

The estimation of quantitative parameters of oligonucleotides immobilization on mica surface

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Abstract. Immobilization of nucleic acids on the surface of various materials is increasingly being used in research and some practical applications. Currently, the DNA chip technology is rapidly developing. The basis of the immobilization process can be both physical adsorption and chemisorption. A useful way to control the immobilization of nucleic acids on a surface is to use atomic force microscopy. It allows you to investigate the topography of the surface by its direct imaging with high resolution. Usually, to fix the DNA on the surface of mica are used cations which mediate the interaction between the mica surface and the DNA molecules. In our work we have developed a method for estimation of quantitative parameter of immobilization of oligonucleotides is their degree of aggregation depending on the fixation conditions on the surface of mica. The results on study of aggregation of oligonucleotides immobilized on mica surface will be presented. The single oligonucleotides molecules have been imaged clearly, whereas their surface areas have been calculated and calibration curve has been plotted.

1. Introduction

Immobilization of nucleic acids on the surface of various materials is increasingly being used in research and some practical applications. Currently, the DNA chip technology is rapidly developing. To create DNA chip a major component is attachment the DNA or DNA fragments - oligonucleotides - on the surface [1-2]. The basis of the immobilization process can be either physical adsorption (non-covalent fixation) [3-6] or chemisorption (covalent fixation) [7-10].

A proper way to control the immobilization of nucleic acids on a surface is to use atomic force microscopy (AFM) which allows one to investigate the topography of the surface by its direct imaging with high resolution [11-15]. In the review [8] the most common strategies for immobilization of biomolecules before the study by AFM have been represented. To fix the DNA on the surface of mica are used polyvalent cations which mediate the interaction between the negatively charged mica surface and the DNA molecules. Primarily, these transition metal ions of the fourth group. However, as shown in [10], it is possible to use monovalent metal cations.

Specific achieve visualization of short DNA fragments are shown for the first time in [13]. The authors showed that by using the AFM could visualize the DNA molecule length of about 25 nucleotides. Such single-stranded DNA is globule as capable of intramolecular interactions bases, especially in the presence of divalent metal cations.

The aim of the present work is to develop a method of estimation the quantitative parameter of immobilization of oligonucleotides: the degree of aggregation, depending upon the fixation conditions on the surface of mica.



2. Materials and Methods

We have used atomic force microscope Solver P47 and probe nanolaboratory Ntegra-Prima («NT-MDT», Zelenograd, Russia) for the study of oligonucleotides immobilized separately each one or combined into aggregates. Investigations were carried out in air using DLC (diamond-like) cantilevers («NT-MDT»). The substrate was fresh cleaved mica surface modified through treatment with metal cations. In the preparation of all solutions used deionized distilled water (Millipore, France). Specimens were 93-mer oligonucleotide ("Syntol", Moscow).

3. Results and discussion

First, it was verified reproducibility AFM images, i.e. the statistical analysis was performed. 10 samples were prepared by the same procedure. Immobilization solution containing 2 ng/μl DNA and 50 mM MnCl₂ was heated at 80° C for 2 minutes to denature the double-stranded structures. Then this solution was placed on the surface of fresh cleaved mica. The solution was kept for 4 min on the mica, and then the substrate was washed with water (100 μl). Each sample (substrate) in ten points scanned and respectively obtained 10 AFM images for each sample. On the obtained AFM images individual objects of different sizes were visualized. These probably as a single molecule of oligonucleotide and their aggregates. Heights of alleged single oligonucleotides were found, and calculated the arithmetic mean values of heights. According to the data the average height of objects situated on the surface of mica was found to be 0.58 ± 0.12 nm.

As an additional criterion for estimate the reproducibility was taken density of single oligonucleotides on the substrate. It was found that the density is 13 ± 2 pcs/μm². This indicates that the reproducibility of the AFM images is satisfactory and such criteria can be recommended to estimate the reproducibility of the obtained AFM images of biomolecules.

When immobilization of oligonucleotide molecules from solution they are arranged on the surface either in a single state or form aggregates. To estimate the number of molecules in the aggregate was the assumption that the surface area of the aggregate S_a should be independent of the number of molecules in aggregate n . In the same method were prepared the samples of different length oligonucleotides and then were visualized by AFM. Next, find a supposedly single oligonucleotides and their surface area was calculated as follows. On the AFM image a single oligonucleotides - a circular form objects - modeled the regular cone height h , base radius R and the generatrix L . Lateral surface of the cone $S_b = \pi RL = \pi R \sqrt{(h^2 + R^2)}$. Then knowing h and R can be calculated S_b . On the AFM image height and radius of the quasi-round form objects can be calculated by plotting their profiles, which are easily obtained using the integrated package of image processing software Image Analysis. Thereby calculating the surface area of the cones simulating single oligonucleotide molecules of different lengths on the AFM images, the calibration curve was plotted (Figure 1). From this calibration curve can be determined aggregate weight, and accordingly, the number of molecules in aggregate.

