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Development of an immunosensor for the determination of rabbit IgG using streptavidin modified screen-printed carbon electrodes

María Díaz-González, David Hernández-Santos, María Begoña González-García, Agustín Costa-García*

Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, Asturias, 33006 Oviedo, Spain

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Abstract

Voltammetric enzyme immunosensors based on the employment of streptavidin modified screen-printed carbon electrodes (SPCEs) for the detection of rabbit IgG, as a model analyte, were described. Alkaline phosphatase (AP) and 3-indoxyl phosphate (3-IP) were used as the enzymatic label and substrate, respectively. The adsorption of streptavidin was performed by deposition of a drop of a streptavidin solution overnight at 4 °C on the pre-oxidized surface of the SPCEs. The analytical characteristics of these sensors were evaluated using biotin conjugated to AP.

The immunosensor devices were based on a specific reaction of rabbit IgG with its biotinylated antibodies, which were immobilised on the modified screen-printed carbon electrodes through the streptavidin:biotin reaction. The immunosensors were used for a direct determination of AP labelled rabbit IgG, and for free rabbit IgG detection using a sequential competitive immunoassay. A calibration curve in the range of 5×10^{-11} to 1×10^{-9} M of rabbit IgG was obtained with a estimated detection limit of 5×10^{-11} M (7.0 ng/ml). These immunosensors were stable for 5 months if they were stored at 4 °C.

Keywords: Alkaline phosphatase; 3-Indoxyl phosphate; Screen-printed carbon electrode; Streptavidin:biotin reaction; Rabbit IgG; Immunosensor

1. Introduction

Applications of immunoelectrochemical sensors for the determination of different biologically active compounds have increased significantly during the last 15 years [1–3]. These devices combine high specificity of traditional immunochemical methods with low detection limits of modern electrochemical systems. Other advantages of the electrochemical immunosensors are their simplicity and possibility to carry out continuous, fully automated assays [2]. Immunoenzyme electrodes constitute a significant portion of the elaborated immunosensors [2,4]. Operation of these instruments is based on the formation of enzyme-labelled immune complexes on the sensitive electrode surface and the subsequent electrochemical detection of the immobilised enzyme [4]. This approach allows applying standard ELISA formats and reactants for the immunosensors. The immunoenzyme electrodes retain the main advantages of solid-phase immunoassays, namely, high sensitivity and specificity, and furthermore, simplify and accelerate the analytical procedure.

For the design of an immunosensor, the crucial step is the immobilisation of immuno-reagents onto the electrode surface. The immobilisation method will determine the sensitivity and reproducibility of the immunosensor. General strategies for the immobilisation of immuno-reagents on solid surfaces include physical adsorption, entrapment in polymer matrix and covalent attachment [5–11]. Proteins have an amphiphilic nature and they therefore have a tendency to adsorb to a solid surface. So the direct adsorption of antibodies on the electrode surface seems to be a promising approach for immunosensor construction. It does not require the use of chemical linkers simplifying the fabrication procedure of the immunosensor [5–7]. The most important driving force for

* Corresponding author. Tel.: +34 985 103488; fax: +34 985 103125.
E-mail address: costa@sauron.quimica.uniovi.es (A. Costa-García).

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protein adsorption has been identified as hydrophobic interaction and electrostatic interaction. However, a drawback of physical adsorption is that immunosensing phases such obtained are disordered and consequently, a part of the binding sites are not accessible to analyte, implying a lack in sensitivity of the immunosensor device. The attachment of reactants to the support through the specific biotin:avidin (or streptavidin) interaction has been revealed as an effective and reliable approach for protein immobilisation [12–15]. This reaction is characterised by a high affinity constant, which is usually the basis for a very stable immobilisation of proteins. Other alternative for oriented immobilisation of antibodies is through protein A or protein G [16,17].

