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Electrochemical biosensing based on universal affinity biocomposite platforms

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Abstract

Rigid conducting biocomposites are versatile and effective transducing materials for the construction of a wide range of amperometric biosensors such as immunosensors, genosensors and enzymosensors, particularly if the transducer is bulk-modified with universal affinity biomolecules.

The strept(avidin)–graphite–epoxy biocomposite could be considered as an universal immobilization platform whereon biotinylated DNAs, oligonucleotides, enzymes or antibodies can be captured by means of the highly affinity (strept)avidin–biotin reaction. Universal affinity biocomposite-based biosensors offer many potential advantages compared to more traditional electrochemical biosensors commonly based on a biologically surface-modified transducer. The integration of many materials into one matrix is their main advantage. As biological bulk-modified materials, the conducting biocomposites act not only as transducers, but also as reservoir for the biomaterial. After its use, the electrode surface can be renewed by a simple polishing procedure, establishing a clear advantage of these approaches relative to classical biosensors and other common biological assays. Moreover, the same material is useful for the analysis of many molecules whose determinations are based on genetic, enzymatic or immunological reactions. The different strategies for electrochemical genosensing, immunosensing and enzymosensing, all of them being dependent on the presence of a redox enzyme marker for the generation of the electrochemical signal, based on this universal affinity biocomposite platform are all presented and discussed.

Keywords: Graphite–epoxy biocomposite; Protein G; (Strept)avidin; Electrochemical biosensing

1. Introduction

Deposition of biological macromolecules with controlled spatial resolution without any loss in their activity is the subject of increasing research efforts owing to the potential application in the field of electrochemical biosensors, biochips and bioreactors (Song and Vo-Dinh, 2004; Oh et al., 2004).

One of the most valuable strategies for the effective immobilization of biomaterial on different substrates is based on the avidin-biotin affinity reaction (Luppa et al., 2001; Song and Vo-Dinh, 2004). Nowadays, the knowledge about this interaction has advanced significantly and offers an extremely versatile tool. The avidin–biotin reaction as an immobilization strategy for biomolecules presents a variety of specific advantages over other single point immobilization techniques. In particular, the extremely specific and high affinity interaction between biotin and the glycoprotein avidin (association constant, $K_a = 10^{15}$ M) leads to strong associations similar to the formation of a covalent bonding. This interaction is highly resistant to a wide range of chemical (detergents, protein denaturants), pH range variations and high temperatures (Jones and Kurzban, 1995). In addition, the avidin–biotin based-immobilization method maintains the biological activity of the biomolecule being immobilized more successfully than other commonly used methods (Darain et al., 2003; Limoges et al., 2003; Da Silva et al., 2004). Much progress has been done in the modification of biomolecules with biotin. A wide range of macromolecules including proteins – both enzymes and antibodies – (Snejdarkova et al., 1993;

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Hoshi et al., 1995; Amounas et al., 2000) polysaccharides and nucleic acids or short oligonucleotides can be readily linked to biotin without serious effects on their biological, chemical or physical properties. As such, avidin should be considered as a universal affinity molecule capable to attach different biotinylated biomolecules.

Numerous coupling strategies have been specially developed for the immobilization of antibodies on different surfaces through the formation of defined linkages. However, by using the common coupling strategies it is not possible to ensure totally free specific binding sites of the immobilized antibody. Some spatial orientations on the surface may prohibit the formation of an antibody–antigen complex. Several approaches have been developed in order to achieve an improved antibody orientation, which leads to a better binding capacity. One of them is based on avidin–biotin interaction. Another one is based on the antibody bonding through Fc fragment to protein A or G (Compton et al., 1989; Lu et al., 1996; Zatta, 1996; Reinecke and Scheper, 1997; Valat et al., 2000; Yakovleva et al., 2003; Oh et al., 2004). These immobilization strategies allow the binding-sites of the antibodies to be oriented away from the solid phase. As a difference with avidin, protein G is able to link the Fc region of many immunoglobulins (Akerstrom et al., 1985; Janis and Regnier, 1989; Larsson, 1990) thus in this case it is not necessary to have the antibody modified with biotin. As an antecedent, we have previously demonstrated the utility of protein A biocomposite (ProtA–GEB) for the universal attachment of antibodies with different specificities (Zacco et al., 2004).

