Observation of Hybridization and Dehybridization of Thiol-Tethered DNA Using Two-Color Surface Plasmon Resonance Spectroscopy

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Biosensor arrays of immobilized single-stranded DNA (ss-DNA) have the potential for quickly sequencing and assaying DNA samples by selective hybridization.[1,2] Applications of this technology include the sequencing of the entire human genome (The Human Genome Project) and the diagnosis of genetic disease. An immobilized oligonucleotide array contains ss-DNA (probe) molecules with known sequences that are tethered to an interface.[3] When the interface is exposed to a liquid sample containing the target, a ss-DNA molecule of unknown sequence, only those target molecules with sequence complementary to that the tethered ss-DNA probe will hybridize at the array surface. Ideally the biochemically active interface can be regenerated through either chemical or thermally induced dehybridization of the double helix. Optimal performance of this biochemically active interface is achieved by maximizing the number of active hybridization sites (tethered ss-DNA molecules) per unit area while minimizing the number of nonspecifically adsorbed target molecules.

In situ detection of hybridization can be through changes in mass,[4] changes in the optical properties,[5] or changes in the electrochemical properties[6] of the interface. Many of these methods require prior labeling of the DNA target or probe with special isotopes, fluorescence markers, or redox-active tags. Surface plasmon resonance (SPR) spectroscopy is known to be sensitive to the presence of unlabeled DNA at an interface.[7]

We report here the first quantitative *in situ* SPR study of the hybridization and dehybridization of a tethered unlabeled DNA film on a passivated gold surface. For the passivated gold surfaces used in these studies, no nonspecific adsorption of ss-DNA is observed, whereas a bare or partially covered gold surface will readily adsorb ss-DNA. For these studies, we use a novel two-color SPR method[8] that allows us to quantify the number of ss-DNA molecules per unit area for tethered DNA films. These two-component films containing a thiol-derivatized ss-DNA molecule and a diluent thiol, mercaptohexanol ($HS(CH_2CH_2)_3OH$), were prepared using molecular self-assembly techniques developed by Herne and Tarlov.[9] In these films, the 25 base oligomer is tethered to the gold surface via an alkanethiol covalently linked at the 5′ position of the ss-DNA. The mercaptohexanol serves to prevent nonspecific adsorption of ss-DNA. Using *in situ* SPR, we monitored the kinetics of hybridization for these films, determined the total number and percentage of active binding sites, and measured the hybridization activity of the film through five hybridization−dehybridization (melting) cycles.

SPR spectroscopy is an all-optical technique that is sensitive to changes in the dielectric constant at a metal surface.[10] In general, the SPR spectrum can be fit to an optical model that accounts for the thickness and complex dielectric constants of all materials at the interface; however, the thickness and dielectric constant for films <200 Å cannot be determined uniquely from a single SPR measurement.[11] As reported previously, we have developed a novel two-color SPR methodology that allows us to uniquely determine the thickness and dielectric constant of a transparent dielectric film.[8]

The two-color SPR apparatus and the procedures used for the measurements presented here have been described previously.[8,11] All water used was Nanopure[12] (18 MΩ cm). Tris[12] buffer and disodium ethylenediaminetetraacetic acid (EDTA) were used as received from Sigma, and NaCl and KH_2PO_4 were obtained from Fisher. The mercaptohexanol was kindly provided by Prof. Cary Miller (U. Maryland). The thiol-derivatized DNA (KMG2),[13] the underivatized DNA, and the underivatized complement (KMG2C) were obtained from Dr. Keith McKenney (The Institute for Genomic Research, Rockville, MD). Detailed SPR measurements of the film fabrication process will be presented elsewhere.[14]

