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

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Abstract. In the present study, atomic force microscopy (AFM) has been used to investigate the adsorption of horseradish peroxidase (HRP) protein onto the surface of amorphous carbon (graphite). It has been experimentally demonstrated that modification of amorphous carbon surface by magnetron sputtering deposition of ultra-thin (1.3 nm) tungsten coatings allows one to enhance significantly the adsorption of HRP macromolecules onto this surface, as compared with bare carbon.

1. Introduction

Atomic force microscopy (AFM) represents a nanotechnology-based method of molecular detection, which allows visualizing various nanometre-sized objects, including viral particles [1,2], biological macromolecules [3] and their complexes [4] with up to 0.1 nm height resolution [3,5]. This makes AFM an attractive tool in biochemical research.

The macromolecules under investigation must be fixed on the surface of a solid substrate for their visualization by AFM. Regarding biological macromolecules, this can be achieved by their adsorption (non-covalent immobilization) onto the substrate surface. Accordingly, appropriate adsorption efficiency of the macromolecules of interest onto the substrate is required. Despite this efficiency is strongly affected by electrostatic interactions of the macromolecules with the support surface, hydrophobic interactions should also be taken into account, as in a number of cases they determine the possibility of macromolecule adsorption [6]. It depends on the properties of the studied objects. The substrate surface may require modification to provide adsorption of the studied objects.

Magnetron sputtering allows obtaining metal coatings of high purity with excellent adhesion to the coated surface [7], while the porosity of the so-formed coatings is low even at low coating thickness [8,9]. The deposition rate can be varied in a broad range by changing sputtering gas pressure or discharge power (or both of these parameters) [8,9]. These advantages allow employing magnetron sputtering in sample preparation for AFM [2,10]. It was demonstrated that in the case of formation of thin magnetron sputtered metal coating on the support surface, the surface roughness remained virtually unchanged, as compared with the support without the coating [2]. Retaining of atomic smoothness after surface
modification is crucial in the case of supports intended for visualization of nanometre-size objects, such as protein macromolecules [3,5].

Accordingly, magnetron sputtering is suitable for modification of the surface properties of supports for AFM, which has been demonstrated herein with the example of adsorption of horseradish peroxidase (HRP) protein onto the surface of amorphous carbon (graphite). Herein, 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. That is, HRP macromolecules were visualized by AFM on the surface of the support modified with tungsten coating after its incubation in $10^{-7}$ M HRP solution. At the same time, virtually no objects were visualized on the surface of bare carbon even in the case of 10 times higher ($10^{-6}$ M) HRP concentration.

2. Experimental

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: (1) 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; (2) the support was dried in air. In control experiments, protein-free buffer was used.

Ultra-thin tungsten coatings were formed on the surface of carbon supports analogously to the technique described elsewhere [2,11]. 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 $6\times10^{-7}$ 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 sample was 15 cm. The rotation of the substrate 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 carbon supports (either coated with tungsten or not) were imaged using NT-MDT AFM (Zelenograd, Russia) in a semi-contact mode in air with 256×256 resolution.

3. Results and discussion

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

![Figure 1](image)

**Figure 1.** AFM image of amorphous carbon coated with 1.3-nm tungsten film (a) after adsorption of HRP from $10^{-7}$ M solution and (b) control image. Scan size $5\times5$ µm².
As seen from the image in Figure 1a, separate objects with heights of AFM images \( (h) \) from 1 to 2 nm and a number of objects with \( h > 2 \) nm are observed on the surface of tungsten-coated carbon after its incubation in \( 10^{-7} \) M HRP solution. These data are in agreement with previously reported sizes \( (h=1.5 \) nm [3]) of AFM images of HRP (which molecular weight \( M_r \) is 40 kDa [12]), and with the data on the sizes of other proteins with similar \( M_r \) (putidaredoxin reductase, \( h_{max} = 1.8 \) nm [5], \( M_r=45.6 \) kDa [13]; adrenodoxin reductase, \( h_{max}=1.8 \) nm [14], \( M_r=54 \) kDa [15]). Objects with \( h > 2 \) nm apparently correspond to aggregates formed on the support surface by several HRP macromolecules. It should be noted that no HRP adsorption was observed at bare amorphous carbon without tungsten coating, even for ten-times higher (\( 10^{-6} \) M) HRP concentration. The control image in Figure 1b indicates that artefact objects are virtually not observed on the support incubated in protein-free buffer. It indicates that the coating roughness is sufficient for AFM imaging of protein macromolecules.

The studies of HRP solutions performed by Ignatenko et al. [16] revealed that HRP forms oligomers in solutions with concentration exceeding \( 10^{-7} \) M. Accordingly, the compact objects with \( h < 2 \) nm observed in Figure 1a can be attributed to HRP monomers, while objects with greater \( h \) apparently correspond to HRP oligomers. Our data agree with the literature [3, 16].

4. Conclusion
The obtained results demonstrate the influence of surface hydrophobicity on the adsorption of heme-containing HRP protein. The adsorption of HRP onto hydrophobic surface of amorphous carbon is virtually not observed, while it readily adsorbs onto hydrophilic tungsten surface. These results are in agreement with the previously reported data [3], where the HRP adsorption onto hydrophilic chemically activated amino-mica surface was studied by AFM on the single-molecule level; in the latter case, covalent capturing of the protein onto the chemically activated surface was observed to adsorb HRP onto mica from its solution with very low (\( 10^{-17} \) M) concentration.

The magnetron sputtering-based technique described herein can be used for surface modification in the fabrication of analytical chips with surface-immobilized proteins. This is important for the development of novel chip-based methods of protein detection, including highly sensitive ones, intended for use in analytical proteomics as well as for diagnostic applications.

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