet unknown, the aggregation may be partially driven by the need to minimize steric congestion at the surface and to maximize the DNA/antibody and antibody/antibody interactions, again suggesting that the surface conditions (e.g., orientation, position, and coverage of ds-DNA) have a strong influence on these interactions. Our results suggest that, when studying biomolecular interactions at the surface/solution interface, a comprehensive description of the system is warranted only when other factors that can affect the specific interactions between the biomolecules are also considered.

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