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Direct Electrical Measurements on Single-Molecule Genomic DNA Using Single-Walled Carbon Nanotubes

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ABSTRACT

A unique nanoelectronic platform, based on single-walled carbon nanotubes (SWNTs), has been fabricated for measuring electrical transport in single-molecule DNA. We have tested 80 base pairs of single- and double-stranded DNA (ssDNA and dsDNA, respectively) of complex base sequences. About a 25–40 pA current (at 1 V) was measured for the dsDNA molecule covalently attached to the SWNT electrode at its termini. In the absence of base pair stacking, a ssDNA carries a feeble current of ∼1 pA or less. Gate-voltage-dependent *I*–*V* characteristics revealed that the bridging dsDNA molecule acts as a p-type channel between SWNT source and drain electrodes.

Molecular recognition and self-assembly are two most appealing attributes that provoke the scientific world in redeeming the potential use of DNA (deoxyribonucleic acid) as a component of future molecular electronic devices. Despite many expectations that DNA can be a potential ingredient of molecular electronic circuits, transport experiments on this versatile biopolymer have revealed a very intriguing and partly contradictory behavior. Various reports in the literature suggest that DNA may be insulating,[1] semiconducting,[2] or metallic.[3,4] These results demonstrate the high sensitivity of DNA transport experiments to various factors, including the quality of the DNA−electrode interface, the base pair (bp) sequence, the charge injection into the molecule, or environmental effects (humidity, temperature, and counterions). Performing reliable experiments on a single oligo-DNA molecule is extremely delicate. Moreover, interpretation of the native electrical properties on the basis of experimental data is even more difficult.

Porath et al. measured electrical transport through synthetic and non-genomic poly(G)−poly(C) DNA molecule (30 bp) connected to metal nanoelectrodes (10 nm separation) without any functional group at the termini of the double helix.[2] The presence of a voltage gap and steps in the $I-V$ curves indicates the Coulomb blockade effect due to poor coupling between the DNA and the metal electrodes. Iqbal et al. reported dc measurements of covalently attached thiolated dsDNA (18 bp, self-complementary sequence) in gold nanogaps to estimate the electrical resistance of hybridized and denatured DNA molecules.[5] It is more likely that the observed electrical signal of ∼10 nA (at 1 V) arises from a bundle or a series of DNA molecules attached between the 3 μm wide gold electrodes. Cohen and co-workers developed an experimental approach for measuring current through DNA molecules (26 bp), chemically connected on both ends to a metal substrate and to a gold nanoparticle (GNP), by using a conductive atomic force microscope (AFM) tip.[6] Although they claimed that the observed signal was from a hybridized molecule, the measurement technique leaves the scope for several arguments. First, there is a possibility of a tunneling current between the GNP and the gold substrate placed in a very close proximity of less than 5 nm. Second, the attachment of a single complementary strand to the GNP (10 nm diameter) and the transport of charges through a single molecule in the presence of a packed monolayer of ssDNA on the gold substrate are indeed

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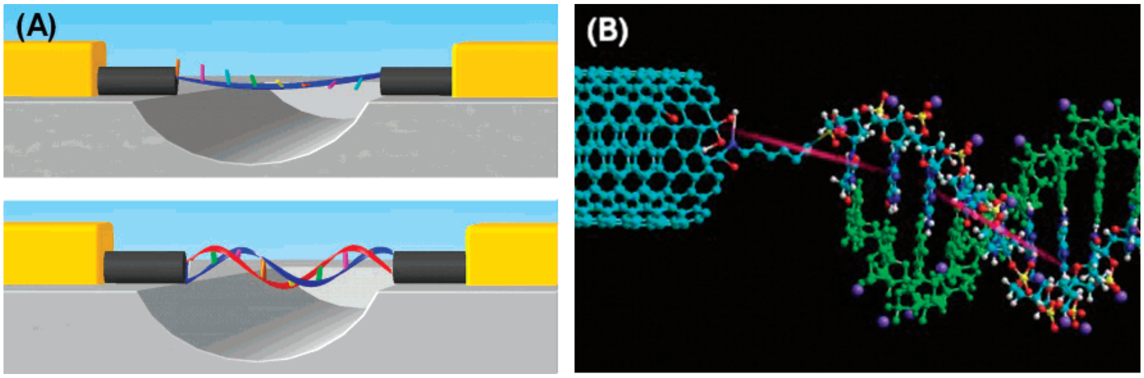

