Eurasian Journal of Physics and Functional Materials

Volume 7 | Number 4

Article 3

Pages: 232 - 238

12-22-2023

Scanning tunneling spectroscopy of homooligonucleotides

T. Sharipov
Ufa University of Science and Technology ,Russian Federation

R. Garafutdinov
Institute of Biochemistry and Genetics Ufa Federal Research Centre of the Russian Academy of Sciences ,Russian Federation

A. Mishra
Durban University of Technology ,South Africa

S. Santer
University of Potsdam ,Germany

R. Shaikhitdinov
Ufa University of Science and Technology ,Russian Federation

See next page for additional authors

Recommended Citation

Sharipov, T.; Garafutdinov, R.; Mishra, A.; Santer, S.; Shaikhitdinov, R.; Balapanov, M.; and Bakhtizin, R. (2023) "Scanning tunneling spectroscopy of homooligonucleotides," *Eurasian Journal of Physics and Functional Materials*: Vol. 7: No. 4, Article 3.
DOI: https://doi.org/10.32523/ejpfm.2023070403

Scanning tunneling spectroscopy of homooligonucleotides

Authors

T. Sharipov, R. Garafutdinov, A. Mishra, S. Santer, R. Shaikhitdinov, M. Balapanov, and R. Bakhtizin

Eurasian Journal of Physics and Functional Materials

2023, 7(4), 232-238

Scanning tunneling spectroscopy of homooligonucleotides

T. Sharipov[*,1], R. Garafutdinov[2], A. Mishra[3], S. Santer[4], R. Shaikhitdinov[1], M. Balapanov[1], R. Bakhtizin[1]

[1]Ufa University of Science and Technology, Ufa, Russia

[2]Institute of Biochemistry and Genetics Ufa Federal Research Centre of the Russian Academy of Sciences, Ufa, Russia

[3]Durban University of Technology, Durban, South Africa

[4]University of Potsdam, Potsdam, Germany

E-mail: sha-t@yandex.ru

DOI: **10.32523/ejpfm.2023070403**
Received: 17.10.2023 - after revision

Short single-stranded DNA molecules of two types, consisting of dC nucleotides only or dA nucleotides only, were immobilized onto the surface of mica and silver substrates and studied by scanning probe microscopy methods. Geometric dimensions of the studied objects were determined. The current-voltage curves of $d(A)_{12}$ and $d(C)_{12}$ oligonucleotides were measured. Their differential electrical resistances were estimated and compared with each other.

Keywords: oligonucleotide; current-voltage curve; atomic force microscopy; electrical resistance; DNA; scanning tunneling microscopy

Introduction

The physical properties of DNA molecules, both natural double-stranded and synthesized single-stranded, are currently being actively studied. The invention of the scanning tunneling (STM) and atomic force (AFM) microscopes opened up possibilities to study various nanoobjects in molecular and submolecular scales [1]. Obviously, DNA molecules and, in particular, oligonucleotides, are

not exception. Chemically synthesized single-stranded DNA with a sequence composed of the same type of nucleotides is of great interest. These structures have unique electron density distribution and charge transfer properties, making them highly valuable for potential applications in bio- and nanoelectronics [2]. Promising fundamental [3, 4] and applied [5, 6] properties make these molecules an object of numerous studies [7, 8].

The data on the electrical resistance of the DNA molecules remains controversial [3, 5, 9]. The discrepancies in the results can be attributed to various factors such as the experimental conditions and the specific characteristics of the DNA molecules being studied [10]. Such factors as the length of the DNA molecule, its nucleotide composition, the sequence of nucleotides in the DNA chain, and the number of chains in the molecule can influence the electrical resistivity. Additionally, studying the surface topography and immobilization of DNA molecules using scanning probe microscopy methods is crucial for the advancement of DNA microarrays [11–13].

The STM method allows to measure the current-voltage curve of a biomolecule using STM. For this, the molecule is placed between two electrical contacts, one of which is a conducting probe of the microscope, and the other – a fragment of the substrate surface of an electrically conductive material.

The aim of the current study was to visualize and measure the electrical resistivity of the oligonucleotides depending on the nucleotide composition. To fix the number of varied parameters, we used the repeated nucleotides of only two types: cytosine $d(C)_{12}$ and adenine $d(A)_{12}$, where "12" is the number of the nucleotides in the sequence.

