Biosensors and Bioelectronics 46 (2013) 37–43

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Multiplexed electrochemical immunoassay of biomarkers using metal sulfide quantum dot nanolabels and trifunctionalized magnetic beads

Dianping Tang [a,*], Li Hou [a], Reinhard Niessner [b], Mingdi Xu [a], Zhuangqiang Gao [a], Dietmar Knopp [b]

[a] Key Laboratory of Analysis and Detection for Food Safety, Fujian Province & Ministry of Education, Department of Chemistry, Fuzhou University, Fuzhou 350166, China

[b] Chair for Analytical Chemistry, Institute of Hydrochemistry, Technische Universität München, Marchioninistrasse 17, D-81377 München, Germany

ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form
14 February 2013
Accepted 16 February 2013
Available online 26 February 2013

Keywords:
Multiplexed immunoassay
Cancer biomarkers
Metal sulfide nanolabels
Electrochemical
Trifunctionalized magnet beads

ABSTRACT

A novel multiplexed stripping voltammetric immunoassay protocol was designed for the simultaneous detection of multiple biomarkers (CA 125, CA 15-3, and CA 19-9 used as models) using PAMAM dendrimer-metal sulfide quantum dot (QD) nanolabels as distinguishable signal tags and trifunctionalized magnetic beads as an immunosensing probe. The probe was prepared by means of co-immobilization of primary monoclonal anti-CA 125, anti-CA 15-3 and anti-CA 19-9 antibodies on a single magnetic bead. The PAMAM dendrimer-metal sulfide QD nanolabels containing CdS, ZnS and PbS were synthesized by using *in situ* synthesis method, which were utilized for the labeling of polyclonal rabbit anti-CA 125, anti-CA 15-3 and anti-CA 19-9 detection antibodies, respectively. A sandwich-type immunoassay format was adopted for the simultaneous determination of target biomarkers in a low-binding microtiter plate. The subsequent anodic stripping voltammetric analysis of cadmium, zinc, and lead components released by acid from the corresponding QD nanolabels was conducted at an *in situ* prepared mercury film electrode based on the difference of peak potentials. Experimental results indicated that the multiplexed immunoassay enabled the simultaneous detection of three cancer biomarkers in a single run with wide dynamic ranges of 0.01–50 U mL^{-1} and detection limits (LODs) of 0.005 U mL^{-1}. Intra-assay and inter-assay coefficients of variation (CVs) were less than 7.2% and 10.4%, respectively. No significant differences at the 0.05 significance level were encountered in the analysis of 10 clinical serum specimens between the multiplexed immunoassay and a commercially available enzyme-linked immunosorbent assay (ELISA).

1. Introduction

Cancer biomarkers, such as α-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), CA 15-3, and CA 19-9, can be found in the body (usually blood or urine) when cancer is present. Their levels in the body are usually used for evaluating therapy and detecting recurrence or metastasis (Xiang and Lu, 2012). Recently, various methods and strategies have been developed for the detection of a single cancer biomarker (J. Tang et al., 2012;L. Tang et al., 2012; Xiang and Lu, 2012; Kim et al., 2012). However, their applications are facing great challenges, because most markers are not specific to a particular tumor. For example, CA 125 is associated with breast cancer and epithelial ovarian tumors, while CA 19-9 is usually relevant to lung cancer, colorectal, pancreatic, and gastric carcinomas. In contrast, most cancers express an elevation of the level of at least two markers associated with their incidence (e.g. breast cancer is associated with CA 15-3, CA 125 and CEA) (Wu et al., 2007). Hence, exploring a simple and sensitive method for simultaneously detecting and quantifying multiple cancer markers in a single run would be advantageous.

