Current–voltage characteristics of diversely disulfide terminated λ-deoxyribonucleic acid molecules

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Current–voltage characteristics were obtained at room temperature on duplex λ-deoxyribonucleic acid (DNA) molecules labeled with disulfide end groups in two configurations: Either the disulfide groups were attached to the 3′ ends of opposite DNA strands, or on the same strand at one 3′ end and at one 5′ end. In the latter configuration, only one strand is attached and contacted to the two Au electrodes utilized in the measurement. The current–voltage characteristics of both configurations are linear and do not reveal significant differences. The disulfide end groups in either configuration thus provide an equivalent contact. Moreover, the strand contacted at both ends by disulfide groups is not preferred for charge transport which, hence, involves the complete double-helical structure. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1595136]

Electrical conductivity measurements on deoxyribonucleic acid (DNA) have produced a wide range of experimental results, attributed to the molecule's properties from fairly conducting to insulating. Variously, an activation gap is found in current–voltage ($I-V$) characteristics, or ohmic behavior is concluded.[1–8] Not only have there been contradictory experimental results but a theoretical consensus has not been reached on the method of charge transport in these biological molecules, whether transport is largely due to the overlapping electron orbitals of the bases forming a so-called ''π-way,'' or whether conduction occurs by the positively charged counterions along the phosphate backbone, or some combination of the two mechanisms.[9] Yet, charge transport through DNA elicits considerable interest, from biological considerations, involving radiation damage and repair,[10] as well as for the potential use of the molecule as a molecular wire in nanoelectronics. While initial measurements of photoinduced charge transfer have shed considerable light on the subject,[11] the direct measurements of the electrical conductivity have been marred by the aforementioned discrepancies. Recent findings point to the possibility that the broad range of experimental conductivity values are due to the ease with which DNA conforms to various environments. Experiments often do not hold constant various factors of importance to DNA conformation, such as the surface of the substrate, contacts to the electrodes, DNA sequence, counterions, and DNA secondary structure (bends, nicks, stacking distance between base pairs, width of the DNA molecule). Here, we report on conditions under which we have found double-stranded λ-DNA to yield reproducible results for direct electrical conductivity measurements. Disulfide end groups are used to provide attachment and electrical contact of the DNA to Au electrodes. More particularly, we investigate the role of disulfide end groups in two cases: (i) double-stranded λ-DNA functionalized with disulfide end groups at the 3′ end of each strand of the duplex, and (ii) double-stranded λ-DNA functionalized with disulfide end groups at the 3′ and 5′ ends of the same single strand.

Photolithography and a lift-off technique were used to fabricate the two-electrode configuration for the measurement of the $I-V$ characteristics. The metallization consisted of a 80 Å Cr sticking layer, followed by a 300 Å Au layer, electron-beam evaporated on *p*-type-doped Si(100) substrates capped with 4500 Å thermal oxide (Fig. 1). The electrodes are separated by 8 μm, and run parallel over a length of 50 μm. The gradual opening of the electrode gap toward the electrical contacts was provided to avoid electric-field concentrations (this geometry, however, also introduces uncertainties in the calculation of the electrical conductivities after measurement). Control of the lift-off profile was crucial to avoid an upturned edge to the electrodes which, in our experiments, prevented the DNA molecules from spanning the electrode gap. After lift-off, the samples were boiled in acetone to remove traces of photoresist, then were etched in a heated ammonium hydroxide/hydrogen peroxide mixture to remove organic residues and, finally, were again boiled in acetone and subsequently in methylene chloride. This rigorous cleaning assured reproducibility in the measurement results, attributable to an enhancement in the number of chemisorbed disulfide–Au sites and hence improved electrical contact.