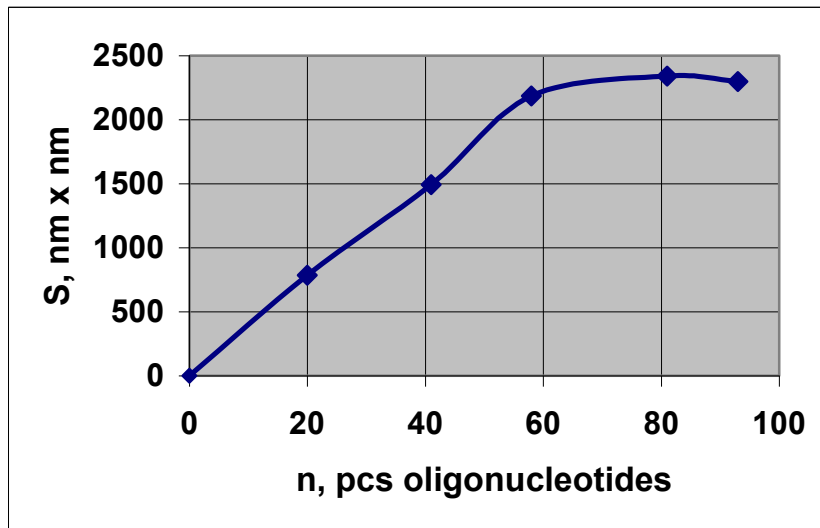


Fig. 1. Calibration curve for determining the number of oligonucleotide molecules in the aggregate

Figure 2 show example of determination of the number of molecules in the aggregate.

h, nm	R, nm	S_b, nm^2
1	27	2290.629

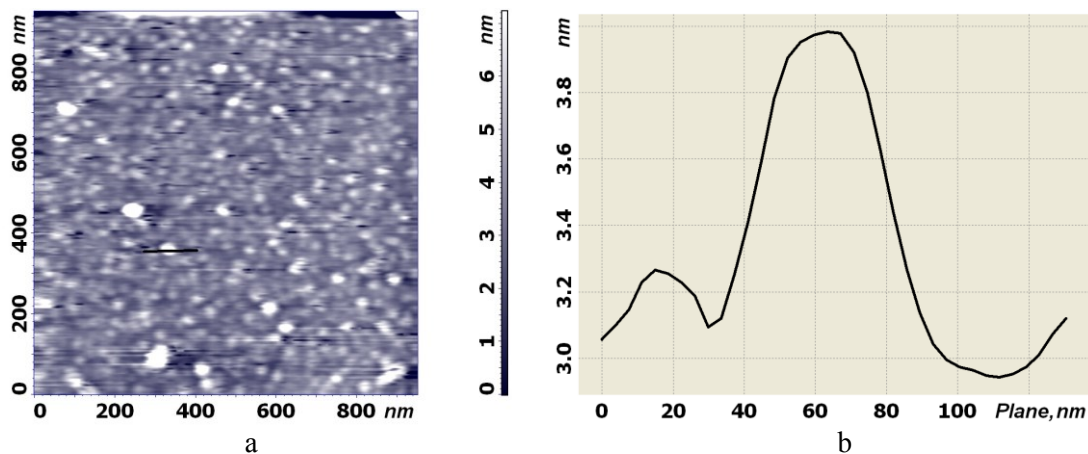


Fig. 2. AFM image of oligonucleotides (a) and corresponding section profile (b).

Figure 2a show the AFM image of oligonucleotides (20 nucleotides length) immobilized on the mica surface. Figure 2b show the section profile obtained along the section line drawn in the figure 2a; in addition here are shown size and lateral area of the selected object. Knowing the area, a calibration curve is determined the size of the aggregate. For an object (Figure 2b), it is rounded is 80 nucleotides, i.e. this aggregate consists of four oligonucleotide molecules length of 20 nucleotides.

In order to find the dependence of the density of single oligonucleotides on the concentration of the immobilization solution components we performed two set of experiments. First, were study the dependence of density of single oligonucleotides ρ on their concentration in the immobilization

solution (Figure 3) at a constant ratio of the number of oligonucleotide phosphate groups and cations Mn^{2+} 1:1. Studied objects were oligonucleotides length of 20 nucleotides.

