Kinetic Control of Hybridization in Surface Immobilized DNA Monolayer Films

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The kinetics of DNA oligomer hybridization to form duplex DNA in which one strand is immobilized on a surface are crucial to a wide range of research areas including work on DNA driven assembly of nanoparticles[1] and biosensor arrays,[2,3] however, few studies have measured the rate of hybridization for surface bound oligomers by surface plasmon resonance,[4,5] fluorescence,[6,7] or other techniques.[8] In this communication we show that the kinetics of hybridization are extremely sensitive not only to the degree of mismatch but also to the position of hybridization relative to the surface. The kinetic data are compared to thermal dehybridization experiments performed for both surface immobilized duplexes and for duplexes in homogeneous solution.

Scheme 1 shows the thiol derivatized DNA probe (row 1) and underivatized DNA targets (rows 2−6) used in this study.

Note that the 18low and 18high targets are complementary to the 25thiol probe at the first 18 and last 18 base pairs, respectively. These targets form duplexes with the 25 thiol probe which have equivalent thermodynamic stability but differ in the position of hybridization. Two other targets, 25comp and 25mismatch, are identical except for the presence of two base pair mismatches at the 10th and 18th base pair of the strand, and will form duplexes with substantially different thermodynamic stability. The 25control target (same sequence as the probe) is entirely noncomplementary.

To make quantitative comparisons, all kinetics experiments were performed on the same immobilized ssDNA probe film. The film is robust enough to survive a series of successive thermal dehybridization experiments while maintaining surface coverage and binding specificity. The ssDNA film has been characterized previously.[4,5] Multiple runs of the same target were repeated intermittently throughout the series of hybridization experiments as were the control experiments to test for nonspecific adsorption. In these, the probe surface was exposed to 1.0 μM solutions of the fully noncomplementary sequence (25control target) in 1.0 M NaCl (TE buffer) for several hours. No adsorption or hybridization occurs.

The experimental setup of the two-color SPR apparatus and the procedures used for the measurements have been described previously.[4,5,9,10] Here, the gold substrate is evaporated directly onto a hemi-cylindrical SF-14 prism ($n = 1.79$). The PTFE flow cell is attached to a programmable Neslab (RTE-111) water bath which varies the cell temperature from 20 to 80 °C. The solution temperature is detected independently by a thermocouple in the cell and the temperature at the surface of the gold is confirmed using critical angle measurements.[11]

Scheme 1. Oligomer Nomenclature and Sequence

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Name	Sequence
25thiol	HSC6-AGA TCA GTG CGT CTG TAC TAG CAC A -3'
25comp	3'- TCT AGT CAC GCA GAC ATG ATC GTG T -5'
18high	3'- AC GCA GAC ATG ATC GTG T -5'
18low	3'- TCT AGT CAC GCA GAC ATG -5'
25mis	3'- TCT AGT CAC **A**CA GAC AT**C** ATC GTG T -5'
25contro	3'- AGA TCA GTG CGT CTG TAC TAG CAC A -5'

All solutions were prepared using Nanopure (18 MΩ resistance) purified water. NaCl was obtained from Fisher (A.C.S. grade); Tris, Tris HCl, and disodium ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma (Reagent grade). All custom DNA oligomers were obtained from Synthegen (reverse phase HPLC purification) and stored in 100 ng aliquots as a frozen solid until used.

For the kinetics and melting experiments, salt solutions of 1.0 M NaCl and 0.1 M NaCl were buffered with Tris EDTA (pH 8.0, 10 mM Tris, and 1mM EDTA). Double-stranded dsDNA thiol, used for the fabrication of the immobilized dsDNA thiol monolayer film, was prepared in 1.0 M NaCl (TE buffer) by combining two single-stranded oligomers, 25thiol and 25comp, at room temperature in a 1:1 stoichiometic ratio. UV absorbance of the mixture confirmed that duplex formation is essentially immediate at room temperature.

The monolayer dsDNA thiolate film was prepared by exposure of freshly piranha cleaned gold to 1 μM dsDNA thiol in 1.0 M NaCl TE buffer (pH 8.0) solution for 10 h. Heating produces a ssDNA thiol film used as the substrate for all subsequent hybridization experiments. Surface hybridization experiments were performed by exposing the immobilized ssDNA thiolate (probe) film to 1 μM solutions of the target DNA in 1.0 M NaCl (TE buffer) for at least 10 h. Dehybridization of the surface immobilized dsDNA was achieved by heating in 0.1 M NaCl (TE buffer) from 20 to 80 °C and then cooling to 20 °C at a rate of 0.3 °C/min. Between runs, immobilized probe film was rinsed with both 1.0 M NaCl (TE buffer) and 0.1 M NaCl (TE buffer). All melting curves for homogeneous solutions of dsDNA were obtained from circular dichroism spectroscopy (Aviv Model 26DS) at similar heating rates and solution concentrations.