In order to determine the amount of tethered DNA on the surface, we used two-color SPR to measure both the dielectric constant and thickness of the tethered single-component ss-DNA film (Figure 1). The measured film thickness in the 1.0 M KH_2PO_4, 176 Å, corresponds to the maximum possible length of a 25 base ss-DNA segment (∼166 Å) plus the length of the 6 carbon alkanethiol anchor (∼10 Å). This estimated maximum length of the ss-DNA is based on 83 Å for the length of the corresponding hybridized DNA.[15] Accounting for the contribution from the buffer solution, we determine that the DNA contributes $\Delta n = 0.009$ to the refractive index of the DNA layer. Using the relationship between refractive index and concentration $\Delta n/\Delta c = 0.14$ cm^3/g,[16] there are 0.064 g/cm^3 in the DNA layer, or 112 ng/cm^2 $= 9.0 \times 10^{12}$ ss-DNA molecules/cm^2. For the two-component tethered DNA mercaptohexanol film, the final coverage of ss-DNA was determined to be $(5.2 \pm 0.8) \times 10^{12}$ molecules/cm^2 (70−100 fmol/mm^2).

To check for nonspecific binding, the freshly made two-component monolayer film was first exposed to a 0.5 μM

(1) Tinoco, I. J. *J. Phys. Chem.* **1996**, *100*, 13311−22.
(2) Drmanac, R.; Drmanac, S.; Stezoska, Z.; Paunesku, T.; Labat, I.; Zeremski, M.; Snoddy, J.; Funkhouser, W. K.; Koop, B.; Hood, L.; Crkvenjakov, R. *Science* **1993**, *260*, 1649−52.
(3) O'Donnel-Maloney, M. J.; Smith, C. L.; Cantor, C. R. *TIBTECH* **1996**, *14*.
(4) (a) Fawcett, N. C.; Evans, J. A.; Chien, L.-C.; Flowers, N. *Anal. Lett.* **1988**, *21*, 1099−114. (b) Ebersole, R. C.; Miller, J. A.; Moran, J. R.; Ward, M. D. *J. Am. Chem. Soc.* **1990**, *112*, 3239−41. (c) Okahata, Y.; Matsunobu, Y.; Ijiro, K.; Mukae, M.; Murakami, A.; Makino, K. *J. Am. Chem. Soc.* **1992**, *114*, 8299−300. (d) Su, H.; Kallury, K. M. R.; Thompson, M.; Roach, A. *Anal. Chem.* **1994**, *66*, 769−77.
(5) (a) Garland, P. B. *Q. Rev. Biophys.* **1996**, *29*, 91−117. (b) Piunno, P. A. E.; Krull, U. J.; Hudson, R. H. E.; Damha, M. J.; Cohen, H. *Anal. Chem.* **1995**, *67*, 2635−43. (c) Stimpson, D. I.; Hoijer, J. V.; Hsieh, W.; Jou, C.; Gordon, J.; Theriault, T.; Gamble, R.; Baldeschwieler, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6379−83. (d) Watts, H. J.; Yeung, D.; Parkes, H. *Anal. Chem.* **1995**, *67*, 4283−9. (e) Xu, X.-H.; Bard, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2627−2631.
(6) (a) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chim. Acta* **1994**, *286*, 219−24. (b) Millan, K. M.; Mikkelsen, S. R. *Anal. Chem.* **1993**, *65*, 2317−23.
(7) (a) Piecevic, D.; Lawall, R.; Veith, M.; Liley, M.; Okahata, Y.; Knoll, W. *Appl. Surf. Sci.* **1995**, *90*, 425−36. (b) Bates, P. J.; Dosanjh, H. S.; Kumar, S.; Jenkins, T. C.; Laughton, C. A.; Neidle, S. *Nucleic Acids Res.* **1995**, *23*, 3627−32.
(8) Peterlinz, K. A.; Georgiadis, R. *Opt. Commun.* **1996**, *130*, 260−6.
(9) Herne, T. M.; Tarlov, M. J.; McKenney, K. H. Manuscript in preparation.
(10) Raether, H. *Surface Plasmons, Springer Tracts in Modern Physics*; Springer-Verlag: Berlin, 1988; Vol. 111.
(11) Peterlinz, K. A.; Georgiadis, R. *Langmuir* **1996**, *12*, 4731−40.
(12) Certain commercial products and instruments are identified to adequately specify the experimental procedure. In no case does such identification imply endorsement by the authors.
(13) *The KMG2 sequence is R-CAC GAC GTT GTA AAA CGA CGG CCA G, where R = HS(CH$_2$)$_6$ at 5′ end of the DNA molecule. The complementary sequence is GTG CTG CAA CAT TTT GCT GCC GGT C.*
(14) Peterlinz, K. A.; Georgiadis, R.; Herne, T. M.; Tarlov, M. J. Manuscript in preparation.
(15) (a) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737. (b) Wilkins, M. H. F.; Stokes, A. R.; Wilson, H. R. *Nature* **1953**, *171*, 738. (c) Franklin, R. E.; Gosling, R. G. *Nature* **1953**, *171*, 740.
(16) *This is a weighted average value for the component phosphates (0.09 cm^3/g), sugars (0.14 cm^3/g), and bases (0.19 cm^3/g) of DNA in water.*
(17) Turner, D. H.; Sugimoto, N.; Freier, S. M. *Ann. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 167−92.

