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Magnetron sputtering deposition of ultra-thin tungsten coatings onto amorphous graphite for enhancement of horseradish peroxidase adsorption

I.D. Shumov[1], V.S. Ziborov[2], Yu.D. Ivanov[1], A.I. Archakov[1], T.O. Pleshakova[1]

[1]*Institute of Biomedical Chemistry, 119121, Moscow, Russia*
shum230988@mail.ru

[2]*Joint Institute for High temperatures RAS, 125412, Moscow, Russia*

Atomic force microscopy (AFM) represents a nanotechnological method of molecular detection, which allows one to visualize various nanometer-sized objects, including biological macromolecules, with up to 0.1 nm height resolution [1]. This makes AFM an attractive tool in biochemical research.

To allow their visualization by AFM, the macromolecules under study must be fixed on the surface of a solid support. Regarding biological macromolecules, this can be achieved by their physical adsorption (non-covalent immobilization) onto the AFM support surface. Accordingly, appropriate adsorption efficiency of the macromolecules of interest onto the AFM support is required. Despite this efficiency is strongly affected by electrostatic interactions of the studied macromolecules with the support surface, hydrophobic interactions should also be taken into account, as in a number of cases these very interactions determine the possibility of macromolecules' adsorption [2].

In the present study, the adsorption of horseradish peroxidase (HRP) protein onto the surface of amorphous carbon has been studied by AFM. It has been experimentally demonstrated that modification of amorphous carbon surface by magnetron sputtering deposition of ultra-thin (1.3 nm) tungsten coatings significantly enhances the adsorption of HRP macromolecules onto this surface in comparison with bare carbon.

In our experiments, peroxidase from horseradish (P2088, Sigma, USA) was adsorbed onto amorphous carbon supports (01843-F; Ted Pella, Inc., USA) according to the following procedure: 5 μL of 10^{-7} M to 10^{-6} M HRP solution in Dulbecco modified phosphate buffered saline were incubated on the support surface for 5 min and then washed off with 1 mL of ultrapure deionized water; after that, the AFM support was dried in air. In control experiments, protein-free buffer was used instead of HRP solution.

Ultra-thin tungsten coatings were formed on the surface of graphite AFM supports analogously to the technique described elsewhere [3]. Briefly, Orion-3 magnetron sputtering system (AJA Inc., USA) equipped with tungsten target (thickness 0.25'', diameter 2'', 99.95% purity, Girmet, Russia) was used. Base pressure in the system was not higher than 6×10^{-7} Torr. The sputtering was carried out using argon plasma in DC mode at a constant power of 70 W and 5 mTorr sputtering gas pressure during 10 s. The distance between the target and the specimen was 15 cm. During the sputtering, rotation of the substrate to be coated with constant angle velocity of 40 rpm was provided. The thickness of the so-obtained tungsten films was 1.3 nm as measured by quartz crystal microbalance.

The HRP macromolecules adsorbed on the surface of graphite supports (either coated or not coated with tungsten) were imaged with an NT-MDT atomic force microscope (Zelenograd, Russia) in a semi-contact mode in air with 256×256 resolution.

Figure 1 displays typical AFM images of the surface of graphite supports coated with 1.3 nm tungsten film after their incubation in 10^{-7} M HRP solution (a) and in protein-free buffer (b).

As seen from the image in Figure 1 (a), separate objects with heights of AFM images h from 1 to 3 nm, and a number of objects with h>3 nm are observed on the surface of tungsten-coated graphite after its incubation in 10^{-7} M HRP solution. These data are in agreement with previously reported sizes (h = 1.5 nm [4]) of AFM images of HRP (whose molecular weight M_r is 40 kDa [5]), and with the data on the sizes of other proteins with similar M_r (putidaredoxin reductase, h_{max}=1.8 nm [1], M_r=45.6 kDa [6]; adrenodoxin reductase, h_{max}=1.8 nm [7], M_r=54 kDa [8]). Objects with h>2 nm apparently correspond to aggregates formed on the support surface by several HRP macromolecules. It is to be noted that no HRP adsorption was observed in experiments with

bare amorphous graphite without tungsten coating, even in the case with 10-times higher (10^{-6} M) HRP concentration. That is, the adsorption of HRP onto hydrophilic tungsten coating readily occurs, while it is virtually not observed in the case with hydrophobic surface of bare carbon. The control image in Figure 1 (b) indicates that artifact objects are virtually not observed on the support incubated in protein-free buffer, what indicates that the roughness of the obtained coatings is sufficient for imaging protein macromolecules by AFM.

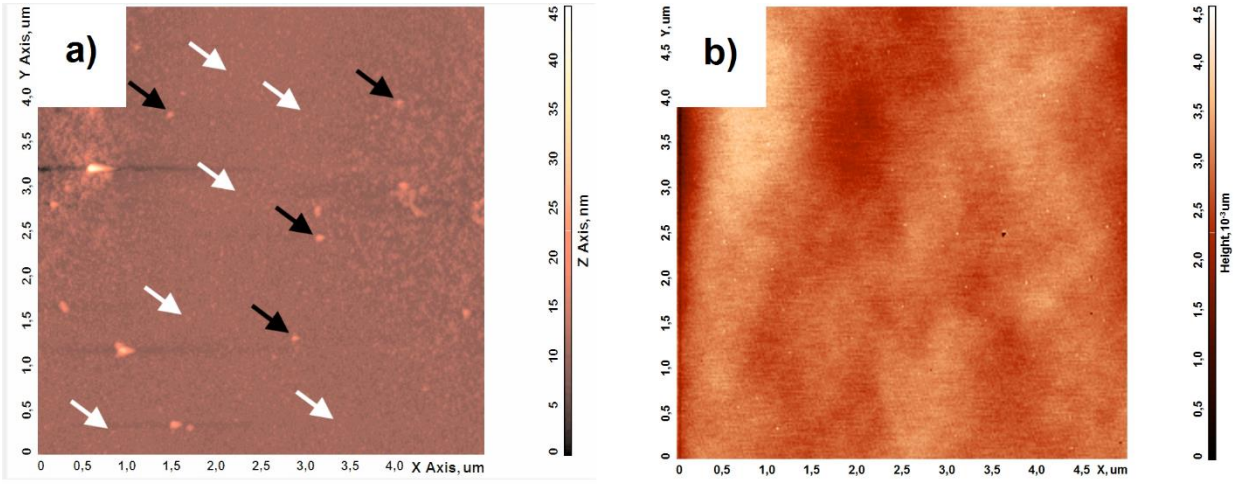

Figure 1. AFM image of amorphous graphite coated with 1.3-nm tungsten film after adsorption of HRP from 10^{-7} M solution (a) and control image (b). Scan size 5×5 μm. Arrows indicate objects with heights of AFM images *h* from1 to 3 nm (white arrows) and *h*>3 nm (black arrows).

The studies of HRP solutions performed by Ignatenko et al. [9] revealed that HRP forms oligomers in solutions with concentration exceeding 10^{-7} M. Accordingly, the compact objects with $h < 2$ nm observed in Figure 1 (a) can be attributed to HRP monomers, while objects with greater *h* apparently correspond to HRP oligomers. Accordingly, our data reported herein are in agreement with the literature data [4, 9].

The data obtained in our present study can be used in proteomic studies and in biotechnological research.

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