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Labeless AC impedimetric antibody-based sensors with pg ml^{-1} sensitivities for point-of-care biomedical applications

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ABSTRACT

This paper describes the development and characterisation of labeless immunosensors for (a) the cardiac drug digoxin and (b) bovine serum albumin (BSA). Commercial screen-printed carbon electrodes were used as the basis for the sensors. Two methods were used to immobilise antibodies at the electrode surface. Aniline was electropolymerised onto these electrodes to form a thin planar film of conductive polyaniline; the polyaniline film was then utilised as a substrate to immobilise biotinylated anti-digoxin using a classical avidin-biotin affinity approach. As an alternative approach, poly(1,2-diaminobenzene) was electrodeposited onto the carbon electrodes and this modified surface was then sonochemically ablated to form an array of micropores. A second electropolymerisation step was then used to co-deposit conductive polyaniline along with antibodies for BSA within these pores to produce a microarray of polyaniline protrusions with diameters of several μm, containing entrapped anti-BSA.

The resulting antibody grafted electrodes were interrogated using an AC impedance protocol before and following exposure to digoxin or BSA solutions, along with control samples containing a non-specific IgG antibody. The impedance characteristics of both types of electrode were changed by increasing concentrations of antigen up to a saturation level. Calibration curves were obtained by subtraction of the non-specific response from the specific response, thereby eliminating the effects of non-specific adsorption of antigen. Both the use of microelectrode arrays and affinity binding protocols showed large enhancements in sensitivity over planar electrodes containing entrapped antibodies and gave similar sensitivities to our other published work using affinity-based planar electrodes. Detection limits were in the order of 0.1 ng ml^{-1} for digoxin and 1.5 ng ml^{-1} for BSA.

1. Introduction

The principle of immunoassays was first established in 1959 (Yalow and Berson, 1959) and their work led to the development of the widely used radioimmunoassay to determine insulin-binding antibodies in human serum, using samples obtained from subjects that had been treated with insulin. Later, within unconnected work (Clark and Lyons, 1962), the concept of a biosensor was pioneered. These workers exploited the selectivity of enzymes for analytical purposes via a methodology which involved immobilising enzymes on the surface of electrochemical sensors and measuring the oxygen consumption by the glucose oxidase enzymatically catalysed oxidation of glucose. This basic idea has remained virtually unchanged since the original design, although the field has undergone continual technological developments over the last 40 years.

The incorporation of antibodies into conducting polymer films was first reported (John et al., 1991) in 1991. Pyrrole was galvanostatically polymerised onto a platinum wire substrate from a solution which contained anti-human serum albumin (anti-HSA). The antibody was incorporated into the polypyrrole film and the polypyrrole anti-HSA electrode found to give a specific electrochemical response to HSA. Since this is early work, there has been burgeoning interest in the development of electrochemical immunosensors — as detailed in several recent reviews (Rodriguez-Mozaz et al., 2006; Diaz-Gonzalez et al., 2005; Cosnier, 2005).

Antibody–antigen interactions are complex by their very nature and it is thought necessary that the affinity reaction be minimally perturbed by the fabrication procedure to give reproducible

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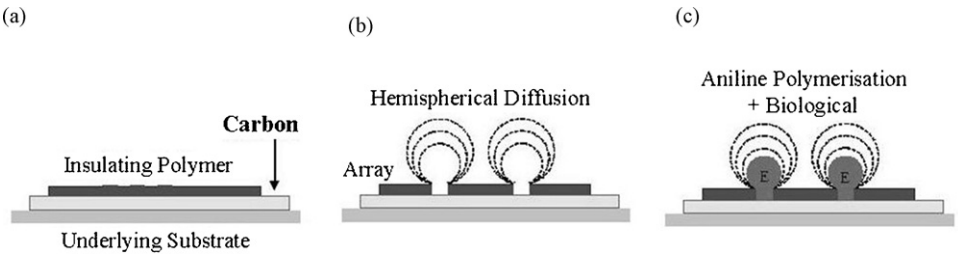

Fig. 1. Formation of polyaniline microarrays: (a) deposition of insulating layer, (b) sonochemical formation of pores and (c) polymerisation of aniline.

response characteristics. We have previously shown that up to 2–3 μg antibodies for BSA and digoxin may be successfully incorporated into conducting polymer films by entrapment in a growing polypyrrole film with no detrimental effect to antibody activity (Grant et al., 2003). Electrochemical interrogation of these films demonstrated selective interactions with the target antigens. Further work utilised an AC impedance protocol (Grant et al., 2005) as the method of interrogation for these films and led to the development of immunosensors for digoxin and bovine serum albumin. Later work by our group utilised polyaniline coated screen-printed planar carbon electrodes as substrates for immobilisation of antibodies utilising the classical avidin-biotin interaction. This enabled the construction of immunosensors for the fluoroquinolone antibody ciprofloxacin (Garifallou et al., 2007) and myelin basic protein—a marker for conditions such as stroke and multiple sclerosis (Tsekenis et al., 2008).

