Immobilization of DNA onto Gold and Dehybridization of Surface-Bound DNA on Glass

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Abstract

A thorough understanding of surface-bound DNA is crucial to the development of biosensors and other diagnostic techniques. This study analyzed procedures for attaching single-stranded DNA (ssDNA) to gold slides via a thiol linker. Contact-angle goniometry was used to determine that thymine (T_{20}) ssDNA attached more efficiently to the gold surface than did adenine (A_{20}) ssDNA because the T_{20} strands bound through the thiol groups, while the A_{20} mainly physisorbed onto the gold through the nucleobases. This work also used fluorescence microscopy to study the conditions that promote complete dehybridization of DNA immobilized on glass slides. When the glass slides were exposed to water or a 20 mM salt solution, a majority of dehybridization occurred within the first 2 hrs and full dehybridization occurred after 7 hrs of exposure.

Introduction

Surface-immobilized DNA is an important aspect of biosensor technology.[1–7] Due to its selective hybridization, DNA has been used in microarrays and other diagnostic techniques that screen for gene expression, genetic diseases, and mutations, such as the mutations associated with breast cancer.[1,5,7] However, in order to take full advantage of DNA's capabilities, the hybridization and dehybridization dynamics at various interfaces and surfaces must be thoroughly understood. Various techniques have been employed to study DNA at the interface, including surface plasmon resonance (SPR),[8] x-ray photoelectron spectroscopy (XPS),[9,10] and Fourier transform infrared (FTIR) spectroscopy.[10] This study focused on fluorescence spectroscopy and contact-angle goniometry to complement work using sum frequency generation (SFG).

SFG is a nonlinear optical probe that can be used to study DNA without labels.[11–14] For SFG to occur, two laser beams, typically one in the visible light spectrum and one in the infrared spectrum, are spatially and temporally overlapped on a surface-bound sample to produce a signal at the sum of the two incident frequencies.[15–17] The generated sum frequency signal contains molecular information about the sample's structure and orientation.[15,17] Due to the selection rules, SFG is inherently surface specific, providing a valuable tool to study surface-bound DNA. To observe hybridization and dehybridization in situ, the surface-bound DNA must be exposed to a solution of its complementary strand. Thus, to examine this phenomenon via SFG, the DNA must be attached to a material that generates a usable signal when an aqueous/solid interface is observed. Some materials, such as silica, do not always yield enough SFG signal to adequately distinguish between single- and double-stranded DNA. Gold, on the other hand, is a much more promising candidate, because its large nonresonant properties yield higher signal-to-noise ratios. This study therefore examined procedures for attaching ssDNA onto gold slides in hopes of improving SFG studies of immobilized DNA. Contact-angle goniometry was also used to optimize the procedure by analyzing the hydrophobicity and hydrophilicity of the surface before and after DNA deposition.

In addition to examination using SFG, DNA can also be studied using fluorescence microscopy.[7] To observe hybridization using fluorescence microscopy, ssDNA is immobilized on a surface and exposed to a complementary strand that has been labeled with a fluorescent molecule.[7,18–21] Fluorescence imaging has been used to determine the conditions in which hybridization occurs and is well suited for study of the melting, or dehybridization, of DNA duplexes. Understanding the conditions that cause DNA dehybridization not only provides information about the duplex's stability but is also crucial to developing reusable biosensors. This work used fluorescently labeled DNA to study the dehybridization behavior of DNA immobilized on glass slides under various salt conditions.

Background

Several studies have investigated functionalizing DNA to gold slides. Wolf et al. and Herne and Tarlov both developed procedures for immobilizing ssDNA onto gold-covered slides.[22,23] They attached a thiol linker, consisting of HS-$(CH_2)_6$-, to the 5' end of the ssDNA and used this linker to bind the DNA to gold.[22,23] This work used a similar method to attach DNA to gold, in order to understand the effects of various sequences and make the procedure suitable for SFG studies. In addition to examining DNA on gold, past studies have looked at immobilized DNA using SFG.[11–14] Sartenaer et al. attached ssDNA onto platinum substrates using a thiol linker, much like Wolf et al. and Herne and Tarlov, and analyzed these substrates using SFG. They found that the thiol linkers arrange in an ordered monolayer on the platinum, but the oligonucleotide chains attached to the linkers arrange in a disordered fashion.[13] Howell et al. also used SFG to investigate how various strands of DNA interact with gold slides. They exposed unmodified thymine strands, thiol-modified thymine strands, and thiol-modified adenine strands to gold slides. While the thiol-modified thymine strands bound perpendicularly to the gold, the unmodified thymine strands and the thiol-modified adenine strands had a tendency to lie flat on the gold surface.[12] This study complemented previous efforts to examine surface-bound ssDNA on gold using contact-angle goniometry.

