Langmuir
The ACS Journal of Surfaces and Colloids

NOVEMBER 24, 1998
VOLUME 14, NUMBER 24

Letters

Orienting DNA Helices on Gold Using Applied Electric Fields

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Received July 14, 1998. In Final Form: September 24, 1998

Gold surfaces modified with thiol-derivatized DNA duplexes have been investigated as a function of applied electrochemical potential via atomic force microscopy (EC-AFM). At open circuit, monolayers of well-packed DNA helices form with a film depth of 45(3) Å. On the basis of the anisotropic dimensions of these 15 base pair duplexes (20 Å in diameter versus 50 Å in length), this corresponds to an average ~45° orientation of the helical axis with respect to the gold surface. Under potential control, the monolayer thickness (and therefore the orientation of the helices) changes dramatically with applied potential. At potentials negative of ~0.45 V (versus a Ag wire quasi-reference electrode) film thicknesses of ~55 Å are observed, whereas at more positive potentials the monolayer thickness drops to a limiting value of ~20 Å. These results are consistent with a morphology change in which the helices either stand straight up or lie flat down on the metal surface, depending on the electrode potential relative to the potential of zero charge (pzc). This voltage-induced morphology change is reversible and effectively constitutes a nanoscale mechanical "switch".

The self-assembly of monolayers on solid surfaces has been an intensely studied phenomenon for more than 15 years.[1] While most of this work has focused on *n*-alkanethiols on Au(111),[2] surfaces modified with both single- and double-stranded DNA have been prepared and investigated for potential use in biosensing[3] and biochemical imaging.[4,5] In addition, the electrochemical properties of redox-active intercalators bound to DNA-modified electrodes have been analyzed to probe charge-transport phenomena through the extended π-stack of

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10.1021/la980874n CCC: $15.00
Published on Web 11/04/1998

DNA.[6] To draw meaningful conclusions from these studies and to design heterogeneous DNA-based biosensors, it is essential to understand the surface structures of these modified gold substrates. Moreover, given the very high charges that characterize DNA-modified substrates (e.g., −30 for each 15-mer duplex), it is important to determine the effects of applied electric fields on the DNA surface structure, as many of the biosensing applications involve electrochemical detection schemes.[3]

The physical properties of densely packed aliphatic and aromatic thiol-terminated self-assembled monolayers (SAMs) on Au(111) have been studied extensively;[7] however, the corresponding DNA films are not nearly as thoroughly characterized. Here we report the results of an electrochemical atomic force microscopy (EC-AFM) study of double-stranded DNA oligonucleotide monolayers on Au(111). We have found that the DNA duplexes form well-packed films on the surface and undergo a dramatic morphology change as a function of applied electrochemical potential.

Experimental Section

Preparation of Thiol-Modified Oligonucleotides. Oligonucleotides immobilized on a controlled pore glass resin were treated in succession with carbonyldiimidazole and 1,6-diaminohexane (1 g/10 mL of dioxane, 30 min/each) at the 5′-hydroxy terminus before cleavage from the resin.[8] After deprotection, the free amine was treated with 2-pyridyldithiopropionic acid *N*-succinimide ester to produce a disulfide. Sequences were purified by reverse-phase HPLC, converted to free thiols using dithiothreitol, and repurified before hybridization to their complements. Derivatized oligonucleotides were characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and HPLC retention times. Before deposition onto gold surfaces, the presence of free thiol was confirmed using a spectroscopic assay based on Ellman's reagent.[9] Commercial Au(111) films on mica (Molecular Imaging) were flame-annealed in hydrogen and then modified by incubation in 0.1 mM solutions of thiol-deriviatized DNA (50 mM phosphate buffer (pH 7), 0.1 M $MgCl_2$) for 1−12 h. Samples were thoroughly rinsed in phosphate buffer before mounting. The deposition time required to obtain a desired surface coverage was somewhat batch-dependent, and longer times were typically required if the sample had not been made immediately before deposition. The extent of DNA coverage was checked before each experiment by examining the electrochemistry of ferrocyanide at the modified surfaces. As $Fe(CN)_6^{4-}$ is electrostatically repelled by the negatively charged DNA, its voltammetric response reports on the quantity of DNA bound to the surface. Although somewhat qualitative, we have found that this electrochemical assay yields surface-coverage values comparable (± ∼10%) to those determined directly by radioactive-tagging experiments.

