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Direct conductance measurements of short single DNA molecules in dry conditions

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Abstract
We present a study of electronic transport in short (12-base-pair) DNA duplexes covalently bonded (via thiol groups) to two gold electrodes obtained by a mechanically controllable break junction (MCJB) technique in dry conditions. A large number of DNA junctions have been repeatedly formed in order to obtain a conductance histogram that reveals a peak which corresponds to the conductance of a single DNA molecule. We observed that the conductivity of a DNA increases upon increasing the content of G:C base pairs in the duplex. With our method we are able to obtain a reliable value of a single DNA conductance and subsequently measure its current–voltage (I–V) characteristics. In contrast to the electronic transport measurements performed with long DNA sequences (hundreds of base pairs) where the obtained conductance values vary a lot with environmental conditions, our values obtained for the short DNA sequences are consistent with the values reported for comparable sequences in aqueous solution.

1. Introduction
The unique properties of DNA, such as self-recognition and spatial structuring, render it an excellent candidate for use as a building block in molecular electronics, for example as an 'active' scaffold [1, 2]. Moreover, due to its property to mediate charges along the base pair stack, DNA can be used in sensor applications [3]. Charge transport in DNA has been intensively studied using photochemical [4–7], biochemical [8–11], electrochemical [12–14] and direct electrical measurements [15–24]. These pioneering direct electrical measurements on dry DNA yielded very ambiguous results; depending on the experimental approach the DNA was found to be insulating [15–19], semiconducting [20, 21] or conducting [22, 23]. There is now increasing consensus that DNA molecules longer than 40 nm (in dry state, lying on a substrate) are insulators [17]. However, for short DNA duplexes (8–26 bp) the situation is different and the most direct charge transport measurements, regardless of the measuring environment, demonstrate significant conductance [25–29]. Although many theoretical models for this charge transfer have been discussed in the literature, the exact mechanism is not yet clear. It is evident that many experimental obstacles must be overcome before the possible intrinsic conducting properties of DNA can be discussed.

We start by focusing on the main problems involved in performing direct charge transport measurements in DNA molecules. Due to the fact that the observable electronic properties of DNA are very sensitive to factors such as the length of the molecule, the base sequence, the helical conformation, the environmental conditions, the surroundings (either on a substrate or suspended) and the way the molecule is connected to the electrodes, unambiguous determination of the intrinsic conductivity of DNA requires each of these issues to be addressed separately. The problem of metal–molecule contacts and their influence on the observed electronic transport through the molecule is a general problem in all direct transport measurements on the molecular scale. A reliable way to achieve a well-defined molecule–electrode contact is to provide the molecule with two linker groups, one at each end, which can bind covalently to the electrodes [30]. Furthermore, the conductance of the linker itself must be considered. For example, commercially available DNA linkers usually contain an insulating alkane chain, which may act as a bottleneck in the conductance measurements. In this study, we used a 3′-thiol-modified DNA where the linker group contained a three-carbon...
alkane chain, which has a relatively high conductance, of the order of $10^{-11} G_0$ [31, 32]. Even in this case differences in the measured molecular conductance can be observed due to the different contact geometries in the molecular junction. In order to overcome this problem, Xu and Tao introduced the scanning tunnelling microscope (STM) break junction technique in order to quickly and repeatedly form thousands of molecular junctions with different contact geometries [33]. By performing a statistical analysis on the individual conductance curves, this method determines the conductance of a single molecule with the most probable contact geometry. This technique has been further developed by various groups and it has now become an established method to provide reproducible measurements of the conductance of a single molecule. It has also been applied to study DNA molecules in an aqueous environment [25, 26]. Since the charge transport studies performed using DNA duplexes of a similar size show that humidity has a great influence on the DNA conductance [27], it is interesting to apply the reliable statistical break junction method in dry or inert gas surroundings in order to compare the obtained conductivities. These values should ideally converge for similar DNA duplexes, and then at least one of the above-mentioned problems would be solved.