Kinetic Studies. Figure 1A shows a plot of the hybridization kinetics for three different target oligomers (25comp, 18 high, and 18 low) binding to the immobilized 25thiol probe. At 0.5 h, the hybridization efficiencies for the 25comp and 18high targets are the same when the SPR response is scaled to account for the difference in mass of the targets: the hybridization efficiency is 25−35% in both cases. However, the hybridization efficiency for the 18low target is substantially suppressed relative to the response for 18high, a target with essentially equal molecular mass and, when hybridized to form a partial duplex with the 25thiol, identical melting temperature in solution. That is, for target/probe duplexes of equal thermal stability in homogeneous solution, we observe a significant difference in the initial kinetics of duplex formation depending on where the target/probe hybridization occurs along the immobilized ssDNA probe strand. Interestingly, the initial rates of hybridization, in the first several minutes of the process, are not the same for these three probes. The rate of hybridization is fastest for the 18high probe and slowest for the 18low. It is important to note that despite the very slow hybridization kinetics, the final coverage of the 18low reaches approximately the same

(1) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849−1862.
(2) Mir, K. U.; Southern, E. M. *Nature Biotech.* **1999**, *17*, 788−792.
(3) Southern, E.; Mir, K.; Shchepinov, M. *Nature Gen.* **1999**, *21*, 5.
(4) Peterlinz, K. A.; Georgiadis, R.; Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3401−2
(5) Georgiadis, R.; Peterlinz, K. P.; Peterson, A. W. *J. Am. Chem. Soc.* **2000**, *122*, 3166−3173.
(6) Fotin, A. V.; Drobyshev, A. L.; Proudnikov, D. Y.; Perov, A. N.; Mirzabekov, A. D. *Nucl. Acids Res.* **1998**, *26*, 1515−1521.
(7) Forman, J. E.; Walton, I. D.; Stern, D.; Rava, R. P.; Trulson, M. O. *ACS Symp. Ser.* **1998**, *682*, 206−228.
(8) Okahata, Y.; Kawase, M.; Niikura, K.; Ohtake, F.; Furusawa, H.; Ebara, Y. *Anal. Chem.* **1998**, *70*, 1288−1296.
(9) Peterlinz, K. A.; Georgiadis, R. *Langmuir* **1996**, *12*, 4731−4740.
(10) Peterlinz, K. A.; Georgiadis, R. *Optics Comm.* **1996**, *130*, 260.
(11) Grassi, J. H.; Georgiadis, R. *Anal. Chem.* **1999**, *71*, 4392−4396.

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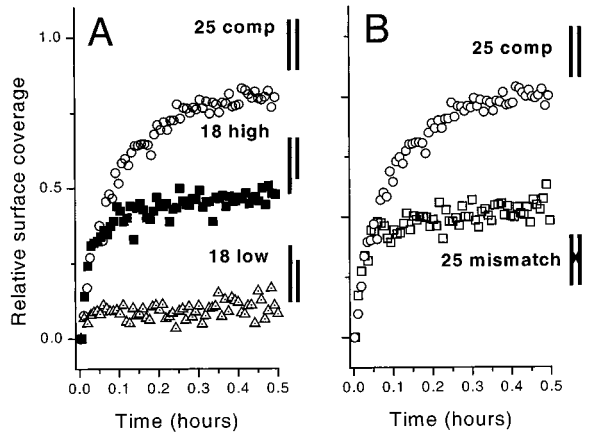

Figure 1. (A) Comparison of hybridization kinetics for the 25thiol immobilized probe film as a function of target oligomer length and binding location. Data are shown for probe oligomers 25comp (circles), 18high (squares), and 18low (triangles) for 1 μM ssDNA in 1.0 M NaCl (TE buffer). The data, relative coverage calculated from analysis of full SPR angular reflectance curves, are averaged for multiple runs and are shown here for the first 0.5 h. (B) Comparison of hybridization kinetics on the same probe film for target oligomers 25comp (circles) and 25mismatch (squares). After 4 h, the relative coverage is 1, 0.76, 0.35, and 0.7 for 25full, 18high, 18low, and 25mismatch, respectively. After 14 h, the relative coverage for the 18low probe oligomer reaches the same level as for the 18high (not shown).

value as that for 18high although the process takes about 10−14 h (not shown).

A comparison between the 25comp and 25mismatch hybridization kinetics is shown in Figure 1B. The large difference in the kinetics of hybridization reflects the difference in the thermal stability due to the presence of base pair mismatches in the 25mismatch oligomer.