Atomic Force Microscopy. All AFM images were collected using a MultiMode atomic force microscope running on the NanoScope IIIa controller (Digital Instruments, Santa Barbara, CA). A glass AFM electrochemistry chamber (Digital Instruments, Santa Barbara, CA) and a fluid volume of approximately 50 μL were used for the experiments. Si_3N_4 cantilevers (spring constant: 0.06 N/m) with integrated, end-mounted, oxide-sharpened Si_3N_4 probe tips were used. The applied vertical force of the AFM probe during imaging was minimized to beneath 200 pN. Continually adjusting the cantilever deflection feedback set point compensated for thermal drifting of the cantilever, and a consistent minimum force was maintained. AFM height calibrations were carried out on a NIST-traceable 180-nm height standard and then confirmed by measuring a single-atom step in the Au gold surface. The AFM images were recorded in either "Height" (constant force) or tapping mode. Potentials were controlled by a Princeton Applied Research Model 173 potentiostat/galvanostat, using silver and platinum wires for the pseudoreference and auxiliary electrodes, respectively.

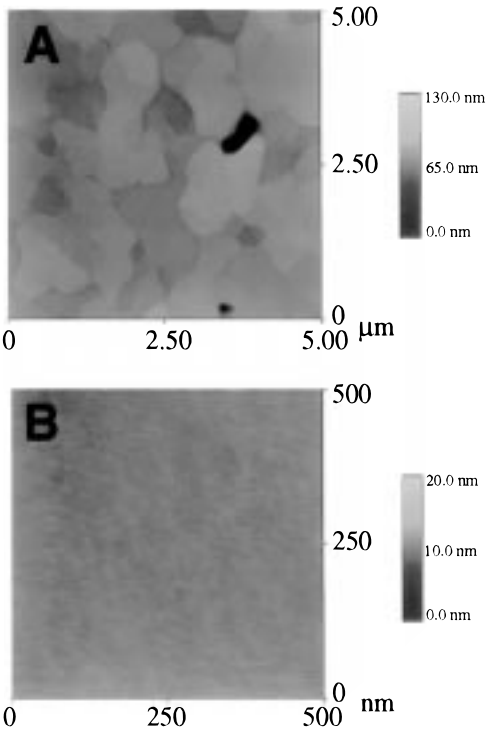

Figure 1. (A) AFM image (5 μm × 5 μm) of DNA-modified gold (sequence: 5′-AGTACAGTCATCGCG) recorded under fluid solution (0.1 M potassium phosphate buffer, pH 7). (B) Image (500 nm × 500 nm) from the same substrate area.

Results and Discussion

DNA-modified gold surfaces were prepared using a variation of a literature procedure.[6,10] Fifteen-base-pair, single-stranded oligonucleotides were synthesized by automated solid-phase techniques and manually derivatized with a thiol-terminated aliphatic linker. After hybridization to unmodified complements, the thiol-modified duplexes were deposited on Au(111) for 1−12 h. The metal substrates were then thoroughly rinsed with buffer (5 mM phosphate, 50 mM NaCl, pH 7, 0.1 M $MgCl_2$) and mounted in the microscope.

An AFM image of the resulting surface is shown in Figure 1. Several large, flat grains of the gold surface under the DNA film are clearly visible. While consistent with a well-packed monolayer, no local order is evident; lateral force instabilities (typically on the order of 1−10 nN) associated with scraping the probe tip across a soft overlayer are well-known to disrupt the local packing order.[11] The large structural anisotropy of the 15-base-

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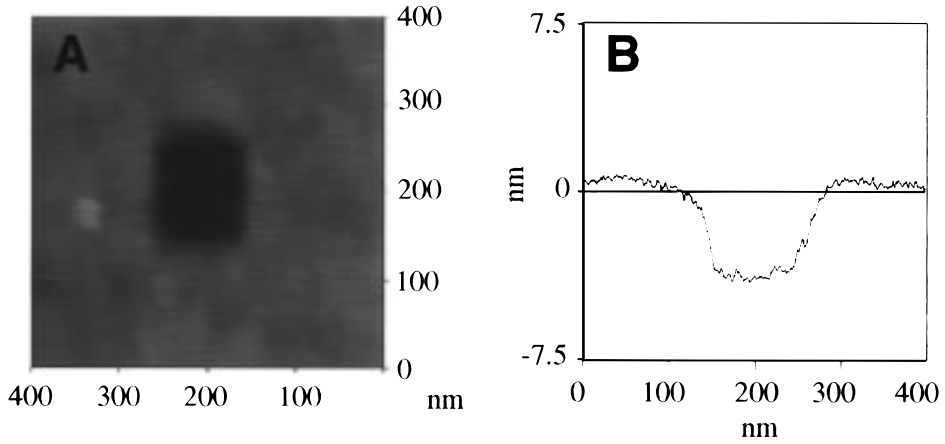


