NEW APPROACH IN ELECTROCHEMICAL IMMUNOMAGNETIC BIOSENSORS FOR PROTEIN DETECTION

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Summary

The simple, sensitive, and selective method for electrochemical detection of ovalbumin is presented. This method is a combination of the selective immunocomplex formation on the surface of magnetic carriers for easy manipulation and preconcentration with a very sensitive electrochemical detection. The evaluation of antigen content is based on electrochemical detection of quantum dots as labels of secondary antibodies, which allows detecting of low amounts of target antigen.

1. Introduction

The detection of low concentrations of target antigen (i.e. proteins) is nowadays of great importance in biosensing area. New trends in this field combine selective immunochemical reaction with the quantum dots (QDs) as a label of secondary antibodies. This approach represents highly selective and sensitive detection method.

QDs are nanoscaled inorganic crystals characterized by very interesting optical and electronic properties[1]. These nanocrystals have a core-shell structure and diameter from 2 to 10 nm. The core is usually composed of elements such as Cd, Pb, or In. The shell usually consists of ZnS (ref.[2]).

Considering the composition, QDs have a great potential for electrochemical detection. Electrochemical techniques are known to have unique advantages in terms of both economic features and high sensitivity, e.g. in the determinations of low levels of heavy metals in different samples[4]. Anodic stripping voltammetry (ASV) in particular utilizes the efficient preconcentration of analyte, which can be combined with sensitive detection step using pulse techniques, like square-wave voltammetry (SWV) or differential pulse voltammetry (DPV). Such techniques effectively discriminate the faradaic current from the background current and they are often used for the identification of the redox processes and the determination of the corresponding current values[1]. Nowadays, the square-wave anodic stripping voltammetry (SWASV) is widely applied in electroanalysis of various species[3].

Additionally, there is a continuous push to use miniaturized electrochemical sensors and integrate as many electrodes as possible on one substrate. Therefore, the use of screen-printed sensors for electrochemical detection of ovalbumin in small sample volumes is presented in this contribution.

2. Experimental

2.1. Voltammetric measurements

All voltammetric measurements were performed with PalmSens interface (PalmSens, Netherlands). With regard to minimization of sample volume the detection of QDs were performed with mercury film screen-printed carbon electrode (ItalSens, Italy). Each screen-printed electrode was pretreated by applying the potential of –1.1 V for 300 s before use. Afterwards, the square wave voltammetric scans were carried out until low and stable background was obtained.

Prior to voltammetric scan, the nanometer-sized quantum dots Qdot®565 ITK Carboxyl Quantum Dots CdSe/ZnS (Life Science) were firstly dissolved with 50 µl HCl. Different concentration of HCl (0.1 M, 1 M, and 3 M) and different time (1, 3, 5, and 10 min) for QDs dissolution were tested. After optimization of QDs detection, the calibration curve of the quantum dots was ascertained using SWASV technique with 2 min of heavy metals accumulation.

2.2. Ovalbumin detection

The immunocomplex for specific immunocapture of ovalbumin was formed onto surface of SiMag-carboxyl magnetic particles (Chemicell, Germany). The anti-ovalbumin antibodies (Tetracore, USA) were covalently immobilized onto surface of magnetic particles. The secondary antibodies were prepared by conjugation of anti-ovalbumin antibodies with Qdot®565 ITK Carboxyl Quantum Dots. The evaluation of ovalbumin concentration was than based on electrochemical detection of QDs presence in analysed sample. Finally, the calibration curve for ovalbumin detection was ascertained.

3. Results and discussion

In this contribution, the electrochemical immunomagnetic assay of ovalbumin with subsequent

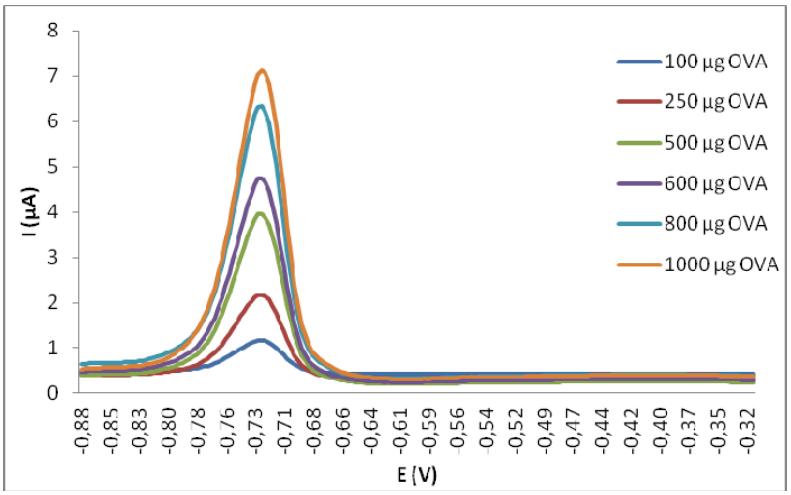

Fig. 1. **SWASV voltammograms of calibration in electrochemical immunomagnetic assay for ovalbumin – system anti-ovalbumin–ovalbumin–anti-ovalbumin labeled with QDs Q565 in 0.1 M HCl for 2 min heavy metals acumulation**

square-wave anodic stripping voltammetry of quantum dots is presented. This technique takes advantage of that quantum dots are composed of heavy metals such as cadmium and lead, which are easily electrochemically detected.

On the basis of optimal detection conditions, this technique was also used for the evaluation of the conjugation efficiency of secondary antibodies with QDs. This conjugate was subsequently used as secondary antibody for determination of the presence of ovalbumin as model system in the sample. The trend of increasing electrochemical response with increasing concentration of quantum dots and therefore ovalbumin is evident.

The use of magnetic particles of nanometer size enables highly efficient separations of target biomolecules due to their large surface area for specific ligand immobilization. They also allow to preconcentrate and separate target ligands on the surface of transducer with an aid of magnetic field.

4. Conclusions

Nowadays, the quantum dots have many interesting optical features for biosensing applications and have emerged not only in optical sensing strategies, but also in electrochemical sensing approaches. Electrochemical immunomagnetic sensor for detection of ovalbumin as a model protein, which is based on QDs determination, is presented. Such biosensor is attractive due to good availability of screen-printed electrodes, simple use, fast analysis, low detection limits and possibility of miniaturization and can be adopted for another protein detection with slight modification only.

This work was financially supported by the Czech Science Foundation P206/12/0381 and 7. FP EU project „NaDiNe“ No 246513.

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