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A label free electrochemical immunosensor for sensitive detection of porcine serum albumin as a marker for pork adulteration in raw meat

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ABSTRACT

A label free electrochemical immunosensor for sensitive detection of porcine serum albumin (PSA) is reported in this work. The immunosensor was constructed by first electrochemically reducing 4-carboxyphenyl diazonium salt, which had been electrochemically generated *in situ*, to a stable 4-carboxyphenyl layer on carbon nanofiber-modified screen printed electrode. Antibodies were covalently attached onto the electrode using carbodiimide chemistry between the carboxylic groups of the 4-carboxyphenyl layer and amine groups of the antibody. Using the strong affinities of serum albumins towards anions, the increase in cathodic peak current in anionic redox probe after immunocomplex formation with antibodies was used for PSA detection. The reported immunosensor demonstrated a linear range from 0.5 to 500 pg/mL for the measurement of PSA with detection limit of 0.5 pg/mL in buffer solution. Cross-reactivity studies have shown excellent specificity with satisfactory recovery of PSA in fresh meat samples without the need of sample dilution.

1. Introduction

Food allergies are becoming a worldwide concern as up to 220–250 million people may suffer from food allergy including children. The incidences of allergy that derive from food are increasing both in developed and developing nations and most notably amongst children (Pawankar, 2011). According to World Allergy Organization, food allergy has a deep impact on socio-economic status and affects the quality of life of patients, which can be life-threatening in extreme cases (Pawankar, 2011).

Serum albumins, proteins that are found in blood serum of vertebrate animals, have many roles and are extremely stable in both their sequence and structure. Serum albumins possess α-helical proteins with a number of disulfide bridges to conserve the structure. The molecule may undergo structure conformation easily in order to bind numerous types of ligands. Serum albumin is also an allergen linked to meat allergy and porcine serum albumin (PSA) is recognized as a significant allergen found in pork meats. So far, there has not been many studies and reports on pork allergy although beef allergy has been well investigated (Kim, Lee, Song, & Ahn, 2011). For instance, pork allergy study was carried out in cross-reactivity work to cat epithelial antigen, called pork-cat syndrome (Drouet & Sabbah, 1996) and cross-reactivity between pork kidney and pork and lamb gut antigens (Llátser, Polo, De La Hoz, & Guillaumet, 1998). Although the pork-cat syndrome has been established for more than two decades, it was not until recently it is considered as an allergy in the US (Posthumus et al., 2013).

Due to its cheaper cost than its meat counterpart (such as mutton and beef), pork is often incorporated in food products and has been identified as potential adulterant in raw meat. Furthermore, mixing pork or pork-associated products is an extremely sensitive issue in Jewish and Islamic laws since pork is not acceptable in Kosher and Halal food laws. Currently, several analytical techniques are available to identify pork in raw meat, such as enzyme-linked immunosorbent assay, electrophoresis on polyacrylamide gel, polymerase chain reaction, polymerase chain reaction-restriction fragment length polymorphism, random amplified polymorphic DNA-polymerase chain reaction and single nucleotide polymorphism (Giarettaa, Di Giuseppeb, Lipperta, Parenteb, & Marob, 2013). However, these methods are time-consuming, laboriously demanding and require skilled workers for handling instruments and preparing samples.

Biosensor, and in particular electrochemical immunosensors, are alternative detection tool that are highly feasible for on-site usage in the detection of PSA. (Ahmed, Hossain, & Tamiya, 2008; Ahmed, Nahar, Safavieh, & Zourob, 2014; Nahar et al., 2014;

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Safavieh, Ahmed, Ng, & Zourob, 2014; Safavieh et al., 2014; Tlili et al., 2013) Moreover, electrochemical immunosensors are highly sensitive and selective due to the high affinity of antibodies towards their target analytes (Lim & Ahmed, 2015). Especially with the rapid development in the area of nanotechnology, electrochemical immunosensors have lately gained an intense amount of attention due to their ease of miniaturization, economical cost and compatibility with microfabrication technology. Numerous research works have demonstrated that incorporation of nanomaterials in immunosensor fabrication remarkably improved its sensitivity and specificity leading to detection of target analytes down to the picogram level.

In this work, a 4-carboxyphenyl layer was electrografted on a surface of carbon nanofiber-modified screen-printed carbon electrodes for covalent attachment of antibodies by carbodiimide chemistry for the detection of PSA (Fig. 1). Label free detection of PSA was then carried out in potassium ferrocyanide/ferricyanide solution, employing the fact that PSA has strong affinity for anions while the large surface area of carbon nanofiber increased the antibody immobilization capacity and electronic conductivity thus improved the sensing capacity of the electrodes.

2. Materials and methods

2.1. Reagents and materials

Albumins from pig, rabbit, sheep, bovine and egg white, *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide (NHS), 4-aminobenzoic acid, hydrochloric acid, potassium ferrocyanide, potassium ferricyanide and hexaammineruthenium(III) chloride (RuHex) were purchased from Sigma–Alrich (MO, USA). Pig serum albumin antibody was obtained from Bethyl Laboratories Inc., (Montgomery, USA). Other reagents used were sodium phosphate dibasic, sodium dihydrogen phosphate dehydrate, potassium chloride, monosodium phosphate (Wako Chemicals, Japan) and sodium nitrite (Harris Reagent, UK).

