Cent. Eur. J. Phys. • 6(3) • 2008 • 422-426
DOI: 10.2478/s11534-008-0072-7

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Central European Journal of **Physics**

Scanning tunneling spectrosopy study of DNA conductivity

Research Article

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Received 16 November 2007; accepted 21 February 2008

Abstract: We used STM to study the conductivity of 32 nucleotide long DNA molecules chemically attached to a gold surface. Two oligonucleotides containing all four base types namely G, A, C, T, one single stranded and one double helical, all showed conductance data significantly higher than DNA containing only T and A that were either single stranded d(T32) or double helical d(T32).d(A32) in confirmation. Within each sequence group, the conductivity of the double helical form was always higher than that of the single strand. We discuss the impact of structure, particular base stacking and affinity to the phase transition.

PACS (2008): 87.14.gk, 81.07.-b, 81.07.Nb, 81.40.Rs

Keywords: molecular electronics • DNA • scanning tunneling microscopy • conductivity • charge carrier transport

DNA is an important and promising molecule, not only due to its genetic function, but also as a molecular scaffold for nanotechnology [1]. The double helical DNA architecture, is well stacked consisting of near parallel bases stacked with their π-electron systems overlapping. Such π-electron systems may be good candidates for long distance and one-dimensional (linear) charge transport. Investigations of DNA conductivity and its physical origin have significant implications towards the study of DNA damage and repair in biological systems, application of DNA in electronic nano-devices, and DNA-based electrochemical biosensors. The electronic transport of various types of organic molecules operating through π-conjugated systems, including DNA, has been the subject of several recent studies, both theoretical and experimental [2–5]. Despite significant achievements, results on DNA conductivity published by different research groups present often conflicting and controversial explanations of

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the DNA-mediated charge transport [6–8].

Two basic experimental approaches have been pursued to investigate the conduction through molecules: (i) contacting isolated single molecules or molecules in thin films, (ii) studying transport in thick films and devices, such as organic thin-film transistors or light emitting diodes. The optimal experimental setup is to position isolated molecules between two electrical contacts but it is very difficult to realize and especially difficult to verify. Working with ordered arrays of parallel π-conjugated molecules where in principle, the coupling to the substrate can be precisely known, offer the possibility to address the individual molecule by making use of the scanning tunneling microscope (STM) tip. STM experiments have already been proven as well suitable tools for the investigation of ordered monolayer films allowing electrical contacting of one or a few molecules and to obtain images of the structural characteristics of the film [9, 10].

In this work, we used the STM to study the conductivity of different DNA sequences in single- and double-stranded forms covalently bonded to a gold surface under ambient conditions (i.e. air, room temperature). It is known that water molecules surrounding DNA strongly participate in DNA structure holding. Working under vacuum, the water molecules are expelled and the DNA structure is reserved mainly by the effect of inner shell water molecules and this results in drastic changes.

Our DNA molecules were short and very precisely prepared as self-assembled 32-mer oligodeoxynucleotides making monolayers and were immobilized by attaching to a gold surface through the formation of disulfide bridges via the C6 spacer at the DNA 5'-end as shown in Fig. 1. The 40 nm thin gold layer was prepared by evaporation of pure gold (99.99% purity) on a silicon <100> wafer which was cut into 1 cm×1 cm pieces. Synthesized were two types of oligonucleotides, two 32-mers with uniform sequences, d(32T), d(32A), and two 32-mers with non-self-complementary sequences containing all four nucleotides, G, A, C, and T in equimolar ratio:

5'-d(CAGCAGGGTCAGTCAATCTATCGTCCGTAATG)-3'

(labeled further as d(GACT)), and the complementary sequence

5'-d(CATTACGGACGATAGATTGACTGACCCTGCTG)-3'

The spacer was incorporated to the 5'-end of d(32T) and d(GACT) oligonucleotides. These two molecules were subsequently chemically attached to the gold surface in their single stranded forms Complexation with their complementary strands, resulted in chemically intact DNA molecules, via double helice formation. All DNA samples were prepared as triethylammonium salt (TEA); bulky TEA counterion with low mobility was selected to reduce ionic conductance of the samples.

