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Tunnel Conductance of Watson-Crick Nucleoside-Base Pairs from Telegraph Noise

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Abstract

The use of tunneling signals to sequence DNA is presently hampered by the small tunnel conductance of a junction spanning an entire DNA molecule. The design of a readout system that uses a shorter tunneling path requires knowledge of the absolute conductance across base-pairs. We have exploited the stochastic switching of hydrogen-bonded DNA base-nucleoside pairs trapped in a tunnel junction to determine the conductance of individual molecular pairs. This conductance is found to be sensitive to the geometry of the junction, but a subset of the data appears to come from unstrained molecular pairs. The conductances determined from these pairs are within a factor two of the predictions of density functional calculations. The experimental data reproduces the counterintuitive theoretical prediction that guanine-deoxycytidine pairs (3 H-bonds) have a smaller conductance than adenine-thymine pairs (2 H-bonds). A bimodal distribution of switching lifetimes shows that both H-bonds and molecule-metal contacts break.

Introduction

Electron tunneling has been proposed as a sensitive local probe to identify individual bases in DNA, a possible basis for direct electronic sequencing.^{1, 2} We have found that current-distance curves collected from junctions in which bases attached to a probe are hydrogen bonded to nucleosides³ or even to intact DNA on an electrode surface⁴ faithfully report the base composition of the target. A careful analysis of these signals shows that they do not arise from single-molecule interactions.⁵ Rather, the overall conductance of the tunnel gap is probably set by through-space tunneling across a large-area junction containing several (two to ten) molecular pairs. Conducting-AFM measurements provide a rough estimate of the conductance of base-nucleoside pairs as lying somewhere near 100 to 300 pS.⁵ Can we measure the conductances of *single* base-nucleoside pairs directly and accurately? Breakjunction techniques for measurement of single molecule conductance^{6–8} are difficult to apply to a system as complex as a base-nucleoside hydrogen bonded pair and they do not report the conductance as measured in the type of fixed junction that would be required for DNA sequencing.⁹ Stochastic switching of bonds between molecules and the electrodes of a tunnel junction offers another approach to measuring single molecule conductance. Ramachandran et al.¹⁰ demonstrated that, for tunnel-junctions using gold electrodes with thiol-attachment chemistry, fluctuations in the molecule-metal contact result in the stochastic-switching of STM images of molecules embedded in a monolayer. Fluctuations in the C-Si bond were monitored in an

STM¹¹ while the transient binding and unbinding of a carboxylate-EDC complex was monitored via conductance fluctuations in a carbon nanotube.¹² Haiss et al.^{13, 14} showed that the time course of bond-fluctuations in a gold-thiol-molecule-thiol-gold tunnel junction could be followed by the simple expedient of placing a gold STM probe above a gold surface functionalized with bis-thiolated alkane molecules. As the molecules spanning the gap bound and unbound to make and break the junction, the tunnel current showed a characteristic fluctuation between two-levels. The two-level nature of the signal is a strong indication that a single molecule is trapped in the gap, as switching of multiple molecules would generate more complex signals. The single-molecule conductance deduced from the amplitude of these tunnel current fluctuations yielded values for the conductance of alkane thiols that were in good agreement with the break junction method.^{7, 13, 14} Here, we report on telegraph-noise measurements made by forming a fixed gap between a probe functionalized with a DNA base and a surface functionalized with nucleosides.

Experimental Methods

We synthesized the nucleosides 5'-S-acetyl-5'-thiodeoxycytidine and 5'-S-acetyl-5'-thiothymidine following published protocols.^{15–17} These compounds were employed to prepare the nucleoside monolayers on freshly-prepared Au(111) substrates using the method developed in our lab.^{3, 5} In brief, a 1 mM solution of thionucleoside in methanol solution (2.5ml) was first treated with Pyrrolidine (0.2 ml) for one hour to remove the thiol protecting group. A freshly hydrogen-flame annealed gold substrate (Agilent, Tempe, AZ) was immersed in this solution for two hours. The sample was rinsed thoroughly to remove physisorbed molecules. The thickness of the nucleoside SAM was measured by ellipsometry yielding 0.92 ± 0.1 nm for thiodeoxycytidine and 0.81 ± 0.08 nm for thiothymidine. The modeled heights for upright monolayers were 0.96 nm (thiodeoxycytidine) and 0.97 nm (thiothymidine) so the measurements suggest that the films are a little less than a full monolayer. STM images showed the layers to be disordered. Further details of the characterization are given elsewhere.^{3, 5}