Materials and methods

$d(A)_{12}$ and $d(C)_{12}$ oligonucleotides were synthesized on an automatic synthesizer ASM-800 ("Biosset", Russia) by amidophosphite method. Water of the highest quality category (>18 MΩ, "Millipore", France) was used to prepare the solutions. The concentration of solutions was determined by the optical density of the aqueous solution at 260 nm on the BioSpec-Mini spectrophotometer ("Shimadzu", Japan).

Scanning probe microscopy (SPM) study was carried out in air using Solver P47 (STM mode) and Ntegra-Prima (tapping mode AFM) devices ("NT-MDT Spectrum Instruments", Russia). For AFM studies, the probes were silicon cantilevers NSG10 ("NT-MDT Spectrum Instruments") which have the curvature radius of 10 nm and force constant of 11.5 N/m. For STM studies the probes were wolfram tips, obtained by the method of electrochemical etching. In STM experiments, silver thermally deposited on the mica surface in vacuum was used as substrate. In AFM studies, the substrate was the surface of fresh mica cleavage.

The objects of the study were the oligonucleotides molecules consisting of 12 identical units – of deoxycytidine (deoxyadenosine). A solution of $d(C)_{12}$ / $d(A)_{12}$ oligonucleotide at a concentration of 5 ng/µl was heated at a temperature of 75–80 °C for 6–7 min for denaturation and then a drop of this solution of 5 µl

was deposited on a silver or mica substrate. The drop dried up in 30–40 min.

Results and discussion

First, tapping mode atomic force microscopy studies were performed. $d(C)_{12}$ oligonucleotide molecules were imaged on the mica surface by AFM. The objects of spherical shape are clearly visible on the AFM-image (Figure 1a). Some of them are the individual molecules of the oligonucleotides, the other – clusters (we will call them aggregates) of the oligonucleotide molecules. These aggregates have different thickness and width due to the different number of $d(C)_{12}$ molecules in the aggregate.

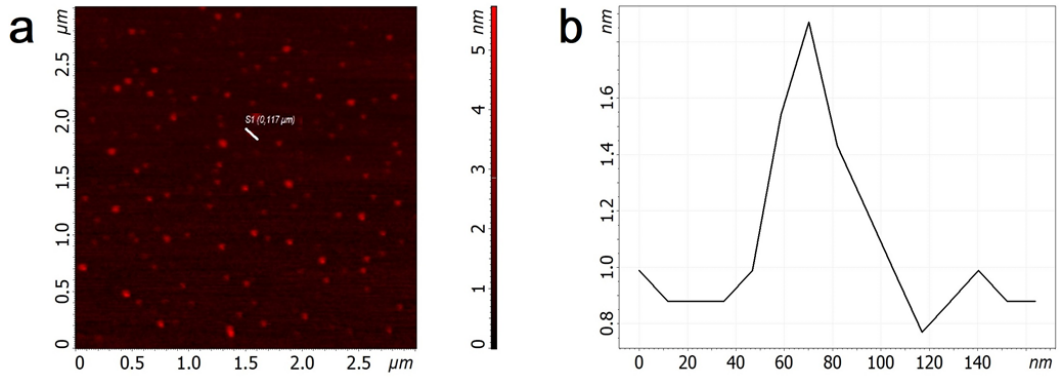

Figure 1. a) AFM imaging of $d(C)_{12}$ oligonucleotides on the mica surface; b) cross-section profile along the line marked at Figure 1a.

We are interested in the smallest of the objects observed on the AFM image, which, in our opinion, are single molecules. A cross-section profile, which displays the geometric parameters, was obtained for them (Figure 1b). Thus, the average size of such objects in laterals was about 36 nm, height was about 0.95 nm, which is in good agreement with the sizes of similar molecules studied by atomic force microscopy [14].

A similar AFM study was performed for $d(A)_{12}$ oligonucleotides. In Figure 2a, these molecules immobilized on a mica substrate are well visualized. In shape, they resemble round objects of different sizes. The smallest objects are about 1.1 nm height and about 30 nm in diameter (Figure 2b). Comparing the objects in Figures 1a and 2a, we can conclude that their shape and size are approximately the same. Which is not surprising, since both types of the studied molecules are single-stranded and consist of the same number of units.