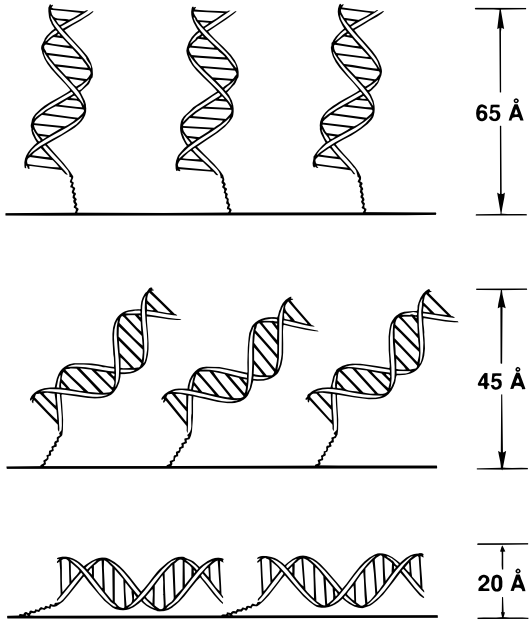
Figure 2. (A) AFM image of DNA-modified gold (sequence: 5′-AGTACAGTCATCGCG) after mechanical removal of a small square area (∼100 nm × 100 nm) of the monolayer. (B) Depth analysis of the area shown in part A.

pair DNA helices (20 Å in diameter versus 50 Å in length), however, provides a convenient handle to gain indirect information concerning the orientation of the duplexes on the surface. A small patch of DNA was removed from the gold by applying a large vertical force with the AFM tip (Figure 2),[12] and height-contrast measurements between the resulting square and the covered surface yield a monolayer depth of 45(3) Å.[13] Including the dimensions of the alkanethiol tether, this corresponds to an average helical packing orientation of ∼45° normal to the surface (Scheme 1).[14]

Although the film is very stable under open-circuit conditions, application of either large positive or negative potentials ($-0.5\ V \leq E_{app} \geq 1\ V$ versus Ag wire) results in rapid desorption of the monolayer, owing to redox reactions that occur at the gold−thiol linkages.[15] After removal of the film, the bare gold patch is no longer detectable, indicating that the mechanical scraping did not alter the structure of the metal within the resolution of this experiment.

We have also investigated the effects of applied electric fields on the surface morphology.[16] Electrostatic effects have been shown[4a,b,17] to modulate the surface coverages of charged species on gold electrodes polarized to either side of the potential of zero charge (pzc).[18] Consequently, for the DNA monolayers one might expect the potential-dependent morphology change illustrated in Scheme 1: at potentials negative of the pzc, the duplexes orient themselves normal to the surface in order to minimize electrostatic repulsion, whereas at potentials positive of the pzc, the duplexes lie flat.

Scheme 1



Our observations fit this model. Figure 3 shows the film thickness as a function of applied electrochemical potential (E_{app}) for a ∼60% complete DNA monolayer. At potentials negative of ∼0.45 V (versus a Ag quasi-reference electrode), the film height increases from its open-circuit value to ∼55 Å (close to the dimension predicted for a fully extended duplex-linker conjugate[14] oriented normal to the gold surface). As the potential is progressively scanned to more positive potentials, the thickness remains relatively constant until $E_{app} \sim 0.45$ V, at which point the height drops dramatically to a limiting value of ∼20 Å (the diameter of duplex DNA). Importantly, this process is chemically reversible;[19] repeated switching (at least four complete cycles) between positive and negative applied potentials results in the formation of thin and thick monolayers, respectively.

(12) Holes in the monolayer were prepared by decreasing the scan size to approximately 100−150 nm, increasing the scan rate to 24−30 Hz, and increasing the vertical force by advancing the set point several units. After about 1 min, the scan size, scan rate, and set point were returned to their previous values, and images featuring a bare gold square were captured.

(13) All images captured for height-contrast analysis were recorded at minimum vertical tip forces. This was accomplished by decreasing the set point until the tip disengaged from the surface and then reintroducing it with the minimum force required to achieve a stable image. In several cases, the film height was also measured in tapping mode, which gave the same result as the contact-mode experiments.

(14) For simplicity, we have assumed that the thiol linker is both fully extended and collinear with the DNA. A molecular model of the tether yields a sulfur/DNA separation of 16.2 Å. However, as the self-assembly of these films is dependent on the much larger DNA helices, the exact conformation of the alkyl linker may vary somewhat. Depending on the effective length of the tether, the actual orientation of the DNA relative to the surface may deviate slightly from this 45° value.

