POSTER VOLTAMMETRIC ANALYSIS OF QUANTUM DOTS-TAGGED ANTIBODIES AS A PART OF ELECTROCHEMICAL IMMUNOSENSOR ON BISMUTH FILM MODIFIED ELECTRODES

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Summary

Specific antibodies can be labelled by quantum dots (QDs) as a suitable alternative to enzymes in immunoassays. Advantages of QDs are known in the field of fluorescent immunoassays. Nevertheless, here we present the possibility of simple and highly sensitive electrochemical detection of QDs by stripping voltammetry on disposable screen printed electrodes. Moreover, modification of working electrode with in situ bismuth film could be successfully used instead of sensors with mercury film. Alpha-fetoprotein (AFP) as a protein from the group of ovarian cancer tumor markers and corresponding IgG antibodies were used as antigen-antibody system.

1 Introduction

The basic principle of the enzyme immunoassay is the specificity of the molecular recognition of antigens by antibodies to form an immunocomplex [1,2]. Instead of less stable enzymes, quantum dots have been used as labels of biomolecules for ultrasensitive detection of biologically active molecules, and therefore we called the method quantum dot-linked immunoassay (QLISA) [3].

Quantum dots are semiconductor nanocrystals of diameters ranging from 1–20 nanometers and have been intensively studied for their fluorescent characteristics in connection to optoelectronics and biological labelling [4,5]. The typical structure of quantum dots is core-shell where the core composed of one semiconductor (eg. CdSe, CdTe, InP, PbS) is surrounded by outer semiconductor layer (eg. ZnS) [6]. Nevertheless, QDs are usually used in conjunction with optical detection, they can be used also in electrochemical detection where the metal component can be measured

by square wave anodic stripping voltammetry (SWASV) after an acid attack, which leads to breaking the nanoparticle and releasing metal ions into the reaction solution [2,7]. Recently, the bismuth film electrodes (BiSPCEs) are used for their environmental friendliness in comparison with commonly used mercury film electrodes (MeSPCEs). The popularity of BiSPCEs consists in simple preparation, well-defined stripping signals of individual metal elements, high reproducibility [8].

2 Experimental

2.1 Apparatus

PEG-COOH capped core-type PbS QDs from Mesoligth Inc. (USA). Standard alpha-fetoprotein and polyclonal rabbit anti-AFP IgG antibodies were obtained from YO Proteins AB (Sweden). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysulfosuccinimide sodium salt were provided by Sigma-Aldrich (USA). SiMAG-carboxyl magnetic particles (1 μm diameter) were produced by Chemicell (Germany).

The electrochemical measurements were performed with a portable electrochemical interface PalmSens (PalmSens, The Netherlands) controlled by software PSTrace 4.7 and connected with the three-electrode screen printed sensors with a carbon working, a platinum auxiliary and a silver pseudoreference electrode (SPCE, DropSens, Spain) and modified with in situ bismuth film (BiSPCE).

2.2 Labelling of polyclonal anti-AFP IgG antibodies by PbS quantum dots

Polyclonal anti-AFP IgG antibodies were conjugated with PbS QDs via standard one-step carbodiimide method slightly modified [9]. EDC and S-NHS (in ratio 6:1) in 0.1M phosphate buffer (pH 7.3) were added to 25 μg polyclonal anti-AFP IgG antibodies. After 10 minute preactivation at room temperature 4 μl of PbS QDs solution (8 μM) were added. After overnight incubation at 4 °C upon gentle mixing labelled anti-AFP^{PbS} IgG antibodies were affinity purified by using the antigen AFP-modified SiMAG-carboxyl magnetic particles.

2.3 Electrochemical verification of functionality of conjugate (ani-AFP$^{PbS-QDs}$ IgG)

The functionality of conjugate labeled by QDs (anti-AFP$^{PbS-QDs}$IgG) was verified by using the magnetic microparticles (SiMAG-COOH, Chemicell, Germany)

modified by AFP antigen. The reaction mixture of specified quantity (2.85 µl, 5.71 µl, 11.4 µl, 22.8 µl, 34.3 µl, 57.1 µl or 114.2 µl) of the conjugate (anti-AFP$^{PbS-QDs}$IgG) and 33,3 µg suspension of antigen modified SiMAG microparticles (corresponds to 500 ng of antigen) was incubated for 1 h at room temperature under gentle mixing in 0.1 M carbonate buffer (pH 9.4) containing 0.1 % BSA and 0.05 % Tween-20. Then it was washed three times with 0.1M phosphate buffer (pH 7.3).

Electrochemical measurement was proceeded after incubation reaction mixture (for 3 minutes) with addition of 20 µl 0.5M HCl, 70 µl 0.1M acetate buffer (pH 4.5) and 10 µl Bi^{3+} (5 mg/l) by square wave anodic stripping voltammetry (SWASV). Addition of Bi^{3+} is achieved creation in situ bismuth film. QDs were monitored and evaluated by the recording of the peak height at potential -0.55 V. Measurement conditions were as follows: deposition potential -1.0 V for 120 s, potential range from -0.15 V to -1.0 V, step potential 0.003 V, amplitude 0.02805 V, frequency 25 Hz.