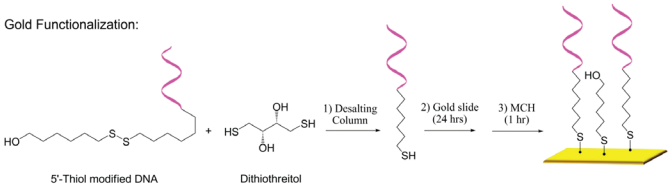

Figure 1. Deprotection and immobilization of thiol-modified DNA onto gold slides.

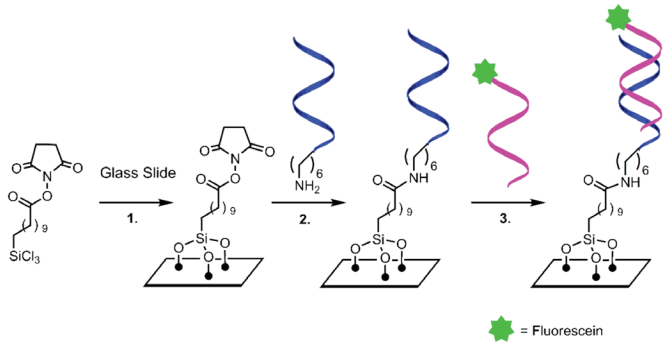

Figure 2. Covalent functionalization of DNA onto glass slides via amide bond formation[25].

A great deal of research has also been done on hybridization using labeled DNA.[7,9,18,19,24] Boman et al., for example, used fluorescence confocal microscopy imaging to determine the optimal exposure time necessary for a complementary strand to hybridize to an immobilized strand of ssDNA. They discovered that DNA hybridization of a surface-bound sequence requires 2 hrs of exposure to the complementary strand.[19] Although a great deal of research has been done on hybridization, there is little literature on the kinetics of DNA dehybridization. Thus, this study gathered complementary information for the dehybridization of surface-bound DNA.

Approach

Gold Slides

Gold slides were made by cutting microscope slides (Fisher Scientific) into fourths and evaporating gold onto them using an Explorer Metal Evaporator (Denton Vacuum). The operating voltage was 85 V, the current was 30 A, and the chamber pressure was 1×10^{-6} Torr. The thickness of the gold layer was monitored by a SQC-122c (Sigma Instruments) and varied between each batch of slides, ranging from 20 nm to 200 nm. A 5 nm chromium adhesion layer was also added under the 20 nm gold layer. Prior to functionalization, the slides were plasma cleaned using a Plasma Cleaner/Sterilizer PDC 32G (Harrick) for 1 min.

The gold slides were exposed to 5' thiol-linked ssDNA (purchased from IDT DNA). Two sequences were immobilized onto the slides: 20-mer adenine, A_{20}, strands (HS-$(CH_2)_6$-5'-A_{20}-3') and 20-mer thymine, T_{20}, strands (HS-$(CH_2)_6$-5'-T_{20}-3'). This ssDNA came with a thiol modifier C6 S-S protecting group. To cleave this group, the ssDNA was exposed to a 0.04 M solution of dithiothreitol (DTT) in 0.17 M sodium phosphate buffer (pH 8) for at least 16 hrs. The cleaved ssDNA was purified using a NAP-5 desalting column (GE Healthcare), and the concentration of DNA was measured with a Cary 300 Bio UV-Vis spectrophotometer (Varian) at 260 nm. The DNA was lyophilized (FreeZone system, Labconco) to remove any remaining buffer from the sample.

