Electrochemical Aptasensor Based on ZnO Modified Gold Electrode

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Abstract
We developed an electrochemical thrombin aptasensor based on ZnO nanorods functionalized by electrostatically adsorption of 30-mer DNA aptamers. The sensor surface was characterized by AFM and SEM. The surface layer assembling was optimized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) with ferricyanide ions as redox markers. The peak current of the ferricyanide and the charge transfer resistance gradually decreased with increasing concentration of thrombin in the range from 3 pM to 100 nM due to formation of aptamer-thrombin complexes and slower diffusion of the marker ions through the surface layer. At optimal conditions, a limit of detection (*LOD*) of 3 pM for EIS measurements and 10 pM for CV response was calculated from the $S/N = 3$.

Keywords: Aptasensor, Zinc oxide, Nanorods, Thrombin, DNA Aptamer, Voltammetry, Electrochemical impedance spectroscopy

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1 Introduction

The progress in the development of biosensors significantly depends on the application of novel matrices for the immobilization of biocomponents and signal generation. In addition to the common requirements to such materials, i.e., the establishment of effective immobilization of the biorecognition elements and easy use, novel nanostructures can enormously enhance the sensitivity and selectivity of the response [1]. In some cases biosensors based on these materials allow reaching a sensitivity sufficient for their direct application without sample pretreatment. This is particularly important for biomedical purposes such as detection of metabolites, pharmaceuticals and cancer biomarkers in whole blood and other biological liquids [2,3]. The necessity in development of such biosensors is dictated by the modern trends on bioanalytical chemistry directed to the human health and drug screening [4].

The use of novel nanomaterials in the assembly of sensing layers is one of the important trends offering unique possibilities for tuning the performance of biosensor depending on particular tasks to be solved [1]. Besides obvious advantages related to an increase of the sensing surface area and the specific concentration of the biorecognition sites, the nanostructuring of the sensing surface can improve the signal transduction and suppress the adsorption of undesired interferences. The improvement of the analytical performance of biosensors with implemented nanomaterials can be also related to the enhancement of the reaction area from the transducer interface to neighboring area without losses of the signal transduction [5].

Carbonaceous materials (carbon nanotubes, graphene, carbon black [5–11]), nanoparticles of noble metals (Au, Ag, Pt) [12–15] and those of the oxides of transient metals [16–18] have been successfully applied in enzymatic, immuno- and DNA sensors. In most cases, their application as support for biopolymer immobilization and signal transduction has been reviewed.

Zinc oxide, ZnO, is another example of such species, which has found increasing attention in the biosensor development in the past decade [19,20]. Zinc oxide is an n-type semiconductor, which is used in optoelectronics, optics and sensor applications due to a wide band gap of 3.37 eV and a large excitonic binding energy of 60 meV at room temperature. ZnO shows some advantages, i.e., high specific surface area, chemical and electrochemical stability, non-toxicity, gelation ability and variation of the morphology and size of the particles depending on the synthesis conditions. High isoelectric point ($IEP = 9.5$) makes ZnO appropriate for electrostatic adsorption of the proteins and nucleic acids.

Various enzyme sensors based on ZnO supporting layers have been described. Most of them were devoted to the determination of enzyme substrates, i.e. glucose