Immunological methods have become the predominant analytical techniques for quantitative detection of cancer biomarkers (Lee et al., 2012). Multianalyte immunoassay, in which two or more analytes are measured simultaneously in a single assay, is requested in clinical laboratories to simplify the work procedure, increase sample throughput, and reduce the overall cost per test (Bronshtein et al., 2012; Wu et al., 2012; Michels et al., 2012; Zong et al., 2012; Arrabito and Pignataro, 2012). Ge et al. (2012) reported a multiplexed sandwich chemiluminescence immunoassay on a 3D origami-based device, comprised of one test pad surrounded by four folding tabs. Juncker et al. utilized microarray-to-microarray transfer of reagents by snapping of two chips for cross-reactivity-free multiplex immunoassay

* Corresponding author. Tel.: +86 591 2286 6125; fax: +86 591 2286 6135.
E-mail address: dianping.tang@fzu.edu.cn (D. Tang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.02.027

(Li et al., 2012). Most recently, Zhu's group designed a new multianalyte electrochemical immunoassay for the detection of two human cardiopathy biomarkers using metal-ions- (i.e., Cd^{2+} and Zn^{2+}) functionalized titanium phosphate nanospheres as distinguishable signal tags (Feng et al., 2012). Among these methods, multiplexed electrochemical immunoassays are suitable for sensor miniaturization and automated detection owing to their high sensitivity, low cost, low power requirements, and high compatibility with advanced micromachining technologies (Liu et al., 2011). Despite many advances in this field, there is still the quest for new schemes and protocols for the improvement of simplicity and sensitivity of multiplexed electrochemical immunoassays.

For the successful development of multiplexed electrochemical immunoassays, the first major concern is how to discriminate each electrochemical signal for one special analyte from the multiple antigen–antibody reactions. Nowadays, the present methods are mainly based on either multilabeling or spatially resolved assay protocols (Du et al., 2011; Hood et al., 2009). Although modification of the individual electrodes with different biological recognition elements would enable the construction of miniaturized immunosensor arrays, the directed immobilization of functional proteins on individual microscopic regions is still a challenge. Hence, multilabel methods based on enzymes (Dill et al., 2006), metal ions (Feng et al., 2012), redox tags (Tang et al., 2011), and quantum dots (QDs) (Kong et al., 2013) have been used for the development of multiplexed electrochemical immunoassays. Among these labeling methods, QDs have shown great potential because of their unique advantages: nanoscale size similar to proteins, broad excitation spectra for multicolor imaging, robust, narrowband emission, and versatility in surface modification (Figerio et al., 2012). Viswanathan et al. (2012) devised an electrochemical immunosensor for multiplexed detection of food-borne pathogens using three metal sulfide (CdS, PbS, and CuS) nanocrystals as distinguishable tags on a carbon nanotube-modified screen printed electrode. Kong et al. (2013) developed an electrochemical immunoassay for the simultaneous determination of two tumor markers using CdS/DNA and PbS/DNA nanochains as distinguishable tags. Due to the difference in the oxidation potentials of these metal components, they exhibited sharp and well resolved stripping voltammetric peaks at various peak potentials. Owing to the advantages of QDs, our motivation in this work is to combine the unique structure of PAMAM dendrimers with the merits of metal sulfide QDs to synthesize a new class of nanolabels for the amplification of electrochemical signals.

The second key issue for the multiplexed immunoassay is the signal-transduction method. Stripping voltammetric analysis is an analytical technique that involves (i) preconcentration of a metal onto a solid electrode surface or into Hg (liquid) at negative potentials and (ii) selective oxidation of each metal species during an anodic potential sweep (Wang et al., 2003; Hansen et al., 2006). Inspiringly, metal ions, e.g. cadmium, copper, zinc, and lead, can be stripped from the corresponding QDs under harsh conditions. The stripped metal ions can exhibit specific voltammetric characteristics at different applied potentials.

