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Direct Conductance Measurement of Single DNA Molecules in Aqueous Solution

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ABSTRACT

We have studied electron transport in DNA duplexes, covalently bonded to two electrodes in aqueous buffer solutions, by repeatedly forming a large number of DNA junctions. The histogram of conductances reveals peaks at integer multiples of a fundamental value, which is used to identify with the conductance of a single DNA molecule. The measured conductance depends on the DNA sequence and length. For $(GC)_n$ sequences, the conductance is inversely proportional to the length (greater than eight base pairs). When inserting $(A:T)_m$ into GC-rich domains, it decreases exponentially with the length of A:T base pairs (m) with a decay constant of 0.43 $Å^{-1}$. This work provides an unambiguous determination of single DNA conductance and demonstrates different conduction mechanisms for different sequences.

The ability to directly measure the conductance of a single DNA molecule wired to two electrodes is exciting because it will allow one to study charge transport, a phenomenon that is associated with oxidative damage of DNA,[1−4] on a single-molecule basis. It will also allow one to read the chemical and biological information of the molecule electronically, which opens the door to biosensor applications[5,6] based on electrical measurement of individually wired molecules. Conductance measurements on DNA have been attempted by many groups, but the reported characteristics of DNA varies from insulator, semiconductor to conductor, and superconductor.[7−11] To measure the conductance reliably, one must provide reproducible electronic coupling between the molecule and the probing electrodes.[12−15] One must also find a signature to identify that the measured conductance is due to not only the sample molecules but also to a single sample molecule. Finally, for biologically relevant molecules such as DNA, it is highly desired to carry out the measurement in aqueous buffer solutions to preserve the native conformation of the molecule. Here we describe a direct conductance measurement of a single DNA molecule (of various lengths and sequences) covalently attached to two electrodes in aqueous solutions.

We have studied two series of DNA sequences, a 5′-$(GC)_n$-3′-thiol linker with $n = 4$, 5, 6, and 7 (8−14 base pairs long) and a 5′-CGCG$(AT)_m$CGCG-3′-thiol linker with $m = 0$, 1, and 2. These thiolated oligonucleotides were purchased in a disulfide form from Integrated DNA Technologies, Inc., and were deprotected with tri-(2-carboxyethyl)phosphine and purified with size-exclusion spin columns before use. These DNA sequences have been extensively studied by NMR, X-ray crystallography, CD spectroscopy, and thermodynamics.[16−18] They are self-complementary sequences and form stable B-form double helixes spontaneously in solution. In very high or low salt concentrations, some of the DNA may also exist in other structures. For example, Z DNA[17] has been observed for poly(GC) in 2.5 M sodium chloride, and the hairpin structure[16] exists for the CGCGATATCGCG sequence when the salt concentration is around 0.01 M. In addition, some imperfect structures, such as frayed and slipped helixes, are concomitant with the perfect duplexes, which have been minimized by controlling the anneal conditions in a 100 mM NaCl + 10 mM phosphate buffer solution (pH 7) in this work.[18] Furthermore, as we will discuss later, the hairpin structure cannot bond to two electrodes and the slipped and frayed structures would be much less conducting than double-helical DNA, so these imperfect structures will not greatly affect the measurement of normal DNA duplexes.

Each double helix is terminated with two $CH_2CH_2CH_2$−SH groups at its 3′ ends that are poised to bind to Au electrodes via strong Au−S bonds. We created individual molecular junctions by repeatedly moving an STM (scanning tunneling microscope) tip into and out of contact with a flat

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Au electrode in the buffer containing 3 μM sample DNA (Figure 1a). The tip was insulated over most of its surface to eliminate undesired leakage currents, permitting the measurement of small signal currents in an aqueous environment.[19] The experiment was controlled by a feedback loop that started by driving the tip into contact with the substrate using a piezoelectric transducer (PZT).[20] Once the contact was established, the feedback loop activated the PZT to pull the electrode out of contact. After breaking the contact, the DNA molecules can bridge the tip and the substrate electrodes to form a molecular junction, which is shown as a series of steps appearing in the conductance (Figure 1b). In the case of the eight-bp DNA duplex, the steps are near-integer multiples of a fundamental conductance value, $1.3 \times 10^{-3}G_0$ or $\sim$0.1 μS (Figure 1c), where $G_0 = 2e^2/h \approx$ 77 μS, and the conductance quantum is roughly the conductance of a single gold atom connected to two electrodes. These conductance steps are due to the formation of 1, 2, 3, ... DNA molecules in the junctions.

