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In-Situ Electrochemical Distance Tunneling Spectroscopy of ds-DNA Molecules

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Abstract

The electrical conductance of ds-DNA duplexes containing 8 to 14 base pairs modified at both ends with a $-(\text{CH}_2)_6\text{-SH}$ linker was measured in a buffered aqueous solution using electrochemically controlled distance tunneling spectroscopy. The tunneling experiment with self-complementary $5'-(\text{GC})_n-3'-(\text{CH}_2)_6\text{-SH}$ ($n=4-7$) duplexes attached covalently to a gold STM tip and a Au(111) electrode shows a wide distribution of currents independent of the ds-DNA length. The voltage-induced horizontal orientation of ds-DNA within the junction results in decreased electrical conductance. The lower currents are also observed for ds-DNA molecules containing a single CA base mismatch.

TOC image

Keywords

DNA conductivity; scanning tunneling distance spectroscopy; electrochemical STM

The nature of electrical transport along the DNA duplex is important in understanding DNA damage in vivo and in exploring the possible use of DNA in molecular electronics and direct detection of DNA hybridization. The ongoing scientific debate places DNA molecules in all possible electrical conductivity ranges - from an insulator to a proximity induced superconductor.¹⁻⁴ It is apparent that the experimental conditions, including the nature of the contact between the electrode and the molecule, as well as the orientation and the structure

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of DNA, influence the measured electrical properties to an extent not encountered in other molecular systems.⁵ Recent studies by Tao et al.^{6, 7}, Xu et al.⁸, Bashir et al.⁹, and Porath et al.¹⁰ involving single DNA molecules in *two-terminal nano-gaps* in the presence and absence of water further indicate that the experimental conditions strongly influence the electrical properties of DNA. Moreover, several reports indicate that the solvent and ions play a critical role in electron transport along a variety of molecules.¹¹ In particular, the DNA structure and flexibility depend significantly on the counter-ion and on the electrostatic interactions between the electrical contact and the molecule.¹² Thus, it is important to measure the electrical properties of single DNA molecules while rigorously controlling the electrochemical parameters of the experiment.

We report here the electrochemical tunneling distance spectroscopy measurements of electrical conductance of series of ds-DNA molecules modified with a $-C_6-SH$ linker attached to 3' ends of DNA in 10 mM phosphate buffer + 100 mM sodium chloride solution. This approach allows us to control the orientation of ds-DNA molecule within the STM junction by means of electrostatic interactions between the molecule and the electrochemically polarized metal contact.

In a tunneling distance spectroscopy with electrochemical gate, shown schematically in figure 1A, the STM tip is first brought into close proximity of the Au(111) electrode at the initial set-point current, i_0 . The STM tip is then lifted while the x-y position is kept constant and the current-distance (*i-s*) curve is recorded. This experimental procedure follows the method previously used by Haiss et al.¹³ to measure electrical conductivity of α,ω -alkanedithiol molecules bridging the STM gap. A similar method of measuring of molecule conductance, starting with the formation of a mechanical contact between the STM tip and a substrate, was previously introduced by Tao et al.¹⁴

Figure 1B shows a typical current-distance (*i-s*) transient recorded in the absence of pre-adsorbed molecules on the Au(111) surface. The initial position of STM tip versus Au(111) surface is established by using a set-point current, $i_0=4nA$, corresponding to a very close (in the range of few Å) tip-substrate separation. As expected for tunneling through water layers in the absence of molecules bridging the STM gap, the current decays quickly to negligibly small values ($<2pA$) corresponding to electrochemical leakage current on Apiezon wax coated STM tips.¹⁵ Figure 1C shows an *i-s* transient recorded under identical conditions but in the presence of 1,9-nonanedithiol on Au (111) electrode surface.¹⁶ The clear plateau on the *i-s* curve is similar to that previously reported by Haiss et al and is attributed to the higher efficiency of super-exchange tunneling along the hydrocarbon chain as compared to tunneling through water at similar distances.¹³ Thus, after formation of a bond between the STM tip and the terminal $-SH$ group of a molecule, the lifting of the STM tip from its initial position changes the absolute distance between the tip and a substrate but does not alter the length of the main tunneling pathway, i.e. the tunneling pathway along the molecule.¹⁷ Indeed, as shown by an arrow in curve 1C, the current decays rapidly upon breaking the contact between the electrode and a molecule. The current recorded at the detachment distance can, therefore, be attributed to the electrical conductivity of a molecule trapped within the gap.^{7, 13, 14}