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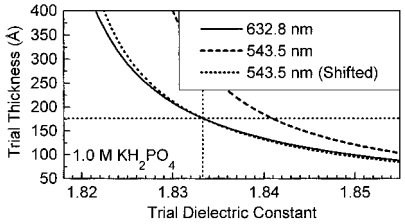

Figure 1. Determining thickness and dielectric constants for a single-component thiol-tethered DNA film from two-color SPR. Shown are the trial film thickness versus trial film dielectric constant, ϵ, curves calculated from SPR spectra of DNA tethered to a gold surface in 1.0 M KH_2PO_4 (buffer, estimated pH = 3.6). The trial curves represent all possible combinations of thicknesses and dielectric constants that equally well describe the SPR spectra. Accounting for the dispersion in the tethered DNA layer, the two curves cross at the true thickness and dielectric constant (at 632.8 nm). In 1.0 M KH_2PO_4, the film thickness is 176 Å (ϵ = 1.833). From a similar experiment in water, the film thickness is 21 Å (ϵ = 1.945). At 632.8 nm the film thicknesses are 1.809 and 1.773 for 1.0 M KH_2PO_4 and water, respectively.

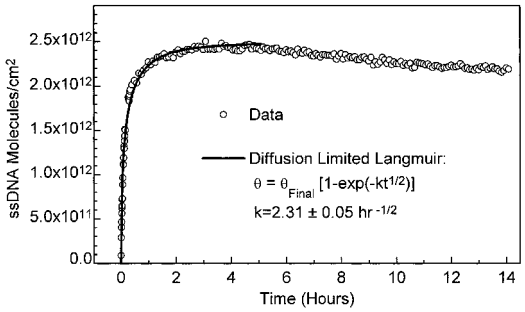

Figure 2. Hybridization kinetics of a tethered DNA mercaptohexanol film. Shown is the coverage vs time for DNA tethered to a gold surface after exposure to a 0.50 μM solution of the complementary DNA fragment in 1.0 M NaCl and TE buffer (see text, pH 8.2). The coverages (○) were calculated from SPR spectra. The kinetics are described well by a diffusion-limited Langmuir adsorption model (—).

solution of nonthiolated KMG2 in 1.0 M NaCl solution with 10 mM Tris and 1 mM EDTA (Tris EDTA or TE buffer, pH 8.2) at 22−24 °C. KMG2 has the same sequence as the surface bound probe and is therefore noncomplementary (i.e., it will not hybridize with the surface-confined DNA). No change in the SPR spectrum was observed indicating that no nonspecific binding occurs. Next, the film was exposed to a 0.5 μM solution of the complement, KMG2C, in 1.0 M NaCl with TE (pH 8.2). The resulting change in the interface due to hybridization was monitored with a series of SPR spectra. The coverage was calculated assuming that the SPR response per unit coverage of the complement corresponds to the same SPR response per unit coverage of the tethered DNA. (The mass difference between the tethered DNA and DNA complement is only 1.6%.) The results, shown in Figure 2, demonstrate that the surface-bound DNA hybridizes over a period of several hours and that the maximum amount of DNA undergoing hybridization is about 2.2×10^{12} molecules/cm^2. This corresponds to a hybridization efficiency (number of hybridized DNA molecules/number of tethered ss-DNA molecules) of 36−50% for the first hybridization cycle. The hybridization kinetics are described well by a diffusion-limited Langmuir adsorption model. From the rate constant, surface coverage, and solution concentration, we calculate[11] a diffusion constant of 8×10^{-8} cm^2/s for the DNA complement.