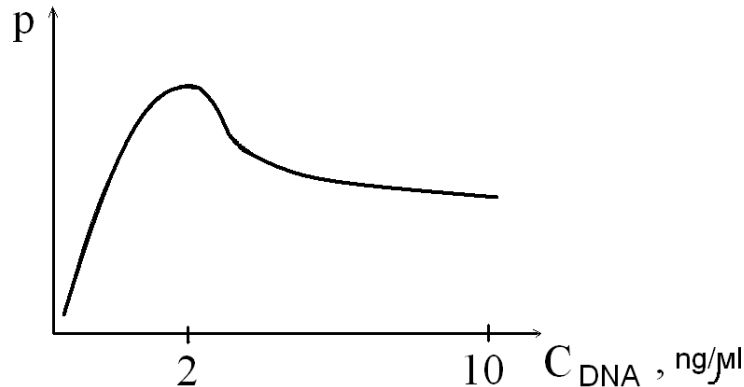


Fig. 3. The dependence of the density of single oligonucleotide molecules ρ on their concentration in the immobilization solution.

It was found that increasing the concentration of oligonucleotide molecules C_{DNA} from 0 to 2 ng/μl, there is a sharp increase ρ . In oligonucleotide concentration equal to 2 ng/μl, the density of single molecules is maximized. Then with increasing concentrations over 2 to 10 ng/μl observed a smooth curve with a gradual decline in the establishment of certain fixed amount per single immobilized molecules. Probably the increasing character of the curve due to the fact that at low concentrations of oligonucleotide molecules are not aggregated and immobilized are separately from each other. A gradual increase in the concentration of oligonucleotides increases the density of single molecules and aggregates. When the concentration of oligonucleotides more than 2 ng/μl aggregation process begins to dominate over the single immobilization process.

There was also investigated the dependence of the density of single oligonucleotide molecules ρ on the Mn^{2+} cations concentration in the immobilization solution $C_{Mn^{2+}}$. This dependence can be described by a function $\rho=f(C_{DNA}/C_{MnCl_2})$ (Figure 4).

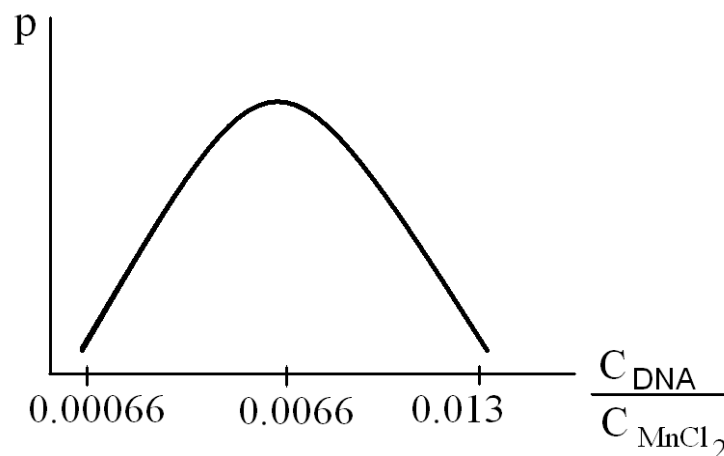


Fig. 4. The dependence of the density of single oligonucleotide molecules immobilized on the mica surface on the Mn^{2+} cations concentration.

When the concentration of Mn^{2+} cations in the immobilization solution increase from 25 to 50 μM (i.e. with value $C_{\text{DNA}}/C_{\text{MnCl}_2}$ from 0.013 to 0.0066) was observed increase in the density of single oligonucleotide molecules that may be due to an increase in the number of binding sites between the negatively charged oligonucleotide molecules and the surface of mica. When Mn^{2+} cations concentration above 50 μM (when the value $C_{\text{DNA}}/C_{\text{MnCl}_2}$ is less than 0.0066) oligonucleotide aggregation process in the solution increases so that the density of single oligonucleotide molecules immobilized on the surface is significantly reduced.

4. Conclusion

We investigate the reproducibility of the AFM images by measuring the height and density of single oligonucleotides immobilized on a substrate. We propose a method for plotting the calibration curve. This calibration curve illustrate dependence of the surface area of the cone, simulating oligonucleotide molecule, on the molecule length and thus the mass. Examples of determining the number of molecules in the aggregate are given. The dependence of the density of single oligonucleotides on the obtained AFM images on the concentration of the immobilization solution components has been studied.

The results of study may contribute to understanding the mechanisms of physical adsorption of DNA molecules and their fragments of different lengths on the surface of mica and may used for the design of DNA chip.

5. References

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