Figure 1D shows an analogous experiment performed in the presence of a low coverage of $-(\text{CH}_2)_6\text{-SH}$ modified ds-DNA preassembled on the Au(111) surface. The 10 mM phosphate buffer + 100 mM sodium chloride solution environment in this experiment ensures that the investigated ds-DNA exists predominantly in B-form double helices.^{7, 18–20} Although the Au – tip distance for DNA detachment varies somewhat from experiment to experiment ($s_{\text{det}}=2.8 \pm 0.6$ nm), significant currents are observed for the tip-distance separation as large as 3 nm. Such large current can only be attributed to the conductivity of DNA molecules trapped in a junction indicating an enhanced electronic coupling *along* the ds-DNA.

We have examined a large number of current-distance curves such as the one presented in figure 1D for several dithiolated ds-DNA molecules. These DNA molecules, purified using HPLC, were purchased from Alpha DNA (Canada). In general, about 1% of *i-s* curves show plateaus similar to those reported in Figure 1D indicating the low surface coverage of a thiolated ds-DNA on the Au(111) surface consistent with observations of Haiss et al. for α,ω -alkanedithiol measurements.¹³ The values of current at the detachment distance (see arrow in curve 1D) for hundreds of current-distance curves were measured and the histogram of these currents for the two different DNA lengths is shown in figure 2.²¹ For all of the self-complementary DNA sequences of the $5'-(\text{GC})_n-3'-(\text{CH}_2)_6\text{-SH}$, $n=4-7$ type, we have observed a wide distribution of current values (see figures 2A, 2B, 3A). *While the % frequency of the particular current values, indicated in figures 2 and 3A, varies from case to case, the general range of values from ca. 30 pA to ca. 1 nA is independent of the length of DNA.* One can consider several possible causes of the observed wide distribution of conductivities. For example, the current plateaus might represent the conductivity of junctions containing different numbers of ds-DNA molecules. In an analogous experiment involving α,ω -alkanedithiol molecules in a two-terminal STM junction, Haiss et al.¹³ reported current distributions showing only up to five average values representing integer multiples of a basic current value. This observation was explained by postulating the insertion of up to 5 molecules within the junction. Our current distributions for dithiolated ds-DNA helices ranging from 30 pA to 1 nA (see fig. 2) would translate into trapping of up to approximately 30 ds-DNA molecules in a junction. The cross sectional area of hydrated ds-DNA is about 20 times larger than the cross sectional area of the α,ω -alkanedithiol. Thus, in view of the results by Haiss et al.¹³, this scenario is clearly unlikely.²² The distribution of conductivities of DNA, essentially independent of the helix length, can also be attributed to the variety of possible conformations of the molecule within the STM gap. The electrochemical STM junction allows us to control the electrostatic interactions between the DNA molecule and electrochemically polarized Au(111) substrate and an Au STM tip. Thus, we can investigate the influence of the orientation of DNA within the gap on the electrical properties of the system.