In this paper we present a study of the conductance of a single DNA molecule. We used short (12-base-pair) double-stranded DNA sequences and performed our measurements with the mechanically controllable break junction (MCBJ) technique. By repeatedly forming metal–DNA–metal structures we obtained conductance histograms for two DNA sequences with different contents of G:C base pairs (42% and 75%). The 3'-thiol-derivatized DNA molecules were covalently attached to the gold electrodes via thiol bonds. After obtaining the most probable value of the DNA conductance, we measured the $I–V$ characteristics of the DNA molecules.

2. Experimental details

The MCBJ technique is a very elegant way to control the spacing between two metallic electrodes with a sub-atomic (<$10^{-10}$ m) resolution [34]. It has been used to study electronic transport in atomic-size metallic point contacts and wires, and it has also been applied in the study of transport in individual molecules [35–38]. The scanning electron micrograph of the free-standing gold bridge in our device is presented in figure 1(a). A schematic view of the experimental set-up is given in figure 1(b). We break the bridge by bending it using a three-point bending mechanism. The central part of this mechanism is a pushing rod that can be controlled with micrometre precision via a linear motor or a piezoelectric element and two counter supports. The Kapton substrate is bent until the gold neck breaks, obtaining two metallic electrodes. By reducing the bending, the electrode spacing can be controlled with sub-atomic precision due to the large mechanical ‘attenuation factor’. The degree of control obtained is comparable to or even better than that of STM [39]. Compared to STM, the MCBJ technique offers the advantage that the spacing of the electrodes is controlled mechanically and can be kept fixed, or can be changed independently of what is on or near the tips of the electrodes. Indeed, our break junctions can be frozen precisely at a certain position, and in the presence of molecules they are stable for several days so that numerous $I–V$ measurements can be performed. The high stability of the set-up is mostly related to the use of a Kapton substrate which possesses very good elastic properties and high mechanical stability.

Figure 1. Schematic view of the mechanically controllable break junction (MCBJ) set-up. (a) SEM micrograph of a break junction. (b) Layout of the MCBJ technique. (c) DNA sequences used in the experiments. The colour coding of AT and GC bases pairs has been chosen to point out their different presence (%) in the chosen sequences DNA1 and DNA2. (d) The schematic view of the break junction during the closing process.
The planar dimensions were 20 mm and hollow site bonding [40]. More detailed information about the nature of the molecular fundamental conductance value, can also be observed. The conductance of a single molecule. In some cases, conductance values are generally located at integer multiples of a fundamental conductance value, which corresponds to plateaus appearing in the histogram plot of the recorded values for the most probable microscopic configurations of the molecules in between the electrodes. The conductance peaks appearing in the histogram plot of the recorded conductance values are generally located at integer multiples of a fundamental conductance value, which corresponds to the conductance of a single molecule. In some cases, additional peaks, which do not fit to an integer multiple of the fundamental conductance value, can also be observed. The additional peaks appearing in the statistical analysis reveal more detailed information about the nature of the molecular junction, for instance the conductance difference between top and hollow site bonding [40].

We fabricated the MCBJ device on a Kapton substrate. The planar dimensions were 20 mm × 6 mm and the substrate thickness was t = 0.5 mm. The top of the substrate was spin-coated with a layer of polyimide. On top of the polyimide, the device was patterned by conventional electron beam lithography. We deposited a gold (60 nm) layer with a thin adhesion layer of titanium (2 nm). After the gold deposition, the polyimide below the bridge was etched away, leaving behind a free-standing gold bridge. The suspended length of the bridge was u = 3–3.5 μm and the width was approximately 100 nm. The distance between the counter supports was L = 12 mm. It is known from the literature [34, 41] that, for homogeneous strain in the bending beam, a geometrical formula can be used to obtain a mechanical ‘attenuation factor’, r = 6tu/L^2. The mechanical ‘attenuation factor’ in our case was of the order of 1–5 × 10^{-5}. The relative change in the distance between the electrodes (∆x) is proportional to the relative vertical displacement of the pushing rod (∆z) via the attenuation factor, r = ∆x/∆z. This provides control of the spacing between the electrodes with a precision of ∼10 pm.

