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Towards a blocking-free electrochemical immunosensing strategy for anti-transglutaminase antibodies using screen-printed electrodes

Daniel Martín-Yerga, Agustín Costa-García *

Nanobioanalysis Group, Department of Physical and Analytical Chemistry, University of Oviedo, 8 Julián Clavería St., Oviedo 33006, Spain

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ABSTRACT

A blocking-free one-step immunosensing strategy using 8-channel screen-printed arrays for the detection of anti-transglutaminase IgA antibodies, celiac disease biomarkers, was developed. A simple but novel immobilization approach to efficiently modify the surface of screen-printed electrodes with a recognition element was employed in order to minimize the non-specific adsorption on the electrode surface, and the optimization of a methodology without a blocking step was carried out. After the functionalization of the electrode surface with tissue-transglutaminase, two different immunoassays, using multi-step and one-step strategies, were optimized. Serum controls from a commercial ELISA kit, anti-human IgA labelled with biotin and streptavidin labelled with CdSe/ZnS quantum dots were employed as bioreagents for the immunoassay. Screen-printed arrays were used as the solid support for the immunosensor and the detection of Cd(II) was performed in situ by anodic stripping voltammetry after an acid attack of the QDs. The electrochemical response from Cd(II) was correlated with the anti-transglutaminase IgA antibody concentration. The analytical characteristics obtained for the multi-step and one-step electrochemical immunosensors allow discrimination between positive and negative serum controls, establishing this biosensor as a useful tool for the determination of celiac disease biomarkers.

1. Introduction

Point-of-care (POC) testing is defined as medical testing at or near the site of patient care [1]. Ideal POC analytical devices should allow simple, rapid, sensitive and selective detection of one or more analytes. They should also be small and inexpensive devices to facilitate their usage by the patient themselves. The most known POC devices are pregnancy tests and electrochemical glucometers for glucose monitoring [2]. Electrochemical biosensors (EBs) are being widely researched in recent years as a clinical diagnostic platform [3–5]. Such biosensors could provide basic tools for POC devices, due to their interesting properties such as high sensitivity and rapid analysis time. In addition, if screen-printed electrodes are used as the transducers, further advantages are obtained such as low cost, low reagent consumption and the ability to perform decentralized tests [6]. Moreover, novel screen-printed multichannel devices are being employed to perform multiple analysis simultaneously [7,8]. Although there has been substantial progress in the development of EBs, the way to POC diagnostic is still hard and long.

Many complex EBs involving several biological reactions and washing steps have been described in the literature [9–11]. Such procedures differ from the ideal concepts of a POC device. Most of these bioassays, and specially for immunoassays, require a blocking step of the electrode surface to reduce nonspecific adsorption of interfering proteins from reagents or samples, which may lead to high background signals decreasing the signal/noise ratio and hence the sensitivity. This blocking step is usually carried out with inert proteins such as bovine serum albumin (BSA) or casein, which increases the procedure complexity and the analysis cost and time. However, this blocking step is usually accepted by immunosensing researchers and only a few EBs without incorporating a blocking step have been reported previously, typically replaced by complex washing strategies [12]. On the other hand, the modification of screen-printed electrodes with the recognition element is frequently performed by modifying the working electrode [13,14]. The hydrophobic character of untreated screen-printed electrodes makes the complete coating of the surface by the recognition element difficult. Consequently, the rest of the electrochemical cell remains available for non-specific adsorption. The complex methodologies involving several reactions (including blocking of non-specific adsorption) and washing steps are some of the main drawbacks for the introduction of electrochemical immunosensors in real-world applications. The development of really simple and fast approaches is expected to have a remarkable significance in the future of these devices.

The development of methodologies employing direct electrochemical labels to replace the most used enzymatic labels may result in several advantages such as the possibility to perform easy multiplexing detection [15] or avoid the often time-consuming enzymatic reaction. For these reasons, the development of simple electroactive labels, especially based on nanomaterials, is a constant concern. Quantum dots (QDs), semiconductor metallic nanoparticles, are being widely used as

* Corresponding author.
E-mail address: costa@uniovi.es (A. Costa-García).

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biosensing labels due to their optical and electrochemical properties [16,17]. The possibility to synthesize QDs with different metal compositions or sizes offers great versatility for biosensing applications [18]. In addition, modification with different coatings allows a simple functionalization with biomaterials and stability improvement [19]. For electrochemical detection, typically an acid attack is carried out to release metal cations to the solution. It has been found that the appropriate selection of the quantum dot size may have an important effect on the number of released metal atoms and the electrochemical signal obtained [20]. In most of the published electrochemical biosensors using QDs, both the bioassay and the subsequent acid attack are performed outside of the detection platform [21–24]. However, the recent development of a methodology by our group for the in situ detection of QDs, in which the bioassay as well as the acid attack and the detection are performed directly on the screen-printed electrodes, has significantly simplified the use of QDs as electrochemical labels for biosensors [25].

Celiac disease (CD) is an autoimmune enteropathy produced by intolerance to gluten. An effect of CD is the production of autoantibodies that cause the destruction of the intestinal mucosa [26]. A small intestinal biopsy is still considered the reference method for the diagnosis of CD, but serological tests for the detection of biomarkers are being of significant help to avoid the most invasive methods [27]. Anti-tissue transglutaminase (tTG) antibodies, particularly IgA antibodies, are serological biomarkers that provide important information concerning the disease with high sensitivity and specificity. The most common method for the detection of CD biomarkers is an enzyme-linked immunosorbent assay (ELISA) [28,29]. However, electrochemical immunosensors appear as an interesting alternative since they have several advantages such as the increase of sensitivity due to the electrochemical detection, lower sample volumes and, typically, shorter analysis time [3,5,30–32].