Gold STM probes were prepared and functionalized with 8-mercaptoadenine (A), 2-amino-8-mercaptoadenine (2-AA) and 8-mercaptoguanine (G) as previously described.⁵ The Watson-Crick base pairings are shown below for (i) adenine-thymidine, (ii) 2-aminoadenine-thymidine and (iii) guanine-deoxycytidine.

We also prepared STM probes and gold substrates functionalized with a monolayer of thiophenol as controls. Further controls employed bare gold probes or substrates. Measurements were carried out on a PicoSTM (Agilent, Chandler) with the sample and probe submerged in 1,2,4-trichlorobenzene. The system was first left to stabilize for three to four hours, and then the probe was advanced towards the surface to achieve a chosen set-point current (I_{sp}) at a tip to substrate bias, V_b . Immediately after the set-point was achieved, the servo-control was broken using custom LabView software, and the tunnel current recorded as a function of time using a digital oscilloscope. With the microscope mechanically-stabilized, the main contribution to gap movement appears to be creep of the piezoelectric (PZT) element, and the Z-voltage was adjusted manually in response to drift once the servo control was broken.

Generally, the required corrections get smaller with time, and the gap is usually completely stable after 5 to 10 minutes of adjustment.

This stability was maintained (less than 20 pA variation in the tunneling baseline) without servo control for up to ten seconds while data were accumulated. At a tunnel conductance of a nS, this corresponds to a tiny fraction of an Å movement in the probe position over this period.

Results and Discussion

In the hydrogen bonded systems, bursts of “telegraph noise” like that shown in Figure 1d were recorded in about half the data collection runs. Usually (>95% of the data collected) the noise reflected stochastic switching between just two distinct levels, indicative of a single base-nucleoside pair fluctuating in the junction. Some bond-breaking possibilities are illustrated in Figure 1. The molecule-metal contacts may “break” (Figure 1b) as in the case of similar recordings from simple bis-thiolated molecules.¹³ The motion in the figure is exaggerated for effect, for the frequent reconnection suggests only a small motion of the contact (which is probably not at the Au-S bond, but rather at the Au-Au bonds that surround the Au atom attached to the S atoms¹⁰ - motion in many such thiol-tethered systems is also affected strongly by interactions with the surrounding molecular matrix.¹⁸) Simultaneous breaking of all the hydrogen bonds (Figure 1c) is yet another possibility for the systems studied here, and it appears that both processes contribute to the observed telegraph noise, as evidenced by a bimodal distribution of switching times (see below).

The result of a typical control experiment (thiophenol probe, thymidine monolayer) is shown in Figure 2a with a current trace for 0.5 s worth of data on the left, and a histogram of the current distribution on the right. The current noise is distributed evenly around the mean. In contrast, the H-bonded systems (Figures 2b, c and d) all show distinct telegraph noise with a corresponding bimodal current distribution. We characterized the frequency with which telegraph signals occurred as follows: once the instrument was stabilized and a signal first recorded, we measured the fraction of each 5 s run occupied by telegraph noise (i.e., the ratio of the time for which the signal switched between two levels to the total time that all signal, baseline plus telegraph noise was collected). These fractions are shown as percentages in Table 1. For control experiments where telegraph noise was not seen at all (“0” in Table 1) we followed the same stabilization procedure and then recorded current data for the same overall experimental duration. Interestingly, thiophenol on the probe or substrate did show rare (few percent) instances of telegraph noise in contact with a base or nucleoside but only at the very lowest bias used. Presumably, interactions between the aromatic benzene ring and the heterocycle can occasionally result in complexation. These rare exceptions aside, the experiments summarized in Table 1 show that the entire metal-thiol-molecule-H-bonds-molecule-thiol-metal system needs to be assembled for telegraph noise to be observed.