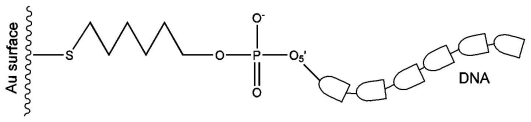

Figure 1. Scheme of attachment of the DNA 32-mers to the gold surface.

The STM measurements were performed with NTEGRA Prima NT MDT system using freshly cut Pt/Ir tips. Both topographic and spectroscopic data were obtained. With STM, for very small voltages of ~10 mV and relatively large current of ~ 0.5 nA, in view of the very small tip-sample distances, the topography of the samples were observed.Topographic images showed a considerable difference between the bare gold substrates and DNA sample molecules . In the case of molecular layers, specific surface patterns were observed. The occurrence of these patterns has been attributed to etching of the bottom gold layers arising from the reaction of thiol-groups with gold atoms, forming dissolvable complexes [11, 12]. All the molecules were ordered in similar geometric structures with the nearest neighbour spacing of 1-2 nm. By ellipsometry, in which the thickness of all the measured molecular layers was established as 8.5-9 nm, we have confirmed that the 32 nucleotide long DNA molecules are oriented approximately perpendicular to the substrate.

$I(V)$ spectra were taken for different feedback voltage and current setpoints, i.e., for different initial sample-tip distances. In all experiments, the STM tip act as the electrical contacts on the "top" side of the assembled monolayer of the DNA molecules, the supporting gold substrate acts as the other (bottom) contact. The distance between the top of the molecules and the STM tip for all the set points was 2.5-4 Å. In view of the experimental setup, it was predicted that the flow of current occurs mainly through the molecule nearest to the tip without significant lateral intermolecular contribution to conduction.

Noise, in the form of height fluctuations, was larger in the case of topographic images of films than in the case of the bare gold. All the organic molecules that have been studied by STM till now showed symmetrical current-voltage ($I - V$) characteristics. The symmetry of the $I - V$ characteristics has been explained in a simple model [13].

$I(V)$ curves were measured at three different set points: 0.1 nA, 0.1 V; 0.2 nA, 0.1 V; 0.5 nA, 0.1 V using the same strategy for all four DNA sequences. Six hundred consecutive $I(V)$ sweeps in both voltage directions (from -1.5 V to +1.5 V and from +1.5 V to -1.5 V) were taken on each sequence. We compared $I(V)$ spectra taken for different molecular systems for the same substrate under the same conditions (set point at the gold terrace). Final

results presented in this paper are based on the average of the curve data set for the subsets collected. Only those curves $I(V)$ were included in the averaging in the analysis that were not affected by appreciable drift of the STM. All the results taken into consideration showed the same trends. Typical examples of $I(V)$ curves measured at set point 0.1 nA; 0.1 V are shown in Figs. 2, 3 and 4. In our experiments we have measured current tunneling through the tip-molecule area for the molecules so close to the tip that their contribution is set at voltage levels above the noise. For tips very close to the molecules (defined by the set point value) at very low voltages the number of molecules contributing to the total current is small. As we could not estimate exactly the number of the molecules, we proceeded to to compare conductances of different DNA sequences for both single- and double-stranded forms. The term conductivity is widely used in equivalent experiments (STM/AFM measured current vs. voltage curves on bundles of molecules) that we have decided to keep its common meaning.

The shape of the $I(V)$ curves can be interpreted as follows: current passes through the molecule and also tunnels through the tip-molecule area. For low volatges the ohmic behaviour was observed due to the Botzmann distribution of the charge carriers and constant position of the Fermi level. As voltage is increasing the current passing through each molecule is higher – nonlinear effect of charge carrier injection takes place (shift of the DNA Fermi level to the electronic tale states and their occupation). Also the number of the molecules contributing to the total current is rapidly increasing so the total current is rising up very sharply at higher voltages.

