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Modified screen printed electrode for development of a highly sensitive label-free impedimetric immunosensor to detect amyloid beta peptides

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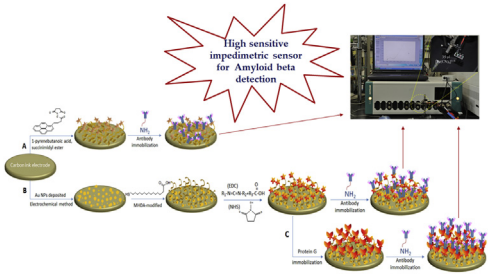
HIGHLIGHTS

- A label-free impedimetric immunoassay for amyloid beta was developed.
- Sensitivity enhanced by elaborate surface chemistry manipulation using SAM of AuNPs.
- Immobilized Protein G enhanced sensitivity by directing optimal antibody orientation.
- Lack of interference from high-abundant high-molecular weight BSA demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

Alzheimer's disease (AD) is a fatal neurodegenerative disease affecting approximately 26 million people world-wide, and the number is increasing as life expectancy increases. Since the only reliable diagnosis for the pathology is histochemical post-mortem examination, there is a rather urgent need for reliable, sensitive and quick detection techniques. Amyloid beta, being one of the established and widely accepted biomarkers of AD is a target biomolecule.

Herein, we present fabrication of a labelless impedimetric amyloid beta immunosensor on carbon DEP (disposable electrochemical printed) chip. Three types of amyloid β impedimetric immunosensors were fabricated in a systematic step-wise manner in order to understand the effects that each surface modification chemistry had on detection sensitivity. We found that compared to a bare electrode, surface modification through formation of SAM of AuNPs increased sensitivity by approximately three orders of magnitude (LoD from 2.04 μM to 2.65 nM). A further modification using protein G, which helps orientate antibodies to an optimum position for interaction with antigen, lowered the LoD further to 0.57 nM. We have demonstrated that the presence of one of the most abundance proteins in biological fluids, bovine serum albumin (BSA), did not interfere with the sensitivity of the sensor. Since the DEP chips are disposable and the detection platform label-free, the developed sensor is relatively fast and cheap. These methods could easily be applied for detection of other antigens, with selection of the detection platform based on the desired for sensitivity.

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1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disease affecting approximately 26 million people world-wide and the number is increasing as life expectancy increases [1]. However, diagnosis remains in the hands of medical doctors who can only at best propose 'probable Alzheimer's or dementia of the Alzheimer type' since there is no current test or procedure that is diagnostic. Although not unilaterally agreed upon, the progressive decline of patients with AD has been correlated with extracellular deposition of amyloid plagues, of which amyloid beta is the major constituent [2]. Amyloid beta (Aβ) has therefore become an important biomarker for the pathology. The main detection method is by enzyme-linked immunosorbent assay (ELISA) techniques, which are less flexible, costly, and labour-intensive [3,4]. The past decade or so has seen tremendous effort put into development of sensitive and selective detection techniques for this and other peptides/proteins. They include FRET-based assays [5]; surface Raman enhanced spectroscopy (SERS) [6]; and several electrochemical platforms [7,8].

Electrochemical impedance spectroscopy (EIS) recently has attracted much interest because it has some important advantages over number of electrochemical methods such as amperometry and potentiometry. With EIS, developed sensing platforms are (i) label-free with detection based on direct specific binding events, (ii) less destructive to the activities of biomolecule due to the small voltage excitation used during detection, (iii) a simple operation and very sensitive, with comparable detection limits to optical-based sensors [9–11]. EIS biosensors have been successfully employed for detection of various biomolecules and biological processes including DNA hybridization, at very low (femtomolar) detection limits [12]. Previously, we reported on an impedimetric immunosensor development using DEP chips, and demonstrated its selective detection using a model protein, chorionic gonadotropin hormone (hCG) (limit of detection (LoD) of 33 pg/mL) [13]. Lien and colleagues also modified DEP chips using a conducting co-polymer, polypyrrole-pyrolecarboxylic acid for hCG detection. The LoD was lowered by an order of magnitude, to 2.3 pg/mL [14]. Most recently, Rushworth and colleagues have reported on specific detection of oligomeric amyloid beta using biotylated peptide of prion protein as the recognition element. The authors have reported an impressive detection limit of 0.5 pM [15].