For the transport experiments, we used 12 bases-long 3′ thiol-terminated DNA oligonucleotides: Seq1 (5′-CTC-GAG-CCT-CGG-C3-S-3′), Seq2 (5′-TCG-AAG-CTT-GAA-C3-S-3′) and their complementary strands Seq1′ (5′-CCG-AGG-CTC-GAG-C3-S-3′) and Seq2′ (5′-TTC-AAG-CTT-CGA-C3-S-3′). All of the oligonucleotides were purchased from EUROGENTEC S.A. The thiol-terminated oligonucleotides were provided in the protected form, C3H6S2C6H4OH. We chose these particular sequences for two reasons. First, we obtained double-stranded DNA containing different percentages of GC base pairs in order to observe the expected differences in electrical conductance: Seq1/Seq1′ (75% GC, referred to from now on as DNA1) and Seq2/Seq2′ (42% GC, referred to from now on as DNA2). Second, these sequences form a stable and specific double-stranded DNA (dsDNA) structure, which was confirmed using Oligo Analyser (IDT SciTools 3.1). The concentrations of all oligonucleotides were determined by measuring UV absorbance at 260 nm. After mixing stoichiometric amounts of the complementary oligonucleotides, the hybridization was performed by heating to 94°C for 10 min and then allowing the mixture to return to room temperature. As a hybridization buffer, we used 1X T4 DNA Ligase Reaction Buffer (New England BioLabs), that is, 50 mM Tris-Cl, 10 mM MgCl2, 1 mM ATP (adenosine triphosphate) and 10 mM DTT (dithiothreitol) at pH 7.5. DTT was used as a reducing agent in order to reduce the disulfide bond and remove the mercaptopropanol-protective group from the DNA. The final concentrations of the dsDNA solutions were 2 μM for each solution.

The freshly fabricated MCBJ samples were cleaned by oxygen reactive-ion etching (RIE) prior to DNA deposition. A droplet of the DNA (concentration 2 μM) solution was introduced onto the junction using a micro-syringe. Subsequently, the sample was placed inside the measurement chamber which was then evacuated (the pressure was of the order of ∼1 mbar when the pump was stopped), letting the drop slowly evaporate. The break junction was then opened in vacuum. For each dsDNA molecule, two different MCBJ samples were measured.

All of the conductance measurements were obtained using a lock-in amplifier (Stanford Research Systems SR830) with a frequency of 13.33 Hz. We typically applied an AC voltage of between 10–50 mV and we measured the corresponding current while opening and closing the contact. In order to increase the sensitivity of the lock-in amplifier to measure lower currents, we used a series resistance of R = 1 MΩ. The pushing rod was moved with a velocity of v = 0.09 μm s^{-1}. We repeated this procedure approximately 100 times for each sample. A home-made LabView (version 6i) program was used to control both the motor and the data acquisition. In this way we could construct a histogram plot of the conductance for many traces of opening or closing. The histograms were constructed from the current versus position traces by converting the currents to conductances and binning the data as a function of conductance without data selection. We emphasize that for the statistical analysis we used 100% of the captured current traces without applying any selection algorithm that could enhance the appearance of the multiple
Figure 2. Typical conductance traces for DNA2 are measured as the MCBJ electrodes are brought into contact. Conduction is expressed as $G_0 = 2e^2/h ≈ 77 \mu S$, where $e$ is the electron charge and $h$ is the Planck constant. $\Delta x$ is the change in the distance between the electrodes. A bias voltage of $V = 50$ mV was used. The arrow indicates the plateaus corresponding to the single-molecule conductance. The conductance reference traces for the bare gold break junction (without DNA) is shown in the inset.

Conductance peaks [42]. Before breaking the contact, the electrodes were each time brought into contact in order to ensure that each measurement started from a different initial configuration. Our in-house MCBJ set-up is very stable and it enables reproducible low-noise electrical measurements.