A new universal affinity and renewable transducing material for electrochemical biosensing is reported. This new transducing platform is based on a graphite–epoxy biocomposite prepared by bulk-modification with avidin (Av–GEB).

The utility of Av–GEB as a universal affinity platform for electrochemical enzymosensing and biosensing of DNA was demonstrated by means of the immobilization of enzymes and biotinylated ODNs (oligonucleotides) on the Av–GEB transducer, respectively. The universal attachment of antibodies onto Av–GEB transducer was performed by both (i) the direct immobilization of biotinylated antibodies and (ii) the previous immobilization of biotinylated protein G as a bridge whereon non-biotinylated antibodies can be easily attached.

Av–GEB surface was characterized not only by electrochemical techniques but also by optical techniques such as fluorescence microscopy. Response parameters as well as ease of preparation, robustness, sensitivity, surface regeneration, costs, and transfer to mass production of these different approaches are discussed.

2. Materials and instrumentation

2.1. Chemicals and solutions

The graphite–epoxy composite and biocomposites were prepared using graphite powder with a particle size of 50 μm (BDH, UK) and Epo-Tek H77 (epoxy resin) and hardener (both from Epoxy Technology, USA). The Av–GEB biocomposite was prepared with Avidin coming from Sigma (product number A9275). The immunological reagents such as protein G (catalogue number P 4589), biotinylated protein G (product number P 8045) rabbit IgG (RIgG), anti-rabbit IgG Fab fragment peroxidase conjugate (anti-RIgG/HRP) (product number A 6667), came from Sigma.

Glucose oxidase–biotin amidocaproyl labeled from *Aspergillus niger* (biotin–Gox) (product number G7779), Peroxidase–biotinamidocaproyl conjugate (product number P9568) and biotin-4-fluorescein (product number B9431) were also purchased from Sigma. Both enzyme (HRP, 1.11.1.7) conjugates used as electrochemical labels – i.e. anti-digoxigenin horseradish peroxidase (anti-Dig–HRP) and the streptavidin–horseradish peroxidase – were purchased from Roche (Germany).

All oligonucleotide stock solutions were prepared with sterilized and deionized water and stored at a temperature of −20 °C until required.

The DNA oligomers were obtained from TIB MOLBIOL (Germany). The oligonucleotide sequences specific for the methicillin resistant *Staphylococcus aureus* (mecA gene), used for the electrochemical genosensing were:

- *mec*A capture probe, biotin-3′-GTC GTT TAC CCT TGA GAT TA-5′;
- *mec*A target related with methicillan resistance *S. aureus* (MRSA), 5′-CAG CAA ATG GGA ACT CTA ATG GAG ATT TTT CCA AAC AAA ATA TAG ATA TT-3;
- *mec*A digoxigenin labeled probe, 3′-GGT TTG TTT TAT ATC TAT AA-5′-Dig;
- *mec*A digoxigenin labeled (mismatch) probe, 3′-GGT TTG T**C**T TAT A**G**C TAT AA-5′-Dig.

Tween 20, hydroquinone, benzoquinone, D-(+)-glucose monohydrate, bovine serum albumin (BSA), sodium dodecyl sulphate (SDS), Tris–HCl, EDTA were purchased from Sigma.

Trisodium citrate, NaCl and hydrogen peroxide were purchased from Merck (Germany). All other reagents were of the highest grade available.

Aqueous solutions were prepared with doubly distilled water. The compositions of these solutions for immuno and enzymosensing were: (i) blocking buffer (0.1 M Tris–HCl, 1.5 M NaCl, 2% (w/v) BSA, 0.1% (w/v) Tween 20, 5 mM EDTA), this solution was prepared at both pH 5 and 7.5; (ii) washing solution (0.1 M Tris–HCl, 1.5 M NaCl, 0.1% (w/v) Tween 20), this solution was prepared at both pH 5 and 7.5. All the immunological reagents were prepared in blocking buffer. The compositions of the solutions for electrochemical genosensing were: (i) hybridization solution (10× SSC, 2× Denhardt's, 200 μg/ml chloroform extracted salmon testes DNA, coming from Sigma); (ii) blocking solution (1× PBS, 2% (w/v) BSA, 0.1% Tween 20, 5 mM EDTA, pH 7.5); (iii) post-enzyme labeling wash solution (10 mM sodium