Comparison of conductivity of two single stranded sequences, d(T32) and d(GACT), and two double stranded, d(T32).d(A32) and d(GACT).d(CTGA), can be summarized as follows. The largest difference was found between conductivity of single-stranded form of oligonucleotides containing only T and the double-stranded form of mixed G, A, C, T sequences. Both samples with the "mixed" sequence, either in single- or double-stranded form, showed larger conductivity than either sample containing the d(T32) sequence (Figs. 2, 3). We observed the double helical sample with the mixed G, A, C, T sequences to be the best conductor from our DNA sample set (Fig. 4). Conductivity of the double-stranded form is larger than that of the single-strand when complementary sequences are compared, which is in agreement with theoretical model of the same system [7].

Based on our topographic data, we assume that the main reason for differences in sampleconductivity arise from the properties of the individual molecules, not as a function of the molecular monolayers.

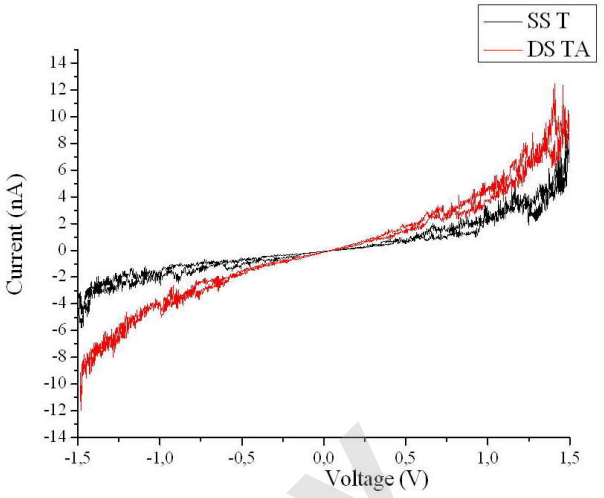

Figure 2. Typical $I(V)$ curve of DNA 32-mer d(T32):d(A32) double helix (DS TA) and d(T32) single-stranded form (SS TA). Set point 0.1 nA, 0.1 V, measured in both voltage directions.

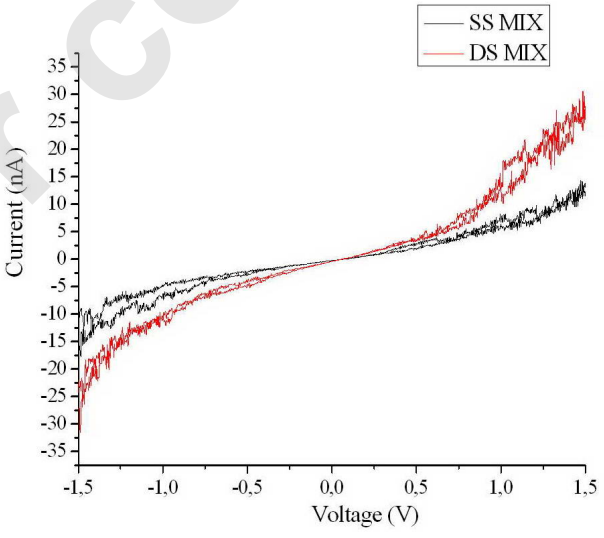

Figure 3. Typical $I(V)$ curves of DNA 32-mer with mixed G, A, C, T sequences: Double helical d(GACT).d(CTGA) (DS MIX) and the single-stranded d(GACT) form (SS MIX). Set point 0.1 nA, 0.1 V, measured in both voltage directions.

Two channels can significantly contribute to the charge transport along the DNA double helix; they include electronic conduction along the base pair sequences and ionic conduction associated with the counterions.

In this work, DNA molecules were synthesized as TEA salts to minimize the concentration of small, and therefore highly mobile, cations as sodium or potassium but ionic conduction cannot be ruled out due to water and solvated