Thereafter oligonucleotides were deposited on the atomically flat silver substrate and previously studied by scanning tunneling microscopy. Oligonucleotides were clearly visualized (Figure 3a) and were located on the surface of the substrate relatively rare, which is very convenient for further spectroscopic study.

Typically, oligonucleotides on STM images are represented as dark objects with small lateral dimensions. This is explained by the fact that they have lower electrical conductivity compared to the electrical conductivity of silver. Cross-section profiles (Figure 3b) allow to estimate the lateral dimensions of these objects, which are on average equal to 19–21 nm. Measuring the depth of the

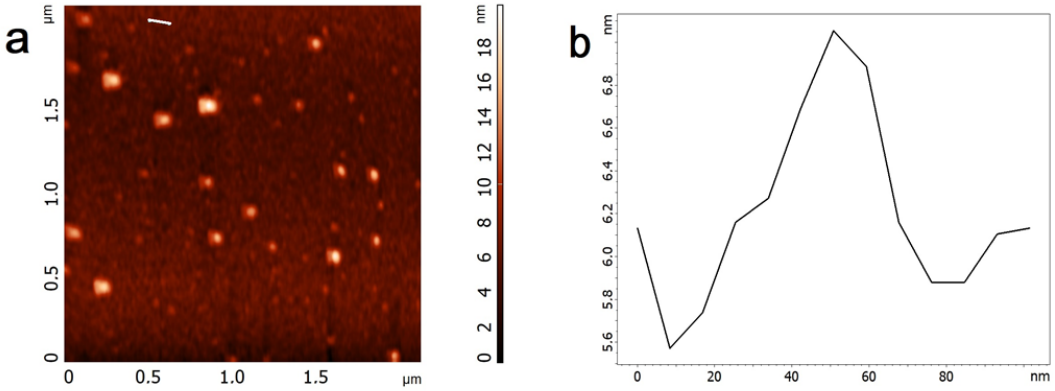

Figure 2. a) AFM imaging of $d(A)_{12}$ oligonucleotides on the mica substrate; b) cross-section profile along the line marked at Figure 2a.

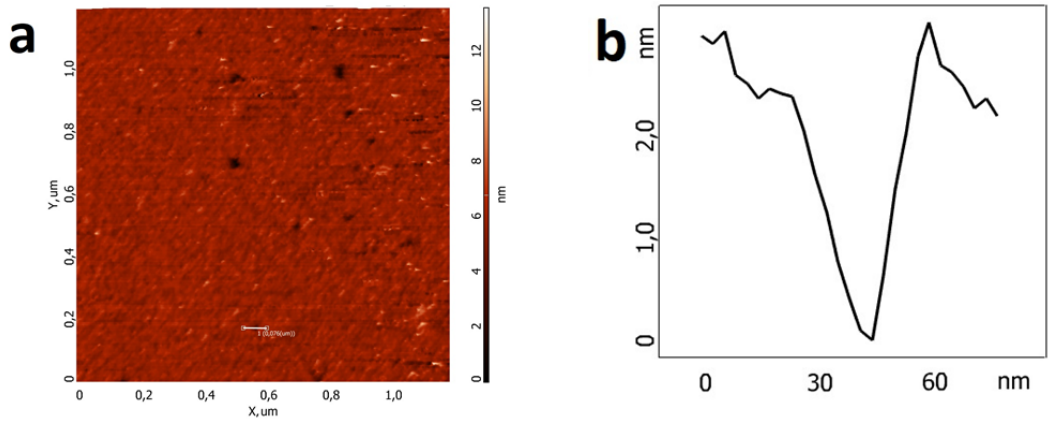

Figure 3. a) STM imaging of $d(C)_{12}$ oligonucleotides on the silver substrate; b) cross-section profile along the line marked at Figure 3a.

observed objects gives a result of about 2.9–3.2 nm. This value is not true, because the vertical dimension values on STM images are indirect. It is a topographic display of the electrical conductivity at each individual point of the scan area.

We used STM technique to visualize oligonucleotides on a substrate surface. To determine the differential electrical resistance of the oligonucleotides, we used scanning tunneling spectroscopy measuring the current-voltage curves. Multiple current-voltage curves were measured at different points where the individual oligonucleotide molecules were expected to be located. To obtain a final current-voltage curve, the measurements were averaged. By applying this method to measure the current-voltage curves of several $d(C)_{12}$ oligonucleotides in a specific scan area (shown in Figure 3a), we obtained an averaged current-voltage curve (Figure 4).