Figure 1. Schematic illustrations. (A) The upper figure represents an arbitrarily shaped ssDNA molecule, which is stretched and attached to a pair of functionalized SWNT electrodes in the presence of a DEP field. The lower figure depicts a covalently attached dsDNA molecule, which has a definite conformation. The nanoelectrodes are separated by a gap of 27 ± 2 nm (the contour length of the 80 bp DNA molecule is ∼27 nm). The bridging DNA molecule suspends over a trench without touching the silicon dioxide surface. (B) Molecular diagram highlighting the covalent bonding between an amine-terminated ssDNA and a carboxyl-functionalized SWNT nanoelectrode via a $(-CH_2-)_6$ spacer. Charge transport takes place through the stacked base pairs in a helical duplex.

questionable. In addition, the high intrinsic conductivity of tris(hydroxymethyl)aminomethane (TRIS) buffer could overestimate the native electrical properties of DNA.

In this letter, we report the development of a novel nanoelectronic platform (Figure 1A) for measuring direct electrical transport in single-molecule DNA of genomic significance. One of the potential applications of the present study would be identification of specific genes based on the hybridization-induced change in electrical signal. Multiple investigations have suggested that a duplex DNA may provide a special pathway for charge transport through the ordered π-electron system of the nucleotide bases.[7] Given that a small perturbation in base stacking significantly diminishes the charge migration, it is expected that a ssDNA molecule will carry only a feeble current due to lack of its structural integrity. Thus, if a ssDNA molecule of known sequence is used as a molecular probe, by monitoring the electrical signal, it is possible to determine if the target strand is present in the test sample. There are several unique aspects of this approach. First, we have exploited single-walled carbon nanotube (SWNT) electrodes for anchoring a DNA molecule of compatible diameter (1−2 nm). Characterization of DNA using carbon nanotubes (CNT) has been pursued in the past, motivated by the prospects of CNT as a unique electrode material.[8−10] A couple of recent reports detailed the techniques of creating a nanogap in a SWNT and bridging the gap by organic molecules.[11,12] The present study extends this concept to overcome the challenge of anchoring and electrically characterizing single-molecule DNA. Second, the application of dielectrophoresis (DEP) in our system provides controlled manipulation of a DNA molecule. DEP is especially useful for stretching a ssDNA molecule, which forms an arbitrarily shaped coil in the buffer solution.[13] The third important issue is the formation of a covalent bond between each terminus of a DNA molecule and the functionalized end of a SWNT electrode (Figure 1B). Establishment of a strong electronic coupling between the trapped molecule and the nanoelectrodes facilitates the charge transport through the system without the Coulomb blockade effect. The fourth important aspect is the presence of a nanotrench, between the SWNT electrodes, that eliminates the contribution of the oxide surface to the charge transport through a DNA molecule. DNA−substrate interactions have been known to be critical in determining the conductivity of an immobilized molecule. The strong interaction between DNA and a silicon oxide surface induces a large compression of the deposited DNA.[14,15] The suspended DNA molecule in the present system mitigates the problem of compression-induced perturbation of charge transport. In addition to electrical characterization in ambient condition, we have studied the influence of water molecules and temperature on the charge-transport properties of a DNA molecule. The gate-voltage-dependent channel current has been measured to determine the polarity of charge carriers in the DNA molecule under investigation. A number of control experiments have also been conducted to confirm that the measured electrical signals indeed originated from the trapped DNA molecules between the nanoelectrodes.