A 1 µM solution of the deprotected ssDNA in a 1 M potassium phosphate buffer (pH 3.8) was deposited onto the slides and left overnight. Some slides were also exposed to a 1 µM solution of DNA that had not been cleaved from its protecting group to determine if that step is necessary for maximum surface coverage. The following morning, the DNA solution was removed from the slides and each slide was rinsed with Millipore water and dried with N_2 gas. The slides were also exposed to a 1.0 mM aqueous solution of 6-mercapto-1-hexanol (MCH) for 1 hr. Figure 1 schematically depicts the deprotection and immobilization process that was utilized.

Contact-angle goniometry was used to assess the surface coverage of the gold slides before and after DNA and MCH deposition. Contact-angle measurements of water were gathered using a FTÅ125 Goniometer (First Ten Ångstroms) after functionalizing the slides. For example, measurements were taken of gold slides prior to functionalization, after only MCH exposure, after only DNA exposure, and after DNA and MCH exposure.

Glass Slides

The glass slides were made by cutting microscope slides (Fisher Scientific) into fourths with a diamond-tipped pen. They were cleaned by being sonicated in methanol for 6 min, rinsed with methanol, and dried in a 110° C oven (Thermo Scientific) for 30 min. The slides were also plasma cleaned using a Plasma Cleaner/Sterilizer PDC 32G (Harrick) for 1 min prior to deposition.

DNA was attached to the slides via the linker 11-(trichlorosilyl)-undecanoic acid N-hydroxysuccinimidyl ester (NHS).[25] This NHS linker was immobilized onto the slides inside a Nexus controlled atmosphere system glove box (Vacuum Atmosphere Co.). A solution was created by dissolving 10 mg of the NHS linker in 5 mL of anhydrous toluene, which was deposited onto the slides and allowed to react for 1 hr. The solution was then removed from the slides, and each slide was rinsed with fresh toluene. The slides were sonicated in toluene for 6 min and rinsed sequentially with toluene, methanol, and water (from a Millipore Milli-Q Biocel A10 instrument). Finally, the slides were placed in a 110° C oven (Thermo Scientific) for 1 hr to anneal.

Once the NHS linker was attached, the slides were reacted with 3'-amine modified 20-mer adenine sequences purchased from IDT DNA Technologies, Inc. (3'-$NH_3C_7H_{14}$-A_{20}-5'). To deposit the DNA, a 10 µM solution of the amine-linked ssDNA in sodium tetraborate buffer (pH 9.0) was pipetted over the glass slides and left to react overnight. The following morning, the DNA solution was removed and the slides were rinsed with Millipore water. They were stored in a 250 mM NaCl solution until hybridization.

To hybridize the immobilized ssDNA for fluorescence microscopy, it was exposed to a 10 µM solution of its complementary strand. This solution was created using the complementary 20-mer thymine sequence, which was modified with 6-carboxy-fluorescein at the 3' end, (3'-6-FAM-T_{20}-5', purchased from IDT DNA) and a 250 mM NaCl

solution. The solution was deposited onto the ssDNA-functionalized slides and allowed to react for 2 hrs. After hybridization, the slides were rinsed with a 250 mM NaCl solution, dried with N_2 gas, and stored in a desiccator until measurements were taken. Figure 2 shows a scheme of the functionalization process. Throughout the hybridization process and storage, the samples were covered in aluminum foil to minimize light exposure and photobleaching.

An analysis of dehybridization conditions was done by exposing the slides to water or a salt solution for various lengths of time. A few slides were left hybridized as controls, while the others were exposed to Millipore water or a 20 mM NaCl solution. All the slides were imaged using a Zeiss 510 Meta confocal laser scanning fluorescence microscope with a 20X objecting and a 488 nm Argon ion laser.

Results

Gold Slides

Gold slides were made using various amounts of gold. Some slides were made with 30 nm, 40 nm, or 200 nm of gold, while others had only 20 nm plus a 5 nm chromium adhesion layer. Contact angles of these slides were all within a few degrees of each other, suggesting that the thickness of the gold layer does not affect contact-angle measurements. The average contact angle for plain gold slides was 21° ± 6° (Table 1).

