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Metallic oxide $CdIn_2O_4$ films for the label free electrochemical detection of DNA hybridization

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Abstract

DNA functionalised semiconductor metallic oxide electrodes have been developed for the direct electrochemical detection of DNA hybridization, without labelling or the introduction of a redox couple. Conductive $CdIn_2O_4$ thin films with controlled properties were deposited on glass substrates using an aerosol pyrolysis technique. The films exhibit a polycrystalline microstructure with a surface roughness of 1.5 nm (r.m.s.) and an electrical resistivity ranging between 1 and $3 \times 10^{-3}\ \Omega\,cm$. These electrodes were functionalised using hydroxylation and silanisation steps, to allow the binding of DNA probe sequences (20 bases). The electrical detection of DNA hybridization with complementary sequences has been performed using electrochemical impedance spectrometry (EIS) measuring the variation of impedance before and after hybridization. Two set-ups were used, a standard set-up including three electrodes and a set-up including two symmetrical electrodes. In both configurations, a significant increase of the impedance modulus, more particularly of the real part of the impedance (160–225% according to the electrochemical cell used) has been obtained over a frequency range of $10–10^5$ Hz. DNA hybridization has also been systematically confirmed using the fluorescence spectrometry. This study emphasizes the high sensitivity of the $CdIn_2O_4$ as a working electrode for the detection of biological events occurring at the electrode surface.

Keywords: $CdIn_2O_4$ film; DNA; Electrochemical impedance spectroscopy (EIS); Label-free detection

1. Introduction

In the field of biochips, the detection of DNA hybridization is commonly performed using fluorescence spectroscopy. This well-proven technique is very sensitive however its use is not simple and easy to manage. Particularly, it requires manipulation of the DNA target to introduce optical labels. Alternative techniques providing direct DNA hybridization detection are under investigation. Notably, electrical techniques such as electrochemical impedance spectroscopy (EIS) and voltammetry offer attractive analytical pathways. These techniques are based on measuring the variation of the dielectric properties of the system [functionalised electrode/electrolyte] accompanying DNA hybridization (Drummond et al., 2003; Lucarelli et al., 2004; Wang et al., 1996). This approach has the advantage of producing electrical readout signal that can be easily analysed in electronic circuits. In order to magnify the electrical signal, the use of redox labelling (Drummond et al., 2003; Lucarelli et al., 2004; Wang et al., 1996; Yang and Thorp, 2001; Xu et al., 2001) or a redox intercalator (Park and Hahn, 2004) is often required. Further, label-free detection without the use of any additional redox labelling is also presently under active investigation using DNA functionalised electrodes (Berggren et al., 2001). The creation of such electrodes requires different kinds of conductive materials or devices together with adequate DNA probe immobilisation strategies.

Conductive polymer-modified electrodes comprising an electro-attractive polymer as the transducer element for hybridization detection have been described by Piro et al. and references therein (Piro et al., 2005). Voltammetry and EIS were used to study the electrochemical changes and mechanisms during DNA hybridization.

On silicon/silicon dioxide substrates, Souteyrand et al. (Souteyrand et al., 1997) and Berney (Berney et al., 2000) have observed variation of capacitance as a consequence of the DNA hybridization using impedance measurements, allowing DNA detection with picomole sensitivity (Berney et al., 2000). Elsewhere, electronic detection of DNA hybridization could be

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obtained using dc measurements on silicon field-effect transistor (FET) arrays (Pouthas et al., 2004). Hamer's group has studied extensively the DNA hybridization induced variation of impedance on working semiconductor electrodes such as n- and p-doped silicon wafers (Cai et al., 2004) and p-doped diamond thin films (Yang et al., 2004). Microlithographically fabricated interdigitated microsensor electrodes (IMEs) have been used with EIS to measure changes related to DNA hybridization (Ghorghe and Guiseppi-Elie, 2003; Hang and Guiseppi-Elie, 2004). In this case, the active zone was the modified (silanised) surface of the glass substrate located between closed interdigitated Pt microelectrodes. In other studies, pure metallic electrodes, notably gold electrodes modified with alkanethiols linkers, have been used to explore the feasibility of a direct capacitive DNA biosensor (Berggren et al., 1999; Guiducci et al., 2004; Estrela et al., 2005).