This conclusion is supported by the following facts. First, in the absence of DNA molecules, no conductance steps significantly below $1G_0$ are observed (Figure 1d). Second, the conductance peaks are located at different conductance values for DNA duplexes with different lengths and sequences. (We will return to this later.) Third, we have recently simultaneously measured the conductance and force of different molecular junctions created using this method for alkanedithiols and bipyridines, which directly shows that the conductance steps are due to the breakdown of individual molecules.[21] We note that the conductance steps do not always occur at the same conductance values, reflecting variations in the microscopic details of the molecule−electrode contacts. A statistical analysis over a large number of molecular junctions is thus necessary. Figure 1c is a conductance histogram constructed from more than 500 individual measurements, which reveals defined peaks near integer multiples of $1.3 \times 10^{-3}G_0$ ($\sim$0.1 μS), corresponding to the conductance of a single eight-bp DNA duplex. The histogram is not sensitive to the moving rate of the PZT, indicating that the DNA molecules adsorbed on the electrodes rather than those in solution are responsible for the formation of molecular junctions. The histogram of the control experiment carried out in the buffer solution containing no DNA shows only a smooth background in the conductance histogram (Figure 1d).

We have obtained the current−voltage ($I-V$) characteristic curves using two different methods. The first method is to monitor the peak positions in the conductance histograms obtained at different bias voltages, which gives a $I-V$ curve averaged over many DNA junctions (open squares in Figure 1e). The advantage of constructing conductance histograms from a large number of measurements minimizes the possibility of mistaking the imperfect DNA mentioned above for regular B-form DNA because the imperfect DNA not only is less conducting but also is fewer in number than the B-form DNA. However, the statistical average of each $I-V$ over many molecular junctions may wash out interesting features of each individual molecular junction. We have thus