The resulting curve exhibits nonlinearity and has a symmetric appearance around zero values of both current and voltage in the voltage range from -1.2 V to +1.3 V. However, in the voltage range above +1.3 V and below -1.2 V, the curve shows substantial asymmetry at zero currents. In the voltage range from -1.0 V to +1.2 V the tunneling current is close to zero. And at voltages of more than +1.2 V and less than -1.0 V, the tunneling current sharply increases. Moreover, we want to note that at voltages above +1.4 V, the tunneling current overcomes 50 nA. Using the current-voltage curve we can calculate the differential resistance

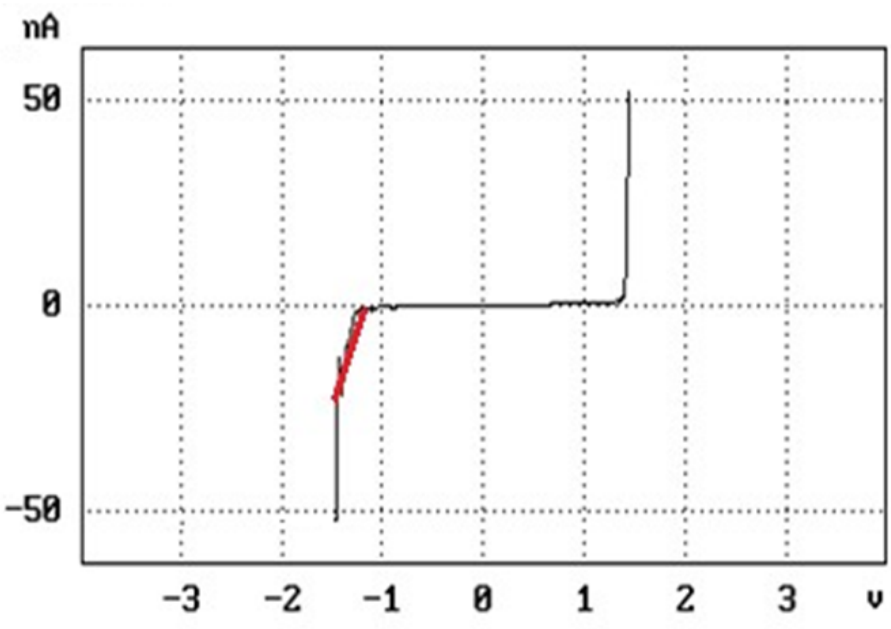

Figure 4. Averaged current-voltage curve of $d(C)_{12}$ oligonucleotides.

of oligonucleotides $d(C)_{12}$. For this, on the current-voltage curve we chose a region where there are no zero current values and at the same time there are no significant current fluctuations. In Figure 4, this region is highlighted with a thickened red line. It was estimated that the differential electrical resistance of a single molecule is approximately equal to R_{dif} (C)=$0.22 \cdot 10^8$ Ω. This value is many times greater than that given in [3]. However, the length of the molecules, the nucleotide composition and the scheme of the experiment are different.

Similar measurements of current-voltage curves were performed for $d(A)_{12}$ oligonucleotide molecules consisting of 12 identical units with an adenine nitrogen base. As in the previous case, we obtained the resulting averaged current-voltage curve (Figure 5):