The device fabrication procedure is similar to that reported in our previous work.[16,17] Electrical contacts to individual SWNTs were made by e-beam lithography followed by deposition of 50 nm of Au on a 10 nm Ti adhesion layer by sputtering. A pair of nanoelectrodes was prepared by creating a controlled nanogap in the middle of a single SWNT by focused ion-beam (FIB) etching.[18] During ion exposure, a trench was formed in the oxide layer on the silicon wafer (Figure 2A). The interelectrode spacing was controlled by adjusting the ion beam current and the exposure time. For an 80 bp long DNA fragment encoding a portion of the H5N1 gene of the avian Influenza A virus,[19] we created a 27 (±2) nm gap between the electrodes (Figure 2B). The choice of 80 bp (corresponds to a contour length of ∼27 nm) is based on the constraint of our FIB system (<25 nm was not reproducible) and the fact that transport through longer single DNA molecules (>40 nm) is apparently blocked.[1] Under applied bias, only electrical noise could be detected between the nanoelectrodes after FIB etching. Carboxylation of the FIB-cut nanotube edges was performed by nitric acid treatment, followed by multiple washing with deionized water until the pH of the sample was neutral.[20] In presence

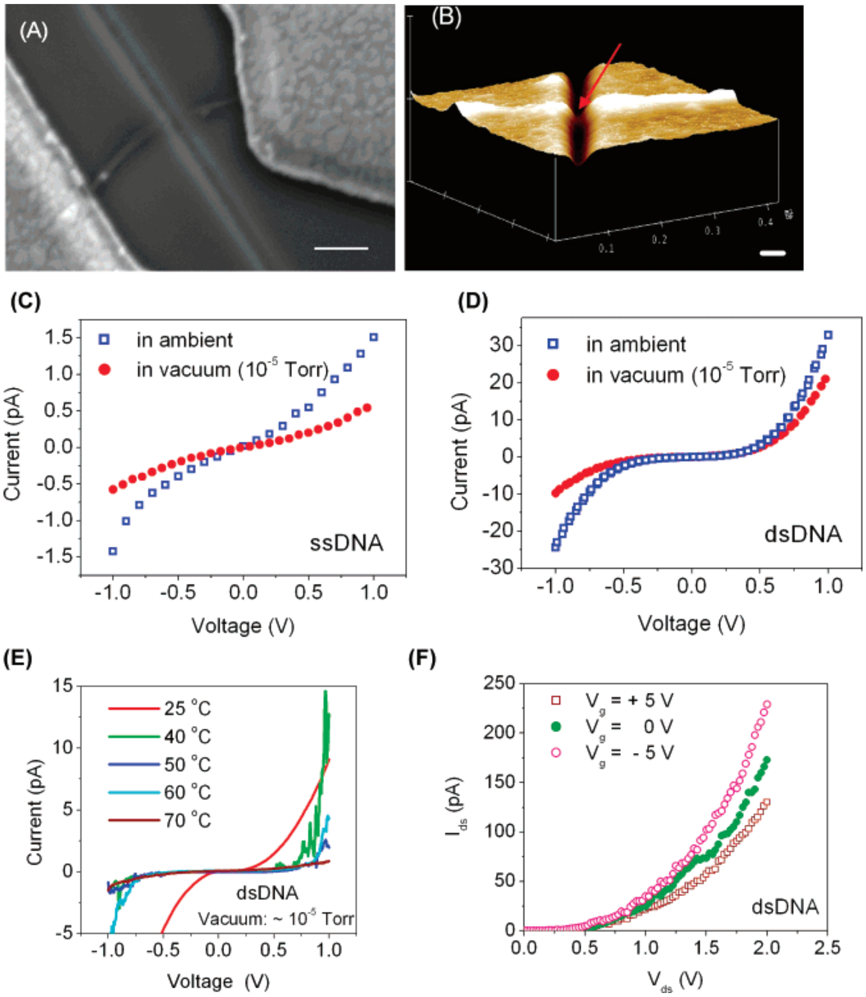

Figure 2. Device morphology and electrical characterization. (A) SEM image of a pair of FIB-etched SWNT electrodes, connected to the bonding pads by Ti/Au microcontact leads. Scale bar = 100 nm. (B) High-resolution AFM image of the DNA molecule between the SWNT electrodes. Scale bar = 30 nm. The red arrow points to the trapped single DNA molecule. (C) and (D) Current flow at room temperature through single ssDNA and dsDNA molecules, respectively, in ambient and in vacuum conditions. (E) Temperature effect on DNA conductivity. The initial increase in current value (from 25 to 40 °C) is in coherence with the small polaron hopping model. Further increase in the device temperature (50−70 °C) resulted in a diminishing current signal at a given voltage. Above the melting temperature of the DNA molecule (75.6 °C), there was no detectable signal. (F) Back-gate biasing effect on the DNA channel conductivity. Depletion in the positive gate bias region and enhancement in the negative bias region indicates that the DNA molecule with a given sequence acts as a p-type semiconductor.