A_{20} vs. T_{20} ssDNA

Two different DNA sequences, A_{20} and T_{20} (both cleaved from the protecting group), were immobilized onto gold slides and examined using contact-angle goniometry. The gold surface alone had a contact angle of 21° ± 6°, which suggests that it is relatively hydrophilic. The surface became very hydrophilic when the A_{20} and T_{20} DNA strands were added, and the contact angle dropped to 12° ± 9° and 9° ± 1°, respectively. The addition of MCH created different surface environments based on the sequence used. The slides with the A_{20} ssDNA became more hydrophobic, with an increase in the contact angle from 12° ± 9° to 34° ± 10°. The slides with the T_{20} ssDNA, however, maintained their hydrophilic surface when MCH was added, and the contact angle only increased a few degrees, to 14° ± 3° (Table 1). Figure 3 shows images of the water droplets used to measure the contact angle before and after T_{20} ssDNA and MCH deposition. When only MCH was added to the gold slides, the contact angle was 27° ± 4° (Figure 4).

Protected vs. Deprotected T_{20} ssDNA

The T_{20} DNA was purchased with a thiol modifier C6 S-S protecting group. To observe whether or not removing this group is necessary, slides were functionalized with protected and deprotected T_{20} strands. After ssDNA was deposited, the slides with protected ssDNA had a contact angle of 10° ± 3° and the slides with deprotected ssDNA yielded 9° ± 1°. Once the MCH was added, the contact angles became slightly higher, with 15° ± 6° for the protected ssDNA and 16° ± 3° for the deprotected ssDNA (Table 2).

Glass Slides

The glass slides with hybridized DNA were exposed to either Millipore H_2O or a 20 mM NaCl solution for various lengths of time that ranged from 1 hr to overnight. After exposure, the slides were imaged with a Zeiss 510 Meta confocal laser scanning fluorescence microscope. Three to seven spots were examined per slide, and the amount of fluorescence intensity was noted. A slide with just ssDNA was also imaged and used as a baseline to factor out background noise.

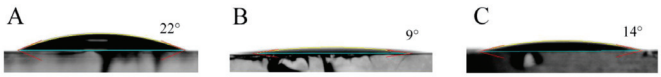

Figure 3. Contact-angle goniometry images of a) gold, b) gold with surface-bound T_{20} ssDNA, c) gold with surface-bound T_{20} ssDNA and MCH.

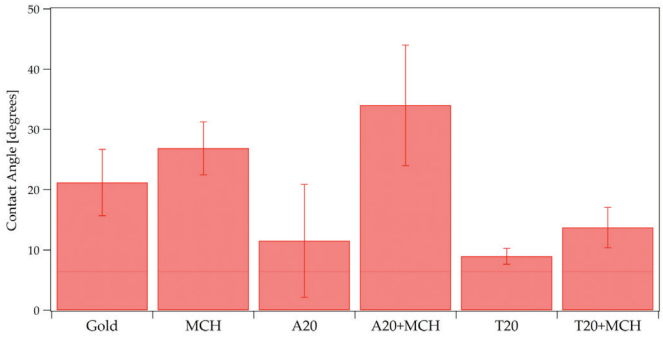

Figure 4. Contact-angle measurements of gold slides before and after exposure to A_{20} DNA, T_{20} DNA, and MCH.

	A_{20} DNA	T_{20} DNA
Gold	21° ± 6°	
Gold + DNA	12° ± 9°	9° ± 1°
Gold + DNA + MCH	34° ± 10°	14° ± 3°

Table 1. Contact-angle measurements of gold slides before and after DNA and MCH deposition.

	Protected T_{20} DNA	Deprotected T_{20} DNA
Gold	21° ± 5°	
Gold + DNA	10° ± 3°	9° ± 1°
Gold + DNA + MCH	15° ± 6°	16° ± 3°

Table 2. Contact-angle measurements of gold slides before and after the immobilization of protected and deprotected T_{20} DNA and MCH.