Herein we design a novel magneto-controlled stripping voltammetric immunoassay for the simultaneous detection of CA 125, CA 15-3 and CA 19-9 using dendrimer-encapsulated metal sulfide QDs as distinguishable signal tags and trifunctionalized MB. Three primary antibodies, i.e, anti-CA 125, anti-CA 15-3 and anti-CA 19-9, are co-immobilized onto the MB, which are used as immunosensing probes for the capture of three target analytes. Three types of QDs containing CdS, ZnS and PbS are used for the preparation of dendrimer-QDs using the *in situ* synthesis method, respectively, which are employed for labeling of three corresponding analyte-specific detection antibodies. Based on a sandwich-type immunoassay format, the formed immunocomplexes are subsequently quantified using square wave anodic stripping voltammetry (SWASV) at an *in situ* prepared mercury film electrode. The electrochemical signals are simultaneously achieved at various peak potentials after metal ions are stripped from the corresponding metal sulfide dendrimer-QDs. The aim of this work is to explore a novel multiplexed electrochemical immunoassay with sensitivity enhancement using functionalized QDs for signal amplification as an alternative to using enzymes.

2. Experimental

2.1. Materials and reagents

Monoclonal mouse anti-human CA 125 (mAb_{1-1}) and CA 19-9 (mAb_{1-3}) antibodies, and polyclonal rabbit anti-human CA 125 (Ab_{2-1}) and CA 19-9 (Ab_{2-3}) antibodies were purchased from LifeSpan BioSciences, Inc. (Seattle, WA, USA). Monoclonal mouse anti-human CA 15-3 (mAb_{1-2}) and polyclonal rabbit anti-human CA 15-3 (Ab_{2-2}) antibodies were obtained from GenWay Biotech, Inc. (San Diego, CA, USA). CA 125, CA 19-9 and CA 15-3 internal standards, comprising graded dilutions of partially purified CA antigens, were obtained from the corresponding ELISA kits (Biocell Biotechnol. Co., Ltd., Zhengzhou, China), and were used for calibration of the developed multiplexed electrochemical immunoassay. Magnetic Fe_3O_4 beads (MBs, particle size: ~100 nm) were obtained from Chemicell GmbH (Berlin, Germany). Bovine serum albumin (BSA, 96–99%), (3-glycidyloxypropyl) trimethoxysilane ($C_9H_{20}O_5Si$, GOPS), and PAMAM dendrimer (1,4-diaminobutane core, generation 3.0 solution, 20 wt% in methanol, and 32 amino surface groups) were supplied by Sigma-Aldrich. All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all runs. Phosphate-buffered saline (PBS, pH 7.4, 0.5 mM), Tris–HCl stock buffer (pH 7.4, 0.02 M) and acetate buffer (ABS, pH 5.6, 0.2 M) were the products of Sigma-Aldrich. The washing buffer was prepared with 0.02 M Tris–HCl buffer containing 0.1% Tween-20, 0.5% BSA, and 0.15 M NaCl.

2.2. Preparation of mAb_{1-1}, mAb_{1-2} and mAb_{1-3}-conjugated magnetic beads (MB-mAb_1)

The trifunctionalized immunosensing probes were prepared by co-immobilization of mAb_{1-1}, mAb_{1-2} and mAb_{1-3} on the MB according to our recently reported methods (Tang et al., 2011;). Briefly, 50 mg of dried MBs was initially added to the mixture containing 4.75 mL dry toluene and 0.25 mL GOPS (98 wt%), and then the mixture was gently stirred for 12 h at room temperature (RT) under nitrogen. During this process, GOPS molecules were covalently linked to hydroxyl groups on the surface of MB via its methoxyl functions. Following that, the MBs were magnetically separated and washed sequently with toluene, ethanol and distilled water. The precipitate was diluted with 2 mL of PBS. Afterward, the incubation solution containing 100 μL of mAb_{1-1}, mAb_{1-2} and mAb_{1-3}, at identical concentration of 1.0 mg mL^{-1}, was added to the suspension. The resultant mixture was gently shaken on a shaker for 6 h at 4 °C. The formed trifunctionalized MB-mAb_{1-1}/mAb_{1-2}/mAb_{1-3} conjugates (abbreviated as MB-mAb_1) were incubated with 5.0 mL of glycine solution (3.0 wt%) at RT for 30 min to block the remaining functional groups. Finally, the as-prepared MB-mAb_1 conjugates were stored in PBS containing 0.1 wt% sodium azide with a final concentration of ~10 mg mL^{-1} at 4 °C until use.