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Biosensors and Bioelectronics 20 (2005) 1580–1586
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Electrical detection of biomolecular interactions with metal–insulator–semiconductor diodes

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Received 28 May 2004; received in revised form 4 August 2004; accepted 4 August 2004
Available online 15 September 2004

Abstract

We report the label-free detection of DNA hybridization using a metal–insulator–semiconductor (MIS) diode or capacitor. Upon immobilization of single-stranded DNA on the gold gate of a MIS capacitor, the capacitance versus voltage characteristics show a significant shift in the direction of negative voltages as expected from the immobilization of negative charges on the gate. The hybridization with the complementary strand gives rise to a further significant shift in the same direction as before, which is consistent with the increase of negative charges on the gate brought about by the hybridization. Fluorescence studies indicate that the immobilization and hybridization of DNA can be electrostatically promoted by electric fields externally applied to the MIS capacitors. The MIS diode detection method is applicable to all biomolecular interactions that affect the surface dipole at the interface between the metal gate and the electrolyte and can be extended to other chemical and biochemical systems such as proteins and cells.

Keywords: DNA hybridization; Metal–insulator–semiconductors; Electrochemical sensors

1. Introduction

The ability to detect biomolecular interactions is of extreme importance in medical, pharmaceutical and biotechnical research and development. One commonly employed method for the detection of such interactions is the optical detection: known probe molecules are immobilized at selected locations, target molecules are labelled with fluorophors and interaction with a complementary probe is evidenced from the presence of fluorescence at the probe's location. This method is expensive and difficult to implement in portable instrumentation. A possible way to overcome these drawbacks is by developing a label-free electrical detection platform.

We report here the sensing of DNA hybridization with metal–insulator–semiconductor (MIS) devices. The dependence of the capacitance of MIS diodes upon an applied voltage, referred to as capacitance–voltage (*C–V*) characteristics, is well known (see for instance Sze, 1981). When the capacitance of the MIS diode is measured by placing the gate metal in contact with an electrolyte and the voltage is measured with respect to a reference electrode in contact with the electrolyte, a shift along the voltage axis of the *C–V* characteristics is expected when compared to the 'dry' characteristics. This is due to the surface dipole magnitude χ at the interface between the metal gate and the electrolyte, the potential across the electrochemical double layer φ_0 and other potential drops (e.g. the reference electrode). The presence of immobilized chemical species results in further change of χ and therefore in a further shift of the *C–V* characteristics. These changes are brought about by different microscopic phenomena, such as the charge distribution in the immobilized chemical species, interaction between the functionalized gate and the electrolyte, like chemisorption or physisorption of electrolyte molecules. This in turn affects φ_0. When the probe molecules interact with their bioconjugate, further changes in χ and φ_0 occur. For instance, when a gate functionalized with a given strand of DNA probe is exposed to a target with

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doi:10.1016/j.bios.2004.08.010

the complementary strand, hybridization occurs. Since the total negative charge carried by the hybridized molecule is approximately twice that of the single-stranded oligomer, χ and φ_0 change. By contrast, when the functionalized gate is exposed to a non-complementary strand, no binding occurs and the above parameters are unchanged. Hence, the shift, or other changes in the *C–V* characteristics, can be used to detect DNA hybridization.

If there are areas of the gate metal that are not covered by the probe molecules and are therefore exposed to the electrolyte, the effectiveness of the method can be reduced. For this reason, molecules that are inert to the target and carry a much lower charge (spacer molecules) can be used to passivate these areas. If the distance between the probe molecules is larger that the Debye length in the electrolyte (Bard and Faulkner, 2001), the effectiveness of the method is also reduced. The density of probes can be controlled by applying a voltage to the gate metal or the back contact, while the Debye length can be controlled by changing the ionic concentration of the solution.

The technique is applicable to any biomolecular interactions that affect the surface potential at the metal gate/electrolyte interface and can be extended to other chemical and biological systems such as proteins and cells.

2. Materials and methods

2.1. Sample preparation

Boron-doped single-crystalline p-type silicon wafers with resistivity 6–12 Ω cm and a 50 nm of thermally grown silicon oxide were purchased from WaferNet. A dot of 3 mm in diameter, consisting of a layer of Cr 10 nm thick followed by 100 nm Au was evaporated on the oxide and served as the metal gate. A 100 nm Al layer was evaporated on the Si back surface in order to provide an ohmic contact (hereafter referred as back-contact). As some regions of the insulator might be exposed to the electrolyte, the protection of these regions was done by using silicone rubber and/or Teflon tape, which are chemically inert and electrically insulating materials. The passivation layer also partly covers the metal gate in order to avoid penetration of the electrolyte through the edges of the metal. An electrical connection can be made directly to the metal gate and under the passivation layer in order to control the immobilization and/or hybridization times as well as the concentration of immobilized probes and effectiveness of the interaction mechanism. Alternatively a potential can be applied between the back-contact of the MIS structure and the electrolyte.