For measurements of the $I–V$ characteristics we used a voltage generator (Yokogawa 7651) where we obtained the bias voltage sweep. The current through the sample was measured using a current amplifier (Stanford Research Systems SR570). The output signals were monitored with an oscilloscope (Yokogawa DL708e) and captured on a computer via a home-made LabView program.

3. Results

In figure 2 we present several representative conductance traces obtained during the closing of the electrodes of the MCBJ junction in the presence of DNA molecules. The value $\Delta x$ is the change in the distance between the electrodes, which was calculated from the attenuation factor ($r$) and the vertical movement of the pushing rod ($\Delta z$). The arrow in figure 2 indicates the region where we observed the first plateaus of conductance corresponding to one or a few molecules bridging the junction. Note that the conductance behaviour in the presence of DNA molecules deviates clearly from the characteristics observed for the bare gold junction, which are shown in the inset of the figure 2. In contrast to the conductance traces observed during the junction opening process, where the observed plateaus are typically shorter and appear less frequently, the junction closing process provides a high yield of conductance plateaus corresponding to a single molecule or a few molecules bridging the electrode gap. This can be intuitively understood for the case of junction closing: the molecules stick out from the electrode and single molecules can easily be trapped between the approaching electrodes. In the case of junction opening, the gold bridge is abruptly broken, leaving a shorter time for the formation of a single molecule bridge. We also note that the onset plateaus in the conductance traces could appear sharper when measuring short molecules [33] if compared with the ones observed in DNA measurements [25]. We think that this is due to the fact that DNA is a quite large and dynamic macromolecule and this can influence the recorded behaviour.

The conductance traces (similar to those shown in figure 2) were added together to obtain the corresponding conductance histograms shown in figure 3. The peaks appearing in figures 3(a) and (b) correspond to the single-molecule conductance of DNA1 and DNA2, respectively. In figure 3(c) we present the corresponding histogram for a control experiment under the same conditions but where no DNA was present; no conductance peaks are observed. Although for both DNA1 and DNA2 we observed fine structure in the main peak corresponding to the molecular signature (as previously observed in conjugated molecules [33]), we decided to focus our quantitative analysis only on the main peak. The peak width reflects the fluctuations in the molecular junction [43–45]. For the quantitative analysis of the conductance peaks, we subtracted the histogram of the background (figure 3(c)) from the histograms of the molecules (figures 3(a) and (b)) and fitted two Gaussian functions to the remaining conductance peaks. The corresponding two conductance values (10.6 and 16.4 nS for DNA1, 6.9 and 11.5 nS for DNA2) can be interpreted as a fluctuation in the
molecule–electrode junction caused by the switching between the different binding sites on gold (hollow, on top, or bridge site) [40, 46]. By fitting only with one Gaussian peak we can obtain the average conductance values $G_1 = 1.7 \times 10^{-4} G_0$ (or 13 nS) for DNA1 and $G_2 = 1 \times 10^{-4} G_0$ (or 8.5 nS) for DNA2 which can be used to compare the conductivity of the DNA sequences.

Since our MCBJ electrodes can be controlled at subatomic resolution, we were able to monitor the time evolution of the single-molecule junction. We stopped the motor at the position of a plateau (the value of the conductance peak) and measured the conductance as a function of time while sequentially increasing the bias voltage. The resulting conductance versus time curves for DNA1 and DNA2 are presented respectively in figures 4(a) and 5(a). In order to see more clearly what is happening upon increasing the voltage, we plotted the corresponding conductance histograms of the DNA1 which can be used to compare the conductance histograms in figure 3(b). We note that the values of such peaks are close to a single-molecule conductance peak during the measurement. (b) The conductance histograms for DNA1.