The specimens used in our study were 16 μm long, linear viral λ-DNA molecules, obtained from Sigma-Aldrich, and functionalized at their ends with disulfide groups. Figure 1 shows a sketch of the DNA molecules utilized in this study. In one type, the disulfide end groups were attached to a different strand of the duplex (called here type I DNA), in the

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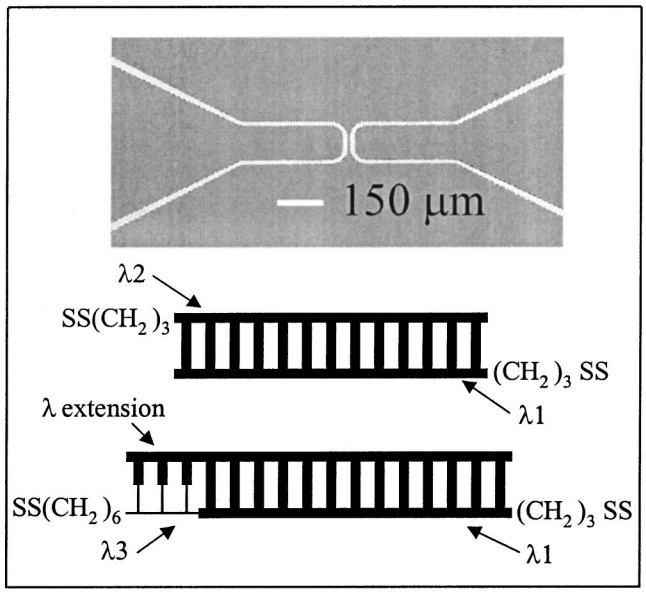

FIG. 1. Below: Sketch of the two disulfide-labeled λ-DNA configurations. For the top configuration, the disulfide groups are attached on opposite strands, for the lower configuration, on the same strand of the duplex DNA. λ1, λ2, λ3, and λ extension are oligonucleotides utilized in the synthesis. Above: The Au electrode geometry, on Si/SiO_2 substrates.

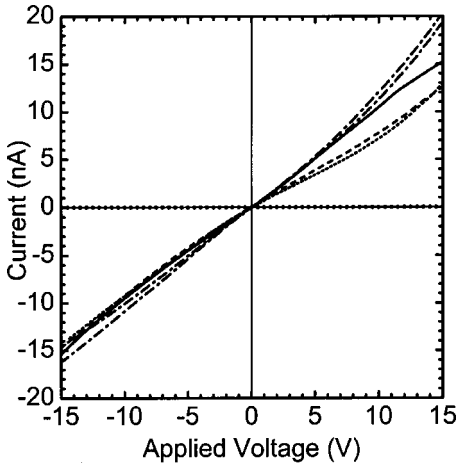

FIG. 2. $I-V$ characteristics on λ-DNA measured at room temperature. Solid line: DNA with disulfides on opposite strands; Dashes: DNA with disulfides on the same strand, device A, swept from -15 V to 15 V; Dots: Same as device A, but swept from 15 V to -15 V; Dashes–dots: DNA with disulfides on the same strand, device B, different sweeps from 15 V to -15 V; Filled circles: Electrodes without DNA (zero line).

other type, the disulfide end groups were attached to the same strand, using a 25-base pair extension (type II DNA). We do not expect the disulfide-Au bond to differ in both cases. Also, the short extension is not expected to modify the charge transport in the ~48 000 base pair long λ-DNA. Hence, a comparison of the $I-V$ characteristics between types I and II DNA allows us to isolate the role of strand-to-strand charge transport.

Type I DNA was synthesized using a hybridization method adapted from Braun *et al.*[12] In its linear form, λ-DNA possesses, at both ends, two short overhang regions of 12-bases long. Single strands of 12 base length, phosphorylated at the 5′ end and modified with terminal disulfide C3 S-S groups at their 3′ ends, complementary to the 12 unpaired bases of each overhang region, were obtained from Integrated DNA Technologies (λ1 and λ2). These single strands were hybridized to the λ-DNA overhang regions, and then ligated to the λ-DNA using T4 ligase (MBI Fermentas) to complete the phosphate backbone, as indicated in Fig. 1. The solution was filtered (Microcon-100) to remove excess unhybridized fragments. To synthesize the type II DNA, three different single-strand oligonucleotides along with λ-DNA were necessary, as reflected in Fig. 1. In the first step, a 12 base 5′ phosphorylated oligonucleotide, functionalized on the 3′ end with a disulfide (λ1) was hybridized and ligated to the complementary 12-base sticky end of λ-DNA, as described herein, and the solution filtered. The second oligonucleotide added to this modified λ was a 5′ phosphorylated 25 base fragment (λ extension) with part of its sequence complementary to the other 12-base sticky end of the λ substrate. After ligation of this oligonucleotide and subsequent filtration, the extra 13 overhanging bases of the λ extension served as another sequence to hybridize a third oligonucleotide to. This final oligonucleotide consisted of a complementary 13 base sequence, functionalized on the 5′ end with a C6 S-S group (λ2). The hybridization was followed by another ligation and filtration.