Unlike in two-terminal approach used previously to measure conductivity of DNA^{6–9}, the electrochemically controlled STM allows for the electrochemical potentials to be applied independently and simultaneously to both the STM tip (E_{TIP}) and the Au (111) substrate (E_{Au}).¹⁴ Figure 3 shows the distribution of current plateaus obtained from the current-distance curves for the self-complementary ds-DNA duplex of the $5'-(\text{GC})_7-3'-(\text{CH}_2)_6\text{-SH}$ sequence under the same bias voltage, $E=0.4$ V, but using different combinations of electrochemical potentials applied to the STM tip and the Au(111) substrate. In figure 3A,

$E_{\text{TIP}} = +300$ mV, $E_{\text{Au}} = -100$ mV. Under these experimental conditions the STM tip is charged positively ($E_{\text{TIP}} > E_{\text{PZC}}$) and the Au(111) is charged negatively ($E_{\text{Au}} < E_{\text{PZC}}$) with respect to the potential of the zero charge of the electrode.^{19,20} It was shown previously by two independent groups that under these conditions the negatively charged DNA is repelled from the negatively charged Au(111) surface.^{19,20} Thus, the DNA molecule can conceivably be stretched in the course of lifting of the STM tip. As can be seen in figure 3A, similarly to experiments shown in figure 2, a wide distribution of currents ranging from about 20 pA to 950 pA is observed. The same bias voltage between the STM tip and the Au(111) substrate (0.4 V) can be accomplished if $E_{\text{TIP}} = -100$ mV and $E_{\text{Au}} = +300$ mV vs. SSCE. Under these conditions, as shown previously, the DNA is horizontally oriented towards the Au(111) surface because of electrostatic interactions of DNA with the positively charged Au(111) surface.^{19,20} At the same time the, Au STM tip is negatively charged thus repelling DNA duplexes. We have observed two important features of current-distance curves recorded under these conditions as shown in figure 3B. First, the detachment distance for all experiments was smaller ($s_{\text{det}} = 1.0 \pm 0.5$ nm) indicating that the DNA can not be stretched under these conditions. Second, the values of current plateaus at the detachment distance were always *smaller* than the currents observed for fully stretched DNAs. These observations lead to the conclusion that the horizontal orientation of ds-DNA results in low conductance even though the absolute geometric length of the STM gap is much smaller than for the stretched DNA. We postulate that the negatively charged STM tip repels the DNA molecules and thus the electrically efficient contact between the STM tip and the DNA can not be formed. This suggests that the ca. 1nA current (at 0.4 V bias voltage) observed for several DNA sequences represents the “along the DNA” pathway of electrical conductivity. Thus, the above results suggest that the electron transport along the base pair stack is responsible for observed conductivity of DNA.

Therefore, the low current values (< ca. 1nA) observed for several DNA sequences (Figures 2, 3A and 4A) should be attributed to poor electrical contact between the STM tip and DNA or unfavorable DNA conformation within the STM gap or the combination of these factors. It is worth noting that the ability to stretch DNA molecule to ca. 3 nm does not necessary indicate that the DNA is in its “most conductive” conformation. Indeed, for experimental conditions for histograms 2A, B, 3A and 4A, B there is not correlation between the “breaking distance” and the magnitude of recorded plateau current. To further confirm our hypothesis that the electron transport along the base pair stack is responsible for the observed length-independent conductivity of DNA, we have performed preliminary experiments probing the influence of CA mismatch on the electrical conductivity of DNA in STM junction. Barton et al. have shown in several experiments, both using photoinduced electron transfer and electrochemical methods, that the efficient pathway of electron transfer along DNA involves a base pair stack.²³ These authors have shown that the single point CA mismatch causes local disruption in the DNA base stack and attenuation of the long range electrochemical electron transfer while the helical structure of the duplex remains preserved.

Figure 4 shows the current distribution for conductivities of 12-mer ds-DNA with and without a single point mismatch. While the distribution of currents for the fully matched 12-mer ds-DNA exhibits 7 peaks (at 31, 47, 83, 128, 186, 390, 1067 pA), the identical ds-DNA containing CA mismatch shows only 3 peaks (at 23, 45, and 100 pA). Thus, the CA

mismatch causes at least 10 fold decrease of electrical conductivity of a molecule. It is worth noticing, however, that the distribution of the current values observed for mismatched DNA molecules is similar to current distribution previously attributed to the unfavorable STM gap configuration shown in figure 3B. Thus, it is possible that the “through base pair stack” pathway of electron transfer is eliminated altogether in the presence of the intervening mismatch. Studies in this direction are underway in our laboratory.

