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Au-nanoparticles as an electrochemical sensing platform for aptamer–thrombin interaction

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ABSTRACT

A novel electrochemical method for the detection of bioaffinity interactions based on a gold-nanoparticles sensing platform and on the usage of stripping voltammetry technique was developed. The oxidation of gold surface (resulted in gold oxide formation) upon polarization served as a basis for analytical response. As a model, thrombin–thrombin binding aptamer couple was chosen. The aptamer was immobilized on a screen-printed electrode modified with gold-nanoparticles by avidin–biotin technology. Cathodic peak area was found proportional to thrombin quantity specifically adsorbed onto electrode surface. Sigmoid calibration curve as is typical for immunoassay was obtained, with thrombin detection limit of 10^{-9} M. Linear range corresponds from 10^{-8} to 10^{-5} M thrombin concentration or 2×10^{-14} to 2×10^{-11} mol/electrode ($R = 0.996$). Binding of thrombin to an aptamer has also been detected using the ferricyanide/ferrocyanide redox couple as electrochemical indicator.

1. Introduction

Metal nanoparticles are widely used in bioelectrochemistry (Tansil and Gao, 2006). Au-nanoparticles (AuNP) serve as label in "sandwich" manner stripping analysis (Wang et al., 2001; Liu and Lin, 2007) as well as effective electrocatalysts for nanostructuring of electrode surface (Tang et al., 2006; Chai et al., 2008; Shumyantseva et al., 2005, 2007a).

Bioaffinity interaction is of great importance for early clinical diagnosis with the increasing application of proteomic researches and strategies (Ivanov et al., 2006). Biorecognition plays an important role in human metabolism. Well-known and functionally important couples are antigen–antibody, protein–protein, enzyme–substrate, and target DNA–complementary DNA.

Selective express detection of proteins is based on specific binding with antibodies or aptamers. Aptamers are artificial oligonucleotides, selected in vitro for their ability to bind to proteins, small molecules and whole cells. Aptamers find their application as biorecognition elements in different analytical methods: chromatography, capillary electrophoresis, mass spectrometry as well as in biosensors design (Tombelli et al., 2005, 2007; Navani and Li, 2006). Analytical characteristics of aptamers are compared with that of antibody for the biosensor construction (Schlecht et al., 2006). However, aptamers have many advantages over antibodies, including simpler synthesis, easier storage, reproducibility (Zheng et al., 2007) and simple modification for further immobilization procedure. Some approaches of aptamers design for biotechnology, diagnostics and therapy have been reviewed in Famulok et al. (2007).

The direct detection of antigen–antibody, protein–aptamer or ss-DNA–complementary ss-DNA interaction has been realized by various techniques, such are quartz crystal microbalance (QCM) (Ayela et al., 2007; Hianik et al., 2005; Minunni et al., 2004), surface plasmon resonance (SPR) (Wang et al., 2004; Bich et al., 2008; Kim et al., 2007) and impedance spectroscopy (Li et al., 2008; Xu et al., 2005; Vagin et al., 2002). Electrochemical registration of aptamer–protein binding was achieved due to intrinsic electroactivity of protein (lysozyme (Kawde et al., 2005)), or by adding different redox indicators in solution (ferricyanide (Kim et al., 2007), methylene blue (Hianik et al., 2005; Bang et al., 2005)) or else by aptamer labeling with ferrocene (Radi et al., 2006), methylene blue (Lai et al., 2007), enzymes (Ikebukuro et al., 2005; Shlyahovsky et al., 2007), metal nanoparticles (Zheng et al., 2007). The labeled aptamers are very promising for "reagentless" aptasensors development. Different strategies for the development of an electrochemical thrombin aptasensor were presented in Mir et al. (2006). A thiol-terminated aptamer was immobilized on a gold electrode. In a straight configuration thrombin was detected electrochemically by quantification of a thrombin-catalyzed reaction that produces *p*-nitroaniline. In a sandwich format

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horseradishperoxidase (HRP) labeled aptamer was allowed to bind to the other thrombin exosite. The activity of HRP was related through calibration to the quantity of thrombin. In a third strategy thrombin was directly immobilized on the electrode surface. The sensor was incubated first with biotin labeled aptamer and then with streptavidin–HRP. Electrochemical detection of HRP was again performed, using H_2O_2 and a diffusional osmium based mediator.

Combination of biorecognition material with metal nanoparticles for electrochemical registration of affinity interaction—is promising and fast upcoming sensor's design direction.

There are two main electrochemical approaches to detect the presence of AuNP. The first one is based on replacement of the sensor into acidic medium (HCl/HNO_3 or HBr/Br_2) for metal dissolution and stripping voltammetry as described in Pumera et al. (2005) and Authier et al. (2001). Another one is electrochemical oxidation of Au under polarization (Ozsoz et al., 2003; Li et al., 2007). Many aspects of electrochemical applications of gold-nanoparticles for genosensors and immunosensors are reviewed in Guo and Wang (2007): the synthesis of AuNP via designing and choosing new protecting ligands; and applications in electrochemistry of AuNP including AuNP-based bioelectrochemical sensors, such as direct electrochemistry of redox-proteins, genosensors and immunosensors, and AuNP as enhancing platform for electrocatalysis and electrochemical sensors

From this review, electrochemical detection of bioaffinity interactions is impossible without additional electro active labels. In our investigation we propose to use gold-nanoparticles included onto the surface of screen-printed electrodes as sensor elements for bioaffinity recognition events.

