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A carbon nanofiber-based label free immunosensor for high sensitive detection of recombinant bovine somatotropin

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ABSTRACT

A carbon nanofiber-based label free electrochemical immunosensor for sensitive detection of recombinant bovine somatotropin (rbST) was developed. In this immunosensor design, a mild site-directed antibody immobilization *via* interaction of boronic acid and oligosaccharide moiety found on Fc region of an antibody was performed to preserve the biological activity of antibody and improve the sensor's sensitivity. Electrochemical characterization of the immunosensor fabrication was carried out by differential pulse voltammetry (DPV) in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ probe. A comparison study between different transducer platforms showed carbon nanofiber gave higher current signal response than single-walled carbon nanotube. In this work, calibration curve was obtained from the decrease of DPV peak current of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ after immunocomplexed was formed. A linear relationship between DPV current change signal response and rbST concentrations from 1 pg/mL to 10 ng/mL (correlation coefficient of 0.9721) was achieved with detection limit of 1 pg/mL. Our developed immunosensor demonstrated high selectivity in cross-reactivity studies and a good percentage recovery in spiked bovine serum sample.

1. Introduction

Somatotropin, also known as growth hormone, is a peptide hormone synthesized and secreted by anterior pituitary glands of humans and animals that function to stimulate growth and development (Dervilly-Pinel et al., 2014). In dairy cows, somatotropin also enhances milk production and carcass composition but isolation and purification of bovine somatotropin from slaughtered cows was inadequate and not deemed cost-productive for commercial use. In the early 1980s the breakthrough of biotechnology had advanced recombinant DNA technology that resulted in mass production of recombinant bovine somatotropin (rbST). Despite the use of rbST being legal in several countries including United States of America, many countries (such as the European Union, Canada, and Australia) still ban its administration to dairy cows due to concerns regarding the welfare of animals and most importantly safe consumption of milk by human. The primary concern regarding safety of milk being treated by rbST for human consumption is the elevation of hormones such as bovine IGF-1 and its linked to certain tumors. As IGF-1 cannot be destroyed by heat treatment process, the impact it has in human digestive tract still remain unknown (Dervilly-Pinel et al., 2014). This therefore necessitates for a sensitive and reliable detection method to alleviate misuse of rbST.

Electrochemical immunosensor, which is based on antibody–antigen binding, has received widespread recognition and interest due to its cost, simplicity, sensitivity, simple construction and feasibility of miniaturization (Thévenot et al., 1999; Ahmed et al., 2008, 2012, 2014a, 2014b; Saito et al., 2008). To increase the possibility of antibody–antigen binding and hence the sensitivity of an immunosensor, antibody can be immobilized at a specific site rather than at random orientation. Immunoglobulin, being a glycoprotein, possesses a branched oligosaccharide N-linked to asparagine 297 found in the Fc region. Because oligosaccharide moiety is facing away from the paratope (antigen–binding sites), this sugar moiety can be used for site-specific antibody immobilization without affecting antibody–antigen binding reaction (Sutton and Phillips, 1983). Ho et al. (2010) reported a simple method using boronic acid to form reversible cyclic covalent complexes with adjacent 1,2 or 1,3 diols (Springsteen and Wang, 2002) for immobilization of anti-biotin antibodies. The simplicity of this technique and mild processes involved became the prime motivation for our immunosensor work strategy.

In the field of biosensor, undoubtedly nanotechnology plays a significant role in its development towards enhanced signal

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response and ultimately lower detection limits. Since the discovery of carbon nanotube (CNT) and carbon nanofiber (CNF), numerous studies were published in literature focusing on their preparation, properties and biosensing applications. CNT can be conceptualized as a sheet of graphene rolled up into a cylindrical tube with diameters ranging from fractions of nanometers to tens of nanometers and lengths from a few micrometers up to several centimeters (Trojanowicz, 2006; Dasgupta et al., 2011). Single-walled CNT (SWCNT) comprises of a single layer of graphene sheet forming a cylinder whereas multi-walled CNT (MWCNT) consists of multiple layers of CNT distanced at 0.034 nm apart from each layer (Loos, 2015). CNF comprises of graphene layers that are arranged as stacked cones, cups or plates in cylindrical shape with lengths measured in micrometers and diameters between tens of nanometers up to 200 nm (Vamvakaki et al., 2006). Although the mechanical strength and electric properties are similar to CNT, it is the unique feature of CNF in that the whole surface area can be utilized for antibody immobilization makes CNF an ideal choice for electrochemical immunosensor fabrication. Incorporation of screen-printed electrode (SPE) technology with electrochemical system has paved ways for more applications in the areas of food, environmental, industrial and medical analyses. SPE are preferred due to the advantages of disposability, simplicity and high consistency in analysis performance (Li et al., 2012). Another advantage of SPE was highlighted in a work by Minhaz's group (Lim et al., 2014) that used inert carbon as their counter electrode, instead of platinum usually used in conventional electrode, and acidic solution as their electrolyte. In acidic medium, platinum dissolves into the working solution and this may affect the activity of their system.

