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Reagentless biosensing using electrochemical impedance spectroscopy

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Abstract

The use of electrochemical impedance spectroscopy (EIS) and the conducting polymer, poly (pyrrole), as an integrated recognition and transduction system for reagentless biosensor systems was demonstrated with two different systems. The first system being an immunoassay for detection of luteinising hormone (LH) with the antibody being entrapped with in the poly (pyrrole) matrix and the second, a construct for DNA hybridisation discrimination able to differentiate single- and double-stranded DNA based on the interaction of the DNA with poly (pyrrole). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Biosensor; Immunoassay; DNA; Poly (pyrrole); Reagentless

1. Introduction

Traditional enzyme electrode systems invoke a 'shuttle' mechanism, whereby reactions at the active site, following the binding of the substrate, create diffusing electron carrier species that migrate to the electrode where they are detected. A reagentless system where the recognition element and the transduction component are integrated at a single interface on the electrode surface offers a number of advantages including speed of assay and deskilled analysis. Therefore, a number of approaches have to be developed to immobilise the biological recognition element and any required mediators at a functionalised surface while retaining efficient electron transfer [1]. While enzyme-based reagentless systems have been developed, affinity based systems, e.g. antibody/antigen, have proven to be more difficult to realise because, unlike enzymes, no reaction cascade occurs following binding. Two-electrode electrochemical impedance spectroscopy (EIS) may overcome this problem since it allows visualisation of the changes in the charge transfer process within the polymer after the affinity interaction.

2. Experimental

Two sensor systems were developed based on poly (pyrrole), one detecting luteinising hormone (LH) using antibodies entrapped in a polymer film and the second discriminating double- and single-stranded DNA using poly (pyrrole) as reporter. The former system was created by mixing 0.25 mg/ml of anti-LH with pyrrole monomer to give a final pyrrole concentration of 0.5 M. This was electropolymerised onto the surface of an interdigitated electrode (IDE) using cylic voltammetry between −0.9 and +0.9 V vs. Ag/AgCl with a Pt counter electrode; the film was deposited over 15 cycles. Following film deposition, the polymer was polarised at −0.1 V for 1 min to fully oxidise the film. EIS measurements were then performed using a frequency sweep between 5 Hz and 13 MHz in phosphate buffer. The electrode was then exposed to the appropriate ligand (e.g. LH) and cycled through a single redox between −0.1 and −0.9 V and the film was then probed using EIS.

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DNA sensor surfaces were produced by absorbing 10mer oligonucleotide sequence onto the electrode surface, either single-stranded or pre-hybridised with the complementary strand. The electrode was then placed into pyrrole monomer solution in phosphate buffer and cycled between −0.9 and +0.9 V for five cycles. The electrode was then examined in deionised water using EIS at frequencies between 5 Hz and 13 MHz.

3. Results and discussion

Bode plots of EIS measurements of a poly (pyrrole) film containing an antibody or any other biomolecule showed a dominant peak in the phase angle at low frequencies with a second peak developing at high frequencies. It is believed that the low frequency peak represents polaronic conduction while the second peak represents electronic conduction. The peak profile was reproducible with independent films produced using the same counter-ion showing little or no difference in terms of position and magnitude. Exposure of an anti-LH-loaded film directly to LH did not result in a change in the EIS spectra, but following a redox cycle, a significant change emerged with regard to the phase angle peak (Fig. 1); this was not observed when the antibody-loaded film had been exposed to a baseline buffer or to a control (urease) solution. The effect of the redox cycling suggests that there is a realignment of the polymer chains following the binding interaction; a similar effect was observed for high molecular weight analyte detection using cyclic voltammetry [2].

Fig. 2 shows the EIS spectra following the electrochemical cycling of an IDE coated with adsorbed single- and double-stranded DNA in pyrrole monomer. There is a significant difference between the two spectra, which is likely to be due to the different reporter interactions of poly (pyrrole) with the double-stranded chain vs. the single-stranded DNA chain indicating that the polymer may act through a specific intercalation or groove binding route in the case of the double-stranded DNA.