In this work, we fabricated a labelless EIS immunosensor for amyloid beta peptide, isoforms 40 and 42. We have used disposable electrochemical printed (DEP) chips, which have been used in development of various DNA- and immuno-biosensors, giving very good reproducibility [13,14,16]. We developed this sensor in a systematic step-wise fashion so that we could also better understand the effects of surface chemistry modification on sensor sensitivity. The developed sensors were very reproducible (coefficient of variation <8%). Although the immunosensor's sensitivity (LOD ~ 0.57 nM) is still lower than the recently reported prion-based sensor [15], it is a good proof-of-principle antibody-based EIS. With further improvement in surface chemistry modification, it offers much promise. Besides, since the antibody–antigen chemistry is well-understood and the fabrication relatively simple and rapid, this sensor can easily be adaptable for application to other antigens.

2. Experimental

2.1. Reagents

1-pyrenebutanoic acid, succinimidyl ester was supplied from Eugene, Oregon (USA). Chloroauric acid ($HAuCl_4$), Bovine serum albumin (BSA) and Dimethyl Sulfoxide Dehydrated (DMSO) were purchased from Sigma Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) hydrochloride was supplied by Dojindo (USA). N-hydroxysuccinimide (NHS) and 16-mercaptohexadecanoic acid (MHDA) were provided by Wako. Amyloid beta (Aβ) peptides (trifluoroacetate salt) were purchased from Peptide Institute Inc., (Osaka, Japan). N-terminal human monoclonal Aβ antibody (anti mAβ) was from Calbiochem (CA, USA). All other reagents used were of the analytical grade or the highest commercially available purity and used as supplied without further purification. All solutions were prepared with deionized water of resistivity no less than 18 MΩcm.

2.2. Electrodes

Commercial disposable electrochemical printed (DEP) chips were obtained from BioDevice Technology Ltd., Japan (http://www.biodevicetech.com). The chips were fabricated by screen-printing technology and designed as system with three electrodes containing carbon ink working, carbon ink counter and Ag/AgCl ink reference electrodes. The carbon ink contained 75% (w/w) graphite powder and 25% (w/w) mineral oil (Sigma). The surface area of the working electrode is 2.64 mm^2.

2.3. Instrumentation

An AutoLab PGSTAT 30 system (EcoChemie B.V., Ultrecht, The Netherlands) was used to perform electrochemical impedance spectroscopy measurements. The spectra was recorded in 0.1 M KCl solution containing 5 mM of $K_3[Fe(CN_6)]/K_4[Fe(CN_6)]$ within frequency range from 100 kHz to 50 mHz. An ac probe amplitude of 10 mV was applied to the system around the open circuit potential.

2.4. Gold nanoparticles fabrication

The carbon ink electrode of DEP chip was modified first by deposition of gold nanoparticles (AuNPs) on working electrode using both potential step voltammetry (PV) and cyclic voltammetry (CV). Tetrachloroauric acid ($HAuCl_4$) was diluted in 100 mM phosphate buffer solution (PBS) to a final concentration of 1 mM. Then 35 μL of the 1 mM $HAuCl_4$ solution was dropped onto DEP chip electrode surface covering all three electrodes (including Ag/AgCl, counter and working electrodes). With the PV method, −0.4 V was applied for different time periods: 5 s, 20 s and 90 s. With the CV method, −600 to +500 mV vs. Ag/AgCl were cycled for 5, 10 and 20 cycles at scan rate of 50 mV/s. Following deposition, the AuNPs-coated electrodes were washed several times in 10 mM PBS, pH 7.4 solution containing 0.05% Tween 20 followed by deionized water, and drying under nitrogen (N_2) stream. This AuNPs-coated electrode was then ready for immobilization of anti mAβ.

2.5. Electrode fabrication and sensor development

Immunosensors detect signals resulting from specific immunoreactions between antibodies immobilized on a transducer and the target antigens. In order to have good sensitivity for detection, the concentration of immobilized antibodies as well as their orientation on the transducer surface should be as optimal as possible in order to interact with as many target antigens as possible. Therefore, surface modification for best possible antibody immobilization is desirable. In our work, three methods were used to fabricate the immunosensor based on the physio-chemical moiety of carbon DEP chip (methods A, B and C). The schematic diagram of these methods is presented in Fig. 1. In method A,