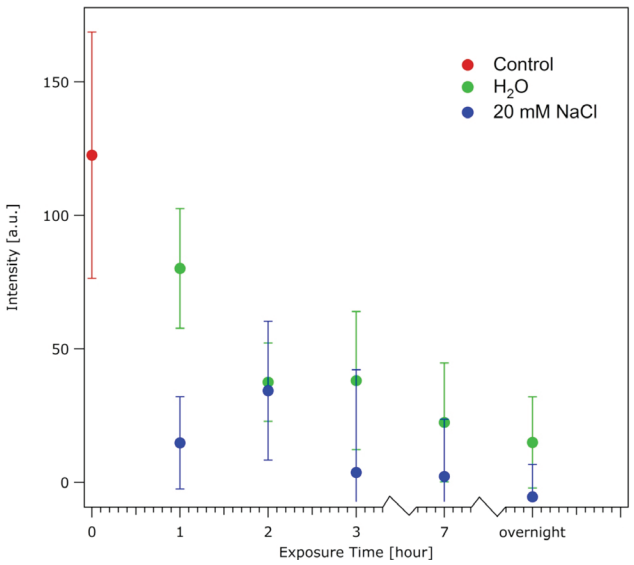

Figure 5. Fluorescence intensity after various hours of exposure to H_2O or 20 mM NaCl

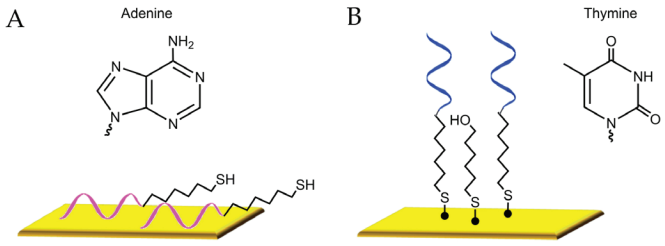

Figure 6. a) A_{20} sequence interacting with the gold substrate nonspecifically (structure of adenine is also shown), b) T_{20} sequence specifically binding to the gold substrate (MCH spacer and the structure of thymine are also shown)

The control slides of fluorescently tagged double-strand DNA, which were not exposed to any dehybridization conditions, had the highest fluorescence intensity. Within 2 hrs of exposure, the fluorescence intensity greatly dropped, indicating that dehybridization occurred. After 7 hrs of exposure, slides exposed to either solution had zero fluorescence, which suggests that complete dehybridization had occurred (Figure 5).

Discussion

Gold Slides

Changes in contact angle reflect the changes occurring on the surface.[26,27] The drop in contact-angle measurements after exposure to ssDNA indicates that the ssDNA interacted with the gold surface. Since DNA molecules are negatively charged, they create a more hydrophilic environment compared with plain gold surfaces, which causes a decrease in contact-angle measurements.

A_{20} vs. T_{20} ssDNA

Herne and Tarlov predicted that MCH adsorbs onto gold films between chemisorbed DNA strands and prevents any physisorbed DNA from remaining on the gold surface.[22] The sudden increase in contact angle that accompanied the addition of MCH to the A_{20} DNA suggests that most of the A_{20} DNA was nonspecifically bound to the gold via physisorption of the bases (Figure 6). Therefore, MCH replaced most of the A_{20} DNA, because the DNA did not bind to the gold through the thiol group. This phenomenon was also observed by Howell et al. when they examined thiolated A_5 and T_5 strands attached to gold using SFG.[12]

The T_{20} DNA, on the other hand, did not experience such a large increase in contact angle when MCH was added. This suggests that the T_{20} sequence was immobilized to the gold surface via the thiol group (Figure 6). When MCH was added, it attached between the chemisorbed DNA strands and only replaced a small percentage of the DNA, hence the slight increase in contact angle.

Since the T_{20} sequence binds to the gold slides through the thiol group, these surfaces are better suited to be studied using SFG. If the gold surface's electronic properties do in fact enhance the signal at the aqueous/solid interface, the gold slides can be used to study hybridization and dehybridization of DNA in real time via SFG.