In this work, we reported a simple label-free method for sensitive detection of rbST based on site-directed immobilization of antibodies. CNF SPE was first modified with carboxyphenyl film and then activated by carbodiimide/succinimide (EDC/NHS). A monolayer of 4-aminophenylboronic acid coating was then fabricated onto the electrode to allow orientation of antibody *via* bonding of boronic acid-saccharide of oligosaccharide moiety located on the Fc region of antibody.

2. Experimental

2.1. Reagents and materials

Anti-bovine growth hormone (anti-BGH) antibody and recombinant bovine growth hormone (rbST) were purchased from Abcam (USA). Bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 4-aminobenzoic acid (ABA), hydrochloric acid, Na_2HPO_4, $NaH_2PO_42H_2O$ and KH_2PO_4, potassium ferrocyanide, potassium ferricyanide, lysozyme, adult bovine serum (Sigma-Aldrich, USA), sodium nitride (Phillips Harris Reagent, UK), 3-aminophenylboronic acid (APBA) (Santa Cruz Biotechnology, USA), and hCG (Abdserotec, UK). All solutions were prepared and diluted using double distilled water throughout this work.

2.2. Instrumentation

Electrochemical measurement of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were analyzed using an Autolab PGSTAT101 III (Metrohm, Netherlands) that works in conjunction with its Nova 1.10 software. The disposable carbon, SWCNT-modified, graphene-modified and CNF-modified carbon working SPE were obtained from DropSens (Spain) that made up of a carbon counter-electrode and silver reference electrode. Nanophotometer P360 (Implen, Germany) was used for UV measurement. All measurements were made at room temperature (21 ± 1 °C).

2.3. Preparation of aminophenylboronic electrode (APBA/CNF-SPE)

Aminophenylboronic-modified electrode was prepared based on work by Ho et al. (2010) with slight modification. In an iced water bath, 20 mg of ABA were dissolved in 2 mL of 1 M HCl. 2 mM sodium nitrite aqueous solution was then added in a drop-wise manner with constant stirring to produce the diazonium salt. The solution was allowed to stir for 5 min. Electrochemical functionalization of the working CNF electrode was carried out by placing 40 µL of the solution onto the SPE using one CV cycle ranging between 0.0 and −1.0 V at a scan rate of 200 mV/s. Upon functionalization of SPE, the electrode was rinsed sequentially with distilled water and methanol and dried at room temperature. 10 µL of an EDC/NHS (0.1 M each) solution dissolved in DMSO was dropped onto the modified electrode and allowed to react at room temperature for 1 h. The electrode was washed with distilled water and methanol and then dried. 10 µL of 50 mM APBA was deposited onto the electrode for 3 h producing aminophenylboronic electrode (APBA/CNF-SPE). The resulting modified electrode was washed with distilled water and methanol, and dried before being used for site-specific antibody immobilization.

2.4. Site-directed immobilization of anti-BGH antibody

50 µL of a 10 µg/mL of anti-BGH solution in PBS (10 mM, pH 7.4) was placed onto the APBA/CNF-SPE and incubated overnight at 4 °C. The electrode was then washed with PBS solution (10 mM, pH 7.4). To prevent non-specific adsorption, 50 µL of blocking solution (0.1% BSA in PBS solution of pH 7.4) was deposited and left

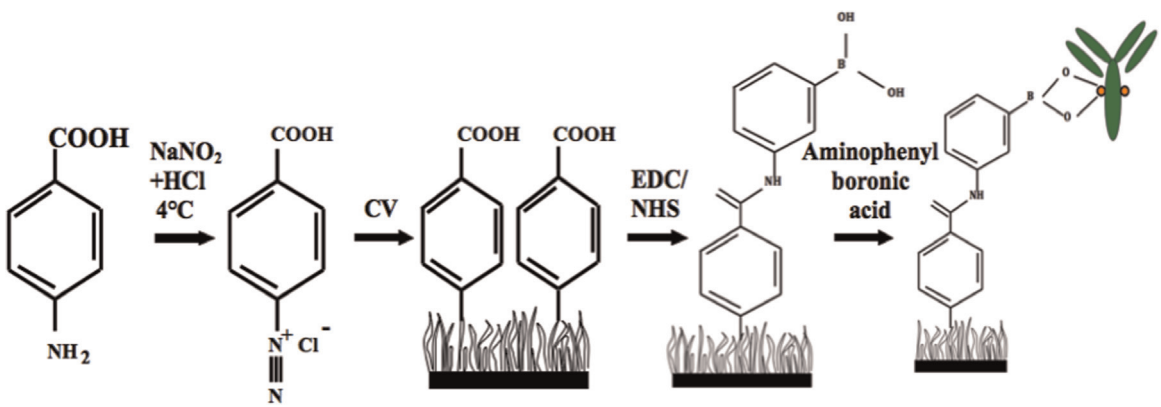

Fig. 1. Schematic representation of the fabrication of Ab/APBA/CNF-SPE electrochemical immunosensor.