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Electrochemical quantum dots-based magneto-immunoassay for detection of HE4 protein on metal film-modified screen-printed carbon electrodes

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ABSTRACT

A novel enzyme-free electrochemical immunosensor was developed for highly sensitive detection and quantification of human epididymis protein 4 (HE4) in human serum. For the first time, core/shell CdSe/ZnS quantum dots were conjugated with anti-HE4 IgG antibodies for subsequent sandwich-type immunosensing with superparamagnetic microparticles functionalized with anti-HE4 IgG antibodies, which allow rapid and efficient HE4 capture from the sample. Electrochemical detection of anti-HE4 IgG – HE4 – anti-HE4 $IgG^{CdSe/ZnS}$ immunocomplex was performed by recording the current response of Cd(II) ions, released from dissolved quantum dots at screen-printed carbon electrode (SPCE), modified with mercury or bismuth film. The linear range of the detection was from 20 pM to 40 nM with limit of detection of 12 pM using three times the standard deviation of blank criterion at mercury-film SPCE and from 100 pM to 2 nM with limit of detection of 89 pM at bismuth-film SPCE. Proposed electrochemical immunosensor meets the requirements for fast and sensitive quantification of HE4 biomarker in early stage of ovarian cancer and due to the proper sensitivity and specificity presents a promising alternative to enzyme-based probes used routinely in clinical diagnostics.

1. Introduction

For ovarian cancer, where reliable diagnosis and differentiation between benign and malignant forms significantly influence survival rate, the analysis of as low as possible level of serum cancer biomarkers is essential [1]. Such analysis is routinely based on Enzyme Linked Immunosorbent Assay (ELISA). The selection of an appropriate antibody label is crucial to the sensitivity of the detection. Commonly used labels of antibodies are enzymes, primarily horseradish peroxidase [2,3], alkaline phosphatase [4] or β-galactosidase [5]. Although all of them generally provide adequate sensitivity, nowadays nanomaterials such as materially diverse nanoparticles [6] and nanotubes [7,8] are increasingly used, as well as various types of quantum dots (QDs) [9], which provide indisputable benefits, particularly when exploited in electrochemical analyses.

Quantum dots in the form of semiconductor nanocrystals, which are composed of heavy metals,have already been employed as labels of various biomolecules due to their unique optical properties [10,11], usable in many applications from fluorescent [12,13], western blot [14] or electrochemiluminescence methods [15] to the recently presented inductively coupled plasma mass spectrometry (ICP-MS) [16]. The metallic core-shell composition of quantum dots is favorable for electrochemical detection in biosensors [11,17]. Comparing to the above-mentioned methods, the electrochemical detection of quantum dots is versatile, robust, cost-effective and easy to manipulate and miniaturize [11,18]. Moreover, its combination with screen-printed electrodes modified with different metal films (mercury, bismuth, gold, antimony and copper) [19] and square wave anodic stripping voltammetry (SWASV) represents a significant contribution in sensitive detection methods for quantum dots [20–23]. Although mercury film electrodes offer high sensitivity, less toxic electrode materials are being tested as a viable alternative. Bismuth film electrodes are promising analogues to mercury counterparts due to their favorable electrochemical properties and easy preparation by the electrodeposition of Bi(III) ions on the surface of the selected substrate (glassy carbon, carbon paste, screen-printed carbon ink, gold, etc.) [24,25].

Human epididymis protein 4 (HE4) as a newly approved biomarker of ovarian cancer is currently of great importance not only for its potential to distinguish malignant and benign forms but also for its ability to reflect the differentiation stage of this cancerous disease [26–28].

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Only a few reports on the electrochemical detection of HE4 protein have been published so far. Lu et al. described an electrochemical immunosensor for HE4 based on an indium tin oxide electrode with electrodeposited chitosan-titanium carbide and gold nanoparticles [29]. An electrochemical immunomagnetic biosensor based on sandwich-type ELISA with alkaline phosphatase-labeled antibodies was developed by Čadková and co-authors [30]. Recently, screen-printed carbon electrodes modified with graphene sheets and gold nanoparticles were applied for electrochemical enzyme-based sandwich immunoassay for HE4 analysis, where horseradish peroxidase was used as enzyme label [31]. Here we present its improvement by the inclusion of quantum dots as the sensitive antibody label instead of an enzyme. This novel HE4 biosensor exploited the specificity of classical immunospecific reaction and the signal sensitivity provided by quantum dots (quantum dot-linked immunosorbent assay - QLISA). Electrochemical detection was performed using two configurations of metal film-modified screen-printed carbon electrodes. First sensor utilizes a recent approach in preparation of mercury film electrodes, traditionally used for sensitive stripping analysis of heavy metals [32,33]. Mercury compound is immobilized directly on the surface of screen-printed carbon working electrode and it is reduced to mercury film during accumulation of heavy metals at negative potentials. In such configuration, manipulation with liquid mercury or mercury salt solutions is avoided while desired electrochemical properties of film electrodes are still maintained. Second sensor was an in situ bismuth-film screen-printed carbon electrode, which has manifested nowadays as the most favorable non-toxic replacement of mercury electrodes in stripping analysis of heavy metals [33,34]. Analytical performance of both configurations in HE4 detection was compared and critically discussed.