Alignment of the DNA molecules between the Au electrodes was achieved through manipulation by ac electric fields. Confocal microscopy using a DNA fluorescent intercalating dye visually ascertained the trapping of DNA molecules by this method. An electric field of 10^6 V/m at a frequency of 1 MHz was found to be optimal.[13,14] Alignment by flow, instead of ac fields, also allowed for the trapping of DNA molecules between electrodes, although with less reproducibility. With the ac voltage applied to the electrodes, a 2 μL drop of the appropriate DNA solution (1–5 nM) was centered over the electrodes. The sample was kept within a hydration chamber where the relative humidity was maintained between 85%–90% to control droplet evaporation during the 25 min alignment. The sample was left for an additional 25 min in the chamber to allow for sufficient Au-S bond formation, after removing the ac voltage. The controlled humidity, long alignment time, and bond formation time, were necessary to obtain repeatable trapping of the DNA and consistent electrical contacts. The alignment was followed by a rinse with deionized water to remove both unbound DNA and buffer solution. The sample was then dried under a flow of nitrogen gas.

Figure 2 shows $I-V$ characteristics measured on the samples under an ambient condition and at room temperature. Included are measurements on type I and type II samples, with, for the latter, some curves repeated in the opposite voltage sweep direction (from negative to positive voltage or vice versa), and for different samples (devices A and B), to determine reproducibility. Figure 2 also shows the current measured before DNA deposition, as a zero line. The data shows a close-to-linear $I-V$ characteristic for both DNA types. Assuming a single layer of DNA molecules is in electrical contact with the Au electrode, a dc conductivity of 6×10^{-4} S cm^{-1} to 1×10^{-3} S cm^{-1} can be deduced for ei-

ther type I or type II DNA. Indeed, no clear difference can be discerned between the $I-V$ characteristics for either type: The data for different samples straddle a range that includes both type I and type II DNA, and more importantly, the $I-V$ curve shape does not substantially deviate from the linear behavior for either type. The number of DNA molecules trapped and measured on different samples can differ, due to uncertainties in the trapping procedure and in the electrical contact formation. Hence, the current values for different samples cannot unambiguously be compared. Yet, since reproducible $I-V$ curves could be obtained for both repeated measurements on the same DNA sample in either voltage sweep direction, and for measurements on different electrode devices, we believe that by using our method, $I-V$ curve shapes can be compared, and order of magnitude estimates of DNA conductivity reliably obtained. Due to the measurement uncertainties, the dc conductivity values deduced from the data ($\sim 8\times 10^{-4}\,\mathrm{S\,cm^{-1}}$, on average) should be regarded as upper bounds. Still, the dc conductivity values we obtain fall around the literature average.[1–8] Uncertainties arise because of the unknown number of molecules, the two-contact geometry utilized, the nature of electrical contacts, and the gradual opening of the electrode spacing.

The major difference between type I and type II molecules has a local nature, namely the position of the disulfide groups. If either disulfide configuration modified the charge transport, the effect would appear as either a nonlinearity of the $I-V$ characteristic, or as an added resistance. Similarly, if the disulfide attachment led to shunting of either strand of the duplex DNA, a difference in conductivity values would result. Neither is observed, and we can conclude that the location of the two disulfide end groups, on each strand at the $3'$ ends (type I), or on the same strands at one $3'$ end and at one $5'$ end (type II), does not modify the charge transport properties of the molecule as a whole. Moreover, our experiments show that charge transport does not favor a single-DNA strand, but instead relies on the duplex nature of the double helix.

In conclusion, experiments leading to $I-V$ characteristics of duplex-DNA molecules were performed, using a lithographically fabricated two-electrode arrangement. The DNA molecules were electrically contacted to the Au electrodes utilizing disulfide end groups in two configurations, namely with the disulfides either on the same single strand or on opposite strands of the duplex. No difference in $I-V$ characteristics was concluded between the two configurations. We infer that charge transport in DNA is insensitive to which strand is contacted.

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