We present the linear fits give the values $G_1 = (2.1 \times 10^{-4} \pm 0.696 \times 10^{-6}) G_0$ (or 17 nS) for DNA1 and $G_2 = (0.61 \times 10^{-4} \pm 0.46 \times 10^{-6}) G_0$ (or 4.7 nS) for DNA2, which is in agreement with the values obtained from the conductance histograms in figure 3. The small differences between the conductance values obtained by fitting to the histogram peaks and from the linear fit to the $I–V$ curves are due to the above-mentioned possibility of having different molecular junction configurations during the measuring process.

4. Discussion

Although the exact mechanism of the electronic transport through dsDNA has yet to be clarified, it is widely believed to be dominated by hole hopping between the individual G:C base
pairs (or G:C regions) through the $\pi$-orbitals of the base pair stack, where the A:T pairs (or A:T regions) act as tunnelling barriers [47]. The transport properties thus depend not only on the total length of the molecule, but also on the content of C:G pairs and their positions with respect to each other. Our results also confirm the fact that the dsDNA sequence with a higher content of C:G pairs results in a higher conductance.

In our experiments we used two different 12 bp DNA molecules: molecule DNA1, which contains three separate A:T pairs, and DNA2, which contains three islands with two adjacent A:T pairs and one separate A:T pair. We note that a molecule with a very similar sequence to our DNA1 was measured by Hihath et al in liquid using the STM break junction technique [26]. For 11 bp dsDNA containing three isolated A:T barriers, they obtained a value $\sim 4$ nS, whereas our value for DNA1 was $\sim 13$ nS. These values differ only by a factor of 3, which can be due to the specific structure of the DNA sequence or the environment used. Thus, our method leads to better agreement with the results performed in aqueous solution than a similar experiment performed in dry conditions using the static nanoscale gap [27], where a factor of $\sim 30$ difference was found compared to the extrapolated values for a 15 bp C:G sequence observed in [25]. Our DNA2 sequence is not directly comparable to the other measurements performed for dsDNA with a similar length scale due to its high A:T content and the order of the bases in the sequence.

Other experimental studies performed using short dsDNA molecules have shown that adding adjacent A:T pairs into the G:C sequence [25] or replacing the C:G pairs with adjacent A:T pairs [27] yields an exponential decay of the conductance. Although the obtained decay rates differ by a factor of two, according to this one might expect a much lower value of conductance for our DNA2 sequence. Our results show clearly that the DNA1 sequence, which contains a higher content of C:G pairs, has a conductance that is a factor of $\sim 2$ higher than that of DNA2. We believe that one of the reasons for this is that in both cases the A:T pairs were placed into the sequence which was composed only of C:G pairs. This type of sequences has relatively poor recognition properties and it does not form a stable B-form structure as biological DNA sequences, since it can form, for instance, a hairpin-type structure. One of the arguments is that, in later work by Tao’s group, where they studied the placing of an A:T pair into the DNA sequence in such a way that it resembled a stable B-form structure [26], they observed that the conductance decreased only by a factor of $\sim 2$ when one C:G pair was replaced by an A:T pair. Moreover, in these studies a large number of A:T pairs were added in the middle of a C:G sequence, leading to a longer barrier length (compared to our DNA2 which contains three barriers of two A:T pairs) or even differences in the helical structure.

5. Conclusions

In this paper we have presented a study of the electronic transport in two short dsDNA sequences (with different contents of the C:G pairs) using the MCJB technique in dry conditions. We have monitored the current through the molecular junction while repeatedly opening and closing the MCJB. From this data we have constructed conductance histograms, which reveal a peak that corresponds to the conductance of a single DNA molecule. The obtained values were $G_1 = 1.7 \times 10^{-4}G_0$ (or 13 nS) for DNA1 and $G_2 = 1 \times 10^{-4}G_0$ (or 8.5 nS) for DNA2. We have observed that the conductivity of DNA increases upon increasing the content of the G:C base pairs in the duplex. Our values of conductance are consistent with those reported for comparable sequences in aqueous solution obtained using the STM break junction method. The main advantage of our method is that we are able to observe almost constant conductance versus time for hours, which allows us to measure the corresponding $I$–$V$ characteristics.

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