of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, the terminal carboxylic acid groups reacted with amino groups that had been introduced at both of the ends of a DNA strand. The amino groups were attached with a C6 linker at the 5′ end and a C7 linker at the 3′ end of the strand.[19] Either a ssDNA or a dsDNA molecule was attached to the functionalized SWNT electrodes by placing a 2 μL droplet of 10 nM respective DNA suspension in 10 mM sodium acetate buffer (pH 5.8). For preparing the dsDNA samples, the template strand with amine modifications both at the 5′ and 3′ ends was hybridized with the unmodified complementary strand at 90 °C for 5 min in 10 mM sodium acetate buffer (pH 5.8) at equimolar concentrations. The formation of a double helix was confirmed by a standard gel electrophoresis experiment. A ssDNA or a hybridized DNA molecule was attached to the SWNT electrodes in the presence of a DEP field with an applied ac bias of 1 V_{pp} (electric field ~ 40 V/μm) at a frequency of 1 MHz.[21,22] The chip was thoroughly rinsed with 10 mM sodium acetate several times to remove any nonspecifically bound DNA and then dried with a high-purity nitrogen stream. Although TRIS−HCl buffer is a more common suspension media, we preferred to use sodium acetate in our experiment as we intended to study the native electrical properties of a DNA molecule without much perturbation from the ionic transport between the electrodes. This selection is based on a control experiment, which showed that after drying a DNA-free TRIS−HCl buffer droplet placed between the SWNT electrodes, there was about a 2 orders of magnitude higher current as compared to that for DNA-free sodium acetate. This observation is in accordance with the recent report by Ostmann et al.[23]

Electrical characterization of the resultant device was performed in a vibration-free chamber using a high-precision semiconductor parameter analyzer (Agilent 4156C). In the absence of base pair stacking, a ssDNA molecule would not be expected to support charge transport. However, we repeatedly observed a baseline current in the range of 0.5−1.5 pA (at 1 V applied bias) through a ssDNA molecule, as exemplified in Figure 2C. The observed baseline current most likely originated from the hydration shell surrounding the ssDNA strand.[23] To confirm the contribution of water molecules, the current−voltage measurements were repeated under vacuum conditions. As the pressure was reduced, the conductivity of the ssDNA strand decreased (Figure 2C), primarily due to the reduction of water molecules in its surroundings. In fact, in ambient conditions, the proton-transfer process in the hydration layer surrounding the DNA promotes the electrical conductivity but diminishes in high vacuum.[24]