In this work, we developed a simple methodology for electrochemical immunosensors eliminating the usual blocking step and performing the in situ detection of QDs as electrochemical labels. This methodology was employed for the determination of anti-tTG IgA antibodies, celiac disease biomarkers. Both, a multi-step bioassay procedure with intermediate washing steps, and a one-step bioassay procedure where all the biological reagents react simultaneously, were optimized. The one-step strategy is much simpler and is carried out in a shorter time than other biosensors published for the determination of celiac disease biomarkers. Another advantage of this work is the use of 8-channel screen-printed electrochemical arrays (8xSPCEs) that further simplifies the procedure and drastically improves the time of analysis. With this work, we get closer to the ideal POC device for celiac disease detection.

2. Materials and methods

2.1. Apparatus and electrodes

Voltammetric measurements were performed with a μStat 8000 (DropSens, Spain) potentiostat interfaced to a Pentium 4 2.4 GHz computer system and controlled by the DropView 8400 2.0 software. All measurements were carried out at room temperature. 8-Channel screen-printed electrochemical arrays were purchased from DropSens (ref. 8x110). Each array is formed by eight 3-electrode electrochemical cells (30 μL volume for each individual cell) with carbon-based working and counter electrodes, whereas pseudoreference electrodes and electric contacts are made of silver. This device has dimensions of 4.0 × 7.9 × 0.06 cm (length × width × height) and the diameter of the working electrodes is 2.56 mm. 8-Channel arrays were connected to the potentiostat through a specific connector, DRP-CAST8X. A graphical scheme and a real image of the 8-channel array are shown in Schematic S1 and Figure S1, respectively. Contact angle measurements were performed using a CAM 200 contact angle goniometer (KSV Instruments Ltd).

2.2. Reagents and solutions

Sodium hydroxide, acetic acid (100%), and fuming hydrochloric acid (37%) were purchased from Merck. Bismuth(III) standard, tris(hydroxymethyl)aminomethane (Tris), bovine serum albumin fraction V (BSA), β-casein (98%, from bovine milk), peroxidase from horseradish (HRP), potassium ferrocyanide trihydrate, potassium ferricyanide and potassium chloride were purchased from Sigma-Aldrich. Human tissue transglutaminase (recombinantly produced in insect cells) was purchased from Zedira (Germany). Qdot® 655 streptavidin conjugate (QD-STV), biotinylated goat anti-human IgA (anti-H-IgA-BT) and Qdot® 655 goat F(ab′)2 anti-human IgG conjugate (anti-H-IgG-QD) were purchased from Life Technologies. Varelisa Celikey tissue transglutaminase IgA and IgG ELISA kits were purchased from Phadia (Germany). Each kit contained six human serum calibrators (0, 3, 7, 16, 40, 100 U mL^{-1}) and a positive and a negative control. Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore Ibérica S.A. (Madrid, Spain) was used throughout this work. All other reagents were of analytical grade. Working solutions of tTG, QD-STV, anti-H-IgA-BT, anti-H-IgG-QD, BSA, and casein were prepared in 0.1 M pH 7.4 Tris–HCl buffer (hereafter called Tris buffer).

2.3. Immunoassay procedure

2.3.1. Immunosensor preparation

The 8-channel SPCEs were rinsed with Tris buffer and left to dry to generate a hydrophilic surface. Then, an aliquot of 4 μL of tTG solution (in Tris buffer) with a concentration of 0.1 μg/μL was dropped to cover completely the surface of the electrochemical cell of each electrode (including counter and quasireference electrodes). It was stored overnight until complete dryness at 4 °C.

2.3.2. Multi-step methodology

Firstly, the prepared immunosensor was washed with Tris buffer. The detection of anti-tTG IgA antibodies was carried out by incubating the immunosensor with 25 μL of serum solutions (1:2 in Tris buffer) for 60 min followed by a washing step with Tris buffer. Then, 25 μL of 7.5 μg/mL anti-H-IgA-BT (with 5 μg/μL of BSA) was added to the sensor for 60 min followed by another washing step with Tris buffer. Finally, 25 μL of QD-STV (10 nM in terms of QDs) was added and left to incubate for 30 min. A last washing step was carried out with ultrapure water. Then, the electrode was connected to the potentiostat for the electrochemical analysis. In Schematic 1A, a diagram of the immunoassay using this methodology is shown.

2.3.3. One-step methodology

In the first place, the prepared immunosensor was washed with Tris buffer. The detection of anti-tTG IgA antibodies was carried out by incubating the immunosensor for 80 min with 25 μL of a mixture of human serum calibrator (10 μL), anti-H-IgA-BT (1 μL, 190 μg/mL), QD-STV (1 μL, 250 nM in terms of QDs) and Tris (13 μL). A last washing step was carried out with ultrapure water. Then, the electrode was connected to the potentiostat for the electrochemical analysis. In Schematic 1B, a diagram of the immunoassay using this methodology is shown.

2.3.4. Electrochemical detection

The measurement step follows a methodology previously developed [25]. After the biological reaction, 1 μL of HCl 1.0 M was added on the working electrode to release Cd^{2+} from QDs and 25 μL of 0.1 M acetate buffer solution pH 4.5 with 1.0 mg L^{-1} Bi(III) was added. A constant potential of + 1.00 V was applied for 60 s to activate the working electrode. The application of this potential may help to the activation of the carbon electrode surface, creating oxygenated functional groups and increasing the electroactive area, as previously described in the literature [33–35]. Furthermore, it is likely that the oxygen gas detaches part of the protein adsorbed on the electrode surface, leaving a more available surface for