Protected vs. Deprotected T_{20} ssDNA

The presence of the protecting group had minor effect on surface coverage. After DNA and MCH deposition, the contact-angle measurements were within a few degrees of each other. This study postulates that the protecting group had little effect on the contact angle because the protecting group is essentially a molecule of MCH attached to the DNA through a disulfide bond. Thus, when the protected DNA immobilized onto the surface, it created the same surface environment as the deprotected DNA and MCH (Figure 7). As a result, if time is an issue, the cleavage of the protecting group can be bypassed without compromising results.

Glass Slides

Since the fluorescence intensity of the hybridized slides greatly decreased after 2 hrs of exposure to H_2O or a 20 mM NaCl solution, this study found that a majority of the dehybridization occurred within the first 2 hrs of exposure. These results parallel the hybridization results found by Boman et al., who concluded that hybridization required 2 hrs of exposure to the complementary strand.[19]

However, in order to achieve complete dehybridization, the slides had to be exposed for 7 hrs. Thus, in terms of complete hybridization and dehybridization, there is a difference in the exposure times. This difference occurred because of the difference in the mechanisms of hybridization and dehybridization. For hybridization to occur, the complementary strand must overcome the forces of diffusion and reach the immobilized strand on the surface. For dehybridization to occur, however, the complementary strand must also break free of the immobilized strand. Since the DNA duplex is a chemically stable structure, more energy is required to disrupt the favorable base-stacking interactions. As a result, extra exposure time is needed when performing dehybridization.

Future research can be done by repeating the dehybridization experiment and altering a different aspect of the dehybridization environment. For example, the pH and DNA chain length can be varied

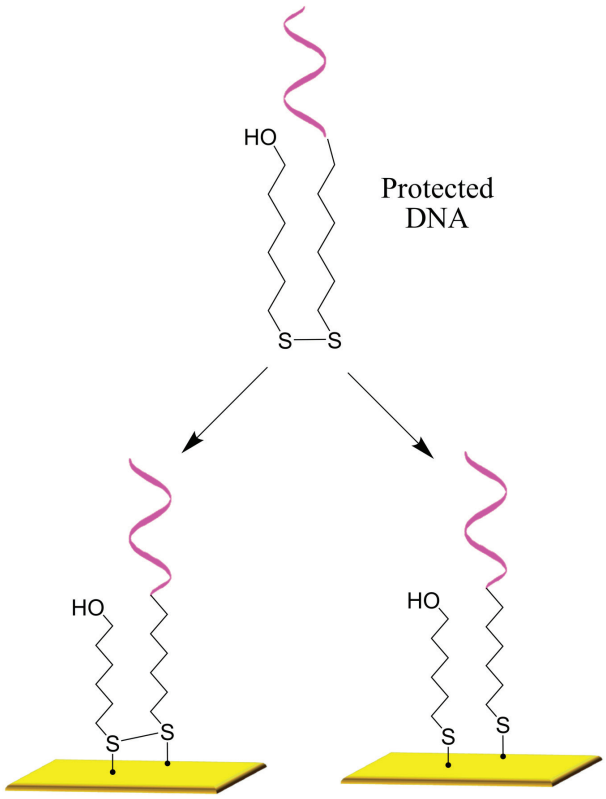

Figure 7. A structural interpretation of how protected DNA can bind to the surface by either maintaining the disulfide bond or breaking it. The final surface environment is essentially identical to the environment created when deprotected DNA is used (Figure 1).

to obtain a more general understanding of the conditions that promote dehybridization. Once dehybridization is fully understood, it can be incorporated into biosensor technology to create reusable biosensors.

Conclusions

In an effort to improve SFG studies, DNA was immobilized onto gold slides and contact-angle goniometry was used to analyze surface coverage. Optimal results were obtained with the T_{20} ssDNA. Unlike the A_{20} ssDNA, which physisorbed onto the gold surface, the T_{20} ssDNA bound to the gold through a thiol linker and successfully immobilized onto the surface. To gain a better understand of immobilized DNA, this study also examined the conditions necessary to dehybridize surface-bound DNA. Dehybridization was studied by immobilizing ssDNA onto glass slides, hybridizing it to a fluorescein-labeled complementary strand, and exposing the glass slides to water or a salt solution for various lengths of time. A majority of the dehybridization occurred within 2 hrs, and full dehybridization was observed after 7 hrs of exposure.

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