In this context, electrodes constituted of transparent and electrically conductive oxides (TCOs) can present advantages over the previously employed types. These electrodes are made of metallic oxide thin films, which can be easily and directly deposited on Si or glass substrates. The films show an interesting and technologically important combination of properties such as visible light transmittance, low electrical resistivity (10^{-3}–10^{-4} Ω cm), excellent adherence on the substrate and chemical stability. DNA grafting onto such materials is possible: similarly to silicon oxide, they can undergo an initial surface hydroxylation prior to the silanisation step. We recently demonstrated the feasibility of DNA grafting onto conductive Sb doped SnO_2 thin films with controlled properties (Stambouli et al., in press). Previously, DNA-modified tin doped indium oxide (ITO) electrodes were used to study the impedance variation related to the DNA hybridization using a redox label such as $Co(phen)_3(ClO_4)_3{\cdot}3H_2O$ to amplify the signal (Yang and Thorp, 2001; Xu et al., 2001).

In this study, we present the first results regarding the label-free detection of DNA hybridization via the use of thin films of cadmium indate $CdIn_2O_4$. This material has been previously deposited and studied in our laboratory for optoelectronics and solar applications (Labeau et al., 1986). $CdIn_2O_4$ material belongs to the ternary compounds family of the transparent conducting oxides (Ginley and Bright, 2000), and it can be positioned in the class of n-type defect semiconductors (Labeau et al., 1986; Wang and Liao, 1995).

2. Experimental

2.1. Thin $CdIn_2O_4$ film synthesis

We have carried out the deposition of $CdIn_2O_4$ thin films directly on glass substrates using an aerosol pyrolysis technique described elsewhere (Labeau et al., 1986). It is related to chemical vapour deposition from an organometallic solution (MOCVD) and is based on the pyrolysis of an aerosol obtained by ultrahigh frequency spraying of a precursor solution on a heated substrate at atmospheric pressure. The solution containing the organometallic precursors were composed of (1) cadmium diacetate dissolved in pure methanol and (2) indium acetylacetonate dissolved in pure acetylacetone. The concentrations of (1) and (2) were the same and equal to 0.05 M. The substrate temperature was kept at 465 °C. The resulting film thickness, measured using ellipsometry, was between 80 and 100 nm.

2.2. Functionalisation, DNA grafting and DNA hybridization

2.2.1. Silanisation

Silanisation was performed using process identical to that used for SiO_2 surfaces, described elsewhere (Peyrade et al., 2004). First, the $CdIn_2O_4$ film surfaces were hydroxylated in a NaOH solution (4 M in ethanol 95%) for 2 h. This treatment creates OH groups at the surface of the film and eliminates organic contamination. These OH groups allow chemically bonding of the functional silane onto the $CdIn_2O_4$ film surface. The silanisation of the films was carried out by liquid phase deposition of a solution of silane in an organic solvent. The samples were placed for 12 h in a solution 0.5 M of 3-aminopropyl-tri-ethoxy-silane APTES (Sigma–Aldrich) in 95% ethanol. This trifunctional silane was chosen: it is stable in solution, then easy to use and leads to a good efficiency, in spite of a longer time of grafting compared to a monofunctional silane. The formation and the thickness of the film are not perfectly controlled, but a rigorous respect of the procedure minimises the non-reproducibility. After two successive rinses with ethanol and deionised water to remove unbound silane, the samples were dried and heated for 3 h at 110 °C. To facilitate strong covalent binding between the NH_2 termination of APTES and the 5′-NH_2 termination of the oligonucleotide, a cross linker molecule (10% glutaraldehyde solution in H_2O) was applied for a period of 90 min. Indeed, the double aldehyde terminations proved very reactive and immediately bound with the two NH_2 terminal functional groups. Yang et al. (Yang and Thorp, 2001) and Xu et al. (Xu et al., 2001), in their work directed at electrical detection of DNA hybridization with redox labelling, have used functionalised ITO electrodes. The mode of DNA immobilisation on the ITO films involved electrostatic adsorption of DNA strands on the silane previously deposited on the oxide surface. By comparison, in this study, the DNA strands are covalently linked to the silane through the glutaraldehyde. This strong link is important in the context of electrical detection and should enhance signal transduction.

2.2.2. DNA grafting and hybridization

Pre-synthesised 20 base oligonucleotide probes were used. A standard-probe sequence was chosen: 5′-NH_2-TTTTTGA-TAAACCCACTCTA-3′ (purchased from Apibio/Biomérieux, France), which was complementary to the DNA target sequence. A non-complementary probe sequence was used, 5′-NH_2-TTTTTTTTTCCAAGAAAGGACCCG-3′ (from Eurogentec, Belgium) to check the specificity of the hybridization. These probes were diluted in a sodium phosphate solution 0.3 M/H_2O to a concentration of 10 μM.

Fifty microlitres drop of this solution were manually deposited on the surface of each sample. The samples were incubated overnight at room temperature. The probes were then