Our group has also pioneered the development of sonochemically fabricated microarrays of conductive polymers (Higson, 1996), the schematic for the formation of which is shown within Fig. 1. Poly(1,2-diaminobenzene) can be electrodeposited on a variety of conductive surfaces to form an insulating layer[15]. We have utilised commercial screen-printed three electrode strips as the basis for these sensors. The working electrodes are initially coated with a thin film (50–70 nm thickness) of an insulating polymer formed by the electrochemical deposition of 1,2-diaminobenzene (Myler et al., 1997). An advantage of this process is that it is self-limiting, making it highly reproducible. Sonochemical ablation is then used to ablate or "drill" holes in this insulating material with diameters of 0.1 to several microns and a density of up to 120,000 pores cm^{-2}. We have used these micropore arrays for the detection of aqueous chlorine (Davis et al., 2007). The arrays may be used as substrates for further electropolymerisation reactions, generating arrays of conducting polyaniline protrusions, consisting of just the polymer or alternatively containing entrapped biological species (Barton et al., 2004). Previous work within our group has utilised these microarrays containing entrapped enzymes for the amperometric detection of glucose (Barton et al., 2004; Myler et al., 2004), alcohol (Myler et al., 2005). and a range of organophosphate pesticides (Pritchard et al., 2004; Law and Higson, 2005) with extreme sensitivity (10^{-17} M).

One difficulty often encountered when using sensors for practical analytical applications is that the species being detected can in some situations be present only at very low concentration while being contained within a complex biological system such as blood. This means that any sensor must display high sensitivities and also low non-specific adsorption of possible foulants or interferents. As can be seen from previous work using enzymes, use of microelectrodes rather than planar electrodes can lead to extremely high sensitivities (Pritchard et al., 2004; Law and Higson, 2005). Attempts therefore were made within this study to determine whether the use of microelectrodes rather than planar electrodes within immunosensors similar to those previously reported (Grant et al., 2003, 2005) would lead to sensitivity enhancements. Microelectrode arrays containing entrapped anti-BSA were constructed and their performance compared with our previous work on planar, entrapped sensors.

A disadvantage associated with the entrapment method used within much of our previous work for antibody immobilisation is that the antigen will often be too bulky to diffuse through the polymer matrix and so only antibodies located at the surface of the polymer film or microelectrode — and suitably orientated, will be available for antigen binding. Other work comparing monolayers of randomly and specifically orientated antibody fragments (Bonroy et al., 2006) showed that immunosensor responses typically double when the fragment is specifically orientated.

Digoxin (Supplementary data, Fig. S2) is a cardiac drug, widely used in the treatment of various heart conditions such as atrial fibrillation and atrial flutter, with a narrow therapeutic range of 0.8–2.0 ng ml^{-1} (Terra et al., 1999). Previous work by our group demonstrated the construction of an immunosensor for digoxin, however, these sensors were only capable of detecting the antigen in the mg–μg ml^{-1} ranges (Grant et al., 2003). The anti-digoxin antibodies used in this early study were entrapped within a planar, electropolymerised film. We therefore within this work also compare results obtained using an affinity method to graft antibodies to the surface of the film with those obtained previously by entrapment (Grant et al., 2003) in an attempt to improve sensitivity.

The focus of this paper is not just to describe the development of particular sensors — but rather to compare the behaviour of sensors fabricated using a variety of methods, namely our earlier work on entrapment in planar polymer films with affinity grafted planar films and also entrapment of the antibodies within conductive polymer protrusions. As will be demonstrated, both methods lead to an enhancement in sensitivity.

2. Experimental

2.1. Materials and equipment

Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, sodium chloride and hydrochloric acid were obtained from BDH (Poole, Dorset, UK). Aniline, polyclonal human anti-IgG (AIgG), biotin 3-sulfo-N-hydroxysuccinimide, the biotinylation kit (part no. BK101), neutravidin, human serum albumin (HSA), BSA, anti-bovine serum albumin (ABSA, developed in rabbit), digoxin, anti-digoxin (developed in rabbit-whole antiserum), sodium acetate, acetic acid, sodium perchlorate, potassium ferrocyanide and potassium ferricyanide were obtained from Sigma–Aldrich, Gillingham, Dorset, UK. All water used was obtained from a Purelab UHQ Deioniser (Elga, High Wycombe, UK). Commercial screen-printed carbon electrodes (Supplementary data, Fig. S1) containing carbon working and counter electrodes and an Ag/AgCl reference electrode were obtained from Microarray Ltd., Manchester, UK. The surface area of the working electrode