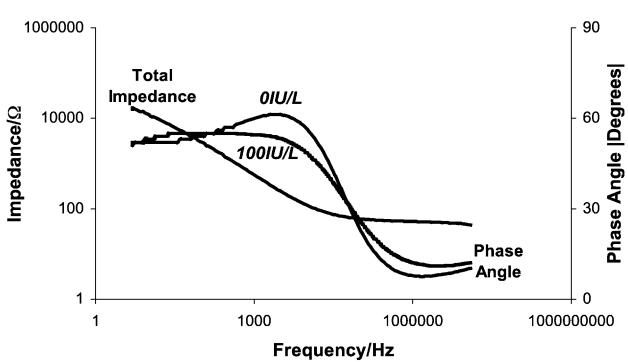

Fig. 1. Bode plot showing change in phase angle of an antibody-loaded film following exposure to luteinising hormone. For clarity, the minor change in the impedance has not been shown.

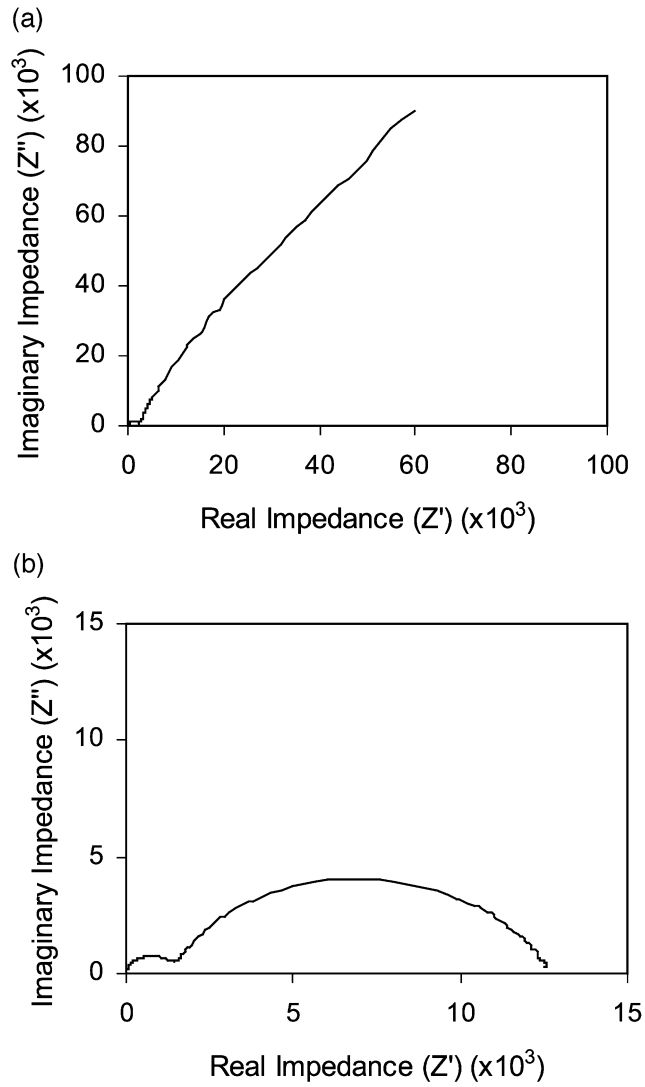

Fig. 2. (a) Plot of single-stranded DNA absorbed at IDE and following cyclic voltammetry in 0.5 M pyrrole in phosphate buffer. (b) Plot of double-stranded DNA following same protocol.

4. Conclusions

While a fully mechanistic understanding of the factors inducing impedimetric change in conducting polymeric films is unclear at present, there is now direct evidence that these changes can be used for reagentless transduction. The lack of a reaction product as an outcome of antigen or

DNA binding may therefore be overcome in this biosensing strategy.

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