The hybridized film was dehybridized or melted by exposing the film to heated TE buffer solutions (pH 8.2) containing varying amounts of NaCl. We dehybridized and rehybridized the DNA film five times, each time testing for nonspecific binding of the noncomplementary DNA, and each time observing only hybridization with the complementary DNA. After the first dehybridization, the amount of DNA hybridizing on

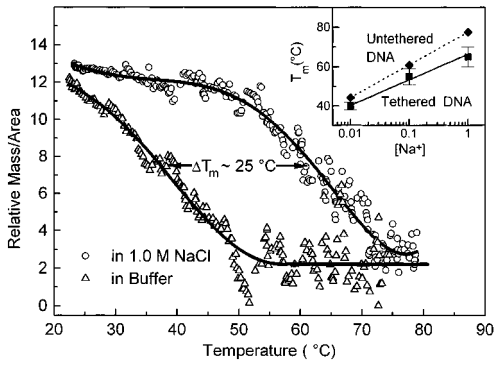

Figure 3. Dependence of dehybridization temperature on ionic strength. Shown is the mass per unit area as a function of solution temperature for hybridized DNA tethered to a gold surface in 1.0 M NaCl with TE buffer (open circles) and in TE buffer with no added NaCl (open triangles). Each point is calculated from a single SPR spectrum. The solid lines are polynomial fits to the data. The inset shows T_m determined from the SPR data (filled squares) as a function of [Na^+]. The [Na^+] dependence for the analogous untethered DNA (filled diamonds), calculated[17] using T_m (°C) = 81.5 + 16.6 $\log_{10}$[Na^+] + 0.41 (%GC) − (675/l), is shown for comparison.

the surface was measured to be 3.7×10^{12} molecules/cm^2 (60−80% hybridization). This increase in the hybridization efficiency with no measurable loss of selectivity indicates that heating the mixed film may induce reorganization resulting in an increase in the number of tethered DNA which are available for hybridization. The hybridization kinetics for all five cycles were observed to follow the same diffusion limited Langmuir kinetics model, and the rate constants varied by at most 16%.

SPR spectra were collected during the heating (dehybridization) and cooling process, and the relative mass per unit area was calculated from the SPR spectra, as shown in Figure 3. Note that the data show more scatter than those for the isothermal experiments. This is most likely due to thermal gradients in the SPR apparatus, which will be eliminated in a forthcoming apparatus. Figure 3 shows the clear difference between dehybridization in a 1.0 M NaCl solution and in a buffer solution with no added NaCl. The inset in Figure 3 shows the melting temperature, T_m, determined from the dehybridization or melting experiments as a function of [Na^+]. The calculated values of T_m for untethered KMG2 DNA are shown for comparison. Note that both curves have a similar logarithmic dependence and that T_m for the tethered DNA is ∼5° C below that of the untethered DNA.

Using two-color SPR spectroscopy, we have quantified the amount of DNA tethered to a surface and have followed both the kinetics of hybridization and the process of thermally induced dehybridization for the tethered DNA film. The hybridization activity of this film (70−100 fmol/mm^2) compares favorably to the highest hybridization efficiencies found in other DNA films.[3] The hybridization process is described well by a diffusion limited Langmuir adsorption model, and the sodium ion concentration dependence of the dehybridization or melting process follows the same trend as the analogous ss-DNA molecule in solution (untethered DNA). In subsequent papers, we will describe details of the film fabrication process studied through *in situ* measurements of functionalized alkanethiol self assembly.

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Supporting Information Available: Experimental details of film fabrication methodology and experimental details and assumptions of data analysis (3 pages). See any current masthead for ordering and Internet access instructions.

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