DNA oligonucleotides were purchased from the Protein and Nucleic Acid Chemistry facility of the Cambridge University Biochemistry Department. All other chemicals used in the sample preparation were purchased from Sigma–Aldrich. All aqueous solutions as well as the rinsing steps were prepared with ultra-pure water.

Single-stranded DNA (ssDNA) consisting of 20 base pairs of Adenine and modified on the 5′ end by HS-$(CH_2)_6$-PO_4-$(CH_2CH_2O)_6$-ssDNA was immobilized on the gold using a concentration of 1 μM in a 1 M potassium phosphate buffer pH 7.0 containing 1 M NaCl and 1 mM ethylene diamine tetraacetic acid (EDTA). After immobilization, the substrate was washed with pure H_2O and 10 mM NaCl containing 10 mM EDTA. Mercaptohexanol, HS-$(CH_2)_6$-OH, was subsequently immobilized as a spacer in a concentration of 1 mM in 1 M potassium phosphate buffer pH 7.0 containing 1 M NaCl and 1 mM EDTA. After the immobilization of the spacer, the substrate was rinsed with H_2O and NaCl/EDTA. Alternatively, both the ssDNA and mercaptohexanol can be simultaneously immobilized onto the gold using a molar ratio of 1:1. Complementary DNA strands consisting of 20 base pairs of Thymine and non-complementary strands consisting of 20 base pairs of Adenine were used in a concentration of 1 μM in 1 M phosphate buffer pH 7.0 with 1 M NaCl. After interaction, the substrate was again washed with H_2O. In some experiements, a positive voltage was applied to the metal in order to accelerate the immobilization and hybridization processes.

2.2. Sample characterization

Fluorescence images of labelled DNA were obtained with a Tokyo Instruments Nanofinder and quartz crystal microbalance measurements were performed using a AffinixQ from Initium. The capacitance versus voltage characteristics of the metal–insulator–semiconductor structures were measured by means of electrochemical impedance using a Gamry Instruments Femtostat with Ag/AgCl and Pt electrodes purchased from BAS. The gold gate is placed in contact with a pH 7.0 electrolyte of 5 mM phosphate buffer containing 5 mM NaCl. A bias voltage between −1 and +3 V is applied on the back-contact of the MIS structure with respect to a Ag/AgCl reference electrode immersed in the electrolyte. A 10 or 20 mV AC voltage with frequency of 1 kHz is superimposed to the bias voltage. The AC current is measured with the help of a platinum wire acting as a counter electrode in a standard three-electrode electrochemical arrangement (Fig. 1). The control of the applied voltage as well as the measurement of current and calculation of the complex impedance is done using a potentiostat connected to a computer. Although only the results obtained at a frequency of 1 kHz will be here presented, all samples were also measured at 100 Hz and 10 kHz. The relative shifts observed for all frequencies are similar. Also, no hysteretic effects were observed in any of the samples.

The capacitance of the MIS diode depends on the voltage applied between the gate and the back contact in a well known way. Different contributions to the capacitance are present: the electrochemical double layer (C_{dl}), the capacitance of the dielectric layer (C_{ox}) and the space charge layer in the semiconductor (C_{sc}). Typical values for the double layer capacitance range between 1 and 50 μF cm^{-2}, while the combined capacitance of the 50 nm oxide layer and C_{sc} is always less

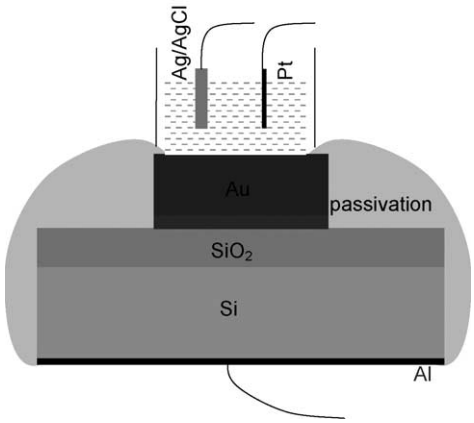

Fig. 1. Diagram of the metal–insulator–semiconductor structures with back-contact and passivation.

than 69 nF cm^{-2}. Since the capacitance contributions are in series, C_{dl} and changes in C_{dl} will have a negligible effect on the total capacitance of the system. By applying an external potential between the metal gate and the back of the semiconductor, three different regimes will be observed: inversion, depletion and accumulation. In the inversion regime, for a frequency of excitation higher than ~10 Hz, the capacitance of the system is $C \approx C_{ox}C_{sc}/(C_{ox} + C_{sc})$ and increases to reach C_{ox} in accumulation regime.