Haiss et al. have found¹⁴ that the measured conductance of a rigid molecule increased approximately exponentially as the tunnel gap was made smaller, an effect they attributed to movement of the molecular levels relative to the Fermi level as the molecule is rotated towards the surface. In the present, more complex system, the measured switching conductances display a variety of dependences on the baseline conductance (G_{bl}) in Figure 2), shown for the three types of molecular pair in Figure 3. We have chosen to plot the switching conductance vs. G_{bl} rather than against the gap value itself because of the rather complex relationship between gap and conductance in these systems.⁵ Plotted this way, an exponential increase in conductance with gap appears as a linear increase of conductance with G_{bl} . In all cases, the switching conductance increases rapidly with G_{bl} at first (gray shaded boxes, Figure 3a-c). Above a critical conductance (similar in all three cases, and marked by an arrow) the switching conductance takes on a broad range of values. The upper limit clearly increases linearly with

$G(bl)$, consistent with the tilting mechanism described by Haiss et al.¹⁴ (though other types of distortion are surely also possible in this more complex system). However, about half of the total data points (green shaded boxes) lie on a plateau of constant conductance. One interpretation of these data is shown in Figure 3d. In the region labeled “1”, the gap is too large to be spanned by molecular pairs at their equilibrium spacing, so molecular-pairs that do span the gap are strained, with a conductance that falls exponentially with strain (i.e., falls linearly as $G(bl)$ falls). Once the gap becomes equal to the length of an unstrained molecular pair, further decreases in the gap result in an increase in molecular switching conductance, possibly owing to the tilting mechanism described by Haiss et al.¹⁴ This is the region labeled “2”. However, the smaller gaps should also accommodate a number of molecular-pairs of equilibrium length at some critical radius of the probe (r_c in Figure 3d), resulting in a plateau of constant conductance (“3”, shown as the green shaded region). The onset of this plateau will occur when the gap can first just accommodate an unstrained molecule, presumably at the point marked by the arrows on Figure 3. Molecules attached at intermediate points in smaller junctions ($0 < r < r_c$) will generate conductances that lie in between these two limits, generating data points above the plateau, but smaller than the maximum values measured. Haiss et al.¹⁴ discuss these possibilities, but conclude that their experiments capture molecules only at the minimum gap, because molecules are pulled into this region by the tip-sample electric field. Our experimental arrangement is different, because one half of the molecular pair is attached to the probe before the junction is made. Thus, we believe that all geometries can be captured in gaps smaller than the critical value. It is also possible that other arrangements such as bent nucleoside-base-pairs could occur. Both geometry (the number of available sites increasing with available tip radius) and energetics (equilibrium molecular pairs have the lowest energy) favor equilibrium pairs, and we find that about half the total data points lie in region 3.

Support for two different types of bonding (stretched vs. equilibrium and equilibrium but tilted) can be found in an analysis of the switching times. The peaks in all the bimodal current histograms are well separated, so the duration of the on-state is easily extracted by recording the intervals for which the current remains above 50% of the switching range in a given run. We plotted distributions of these switching times using logarithmic histograms¹⁹ and data for the G-deoxycytidine pair are shown in Figure 4 (additional data are shown in Appendix B). The distribution of switching times is clearly also bimodal. We fitted Gaussians to the two peaks (“F”=fast, ~8 ms, “S”=slow, ~50 ms) and the fitting parameters are listed in Table 2 for all three base-nucleoside combinations. The fast peak is somewhat faster and noticeably more prominent in junctions held together by two hydrogen bonds (A-thymidine) compared to junctions held together by 3 (G-deoxycytidine, 2AA-Thymidine). Further, the timescales are very similar to the characteristic times for opening of DNA bases in a double helix.²⁰ Thus, the fast process appears to be a consequence of H-bond opening of the complex (Figure 1c). The slow peak does not appear to change with H-bonding. In view of the similarity with the timescale for opening of thiol-gold contacts^{13, 14} we ascribe this feature to fluctuations in the molecule-metal contact (Figure 1b). This assignment was confirmed by carrying out an identical analysis of 1,8-octanedithiol. Only one peak was observed and it is essentially identical to the slow process (Appendix C). The distribution of switching times is broken down as a function of baseline conductance in the 2D plot on the right of Figure 4 (and see Appendix B).