2.2. Instrumentations

Electrochemical analyses were carried out using an Autolab PGSTAT302N (Eco Chemie, The Netherlands) potentiostat/galvanostat that was linked to a desktop computer and used together with NOVA software version 1.6. Disposable screen-printed electrodes comprised of a conventional three-electrode system were acquired from Dropsens (Oviedo, Spain). Working electrodes used were made up of (i) carbon (SPCE) and (ii) carbon nanofiber-modified carbon (CNF SPE), (iii) cadmium selenium (CdSe), (iv) graphene and (v) single-walled carbon nanotube (SWCNT); counter electrode was made up of carbon; and the reference electrode was made of silver.

2.3. Electrochemical immunosensor design

2.3.1. Preparation of functionalized carbon nanofiber electrode

In the preparation of functionalized CNF SPE, 2 mM sodium nitrite solution was firstly mixed with 2 mM 4-aminobenzoic acid in 0.5 M HCl to obtain 4-carboxyphenyl diazonium salt and was stirred at room temperature for 5 min. Electrografted working electrodes were obtained by electrochemically reducing the *in situ* generated diazonium cations by applying cyclic voltamme-

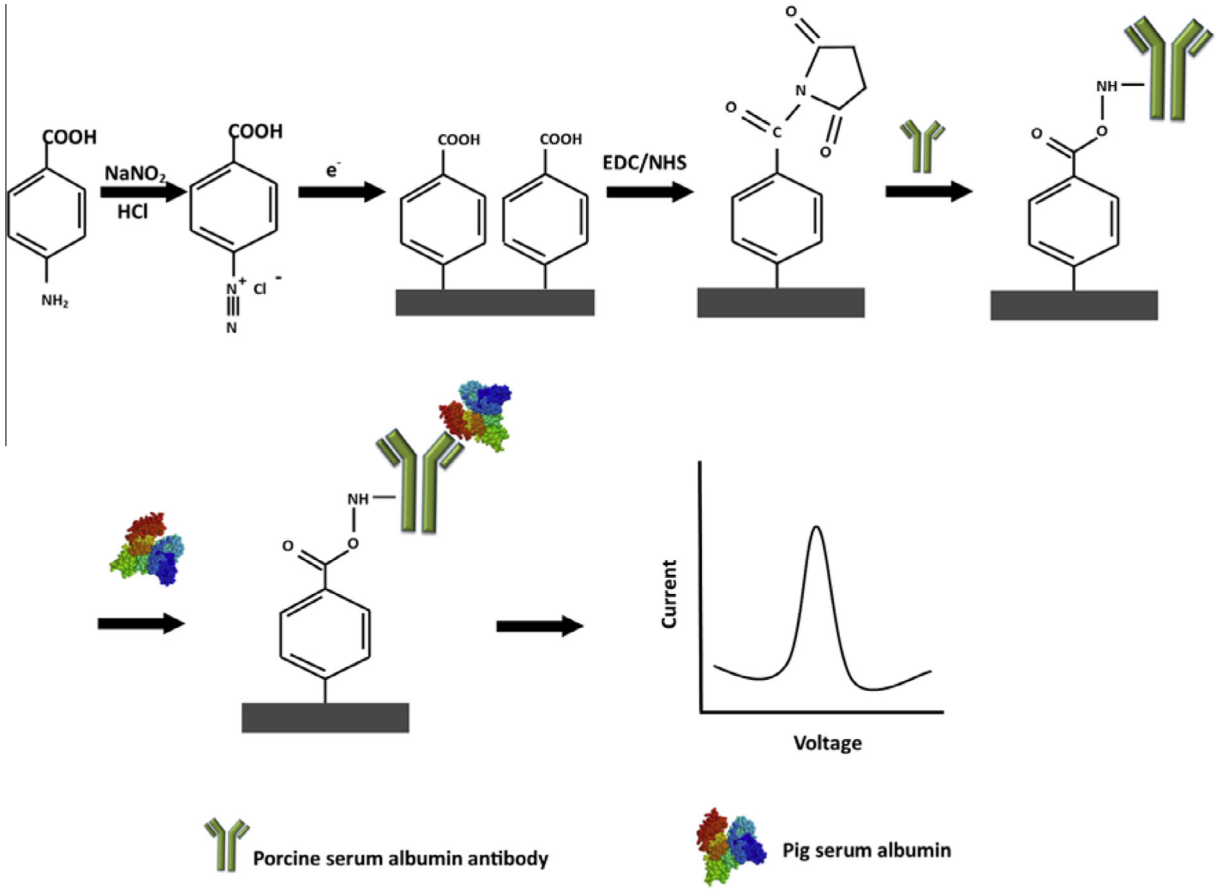

Fig. 1. Modification and immobilization protocol for the fabrication of electrochemical immunonsensor.