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An atomic force microscopy study of DNA hairpin probes monolabelled with gold nanoparticle: Grafting and hybridization on oxide thin films

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Abstract

First and original results are reported regarding the surface evolution of two kinds of oxide film after covalent grafting and hybridization of hairpin oligonucleotide probes. These hairpin probes were monolabelled with a 1.4 nm gold nanoparticle. One kind of oxide film was rough Sb doped SnO_2 oxide film and the other kind was smooth SiO_2 film. Same process of covalent grafting, involving a silanization step, was performed on both oxide surfaces. Atomic force microscopy (AFM) was used to study the evolution of each oxide surface after different steps of the process: functionalization, probe grafting and hybridization. In the case of rough SnO_2 films, a slight decrease of the roughness was observed after each step whereas in the case of smooth SiO_2 films, a maximum of roughness was obtained after probe grafting. Step height measurements of grafted probes could be performed on SiO_2 leading to an apparent thickness of around 3.7 ± 1.0 nm. After hybridization, on the granular surface of SnO_2, by coupling AFM with SEM FEG analyses, dispersed and well-resolved groups of gold nanoparticles linked to DNA duplexes could be observed. Their density varied from $6.6 \pm 0.3 \times 10^{10}$ to $2.3 \pm 0.3 \times 10^{11}$ dots cm^{-2}. On the contrary, on smooth SiO_2 surface, the DNA duplexes behave like a dense carpet of globular structures with a density of $2.9 \pm 0.5 \times 10^{11}$ globular structures cm^{-2}.

Keywords: DNA hairpin probes; DNA hybridization; Gold nanoparticle; AFM measurements; Oxide films

1. Introduction

DNA biochips are in high development since they have shown tremendous promise for medical research diagnosis, process monitoring in the food industry and environmental testing. They rely on the specific hybridization between single stranded DNA (ssDNA) immobilized on a surface (DNA probes) and free complementary ssDNA (DNA target) in solution. The hybridization is strongly influenced by the conformation, the density and the accessibility of the probes on the solid surface. In order to improve the sensitivity, the selectivity and the reliability of such devices, the interface solid surface/ssDNA and the interaction between probes and targets must be characterized precisely with high resolution techniques. Indeed, the commonly used detection techniques such as fluorescence microscopy [1] and ^{32}P-radiolabeling experiments [2] allow global measurements over large surface area. However, these techniques cannot resolve the high lateral resolution required to characterize DNA biochips. A technique with higher lateral resolution is the atomic force microscopy (AFM) which allows obtaining a topographic image of a solid surface with a nanometric resolution. Consequently, AFM become more used to characterize single molecules in air or in solution.

However, in the field of DNA biochips, only few publications deal with AFM studies performed on DNA strands covalently grafted on biochip surfaces. Indeed, most of the

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studies deal with long DNA molecules such as plasmid adsorbed on different substrates: sapphire [3], silicon [4], mica [5–7] or shorter double-stranded DNA (dsDNA) and linear ssDNA either physisorbed on highly oriented pyrolitic graphite (HOPG) [8] or chemisorbed on gold films [9]. By contrast, AFM studies performed on short DNA strands covalently grafted are limited. For example, such a study has been performed on SiO_2 film by Rouillat et al. [10], who investigated the organization of linear DNA strands (25 bases) after covalent grafting and hybridization. To the best our knowledge, no publication has been reported about grafting and hybridization of hairpin DNA probes.

Hairpin probes compose a stem in which both strands are complementary and a loop. The interest of such DNA probe conformation is that these oligonucleotide sequences are very sensitive to detect one or more mismatch [11–13]. Furthermore, these hairpins can be functionalized by different elements such as gold nanoparticle, in association with a fluorescent dye, for detection by fluorescence microscopy [14]. Another interest can be provided by the use of gold nanoparticle labelled DNA: an unexpected rearrangement of the surface-confined probe-target hybrids. This rearrangement significantly enhances the quality of AFM imaging [15].