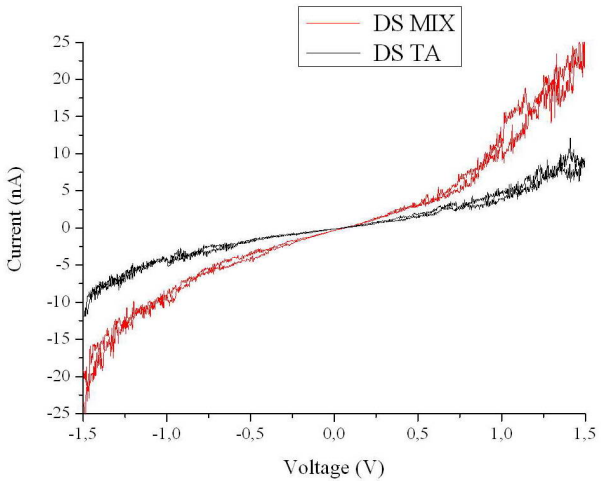

Figure 4. Comparison of $I(V)$ curves of double helical DNA 32-mers with mixed G, A, C, T sequences d(GACT).d(CTGA) (DS MIX) and d(T32):d(A32) (DS TA). Set point 0.1 nA, 0.1 V, measured in both voltage directions.

ions used to dissolve and subsequently deposited on the DNA molecules. However, an ionic conduction mechanism would not require just presence the presence of mobile ions, such as in a liquid or a molten phase, but also their macroscopic reservoir to maintain the observed stable current flow for as long as the voltage was applied. Neither of the two conditions were completely satisfied in our experiments. Thus, in our case, the ionic conduction mechanism should not be a strong source of the DNA conductivity.

On the other hand, any molecule or ion associated with DNA via ionic or covalent bonding might in principle affect the DNA electronic structure, the π-electron distribution of the stacked bases with their energetic states, and hence its electrical conductivity. Ions can act in the molecular system like dopants-supplying the system by charge carrier – electron or hole. The electronic interactions between the π-electron systems of the bases may generate a molecular energy band with electronic states delocalized over the entire length of the molecule. We assume the impurity concentrations from sample to sample to be approximately the same, therefore we did not expect the impurity concentration to be a source of differences in the conductivity between samples.

The question arises, what can be the main origin of the different conductivity of our molecular samples? Besides the charge-carrier concentration, the explanation for the measured conductivity differences in the DNA samples may relate to the the specific electronic structure, molecular chains structural differences and electron-phonon interactions. Single strands of both investigated sequences can be considered to be much less structurally regular than the double helices because of their local and especially long-scale deviations from the regular helical arrangement [14]. Reduced stacking interactions can decrease the potential overlap of the π-electron systems. Furthermore, a more regular structure may promote better long range ordering resulting in a decrease in the dispersion of the polarization energy and narrowing in the distribution of hopping states. Under these conditions, the charge carrier mobility, and thus conductivity, in regular systems increases.

Based on our experimental results we propose that electrical conductivity in DNA strands is higher for the well-defined right-handed double helical forms due to their smaller structural deviations resulting in larger π-electron potential overlap and smaller dispersion of the polarization energy (Figs. 2, 3).

Guanine base is the most easily oxidized. This allows the generation of a charge carrier (hole). Once charges, and especially holes, are created on the uniform DNA chain, the hopping charge transport phenomena can occur among discrete guanine sites or delocalized (e.g., polaron) domains [12]. Furthermore, the stacking distance of the adjacent base pairs also affects the π-electron overlap. The crystal structure analysis of oligonucleotides indicates that the axial rise of residue is 2.88 Å for poly(dG).poly(dC) and 3.22 Å for poly(dA).poly(dT) [15]. The more compact the base stacking is, the more favorable the charge transport is expected to be. Charge transport enhancement in tighter stacked compact samples is in agreement with our results showing higher conductivity of double helical mixed d(GACT).d(CTGA) sequences than d(T32).d(A32) samples (Fig. 4).

Based on our theoretical expectations and experimental data, we can say that the d(GACT).d(CTGA) is the better conductor of all the DNA samples investigated due to the regular structural double helical form with compact base (G-C) stacking.

Obviously, the DNA conductivity is a very complex problem and requires further attention. Detailed understanding of the conduction mechanism remains a challenge and, once achieved, it will undoubtedly provide a better insight into the biological, chemical and physical properties of DNA molecules.

Acknowledgements

This work was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (grants KAN400720701, KAN401770651, KAN 200100801 and COST OC 137) and by institutional project AVOZ 10100520. The authors thank the scientists participating in BIMORE (Bio-inspired Molecular Optoelectronics)

project for valuable discussions.

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