Solution Phase Dehybridization Measurement. Solution phase dehybridization experiments measured by circular dichroism spectroscopy were conducted on both unfunctionalized dsDNA duplexes and on duplexes in which one of the oligomers is functionalized with C_6 thiol covalently attached at the 5′ position. We find that the melting transition temperature, T_m, for the thiolated dsDNA is suppressed by a few degrees (2−4 °C) compared to the nonthiolated duplex of the same sequence and length. Regardless of the presence of thiol functionality, our measurements show that the values of T_m for the two partial duplexes (18high or 18low target hybridized to probe) are identical in homogeneous solution. Theoretical predictions place the value of T_m for both partial duplexes at 10 or 12 °C lower than T_m of the fully complementary 25mer duplex, depending on which algorithm is used.[12]

Surface Dehybridization Measurements. In previous work, we have demonstrated that surface dehybridization of monolayer dsDNA films can be measured by temperature-dependent in-situ SPR spectroscopy[4] and that the value of T_m for immobilized duplexes is suppressed relative to that in free soluion. In this study, we find that the effect of oligomer length on the stability of the duplex is clearly evident in the surface dehybridization experiments, as expected. As measured from SPR experiments, T_m values for the 18high and 18low targets are 10 and 13 °C lower than T_m for the 25comp target, respectively, consistent with the expected lower stability of the partial duplexes relative to the full complementary 25-mer duplex. Within the error in the measurement (±4 °C), the T_m for surface melting of the 18low is the same as that for the 18high target case.

The effect of mismatch can also be seen in the SPR surface dehybridization experiments which show that T_m for the 25mis target is at least 8 deg lower than T_m for the 25comp target (the fully complementary sequence) in agreement with theoretical predictions and with the solution phase results.

Discussion and Conclusions. We find that the kinetics of hybridization are sensitive to the thermodynamic stability of the duplex (presence of base pair mismatches) as well as to the position at which hybridization occurs along the immobilized strand. The first result is not surprising. To understand the latter result we consider both thermodynamic and kinetic aspects of surface hybridization reactions. We have found that surface bound duplexes are generally less stable than the same species in homogeneous solution, as shown by the depressed melting temperature observed in SPR melting experiments.[4] This may be due to reduced availability of binding sites and/or differences in the solution dielectric constant, ionic strength, or pH relative to bulk solution. It is possible that this reduction in duplex stability is most pronounced for the first few base pairs of the immobilized duplex. One possible explanation for the difference in 18high and 18low kinetics is based on thermodynamic arguments assuming that the duplex formed by the 18low target is more destabilized. However, this explanation is not consistent with the SPR thermal melting experiments which show no difference in thermodynamic stability for these two surface immobilized partial duplexes (T_m values within error, ±4 °C) nor with fact that similar coverages are observed for these two probes at long times (>10 h).

The more likely explanation is based on kinetic arguments. The current view of duplex formation for short oligonucleotides is nucleation followed by helix zipping.[13] The same pathway is accessible for the surface-immobilized DNA, except that only one end of the probe DNA is easily available to form the initial nucleation site for hybridization. For the case of 18low target, the first 6 base pairs on the end of the 25thiol probe DNA are noncomplementary to any other span of 6 consecutive base pairs of the target sequence. Thus, for the probe to hybridize at the surface, it must first penetrate further into the DNA film compared with the 18high target. Although the surface coverage of the immobilized ssDNA probe is relatively low (less than about 20% of that calculated for a monolayer of close-packed dsDNA at the maximum packing density for duplexes), it is possible that lateral interactions with nearby probe DNA molecules affect the kinetics. The effect of surface coverage will be explored in future work.

We have used SPR spectroscopy to follow the kinetics and melting of DNA oligomers immobilized in monolayer films and show that the kinetics of hybridization are very sensitive not only to the presence of base pair mismatches but also to the location at which the hybridization occurs along the immobilized strand.

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(12) Turner, D. H.; Sugimoto, N.; Freier, S. M. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 167. Sugimoto, N.; Nakano, S.; Yoneyama, M.; Honda, K. *Nucl. Acids Res.* **1996**, *24*, 4501. Breslauer, K. J.; Frank, R.; Blocker, H.; Marky, L. A. *Proc. Natl. Acad. Sci.* **1986**, *83*, 3746. Wetmur, J. G. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 227. Porschke, D.; Eigen, M. *J. Mol. Biol.* **1971**, *62*, 361.

(13) Craig, M. E.; Crothers, D. M.; Doty, P. *J. Mol. Biol*. **1971**, *62*, 383−401. Cantor, C.; Schimmel, P. *Biophysical Chemistry, Part I*; WH Freeman: New York, 1980. Cantor, C. R.; Smith, C. L. *Genomics*; John Wiley & Sons: New York, 1999.