In these conditions, the total capacitance of the system will be given, in first approximation, by $C = -1/\omega Z''$, where ω is the frequency of the AC signal and Z'' is the imaginary part of the measured impedance.

3. Results and discussion

3.1. Electrical detection

Fig. 2 shows the *C–V* characteristics for three cases: (1) ssDNA with spacers; (2) ssDNA with spacers after exposure to a non-complementary target; (3) ssDNA with spacers after exposure to the complementary target. The voltage is applied between the back-contact and the Ag/AgCl reference electrode and referred to the latter. For this sample, the ssDNA probe was immobilized on the gold gate for 3 h. In order to avoid a situation were the metal gate is floating, a potential of −0.3 V was applied on a platinum wire immersed on the solution containing the DNA with respect to the Al back-contact. Mercaptohexanol was subsequently immobilized for 1 h. The complementary DNA strand was deposited on top of the substrate for 1 h applying a −0.3 V potential as before. As a control, a non-complementary DNA strand in the same concentration and buffer as the complementary one was used. DNA sequences and other conditions were as described in Section 2.1. The characteristics show negligible change upon exposure to the non-complementary strand. Upon hybridization with the complementary strand however there is a significant shift of about 170 mV in the direction of negative voltages, as expected from the increase of negative charges on the gate brought about by the hybridization. In this way, label-free electrical detection of DNA is achieved.

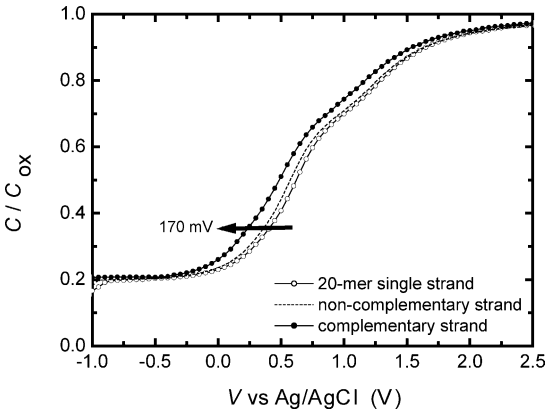

Fig. 2. Capacitance versus voltage curves of a MIS diode for single-stranded DNA (hollow circles), after adding a non-complementary strand (dot line) and after adding a complementary strand (full circles). The capacitance values have been normalized to the capacitance of the oxide layer.

Immunochemically sensitive field effect transistors have for long been described in the literature (see Janata, 1992 for a review), namely in trying to detect the change in charge caused upon antibody–antigen interactions. A fundamental problem with the devices described in the literature is the fact that the change in charge takes place outside the outer Helmholtz layer due to the large size of the immunological molecules involved, so all change in charge is screened by the non-specifically adsorbed ions at the outer Helmholtz layer. One way to overcome this is to use self-assembled monolayers (SAMs), namely of thiolated hydrocarbon chains. Field effect devices without the metal gate have been employed to detect DNA hybridization. The probes were immobilized onto silicon (Cai et al., 2004) or silicon based insulators such as silicon oxide (Souteyrand et al., 1997; Cloarec et al., 2002) and silicon nitride (Berney et al., 2000). We believe that the formation of self-assembled monolayers onto Au substrates via thiolated hydrocarbon chains has some advantages. The chemistry is well know and the SAM formation and can be achieved with one single biochemical step (Nuzzo and Allara, 1983; Wink et al., 1997). The formation of the SAM occurs in two phases. Due to the strong affinity of the sulphur atoms to gold there is initially a fast adsorption process of the thiolated chains. A slower process then occurs where the SAM is stabilized by van der Waals interactions between the hydrocarbon chains, forming an almost crystalline structure (Collard and Fox, 1991). Biomolecules pre-modified with a thiol group can then be easily assembled onto Au substrates simply by placing a solution containing the modified biomolecules in contact with the gold substrate for a certain period of time. The immobilization time and the probe concentration can be controlled by applying a voltage between the gold substate and the solution. The result is the reproducible formation of monolayers of biomolecules. Furthermore, semiconductors and insulator surfaces, such as silicon, silicon oxide and

silicon nitride are subject to uncontrolled modifications and contaminations, which add to the problem of achieving reproducible assays. On the contrary, inert metals such as Au or Pt are immune to oxidation and their surface can be rendered clean and reproducible by chemical etching, chemical or plasma cleaning, thermal annealing, etc.