Figure 2D shows the $I-V$ characteristics of a dry dsDNA molecule previously suspended in sodium acetate buffer. We observed a current signal in the range of 25−40 pA (at 1 V bias) from the single DNA molecule. A nonlinear $I-V$ characteristic was observed, indicating semiconducting behavior of the trapped DNA fragment encoding a specific gene but devoid of any periodic arrangement of the base pairs. Similar results were obtained for more than 20 different samples in ambient conditions. The resistance value obtained from our electrical measurements for an 80 bp dsDNA molecule at 43% relative humidity (RH) was in the range of 25−40 GΩ. For comparison, Tuukkanen et al. reported resistance values for a bundle of 414 bp dsDNA molecules between gold electrodes as 10 TΩ and 40 GΩ in the dry state (30% RH) and in a moist environment (80−90% RH), respectively.[23] In our study, the high vacuum (10^{-5} Torr) measurements showed an increase in resistance (50−65 GΩ) of a dsDNA molecule as compared to that in ambient conditions. This observation is very similar to that for the ssDNA and can be explained similarly by the depletion of water molecules in the hydration shell surrounding the DNA. For dsDNA molecules, another possibility of reduced charge transport is the conformational changes in the double helix at high vacuum.[24]

Next, we studied the temperature-dependent conductivity of DNA using the same platform. In order to avoid oxidation, current−voltage measurements of the dsDNA molecules were performed with a gradual increase in temperature at a vacuum level of $\sim 10^{-5}$ Torr. It can be seen from Figure 2E that when the device temperature was increased from 25 to 40 °C, there was a noticeable increase in current above a certain threshold voltage (0.8 V). This observation can be explained by the small polaron hopping model.[25] According to this model, in the high-temperature regime, shorter hops contribute to the transport process, resulting in an increase in conductivity. However, with further increase in temperature, a gradually diminishing current was observed. We believe that eventual evaporation of water molecules from the hydration shell surrounding the DNA molecule at high temperature and high vacuum condition and subsequent change in DNA conformation became predominant factors in this case. Finally, above the melting temperature of the DNA (75.6 °C in the present case), the noise was too high for any signal to be detected. To verify if there is any hysteresis effect, the devices were cooled down to room temperature and the $I-V$ measurements were repeated. In contrast to the freshly prepared samples, the temperature-treated devices often exhibited a very low current signal ($\leq$1pA) or only noise. We correlate this observation to the thermal denaturation of the DNA helix and/or to the deterioration of the DNA−SWNT interface.

We also examined the back-gate voltage dependence of the current through the DNA molecule, bridging the gap between the nanoelectrodes and thus forming the channel. At a positive gate bias ($V_g = +5$ V), a small depletion was observed (Figure 2F). On the other hand, a negative gate voltage ($V_g = -5$V) resulted in a slight enhancement. The above set of observations implies that the DNA molecule acts as a p-type channel. In our study, the 80 bp DNA sequence includes 34 guanine−cytosine (G−C) pairs.[19] Because of the low ionization potential associated with the G base, it causes localization of holes, and the charge transport is facilitated through the hopping mechanism.[26] Thermal hopping is presumably more dominant over coherent tunneling through the DNA duplex, as is apparent from our temperature-dependent conductivity study described above. One of the possible reasons for weak back-gate coupling is the presence of an air gap between the bridging DNA molecule and the SiO_2 surface in the trench. The gate bias was restricted to ±5 V since an appreciably high leakage current was observed for higher values of V_g. Further studies are needed for realization of the DNA-FETs with improved functionality.