3 Results and Discussion

This work was focused on the compiling the conjugate consisting of polyclonal anti-AFP IgG antibody labelled by core type PbS quantum dots which is subsequently used as a part of QLISA based electrochemical immunosensor for quantification of AFP tumor marker exploitable for an early stage of ovarian cancer. Anti-AFP IgG^{PbS} were prepared by in-lab developed protocol due to its commercial unavailability.

Commercial PbS QDs modified by carboxy groups were chosen because of their strong and stable signal providing in square wave anodic stripping voltammetric detection (SWASV). MeSPCEs (electrode with mercury film) are usually used for analysis of heavy metals and therefore QDs. For initial testing of QDs, the MeSPCEs were used for verification the quality and response of the QDs.

For antibody labelling “in-lab” protocol was used consisting of covalent carbodiimide method of conjugation with subsequent affinity purification of gained anti-AFP IgG^{PbS} IgG antibodies by using the antigen (AFP) modified SiMAG-COOH magnetic particles. This step ensures the protection of the antibody binding site as well as purification of antibodies from unreacted free QDs.

For the verification of the functionality of the conjugate (anti-AFP IgG^{PbS}), of the antigen AFP covalently bound to the magnetic particles was done. Consequently, the resulting immunocomplex was detected electrochemically. The optimal dilution of the conjugate for next application was seeked. It is important for other determining of

antigen in serum. The measurement was done with screen printed electrodes with bismuth film (in situ formed), which are used because of non-toxicity and utilization for easy measurement QDs. The obtained results are shown in fig. 1.

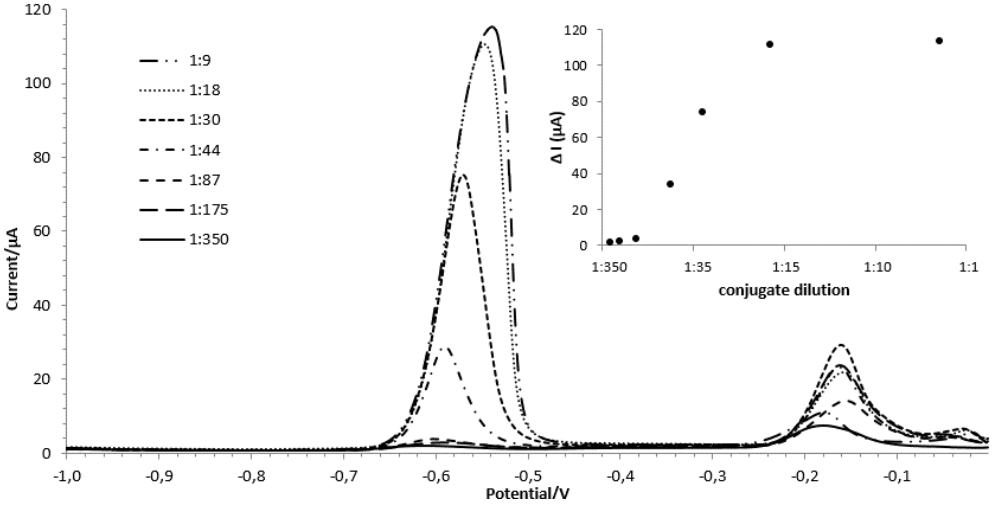

Fig.1. Square wave voltammograms of released Pb(II) from created conjugate anti-AFP^{PbS} onto BiSPCEs (upon experimental conditions described in experimental part).

4 Conclusions

Polyclonal antibodies (anti-AFP IgG) have been successfully labeled by PbS quantum dots. Subsequently, electrochemical response of the conjugate was verified by using BiSPCEs. From these results, we can choose optimal dilution 1:18, which is usable for monitoring of the low concentration of soluble antigen AFP in serum.

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References

[1] Aydin, S., *Peptides*, 2015, *72*, 4-15
[2] Kokkinos, Ch., *Trends in Analytical Chemistry*, 2016, *79*, 88-105
[3] Babu, S., et al. *Biosensors and Bioelectronics*, 2009, *24*, 3467–3474
[4] Foubert, A., et al. *TrAC Trends in Analytical Chemistry*, 2016, *83*, 31-48.
[5] Ung, T. D. T., et al. *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, 2012, *3*.
[6] Reiss, P., et al. *Small*, 2009, *5*, 154-168.
[7] Martín-Yerga, D., et al. *Talanta*, 2014, *130*, 598-602.
[8] Economou, A. *TrAC Trends in Analytical Chemistry*, 2005, 24, 334-340
[9] Hermanson, G., *Zero-length crosslinkers in Bioconjugate Techniques*, 3rd ed., Academic Press, Elsevier Inc., San Diego, 2013, 263-265.