3.2. Effect of external electric fields

The immobilization or interaction of electrically charged molecules, such as DNA, can be promoted by applying a voltage between the metal gate or the back-contact and an electrode in the solution containing the biological molecules. The applied voltage on the metal gate must be attractive for the charges in the biomolecules. The electric field will help offset the repulsion on the negative charges of probe and target DNA molecules (Sosnowski et al., 1997). Also, the orientation of the DNA molecules immobilized on gold can be controlled with applied electric fields (Kelley et al., 1998).

We carried out a systematic study of the effect of electric fields on the immobilization of ssDNA on the MIS structures and its hybridization with the complementary strand, using DNA labelled with fluorescence tags. In order to properly observe fluorescence, especially when using gold substrates with high back-fluorescence, a clear contrast between the areas with and without fluorescing DNA has to be produced. To achieve this, we used Teflon tape to mask the silicon oxide and the edges of the gold dot during the DNA immobilization and/or hybridization process. Prior to analyse the fluorescence of the samples, the tape needs to be removed to order to properly focus the scanning confocal microscope on both areas with and without DNA. After removing the tape, remains of the glue stay on the sample and a few fluorescent tags might attach themselves to the glue during washing. Although at the edges of the tape high concentration of fluorescence is normally present, a clear difference in fluorescence can be observed between the gold regions inside and outside this glue ring.

At an initial stage, an electric field was applied during the ssDNA immobilization process. The voltage was applied between a Pt wire immersed in the solution and the Si back-contact of the MIS structures. Voltages between 0 and −15 V were applied during 10 min at the Pt electrode during the simultaneous immobilization of 2.5 μM of mercaptohexanol and 2.5 μM of ssDNA. The ssDNA used was a 20-mer poly-A sequence modified with a thiolated $(CH_2)_6$ chain and PEG at the 5′ end and a TAMRA fluorescent tag with emission wavelength λ_e = 580 nm at the 3′ end. Under normal conditions, immobilization of ssDNA onto Au substrates for only 10 min will produce an inhomogeneous layer with low DNA density. The fluorescence patterns observed show that, for low voltages, very little DNA is immobilized and upon increasing the voltage larger regions with fluorescence start to be observed. A voltage of around −10 V is necessary to produce large areas of dense DNA and for −15 V homogeneous samples are formed.

One of the problems of applying a voltage via the Si back-contact is that most of the voltage is going to fall on the silicon oxide. Considering that the value of capacitance of the oxide C_{ox} = 69 nF cm^{-2} is much smaller than the typical capacitance values observed for this type of double-layers ($C_{dl} \sim 30$ μF cm^{-2} for gold and $C_{dl} \sim 3$ μF cm^{-2} for gold with a SAM and ssDNA), approximately only 1/400 of the applied voltage will fall on the double-layer for bare Au. As DNA starts to immobilize on the surface, C_{dl} will decrease and therefore the voltage that will fall on it will increase. However, even after the eventual full formation of a DNA monolayer, only approximately 1/40 of the applied voltage will have an effect on the promotion of DNA immobilization. In order to avoid this situation, the voltage should be applied directly on the gold dot with respect to the Pt electrode in the solution. A polyimide tape coated with gold was placed in contact with the gold dot and protected with Teflon tape. The potential can then be applied on the Pt electrode immersed in the solution with respect to the gold coated polyimide tape. The back of the silicon was kept grounded during the electric field assisted immobilization and/or hybridization of DNA.