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(19) In some experiments, we observed a slight hysteresis (∼10−30-mV shift) in the film-height change when scanning the potential in the negative direction. We were unable to distinguish whether this resulted from a kinetic or thermodynamic origin.

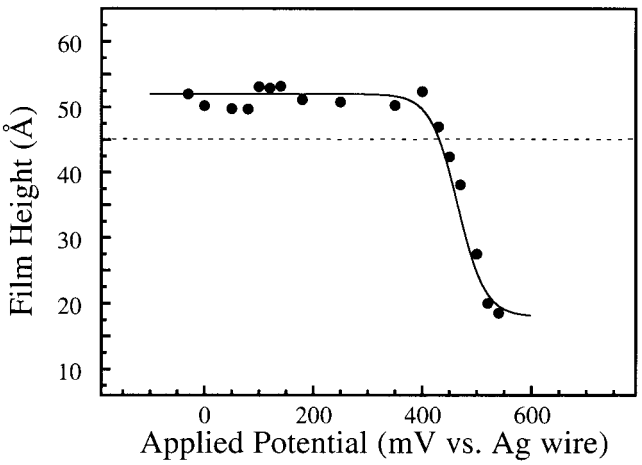

Figure 3. Potential dependence of monolayer thickness measured by atomic force microscopy under electrochemical control. The dashed line corresponds to the open-circuit value.

The (111) surface of gold is well-known to undergo a reconstruction at high potentials[20] which results in changes of the gold surface on the order of ~3 Å. This effect cannot account for the substantial changes in depth we observe. Therefore, we conclude that the film-thickness variations result from an orientation change of the duplexes in the monolayer.[21] Potential-dependent EC-AFM measurements on 100% complete monolayers are qualitatively similar to those shown in Figure 3; however, at potentials positive of ~0.45 V the more densely packed monolayers reach a limiting thickness of only ~35 Å. As the DNA surface density in complete monolayers is too large to accommodate a horizontal orientation of the helices,[6] it is likely that the observed 35-Å value represents the closest DNA/gold separation that is sterically possible. Whether there are specific conditions under which we can induce structure still remains an open question.[27]

The results presented here highlight several unique features of DNA thin films. Densely packed thiol-modified DNA duplexes readily adsorb to gold, with an apparent high and uniform surface coverage. Due to the large charge density of the DNA backbone (2−/base pair without condensed counterions), the orientation of the individual helices is very sensitive to surface charges on the metal substrate. As a consequence, employing EC-AFM, we have characterized a ~40-Å variance in DNA-film thickness using only small changes in the applied electrochemical potential to select the preferred helical orientation. As these changes are reversible, this system effectively constitutes an electrochemical nanoscale "switch". The ability to modulate the thickness of a monolayer using only small changes in potential may provide a unique design feature for the construction of sensors and nanoscale electronic devices.

Acknowledgment. This work was supported by the NSF (M.G.H. and E.M.S.), the Research Corporation (M.G.H.), and the NIH (Grant GM49216 to J.K.B., predoctoral traineeship to S.O.K.).

LA980874N

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(21) Because the Si_3N_4 probe tip possesses a slight negative charge in aqueous solution at pH 7,[22−24] we also considered the possible influence of surface/tip interactions on our film-height measurements. Recently, the electrostatic forces have been directly measured between a tip and a lipid bilayer surface in fluid solution:[23,24] they are relatively small compared to the total applied vertical force and do not appear to alter the surface topography. Bard and co-workers have also used AFM to measure double-layer forces between a silicon tip and bare or chemically modified gold surfaces.[25,26] At small separations, they observe a roughly linear change in the electrostatic force between the tip and substrate over an ~800-mV range of applied potentials. For our system, we estimate that these tip/surface interactions are no more than 5−20 pN, or 5−10% of the total vertical force used to image our surface, and therefore do not significantly affect our results. (This estimation is based on the dimensions of our silicon nitride tip ($r \sim 5-20$ nm) and the *maximum* electrostatic forces measured by Bard for a more highly charged silicon tip.) We also note that the changes in DNA film height occur within a very narrow range of potentials (~75 mV), in sharp contrast to the monotonic response that characterizes electrostatic interactions between the tip and the surface.[25−27] Finally, we have observed no measurable differences in film heights as a function of either pH ($6.5 < \text{pH} > 7.5$) or ionic strength (50−200 mM); both of these factors would be expected to affect our measurements, were electrostatic forces at our tip significant.

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(27) For example, with incomplete monolayers we have observed the formation of rows of DNA that form at positive applied potentials—a result that is consistent with duplexes nucleating at the fold of the $23 \times \sqrt{3}$ reconstruction.