The SPM study of oligonucleotides consisting of repeated nucleotide sequences

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Abstract. Recently, the study of the dependence of the physical characteristics of DNA molecules on their structure has become of considerable interest. Various methods are used to conduct such research, among which scanning probe microscopy (SPM) is becoming increasingly important. In this work, the short single-stranded DNA molecules consisting of dC nucleotides are visualized and their conductivity is investigated by SPM methods. It has turned out that the distribution of DNA molecules on the surface of the substrates is uniform. The measured current-voltage characteristics have made it possible to estimate the differential electrical resistance of individual d(C)12 molecules.

1. Introduction
The study of nucleic acid molecules – DNA and RNA – as physical objects is of considerable interest. The variety of possible structures of nucleic acids (single-stranded and double-stranded state, linear and ring molecules, various conformational forms, variability of nucleotide composition, etc.) determines the presence of a wide range of similar objects. Recently, new data on the dependence of the physical characteristics of DNA molecules on their structure have appeared. Thus, it has been shown that the conformation and hardness of DNA are influenced by the molecular environment [1,2], nucleotide composition and methylation [3-5]. The difference in the nature of DNA destruction under mechanical action (irradiation of aqueous solutions with ultrasound) has been shown [6,7].

One of the actively used methods for studying the physical parameters of nucleic acids is scanning probe microscopy (SPM), which, thanks to its high resolution, allows studying various objects at the molecular and submolecular levels, including DNA molecules. Synthetic single-stranded DNA molecules with a homonucleotide sequence, that is, consisting of nucleotides of the same type, are of particular interest. Such structures assume a special nature of the electron density distribution and charge transfer, so the interest in them is consistently high due to the prospects of using these molecules in nanoelectronics. Attempts to measure the electrical resistance of DNA give conflicting results [8-11]. The ambiguity of the results is influenced by the experimental conditions and the parameters of DNA molecules studied, namely length, nucleotide composition, different nucleotide sequence in the DNA chain, and the number of chains in the molecule [12]. The study of the surface topography and immobilization of the DNA molecules by SPM methods is also important in terms of the development of DNA microarrays [13-16].

The electrical properties of DNA molecules can be studied using one of the varieties of SPM – scanning tunnelling microscopy (STM), by obtaining the current-voltage curves. To do this, the molecule is placed between two electrical contacts, one of which is a conducting probe of the
microscope, and the other is a fragment of the substrate surface of an electrically conductive material. The aim of this work was to visualize and study the conductivity of short single-stranded DNA consisting of nucleotides of only one type, the nitrogen base of which is cytosine.

2. Materials and methods
d(C)_{12} oligonucleotide was 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 was determined by the optical density of the aqueous solution at 260 nm on the BioSpec-Mini spectrophotometer (“Shimadzu”, Japan).

SPM study was carried out in air using Solver P47 and Ntegra-Prima devices (“NT-MDT Spectrum Instruments”, Russia). For atomic force microscopy (AFM) studies, the probes were silicon cantilevers NSG10 (“NT-MDT Spectrum Instruments”) with 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 a substrate. In AFM studies, the substrate was the surface of fresh mica cleavage.

The object of the study was the oligonucleotide molecules consisting of 12 identical units of deoxycytidine. A solution of d(C)_{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 was dried up in 30-40 minutes.

3. Results and discussion
Previously, we have shown the possibility of studying of short single-stranded DNA using SPM [17-19]. In this work, the object was the d(C)_{12} oligonucleotide molecules, which initially were imaged on the mica surface by AFM in the tapping mode. On one of such AFM-images (Fig. 1a), objects of spherical shape are clearly visualized, some of which are individual molecules of oligonucleotides, and others are aggregates representing clusters of single oligonucleotide molecules. These aggregates have different sizes both in normal and lateral directions, which is due to the difference in the number of molecules making them up.

Because the smallest objects observed on the AFM image, which in our opinion are single molecules, were of the greatest interest, a cross-section profile was obtained for them, by which the geometric parameters were determined (Fig. 1b). Thus, the average size of such objects in lateral direction was about 36±1.8 nm, height was about 0.9±0.05 nm, the calculation was carried out for 50 smallest objects observed on the AFM image.

![Figure 1](image-url) 
Figure 1. (a) AFM image of d(C)_{12} oligonucleotides on mica surface; (b) cross-section profile along the line marked at (a).
Then \( \text{d(C)}_{12} \) oligonucleotides were deposited on the atomically flat silver surface and previously studied by STM. Oligonucleotides were clearly visualized (Fig. 2a) and were located on the surface of the substrate relatively rare, which is very convenient for further spectroscopic study.

Usually, oligonucleotides on STM images are represented by 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 (Fig. 2b) estimated the lateral dimensions of these objects, which were on average equal to \( 17 \pm 0.9 \) nm. Measuring the depth of the observed objects gives a result of about \( 3.9 \pm 0.22 \) 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. The above values were calculated for 30 smallest dark objects observed on the STM images.

After obtaining STM images and visualization of oligonucleotides on the substrate surface in the mode of scanning tunnelling spectroscopy, their current-voltage curves were measured in the areas, where individual oligonucleotide molecules were presumably located. Several current-voltage curves are measured at each point. Final current-voltage curve is obtained by averaging. By performing the described method for measuring the current-voltage curves of several oligonucleotides identified in the scan area shown in Figure 2a, we obtained the resulting averaged current-voltage curve (Fig. 3).

The kind of current-voltage curve was nonlinear. It has a symmetric appearance concerning zero values of both current and voltage in the voltage range from \(-1.5\) V to \(+1.5\) V. In the voltage range more than \(+1.5\) V and less than \(-1.5\) V it has an asymmetric form concerning zero values. For voltage applied between the substrate and the STM tip from \(-0.8\) V to \(+1.2\) V, the tunnelling current is close to

![Figure 2.](image)

**(Figure 2.** (a) STM imaging of \( \text{d(C)}_{12} \) oligonucleotides on the silver surface; (b) \( \text{d(C)}_{12} \) oligonucleotide cross-section profile along the line marked at (a).)

![Figure 3.](image)

**(Figure 3.** Averaged current-voltage curve of \( \text{d(C)}_{12} \) oligonucleotides.)
zero. Using current-voltage curve, the differential resistance of d(C)_{12} oligonucleotides can be calculated. To do this, on the current-voltage curve we chose a site, where there were no zero current values and at the same time there were no significant current fluctuations. In Figure 3, it is highlighted with a thick red line. It was calculated that the differential electrical resistance of a single molecule was approximately equal to R_{diff}=0.85\cdot 10^8 \, \Omega.

4. Conclusion
Visualization of samples obtained after immobilization of d(C)_{12} oligonucleotide 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). In addition, the results of an experimental study of electrical conductivity are obtained. The current-voltage curves have been measured. Differential electrical resistance of the d(C)_{12} oligonucleotide molecules has been estimated.

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