The aim of this work was to study AuNP as an electrochemical sensing platform for direct detection of bioaffine interaction. In order to evaluate AuNP surface status differences due to electrode modification, Au/Au–oxide film formation was studied by cyclic (CV) and stripping voltammetry. As a model, the couple thrombin–thrombin binding aptamer was taken. The couple thrombin–thrombin binding aptamer is one of the most extensively studied. Thrombin is the serine protease that plays a main role in blood coagulation (Hianik et al., 2005; Zheng et al., 2007). Specificity of binding was proved with experiments in the presence of trypsin. Correlation between the cathodic peak area and the logarithm of thrombin concentration was found. In case of trypsin signal has not changed. Novel electrochemical aptasensor for protein detection based on AuNP redox properties was developed. Also, as alternative electrochemical method for thrombin detection, square wave voltammetry (SWV) with ferricyanide/ferrocyanide $Fe(CN)_6^{3-/4-}$ redox indicator was used.

2. Materials and methods

2.1. Reagents

5′-Biotin-labeled aptamers (APT) were synthesized on the DNA-synthesizer ASM-800 (Biosset, Novosibirsk, Russia) accordingly to the manufacturer's instruction and purified by reverse-phase chromatography, using Poly-Pak cartridges from Glen Research. Synthesized oligonucleotides had the following sequence:

B-5′-ATGT**CTACTGGTTGGTGTGGTTGGGTAG**-3′, where B denotes biotin. Sequence highlighted in bold (a core sequence) corresponds to the aptamer motif reported by (Macaya et al., 1995). The 4-nucleotides linker has been added to the 5′-terminus of the core sequence to facilitate aptamer immobilization and an interaction of thrombin with the immobilized aptamer. The linker provides a single-stranded 5′-overhang of the duplex formed by the underlined nucleotides of the core sequence. The aptamer stock solution in water had concentration of 2.4×10^{-4} M (2.2 μg/μl) and was diluted by an appropriate buffer to the concentration of 10 μM.

The following reagents were used: avidin from egg white 10–15 U/mg protein, human thrombin, trypsin, potassium ferricyanide, didodecyldimethylammonium bromide (DDAB, 98%), $HAuCl_4 \times 3H_2O$, $NaBH_4$ (all from Sigma–Aldrich, USA), and bovine serum albumin (BSA, 98% pure, Calbiochem, USA). Reagents used in oligonucleotide synthesis and purification were from Acros Organics (Belgium) except for phosphoamidites and ethylthiotetrazole which were from Glen Research (Sterling, USA). All other chemicals were of analytical grade. To prepare buffer solutions, double distilled water was used.

Buffer solutions: electrochemical experiments were carried out in phosphate buffer (PBS): 0.1 M KH_2PO_4 + 50 mM NaCl (pH 7.4). Aptamer immobilization on the electrode surface was performed in the immobilization buffer: 150 mM NaCl + 10 mM HEPES + 0.005% Tween-20, pH 7.4. Binding of proteins was studied in the binding buffer: 50 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, 50 mM KCl, pH 7.5.

2.2. Apparatus

Electrochemical measurements (stripping voltammetry, CV and SWV) were performed using a PGSTAT Autolab potentiostat/galvanostat (Ecochemie, Utrecht, The Netherlands) with the GPES software in a 3-electrode electrochemical cell. Three-pronged screen-printed electrode (SPE, Elcom, Moscow, Russia http://www.elcom-moscow.ru) consisted of the working graphite electrode (d = 2 mm), the auxiliary graphite and Ag/AgCl reference electrodes. SPEs connected to the potentiostat with home made connector.

2.3. Preparation of colloidal Au-nanoparticles in the DDAB chloroform solution (DDAB–AuNP)

Gold-nanoparticles were prepared as described in Shumyantseva et al. (2007b). Briefly, 0.5 ml of 10 mM $HAuCl_4 \times 3H_2O$ aqueous solution was added to the 1ml of intensively stirred 0.1 M DDAB chloroform solution. Thereafter, the freshly prepared 0.4 M aqueous solution of $NaBH_4$ (0.2 ml) was added to the stirred mixture. After 2 h incubation, the colored organic layer was collected and washed with an equal volume of water. The colloidal Au-nanoparticles in the DDAB chloroform solution were characterized by absorptive spectroscopy (λ_{max} = 520 nm). With the stoichiometry of the reaction, the AuNP concentration was estimated as equal to 5 mM.

2.4. Scanning electron microscopy

Images of SPEs after modification with DDAB and DDAB–AuNP were obtained by using the S-3400N Hithachi Scanning Electron Microscope. Samples for scanning electron microscopy (SEM) were prepared by drop casting of 2 μl of 0.1 M DDAB or DDAB–AuNP solutions onto the electrode surface.

2.5. Aptasensor construction

To modify the working electrode by Au-nanoparticles, 2 μl of 5 mM DDAB–AuNP solution was dropped onto the surface and chloroform was allowed to evaporate. After that, the electrodes were kept at 4 °C overnight. For control experiments, SPE/DDAB sensors were made by placing 2 μl of 0.1 M DDAB chloroform solution onto the electrode surface. DDAB is water-insoluble liquid crystal compound that was used to stabilize AuNP, to avoid the loss of AuNP from electrode surface in follow-up determination and