The fast process diminishes rapidly with increasing baseline conductance, while the slow process becomes more important with increasing baseline conductance. The turnover, which starts in the neighborhood of $G(bl)=1\text{ nS}$, begins in about the same place that the molecular switching conductance moves from region 1 to regions 2 and 3 (Figure 3). This is consistent with the idea that the hydrogen bonding is more stable (less strained) when the molecular pairs span junctions smaller than the equilibrium length of the pairs. The “disappearance” of H-bond

openings in very small gaps is unexpected, and possibly reflects the consequences of confining the molecular pair to a very small volume.

If this interpretation is correct, then the conductance of the relaxed molecular pairs will be given by the data in the plateau regions in the green boxes (region 3) on Figure 3. Using these data, we plot the mean currents (error bars are ± 1 sd) as a function of V_b in Figure 5. The current-voltage relationships for all three Watson-Crick bonded pairs are linear, and the slopes yield the conductances listed in Table 2. Density-functional methods²¹ have been used to calculate the conductances of these base-nucleoside pairs⁵ and the results of these calculations are reproduced in the third column (G_{pred}) of Table 2. These conductances correspond to a direct tunneling transition from one electrode to the other as enhanced by the presence of molecular states near the Fermi level (“superexchange”). In calculations, the HOMO dominates the process. It is striking that the conductances obtained from the plateau regions agree with the predicted conductances within a factor of two, remarkably good agreement in the field of single molecule conductance measurements.²² Even more strikingly, the surprising prediction that G-deoxycytidine (3 H-bonds) is less conductive than that of A-thymidine (2 H-bonds) is supported by this analysis. This unusual result is, in the theory, a consequence of the different relative position of the HOMO with respect to the Fermi-level in the two cases, the contribution of two vs. three hydrogen bonds being masked by low transmission through the deoxyribose sugar ring.²¹

Conclusions

We have determined the absolute tunnel conductance for A-Thymidine, 2AA-Thymidine and G-deoxythymidine spanning gold electrodes. The distributions of switching times clearly allow identification of both H-bond and molecule-metal contact breaking. Further, the analysis of switching conductance as a function of baseline conductance facilitates design of an optimal fixed tunnel gap for readouts of DNA basepairs. Clearly, the gap must be smaller than the critical value at the transition from region 1 to regions 2 and 3. The close correspondence between the measurements and theory for the present case of base-nucleoside interactions adds credibility to the DFT calculations. These same calculations predict very small (fS) conductance across an entire DNA molecule.^{5, 21} Thus, electronic sequencing of DNA probably requires readout schemes that involve shorter tunneling paths, and these schemes are currently under investigation in our laboratory.

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Appendix A

Figure A1.

Signals collected for the three base-nucleoside pairs (c.f. Figure 2c, d, e) at higher baseline conductance.

Appendix B

Figure B1.

Switching time distributions for (a) 2AA on Thymidine and (b) A on Thymidine.

Appendix C

Figure C1.

Distribution of switching times for telegraph noise measured in 1,8-octanedithiol. Samples were prepared and measured as described elsewhere.^{13,14} There is only one peak in the distribution and the most probable time (44 ms) corresponds to the “slow peak” (τ_S) in the bimodal distributions observed in the thiol- and hydrogen-bonded systems.

Figure 1.

