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Competitive electrochemical immunosensor for amyloid-beta 1-42 detection based on gold nanostructurated Screen-Printed Carbon Electrodes

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ABSTRACT

Alzheimer's disease is the most common form of dementia, characterized by the progressive accumulation of plaques with amyloid-beta peptide of 42 amino acids as one of the primary constituents. A disposable electrochemical immunosensor for the detection of amyloid-beta 1-42 is developed. Screen-Printed Carbon Electrodes nanostructured with gold nanoparticles generated "in situ" are used as the transducer surface. The immunosensing strategy consists in a competitive immunoassay: biotin-amyloid-beta 1-42 immobilized on the electrode surface and the analyte (amyloid-beta 1-42) compete for the anti-amyloid-beta 1-42 antibody. The electrochemical detection is carried out using an alkaline phosphatase labeled anti-rabbit IgG antibody. The analytical signal is based on the anodic stripping of enzymatically generated silver by cyclic voltammetry. The immunosensor achieved shows a low limit of detection (0.1 ng/mL) and a wide linear range (0.5–500 ng/mL).

1. Introduction

Today, over 35 million people worldwide currently live with dementia, and this number is expected to double by 2030 [1]. Alzheimer's disease (AD) represents 50–75% of all dementias [2]. The major histopathological hallmarks of AD are the progressive accumulation of plaques with amyloid-β (Aβ), and neurofibrillary tangles containing microtubuli-associated tau protein [3]. Aβ peptide comprising of 39–42 amino acids is the primary constituent of these plaques that hinder the communication between neurons causing cell death, cognitive dysfunction, and behavioral abnormalities [4,5]. Among these Aβ peptides, Aβ1-40 is the most abundant, but Aβ1-42 appears to be essential for initiating Aβ aggregation, and is considered central to the amyloid cascade hypothesis of AD [6]. This hypothesis postulates a central initiating role for Aβ1-42 in the subsequent pathological features of AD, such as neuroinflammation, synapse and neuritic dysfunction, tau hyperphosphorylation and development of intraneuronal neurofibrillary tangles. Due to their roles in the pathogenesis of AD, Aβ1-42 seems to be a more useful biomarker for AD than Aβ1-40 [6].

Cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain and biochemical changes in the brain are thought to be reflected in CSF [7].

Nowadays, there are few works described about devices for Aβ1-42 detection and these works are very recently (from 2010 to now) [8–13] There is one previously reported (2008), that, so far, is the only one based on Screen-Printed Electrodes [14]. But, for this sensor the Aβ peptides recognition is based on the saccharide–protein interactions, and the analytical signal is the oxidation peak of tyrosine that Aβ peptides have. So, this sensor cannot discriminate between Aβ1-40 and Aβ1-40.

In this work, the first electrochemical immunosensor based on Screen-Printed Electrode for Aβ1-42 detection is described. The biosensor consists of a competitive immunoassay carried out on a Screen-Printed Carbon Electrode nanostructured with gold nanoparticles. Concentration of antigen labeled, antibody and secondary labeled antibody are optimized and non-specific binding is also studied. Label used is alkaline phosphatase and a mixture of 3-indoxyl phosphate with silver ions (3-IP/Ag^+) is used as substrate. The analytical signal is based on the anodic stripping of enzymatically generated silver by cyclic voltammetry. The linear range of the immunosensor developed allow the diagnosis of

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid-beta; SPCE, Screen-Printed Carbon Electrode; anti-IgG-AP, anti-rabbit IgG antibody labeled with alkaline phosphatase; biotin-Aβ1-42, amyloid-beta 1-42 labeled with biotin; anti-Aβ1-42, amyloid-beta 1-42 monoclonal antibody recombinant rabbit IgG; NPAu, gold nanoparticles; B-AP, biotin conjugated to alkaline phosphatase.

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AD because, although the values are not well established, several authors consider 500 pg/mL as a optimum cut-off value to differentiate between patients with dementia and healthy patients [13,15–18].

2. Experimental

2.1. Apparatus and electrodes

SPCE gold nanostructuration is performed with a μStat 8000 potentiostat (DropSens, Spain) interfaced to a Pentium 4 2.4 GHz computer system and controlled by DropView 8400 1.0 software.

The voltammetric measurements are carried out using an ECO Chemie μAutolab type II potentiostat/galvanostat interfaced to a Pentium 4 2.4 GHz computer system and controlled by the Autolab GPES software version 4.9. All measurements are performed at room temperature.

Disposable Screen-Printed Carbon Electrodes (SPCEs) are purchased from DropSens (Spain). These electrodes incorporate a conventional three-electrode cell configuration, printed on ceramic substrates (3.4 cm × 1.0 cm). Both the working (disk-shaped 4 mm diameter) and counter electrodes are made of carbon inks, whereas the pseudoreference electrode and the electric contacts are made of silver. An insulating layer delimits the electrochemical cell (50 μL) and the electric contacts. The SPCEs are easily connected to the μStat 8000 potentiostat and to the μAutolab potentiostat through the specific DropSens connector (Spain) in each case.

2.2. Reagents and solutions

Tris(hydroxymethyl)aminomethane (Tris), magnesium nitrate, bovine serum albumin fraction V (BSA), β-casein from bovine milk (casein), streptavidin (molecular weight, 66 kDa) and biotin conjugated to alkaline phosphatase (B-AP; dimmer, four units of B per molecule of AP, molecular weight, 160 kDa) are purchased from Sigma (Spain). Standard gold (III) tetrachloro complex ($AuCl_4^-$), silver nitrate, hydrochloric acid (37%) and nitric acid (HNO_3) are obtained from Merck (Germany). Biosynth (Switzerland) supplied the 3-indoxyl phosphate disodium salt.