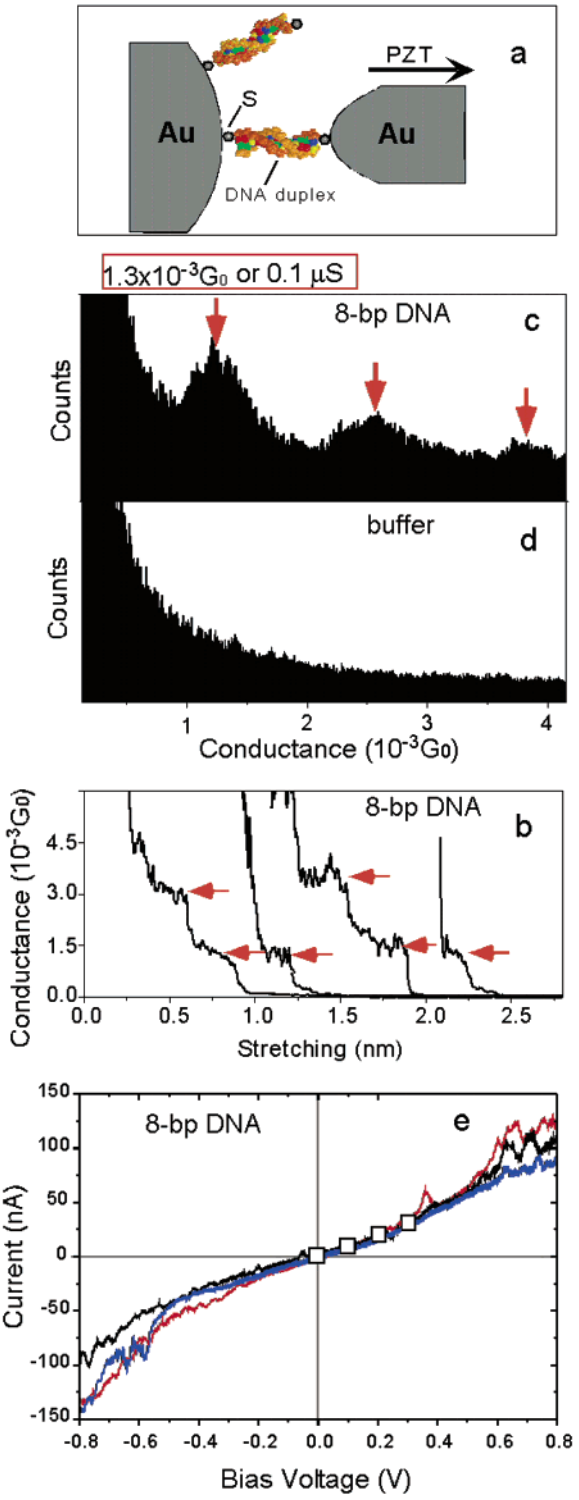

Figure 1. (a) Schematic illustration of a single DNA conductance measurement. (b) Formation of molecular junctions shown as discrete steps in the conductance (eight-bp DNA duplex). (c) Conductance histogram constructed from more than 500 individual measurements revealing well-defined peaks near integer multiples of a fundamental value, $1.3 \times 10^{-3}G_0$ (0.1 μS), which is identified as the conductance of a single eight-bp DNA duplex. (d) Conductance histogram in buffer solution revealing a smooth background. (e) Current−voltage characteristic curves of a single eight-bp DNA. Lines with different colors are obtained by recording current vs bias voltage for three different DNA junctions. The open squares are from the peak positions of the conductance histograms obtained at different bias voltages.

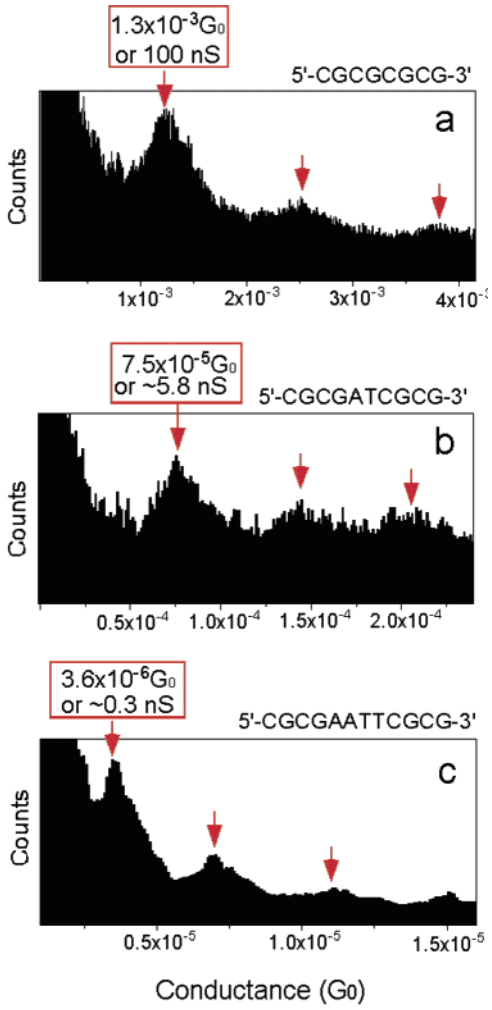

Figure 2. Conductance histograms of three DNA duplexes: (a) 5′-CGCGCGCG-3′-thiol linker, (b) 5′-CGCGATCGCG-3′-thiol linker, (c) and 5′-CGCGAATTCGCG-3′-thiol linker.

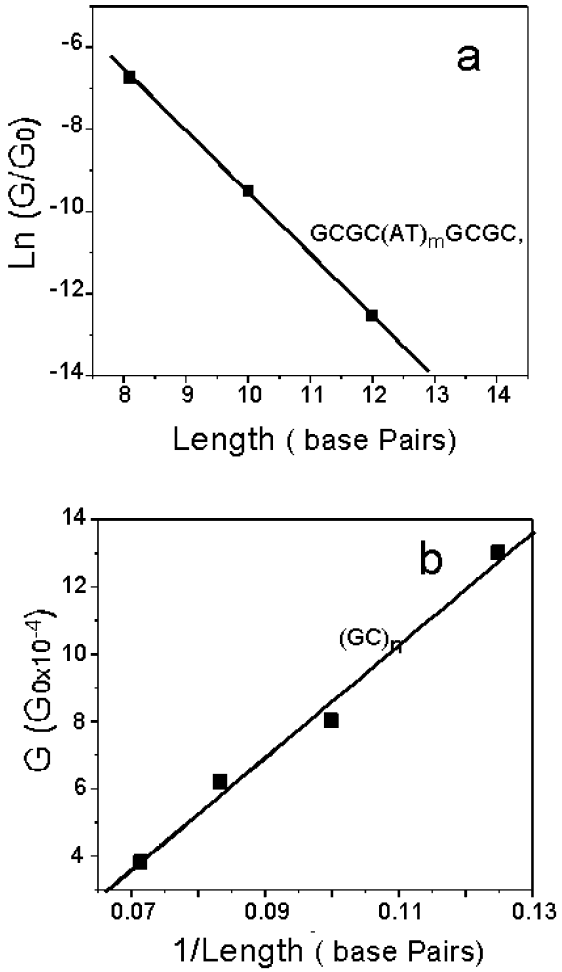

Figure 3. (a) Natural logarithm of GCGC(AT)$_m$GCGC conductance vs length (total number of base pairs). The solid line is a linear fit that reflects the exponential dependence of the conductance on length. The decay constant, β, is determined from the slope of the linear fit. (b) Conductance of (GC)$_n$ vs 1/length (in total base pairs).