(a) An intact junction in which the tunnel gap is spanned by a guanine attached to the probe, hydrogen bonded to a deoxycytidine attached to the substrate. (b) Fluctuations that break the metal-molecule contact will reduce the conductance, as will fluctuations that break the hydrogen bonds (c). (d) An example of the telegraph noise signal produced as bonds break and reform.

Figure 2.

Recordings of tunnel current vs. time (left column) together with the corresponding distributions of current for (a) a control junction with thiophenol on the probe and thymidine on the surface, (b) adenine on the probe and thymidine on the surface, (c) 2-aminoadenine on the probe and thymidine on the surface and (d) guanine on the probe and deoxycytidine on the surface. Solid lines are Gaussian fits to the upper and lower switching level distributions. These fits are used, together with the bias, to determine the molecular switching conductance in a given run. High-current switching data are given in Appendix A.

Figure 3.

Plot of molecular switching conductance vs. baseline conductance for the three base-nucleotide combinations (a–c). (d) illustrates mechanisms for the various regions. For (1) the tunnel gap is larger than the equilibrium length of the molecular pair, leading to a region of rapid increase in conductance (shaded gray in a–c) as the strain required to span the gap decreases. When the gap is equal to or smaller than the equilibrium length of the molecular pairs, they may span the gap in either the equilibrium configuration (3 - shaded green in a–c, $\theta = 0$) or tilted configurations (2 – unshaded data in a–c). The applied biases were 0.05V (squares), 0.1V (circles) and 0.2V (diamonds).

Figure 4.

Distribution of “on” times for G-deoxycytidine (left). The distribution is plotted as a function of $G(bl)$ in the 2D color plot on the right (red = high counts). Parameters obtained from Gaussian fits (τ_F , τ_S and h_F/h_S) are listed in Table 2. Data for the other base-nucleoside pairs and a purely thiol-bonded system are given Appendix B.

Figure 5. Current-voltage curves for 2AA-thymidine (diamonds), A-thymidine (circles) and G-deoxycytidine (squares). Each data point is the mean obtained from data in the plateau regions. The error bars correspond to ± 1 sd.

Table 1

Observed frequency of switching (fraction of time occupied by telegraph noise in a 5s run) for various preparations of the probe (left column) and the surface (top row). Errors are ± 1 sd. “—” represents untried combinations.

	Thio- thymidine	Thio- deoxycytidine	Thiophenol	Bare
8-Mercapto-adenine	47 $\pm$ 8%	-	1 $\pm$ 0.3% *	0
8-Mercapto-2-aminoadenine	51 $\pm$ 7%	-	-	0
8-Mercapto-guanine	-	52.5 $\pm$ 13%	-	0
Thiophenol	2.5 $\pm$ 1% *	-	0	0
Bare	0	0	0	0
Adenine	0	-	-	-
2-Aminoadenine	0	-	-	-

* indicates that the observed switching only occurred at low bias (50 mV). The bottom two rows are for bases physisorbed onto the probes (no thiol attachments).

Measured conductances (G_{meas}) obtained from the conductance plateau region (“3” in Figure 3) compared to calculated values (G_{pred}). Values are best-fits to the I–V curves in Figure 5 with fitting errors shown. Error values in parenthesis are derived from the SDs of the single molecule measurements (spread of data in the green boxes in Fig. 3). Also shown are mean lifetimes (τ_F , τ_S) with associated halfwidths, and ratios of the peak heights, h_F/h_S , for Gaussian fits to the switching time distributions.

Base-nucleoside pair	G_{meas} (nS)	G_{pred} (nS)	τ_F (ms)	τ_S (ms)	h_F/h_S
A-thymidine	0.76±0.01 (±0.03)	1.62	7±3.6	46±11	1.3±0.1
2AA-thymidine	0.80±0.03 (±0.14)	1.43	8.3±3.9	45±11	1±0.1
G-deoxycytidine	0.66±0.007 (±0.07)	0.96	8.4±3.4	47±11	1±0.1