2. Experimental

2.1. Chemicals

Monoclonal and polyclonal anti-HE4 IgG antibodies and standard human HE4 protein were provided by Sino Biological (USA) (www.sinobiological.com). SiteClick™ Qdot® 565 Antibody Labeling Kit and Qdot® 565 ITK™ Carboxyl CdSe/ZnS Quantum Dots were purchased from Life Technologies (USA) (www.thermofisher.com), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), 2-morpholinoethane-1-sulfonic acid (MES), bovine serum albumin (BSA), bismuth(III) nitrate pentahydrate and TWEEN 20 were from Sigma–Aldrich (USA) (www.sigmaaldrich.com). Carboxylate-modified (SiMAG-COOH) magnetic particles (1 μm in diameter) were bought from Chemicell (Germany) (www.chemicell.com). Precision Plus Protein™ unstained standard (10–250 kDa) was produced by Bio-Rad (USA) (www.bio-rad.com). Biochemistry Control Serum Level II was from BioSystems (Spain) (www.biosystems-sa.com). All other chemicals were of analytical grade purity and were supplied by Penta (www.penta.cz) or Lachema (www.lach-ner.com) (Czech Republic).

2.2. Apparatus

All electrochemical measurements were performed with screen-printed three-electrode sensors. First type was C110 from DropSens (Spain) (www.dropsens.com) consisted of carbon working and auxiliary electrodes and a silver pseudoreference electrode, which served as a substrate for preparation of in situ bismuth film electrode (Bi-SPCE). Second type HM1, manufactured by ItalSens (Italy) (www.palmsens.com), comprised carbon working electrode modified with a mercury salt (unspecified by the producer), platinum auxiliary and silver pseudoreference electrode (Hg-SPCE). The sensors were connected to a PalmSens2 compact electrochemical analyzer with PSTrace software (PalmSens BV, the Netherlands) (www.palmsens.com). SDS-PAGE separation was performed in a Mini-PROTEAN® cell (Bio-Rad, USA) (www.bio-rad.com) and gels were evaluated using the ChemiDoc™ XRS + System with Image Lab™ Software (Bio-Rad, USA) (www.bio-rad.com).

2.3. Labeling of anti-HE4 IgG with CdSe/ZnS quantum dots

Polyclonal anti-HE4 IgG (100 μg) was labeled by CdSe/ZnS quantum dots using a commercially available SiteClick™ Qdot® 565 Antibody Labeling Kit according to the manufacturer´s instructions or by the common one-step carbodiimide method adjusted for the required antibody amount, using Qdot® 565 ITK™ Carboxyl CdSe/ZnS Quantum Dots. Labeled anti-HE4$^{CdSe/ZnS}$ antibodies were affinity purified using antigen HE4-modified SiMAG-COOH magnetic particles.

2.4. Anti-HE4 IgG-HE4-anti-HE4$^{CdSe/ZnS}$ immunocomplex formation

The immobilization of monoclonal anti-HE4 IgG antibodies onto the surface of SiMAG-COOH magnetic beads (MBs) and the determination of immobilization efficiency were performed according to the already published protocol in [30]. Briefly, 50 μg of antibodies were immobilized to 1 mg of magnetic particles. A two-step protocol was used, consisting of 30 min of particles activation with EDC and sulfo-NHS, followed by washing and then the addition of antibodies in 0.1 M MES buffer, pH 5.0, and incubation overnight at 4 °C with gentle mixing.

Subsequently, the immunocomplex was formed by incubation of appropriate amount of magnetic particles with immobilized monoclonal anti-HE4 IgG (10 μg anti-HE4 IgG) with different amounts of standard HE4 protein (1 h, room temperature) and followed by incubation with anti-HE4 IgG$^{CdSe/ZnS}$ in 0.1 M phosphate buffer, pH 7.3, containing 0.1% BSA and 0.05% TWEEN 20 (1 h, room temperature) prepared beforehand as described in 2.3. Dilution 1:500 (v/v) was used for labeled antibodies prepared via commercially available kit, or 1:10 (v/v) for antibodies prepared by the carbodiimide method, respectively.

Magnetic particles with the formed immunocomplex anti-HE4 IgG–HE4–anti-HE4 IgG$^{CdSe/ZnS}$ were washed five times with 1 ml of 0.1 M phosphate buffer, pH 7.3, containing 0.15 M NaCl and five times with distilled water. 50 μl of 0.1 M HCl was added to a sample and incubated at room temperature for 3 min, to release Cd(II) ions for voltammetric measurement.

2.5. Electrochemical measurements

Square wave anodic stripping voltammetry (SWASV) was used as the detection technique for the analysis of Cd(II) ions released from CdSe/ZnS by acid hydrolysis at disposable screen-printed carbon electrodes with a mercury salt (Hg-SPCE) or in situ formed bismuth film (Bi-SPCE). First, the electrode surface of Hg-SPCE was pre-treated by applying the conditional potential −1.1 V for 300 s for reduction of immobilized mercury salt to mercury film with subsequent detection of Cd (II) ions under the following conditions: deposition potential −1 V, deposition time 120 s, potential range from −1 V to −0.15 V, frequency 25 Hz and amplitude 0.0285 V.

The in-situ bismuth film was formed after the addition of Bi(III) ions (500 ppb in a final volume of analyzed solution) to each sample just before its application to SPCE. Measurements were performed in the presence of 0.1 M hydrochloric acid and 0.1 M acetate buffer (pH 4.5) and conducted using the experimental conditions mentioned above, but without the prior electrode pretreatment.

3. Results and discussion

The development of an electrochemical magneto-immunoassay using disposable screen-printed electrodes with the potential to become part of a point-of-care device in the early diagnostics of ovarian cancer is of great interest nowadays. Such an electrochemical immunoassay is presented here, based on square wave voltammetric monitoring of the