To confirm that the measured electrical signals indeed originated from the DNA molecule covalently attached between the nanoelectrodes, we performed a series of control experiments. First, we verified if the recorded current was merely due to ionic transport between the nanoelectrodes. We spotted a droplet of DNA-free sodium acetate on the chip under ac bias conditions, as mentioned previously. For multiple experiments, we observed only a noise (Figure 3A), unlike the current curve reproducibly passing through the origin of the $I-V$ plot when the buffer contains ssDNA or dsDNA molecules. Another indirect evidence for the signals arising from the DNA molecules bridging the SWNT electrodes was obtained when we attempted to couple an 80 bp dsDNA (contour length ~27 nm) between a pair of electrodes separated by a distance of about 50 nm (Figure 3B). Lack of success indicates that unless the termini of a DNA molecule are attached to the nanoelectrode pair, no reproducible current signal can be obtained. To observe the efficacy of the amide bond between a DNA molecule and SWNT electrodes, we placed a droplet of dsDNA suspension on a pair of SWNT electrodes without any modification. For most of the devices, there was no signal. Although some devices exhibited a detectable current, the magnitude of the signal was much lower than that observed for carboxyl-functionalized electrodes (Figure 3C).

To summarize, we have developed a novel platform based on SWNT nanoelectrodes for directly probing the dc conductivity in DNA at the single-molecule level. Applica-

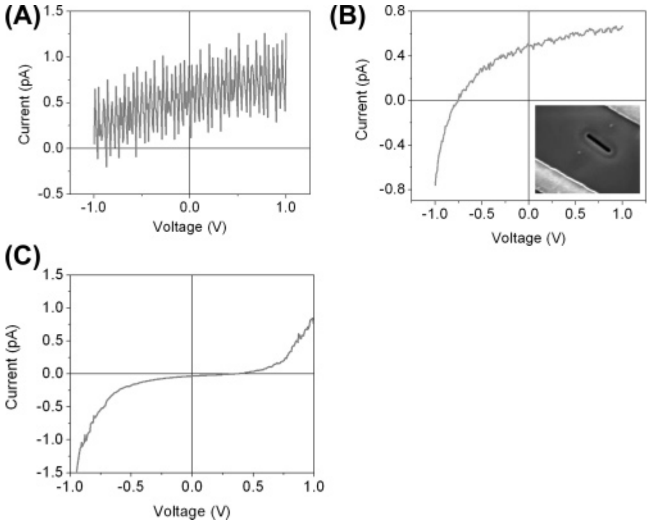

Figure 3. Control experiments. (A) Electrical noise when the DNA-free buffer solution (10 mM sodium acetate, pH 5.8) was spotted onto the electrodes and dried using a nitrogen stream. These data indicate that the observed current signals in Figure 2 are indeed from DNA molecules and not merely due to the ions in the buffer solution. (B) $I-V$ data obtained when we attempted to attach an 80 bp dsDNA molecule (contour length ∼ 27 nm) to a pair of nanoelectrodes with a separation of about 50 nm (inset picture). The lack of signal further confirms that the current flows only when a DNA molecule bridges the gap between the nanoelectrodes. (C) $I-V$ data from a dsDNA molecule attached to a pair of SWNT electrodes without any carboxyl functionalization. There is a lack of reproducibility, and even when there is a signal, the magnitude is much lower than that observed for a functionalized SWNT. This data proves the efficacy of the amide bond, which strongly couples a DNA molecule with the nanoelectrodes.

tion of DEP in our system causes stretching of the DNA molecules and positioning of them between the electrodes. Statistically, for the majority of the devices, we observed a current value in the range of 25−40 pA when a dsDNA molecule bridged the SWNT electrodes. In comparison, a ssDNA molecule carries a much lower current (∼1 pA or less), primarily due to the lack of regular stacking of the nucleotide bases. Application of the back-gate voltage revealed that the bridging DNA molecule forms a p-type semiconducting channel between the SWNT electrodes. The results of several control experiments confirmed that the measured electrical signals indeed originated from the anchored DNA molecules. The present study demonstrates that SWNT can be employed as efficient nanoelectrodes for direct measurements of charge transport in DNA at the single-molecule level.

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Supporting Information Available: Fabrication procedure of nanoelectrodes, selection of the DNA base sequence, gel electrophoresis data in favor of double helix formation, and the control experiments with different buffer solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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