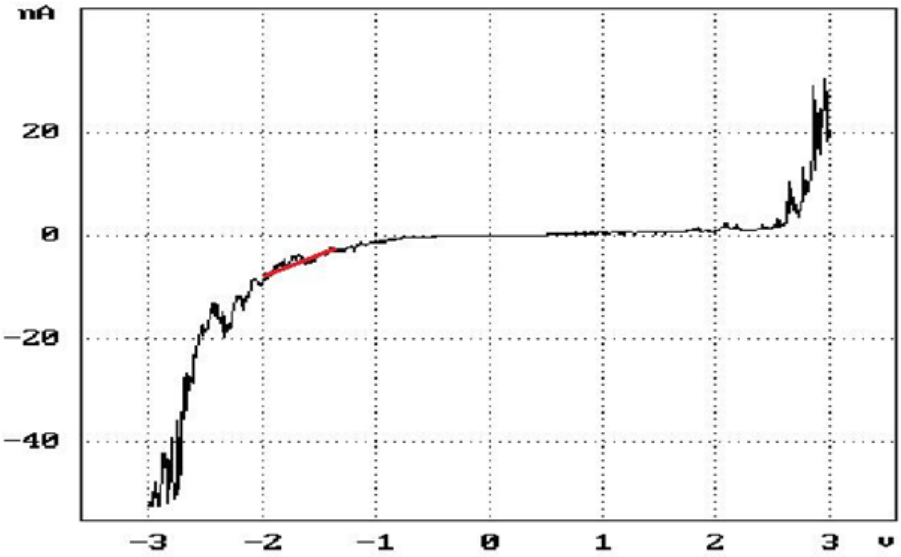

Figure 5. Averaged current-voltage curve of $d(A)_{12}$ oligonucleotides.

It can be seen that with a positive voltage up to +2 V, the tunneling current is

close to zero. And in the voltage range from +2.5 V to +3 V the tunneling current barely reaches 29 nA, which is much less than on the current-voltage curve of $d(C)_{12}$ oligonucleotides (Figure 4). The differential resistance of oligonucleotides was also calculated in the voltage range in which there are no zero current values and at the same time there are no significant current fluctuations. In Figure 5, it is also highlighted by a thickened red line. Taking the values of voltage and current in this area, it was estimated that the differential electrical resistance of an individual molecule is approximately equal to R_{dif} (A)=$0.95 \cdot 10^8$ Ω.

As a result, an attempt to estimate the differential resistances of two types of oligonucleotides consisting of homonucleotides showed that in the same voltage range the $d(A)_{12}$ oligonucleotide, on average, has a higher differential resistance than the $d(C)_{12}$ oligonucleotide. Obviously, the reason for the difference in differential resistance is the different nucleotide composition of the studied molecules.

Conclusion

Visualization of samples obtained after immobilization of $d(C)_{12}$ and $d(A)_{12}$ oligonucleotides on a solid substrate by scanning probe microscopy showed a uniform distribution of DNA molecules either in a single state or in the form of aggregates – clusters of several molecules, regardless of the substrate nature (mica or silver).

The results of an experimental study of differential electrical resistance of $d(A)_{12}$ and $d(C)_{12}$ oligonucleotides are obtained. Averaged current-voltage curves have been measured. The differential electrical resistances of individual molecules of oligonucleotide $d(C)_{12}$ and oligonucleotide $d(A)_{12}$ have been estimated and compared with each other. It was shown that in the same voltage range the $d(A)_{12}$ oligonucleotide, on average, has a higher differential resistance than the $d(C)_{12}$ oligonucleotide.

References

[1] T.I. Sharipov et al., Journal of Nanoparticle Research **25** (2023) 64. [*CrossRef*]
[2] B. Demir et al., Physical Chemistry Chemical Physics **25** (2023) 16570–16577. [*CrossRef*]
[3] H.W. Fink, C. Schonenberger, Nature **398** (1999) 407–410. [*CrossRef*]
[4] D. Ryndyk et al., ACS Nano **3**(7) (2009) 1651–1656. [*CrossRef*]
[5] D. Porath et al., Nature **403** (2000) 635–638. [*CrossRef*]
[6] A.O. Puchkova et al., Journal of Nanoparticle Research **13**(9) (2011) 3633–3641. [*CrossRef*]
[7] S. Záliš et al., The European Physical Journal E **18** (2005) 201–206. [*CrossRef*]
[8] I. Kratochvilova et al., The European Physical Journal E **25** (2008) 299–307. [*CrossRef*]
[9] T.I. Sharipov, R.Z. Bakhtizin, News of higher educational institutions. Volga region. Physical and mathematical sciences. **49** (2019) 133–141. [*CrossRef*] (in

Russian)
[10] M. Iijima et al., Chemistry Letters **34**(8) (2005) 1084–1085. [*CrossRef*]
[11] R.R. Garafutdinov et al., Vestnik Bashkirskogo universiteta **10** (2005) 49–54. [*Google Scholar*] (in Russian)
[12] A.Kh. Ayupova et al., Vestnik Bashkirskogo universiteta **17** (2012) 1677–1682. [*Google Scholar*] (in Russian)
[13] T.I. Sharipov, R.Z. Bakhtizin, IOP Conference Series: Materials Science and Engineering **195** (2017) 012002. [*CrossRef*]
[14] H.G. Hansma et al., Nucleic Acids Research **24**(4) (1996) 713–720. [*CrossRef*]