A wide variety of electrodes have been used as support to fabricate immunosensor devices including carbon paste electrodes, glassy carbon electrodes or gold electrodes. Recently, several immunosensor devices have been developed on screen-printed electrodes. The screen-printing microfabrication technology is nowadays well established for the production of thick-film electrochemical transducers. This technology allows the mass production of reproducible yet inexpensive and mechanically robust strip solid electrodes. Other important features that these electrodes exhibit are related to the miniaturisation of the corresponding device along with their ease of handling and manipulation in a disposable manner. However, as the exact formulations of the commercial inks are unknown, their functionalisation is difficult to control and depends on the ink used [18] and on the binder used [19]. As in the case of conventional electrode supports, the immobilisation of antibodies or antigens on screen-printed electrodes has been carried out by physical or electrostatic adsorption [20–25], by sol–gel entrapment [8] or through the affinity reactions as biotin:(strept)avidin [14,15] or protein A or protein G [17], obtaining different immunosensor devices.

In this work an enzyme-immunosensor device for the determination of a model analyte (rabbit IgG, RIgG) was fabricated on screen-printed carbon electrodes (SPCEs), which were previously modified with streptavidin by physical adsorption. The novelty of the methodology used to adsorb the streptavidin on the electrode surface is that the physical adsorption of streptavidin must be performed at a constant temperature above the room temperature. Moreover, the electrode surface must be previously electrochemically pretreated at an anodic potential in acidic media to improve its adsorptive properties. In this way, reproducible, sensitive and stable sensing phases are obtained. This methodology for coating the surface of a SPCE with streptavidin is very simple, in contrast to other works where the covalent attachment of biotin was performed on SPCEs followed by the reaction with streptavidin [14] or where the covalent attachment of streptavidin was carried out on conducting polymer modified SPCEs [15]. The attachment of an antibody on the electrode surface (goat anti-rabbit IgG) was carried out through the streptavidin:biotin interaction. The immunosensing phases obtained in this way are stable for several months. A sequential competitive assay between analyte and alkaline phosphatase (AP) labelled analyte was carried out on this immunosensor device, obtaining lower limits of detection than others reported in the literature [15].

On the other hand, 3-indoxyl phosphate (3-IP) was used as electrochemical substrate of AP. This substrate has been proposed by our group as a suitable electrochemical substrate for AP [26] and various immnunoanalytical devices with this substrate for the detection of different model analytes were performed on the surface of pretreated carbon paste electrodes [27,28]. The resulting enzymatic product is indigo blue, an aromatic heterocycle insoluble in aqueous solutions. Its sulfonation in acidic medium gives rise to indigo carmine (IC), an aqueous soluble compound which shows a similar electrochemical behaviour than indigo blue. Both 3-IP and IC have already been studied on SPCEs [29,30].

2. Experimental

2.1. Apparatus

Voltammetric studies were performed with an Autolab PGSTAT 12 (Eco Chemie, The Netherlands) potentiostat/galvanostat interfaced to an AMD K-6400 computer system and controlled by Autolab GPES software version 4.7 for Windows 98.

Screen-printed carbon electrodes were purchased from AndCare Inc. (Durham, NC, USA), together with and edge connector (Fig. 1). The AndCare electrodes incorporate a conventional three-electrode configuration, which comprises a disk-shaped working (4 mm diameter), counter and silver pseudoreference electrodes printed on polycarbonate substrates (4.5 cm × 1.5 cm). Both working and counter electrodes were made of heat-cured carbon composite inks. An insulating layer was printed over the electrode system, leaving uncovered a working electrode area of 7 mm × 5 mm and the electric contacts. A ring-shaped layer further printed around the working area constituted the reservoir of the electrochemical cell, with an actual volume of 50 μl.

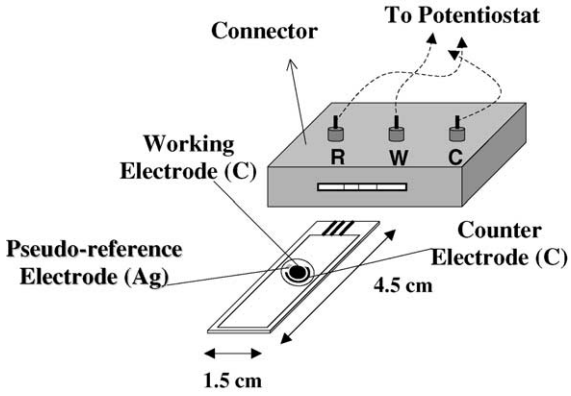

Fig. 1. Schematic diagram of the screen-printed carbon electrode and connector.