used a second complementary method to determine the $I-V$ curves. This method starts by pulling the tip out of contact with the substrate until the lowest conductance plateau, corresponding to the formation of a single DNA junction, is reached. We then freeze the tip position and record the current while sweeping the bias voltage (Figure 1e). This method requires a stable tip−substrate distance, and our homemade setup allows us routinely to hold the tip position for many seconds to several minutes, long enough for us to measure the $I-V$ curves. The $I-V$ curves obtained by the two methods (open squares and lines) are in good agreement with each other and show rather linear behavior below 0.5 V, as shown in Figure 1e.

We have performed similar measurements on other DNA duplexes. Some are shown in Figure 2b and c. The conductance histograms also reveal well-defined peaks, but the peaks are located near integer multiples of different values. For 5′-CGCG(AT)$_m$CGCG-3′ sequences, the natural logarithm of the conductance versus the DNA length (Figure 3a) shows that the data can be described by $G = A\exp(-\beta L)$, where $A = (1.3 \pm 0.1) \times 10^{-3}G_0$, $\beta = 0.43 \pm 0.01$ Å^{-1}, and L is the length of the A:T bridge. In contrast, the conductance of (GC)$_n$ sequence DNA duplexes decreases more slowly and can be fit by $G \approx {}^1/_L$ (Figure 3b). The sequence dependence suggests that charge transport via the stacked base pairs, rather than via the backbones, dominates the conductance. These findings are consistent with the model that describes charge transport in DNA as a tunneling or superexchange process across A:T regions between two G:C domains.[22,23] The systematic dependence of the conductance on the length supports the fact that the measured conductance is due to double-helical DNA rather than imperfect structures.

Is the measured conductance due to the electronic or ionic conduction of DNA? We have coated our STM tip electrode with Apiezon wax such that the ionic leakage current between the two electrodes is <1 pA. The control experiment further confirms that ions in the solution do not significantly contribute to the measured conductance. However, one may argue that the counterions surrounding DNA are responsible for the measured conductance. The counterions could contribute to the conductance either via polarization or faradic processes. The former is ruled out because the measured conductance is independent of the pulling and voltage sweep rates. The later would require the counterions

to exchange electrons with the electrodes at the molecule−electrode interfaces via reduction or oxidation, which occurs at potentials much higher than those used in our experiments. The faradic process would also require the diffusion of ions into the gap to replace the counterions lost in the faradic process, which is limited to <1 pA. Furthermore, ionic conductance would be insensitive to the DNA sequence. These considerations indicate that the counterions are unlikely to be the direct source of the measured conduction. However, the counterions may have an indirect role in the charge transport in DNA.[24]

The experimental techniques that we used here have been previously used to study electron transport through alkanedithiols,[20,21] benzenedithiols,[25] and peptides.[26] There are two differences between those molecules and the DNA duplexes studied here. First, for both alkanedithiols and peptides, the conductance decreases exponentially with molecule length with a decay constant of 0.8−0.9 $Å^{-1}$, suggesting electron tunneling as the conduction mechanism in these molecules. In contrast, the dependence of the DNA conductance on its length is sensitive to the DNA sequence. For $(GC)_n$ sequence, the conductance decreases very slowly with the length. Even in the case of an $(AT)_n$ sequence where conductance decays exponentially with distance, the decay (decay constant ≈ 0.45 $Å^{-1}$) is much slower than that of the alkanes and peptides. This indicates that DNA is indeed much more "conductive" than alkanes and peptides. Second, there is a stepwise decrease in the conductance during the stretching process for all of the molecules, but the steps for DNA are shorter and not as flat. This difference likely reflects the fact that the DNA duplex is much softer than alkanedithiols, benzenedithiols, and oligopeptides. To gain a better understanding of this observation, we are currently performing simultaneous conductance and mechanical force measurements of these molecules.

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