Aβ1-42 monoclonal antibody recombinant rabbit IgG (clone H31L21) specific to amino acids 707–713 is purchased from Life Technologies (Spain). Anti-rabbit IgG (whole molecule) labeled with alkaline phosphatase (anti-IgG-AP) is provided by Sigma (Spain). Aβ1-42 and Aβ1-42 labeled with biotin (Biotin-LC-β-Amyloid 1-42) are purchased from Anaspec (USA).

Ultrapure water obtained from a Millipore Direct-Q™ 5 purification system from Millipore Ibérica S.A. (Spain) is used throughout the work. All chemicals employed are of analytical reagent grade. Working solutions of streptavidin, Aβ1-42 monoclonal antibody, Aβ1-42 and Aβ1-42 labeled with biotin (biotin-Aβ1-42) are prepared in a 0.1 M Tris–HNO_3 pH 7.2 buffer (buffer 1). Working solutions of secondary alkaline phosphatase labeled antibody are prepared in a 0.1 M Tris–HNO_3 pH 7.2 buffer containing 2 mM $Mg(NO_3)_2$ (buffer 2). A solution containing 1.0 mM 3-IP and 0.4 mM silver nitrate is prepared daily in 0.1 M Tris–HNO_3 pH 9.8 buffer containing 20 mM $Mg(NO_3)_2$ (buffer 3), and store in opaque tubes at 4 °C. Casein and albumin lyophilized powder are reconstituted in buffer 1.

2.3. Procedures

2.3.1. SPCEs nanostructuration

Gold nanoparticles are generated "in situ" over SPCEs (SPCEs–NPAu) following a method previously reported by Martínez-Paredes et al. [19], using μStat 8000 potentiostat. The procedure consists in applying a constant current intensity of −100 μA for 240 s in an acidic solution of 0.1 mM $AuCl_4^-$. Then a potential of +0.1 V for 120 s is applied. Finally, the nanostructured electrodes are rinsed with water and are ready to use. NPAu generation is performed at room temperature. Using the μStat 8000 potentiostat, gold nanoparticles can be generated over eight different Screen-Printed Carbon Electrodes at the same time.

2.3.2. Evaluation of the analytical signal improvement using SPCEs–NPAu

The reaction streptavidin–biotin is used to evaluate the effect of the NPAus generated over the SPCE. A drop of 10 μL of 0.1 μM streptavidin [20] solution is placed on the nanostructurated surface of the SPCE solution and incubated overnight at 4 °C. The immobilization of the streptavidin on the electrode surface is achieved by physical adsorption. Then, the electrode is washed with buffer 1, and the free surface sites are blocked with 40 μL BSA solution (2%) during 30 min. The electrode is washed again using buffer 2, and a drop of 40 μL B-AP solution (0.1 nM) is dropped on the streptavidin modified electrode for an hour reaction. After a washing step with buffer 3, the enzymatic reaction is carried out dropping 40 μL of a mixture of 1.0 mM 3-IP/0.4 mM silver nitrate solution on the electrode. The enzymatically silver deposition catalyzed by alkaline phosphatase has been already reported [21]. AP works as the enzymatic label and a mixture of 3-IP with silver ions (Ag^+) as the substrate. AP hydrolyzes 3-IP resulting a indoxyl intermediate. This intermediate reduces the silver ions presents in solution resulting in metallic silver (Ag^0) and indigo blue (I) [21]. Thus, the silver enzymatically deposited on the electrode surface can be detected through the redissolution peak when an anodic stripping scan is carried out. After 20 min of enzymatic reaction, an anodic stripping cyclic voltammetric scan is recorded from 0.0 V to +0.4 V at a scan rate of 50 mV/s.

2.3.3. Immunosensor for the detection of Aβ1-42

The following procedure (Fig. 1) describes an optimized assay. The working area of SPCE–NPAu is coated with 10 μL of 0.1 μM streptavidin [20] solution and incubated overnight at 4 °C. After the overnight incubation step, the electrode is washed with buffer 1. Free surface sites of the streptavidin-modified SPCE–NPAu are blocked with 40 μL casein solution (2%) during 30 min. After another washing step with buffer 1, an aliquot of 40 μL of 300 ng/mL biotin-Aβ1-42 solution is dropped on the streptavidin modified electrode for an hour reaction. After a washing step with buffer 1, the sensing part of the immunosensor is completed. Then, 40 μL of a solution of Aβ1-42 and antibody anti-Aβ1-42 (0.5 μg/mL) is dropped for an hour to carry out the competitive reaction. The competition was established via the binding between analyte (Aβ1-42) and the biotin-Aβ1-42 previously immobilized in the electrode surface, for the limited binding sites of the anti-Aβ1-42. Finally, after a washing step with buffer 2, the immunosensor is incubated with 40 μL of an anti-IgG-AP (1:15,000) solution for 60 min and washed with buffer 3. The enzymatic reaction is performed as is explained in Section 2.3.2: placing a 40 μL aliquot of the 1.0 mM 3-IP/0.4 mM silver nitrate solution on the sensor, and after 20 min, recording an anodic stripping cyclic voltammetric scan from 0.0 V to +0.4 V at a scan rate of 50 mV/s. Buffers employed have been chosen because of the satisfactory results in immunosensors with similar procedure [22,23].

3. Results and discussion

3.1. Analytical signal improvement using SPCEs–NPAu

The use of this gold nanostructuration is based in works previously reported [24]. It is well know that gold nanostructured