Present work shows the use of hairpin oligonucleotides modified by a 1.4 nm gold nanoparticle as probes grafted on two kinds of thin oxide films: antimony doped tin oxide (Sb doped SnO_2) film and silicon oxide (SiO_2) film. These oxide films can undergo the same process of covalent DNA probe grafting on their surface. Sb doped SnO_2 films were deposited at the laboratory using aerosol pyrolysis technique. The films were polycrystalline and exhibited a granular and rough surface [16]. As they are electrically conductive, they can be used as DNA-modified electrodes involved in electrochemical detection based biochips. Indeed, we have demonstrated their relevant and promising use for label-free electrical detection of DNA hybridization by electrochemical impedance spectroscopy [17]. In this reference, the naturally rough SnO_2 surface was modified with oligonucleotide probes exhibiting linear conformation. However, as mentioned above, a better sensitivity to detect mismatch should be expected when using hairpin probes. So, in the aim to better control the SnO_2 surface morphology when modified with gold nanoparticle labelled hairpin probes, we have investigated its evolution using AFM after different modification steps: functionalization, hairpin probe grafting and hybridization with fluorescent complementary target. In order to have a reference surface, thermally oxidized Si (SiO_2) surface was also systematically explored after each step of the process. On the contrary of SnO_2 surface, SiO_2 exhibits a smooth and featureless surface which makes it favourable to AFM study. On both materials, fluorescence measurements have been systematically performed to check hybridization process. We have presented the detailed results elsewhere [18].

2. Experimental

2.1. Thin film preparation

2.1.1. SiO_2 films

SiO_2 films were obtained from cleaned (1 1 1) Si wafers by thermal oxidization performed at 1050 °C in presence of O_2 and H_2 during 190 min. The obtained SiO_2 thickness was 460 ± 4 nm as measured using ellipsometry taking 1.46 as a refractive index. The SiO_2 film surfaces were very smooth and flat (Fig. 1a and b), exhibiting a roughness of 0.23 ± 0.01 nm (Table 1).

2.1.2. Sb doped SnO_2 films

Electrically conductive Sb doped SnO_2 thin films were deposited directly on glass substrates using the aerosol pyrolysis technique which is described elsewhere [19]. This is based on the pyrolysis of an aerosol obtained by ultrahigh frequency spraying of a precursor solution on a heated substrate at atmospheric pressure.

The precursor solution was obtained by dissolving $SnCl_4 \cdot 5H_2O$ salt in pure methanol (solution 0.2 M) and adding a 2% volume of a 0.2 M solution of $SbCl_3$ salt dissolved in pure methanol (Sigma–Aldrich). The substrate temperature during deposition was kept at 420 °C. The solution consumption was about 1.6 ± 0.1 ml min^{-1}. Under these conditions, the deposition rate was about 45 nm min^{-1}. The resulting film thickness was 90 ± 10 nm as measured using ellipsometry taking 1.95 as a refractive index. The electrical resistivity of the films was $2.5 \pm 0.5 \times 10^{-3}$ Ω cm. Compared to SiO_2 film surfaces, SnO_2 (Fig. 1c and d) exhibited important roughness values, i.e., 8.21 ± 2.00 nm (Table 1), which were correlated to the high deposition rate and to the polycrystalline structure [16]. The roughness resulted in a grain agglomeration of 110 ± 20 nm.

2.2. Synthesis of oligonucleotides

The synthesis and the functionalization of the oligonucleotides (ODN) (probes and targets) were carried out by Biomérieux (France). ODN syntheses were achieved on an EXPEDITE 8900 DNA synthesizer (Applied Biosystems) using standard phosphoramidite chemistry at 1 μmol scale. All the sequences are presented in Table 2.

2.2.1. Synthesis of modified hairpin oligonucleotides probes

The 32-mers synthetic oligonucleotides were used as probe precursors. They were modified with a primary amine at their 5′ end and a disulfide group at their 3′ end. Selected sequence of 5′-NH_2-TTTTT GCG ATG GAT AAA CCC ACT CTA CAT CGC-SSdT-3′, allowed the oligonucleotides to auto-hybridize on a stem of 6 bases to form a probe with the following characteristics : a spacer of 5 bases T, a stem of 6 base pairs and a loop of 15 bases. Fig. 2 illustrates the native conformation of the hairpin. The 3′ modification of the probes was introduced via the