The electrochemical tunneling spectroscopy measurements reported above show that the electrical conductivity of DNA depends critically upon the DNA orientation within the tunnel junction and the integrity of a base pair stacking.

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- Following the procedure reported by Haiss et al. (ref.13) a 15 s self-assembly from 0.05 mM methanolic solution was performed. The current plateau is observed for about 1% of our measurements consistent with presence of a diluted submonolayer of 1,9-nonanedithiol on Au(111).
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- We did not observe correlation between the detachment distance and the value of current plateau for a particular DNA sequence and experimental conditions.
- We have reproduced the tunneling results of Haiss et al. (ref. 13) for α,ω -alkanedithiol molecules. For each length we have observed up to 5 average values representing integer multiples of a basic current value.

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Figure 1.

(A) Scheme of the experiment showing flat Au(111) electrode immersed in a deoxygenated electrolyte solution containing 10mM phosphate buffer and 100mM sodium chloride. The Au electrode is polarized to the potential E_{Au} vs. reference electrode (RE, saturated silver/silver chloride electrode) and an Au STM tip is polarized to the E_{TIP} potential. The circuit is completed with Pt counter electrode. The voltage between the STM tip and an Au electrode is $E = |E_{Au} - E_{TIP}|$. The distance tunneling spectroscopy is performed by lifting a STM tip from the initial position while keeping x-y position constant. (B, C, D) The resulting current-distance (i-s) curves recorded using set-point current, $i_0 = 4$ nA, the tip potential, $E_{TIP} = +300$ mV, and the potential of Au electrode $E_{Au} = -100$ mV. A typical i-s curve recorded on (B) bare Au(111) surface, (C) in the presence of a low coverage pre-adsorbed 1,9-nonanedithiol molecules on Au(111) electrode, (D) in the presence of pre-adsorbed ds-DNA helix (self-complementary structure of $HS-(CH_2)_6-3'-(GC)_7-5'$).

Figure 2.

The histogram of current values recorded for different ds-DNA's in STM junction. For clarity, each column represents an average current value together with the % occurrence for each group of values. The current values (in pA) are as follows: (A) 28 ± 6 ; 51 ± 3 ; 88 ± 7 ; 135 ± 14 ; 253 ± 10 ; 313 ± 20 ; 502 ± 69 ; 840 ± 113 ; (B) 31 ± 1 ; 46 ± 9 ; 81 ± 13 ; 148 ± 24 ; 252 ± 18 ; 500 ± 58 ; 1020 ± 89 . $E_{\text{TIP}} = +300$ mV, $E_{\text{Au}} = -100$ mV. Experiments were performed in a 10mM phosphate buffer and 100mM sodium chloride.

Figure 3.

The histogram of current values recorded for ds-DNA of self-complementary sequence of 5'-(GC)₇-3'-(CH₂)₆-SH in STM junction. The current values are as follows: (A) 21±4; 45±9; 81±10; 130±14; 196±17; 302±11; 489±92; 950±141; (B) 25±4; 103±13; 148±11; 199±13. Experiments were performed in a 10mM phosphate buffer and 100mM sodium chloride. The bias voltage between the tip and an Au(111) electrode was 400 mV. The applied electrochemical potentials were: (A) E_{TIP} = +300 mV, E_{Au} = -100 mV, (B) E_{TIP} = -100 mV, E_{Au} = +300 mV vs. SSCE.

Figure 4.

The histogram of current values recorded for 2 different sequences of ds-DNA in STM junction. The current values are as follows: (A) 31±2; 47±6; 83±4; 128±9; 186±31; 390±92; 1067±115; (B) 23±6; 45±4; 100±12. $E_{\text{TIP}} = +300$ mV, $E_{\text{Au}} = -100$ mV. Experiments were performed in a 10mM phosphate buffer and 100mM sodium chloride. The base mismatch is indicated by an arrow.