Fig. 3 shows the fluorescence images obtained for applied voltages between 0 and −1 V versus Au during the immobilization of mercaptohexanol and poly-A with a TAMRA tag for a period of only 10 min. The results clearly show that the electric field promotes the immobilization of DNA. When no voltage is applied (Fig. 3a), only a few isolated fluorescent spots are observed and for −0.3 V more fluorescence starts to be observed (Fig. 3b). Large areas of DNA are present when a potential of −0.5 V is applied (Fig. 3c) and a dense layer of ssDNA is formed upon a field of −1 V applied for only 10 min (Fig. 3d). For higher voltages, electrochemical peeling of the gold dot starts to occur. If a voltage of −1 V is applied during

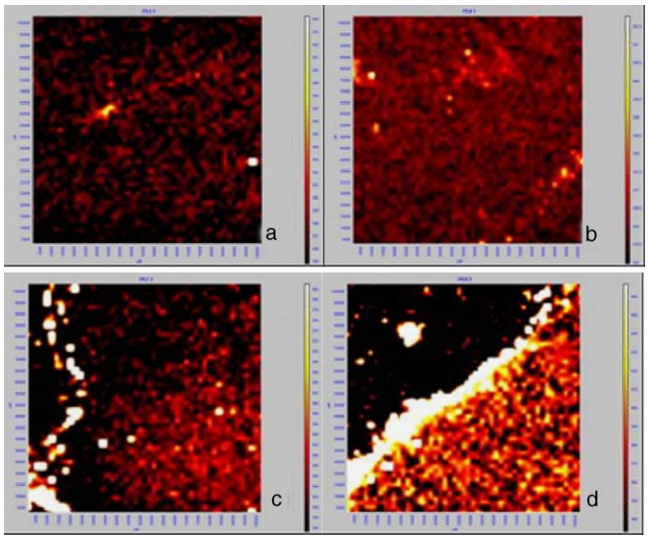

Fig. 3. Fluorescence images taken over an area of 100 μm × 100 μm on MIS diodes after immobilization of ssDNA with TAMRA tags and spacer molecules with a voltage of 0 V (a), −0.3 V (b), −0.5 V (c) and −1 V (d) applied for 10 min between a Pt electrode immersed in the solution and the gold gate.

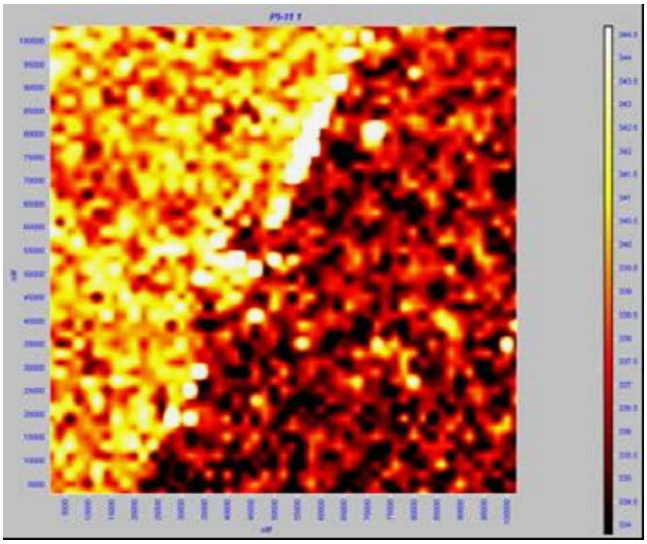

Fig. 4. Fluorescence image taken over an area of 100 μm × 100 μm after hybridization of DNA with the target molecules labelled with HEX tags. The hybridization was performed a voltage of −1 V applied for 3 min between a Pt electrode immersed in the solution and the gold gate.

the immobilization process for a period of only 5 min, high density of fluorescence is observed in some areas of the gold gate, while some other areas show low fluorescence intensity, indicating a partial formation of a dense ssDNA layer.

The effect of an electric field on the hybridization process was also studied. ssDNA with TAMRA tags was immobilized without electric fields on the MIS substrates for 4 h, which should yield an homogeneous and dense surface. The complementary strand with HEX tags (λ_e = 556 nm) was then placed in contact with the substrate for only 3 min with and without an applied voltage. Taking into account the emission wavelengths of the tags used, the fluorescence of each tag can be individually filtered. By probing the DNA fluorescence, it was observed that a few regions on the sample where no voltage was applied show weaker target fluorescence than probe fluorescence, which is consistent with the fact that hybridization does occur but with a low efficiency. On the other hand, when a voltage of −1 V is applied for 3 min, high density of target DNA is observed (Fig. 4) and no significant difference is observed on the intensity of fluorescence of the tags on the DNA probes and the tags on the DNA targets, indicating that high hybridization efficiency occurs in just 3 min when an electric field is applied.

The immobilization and hybridization processes for the particular DNA strands used was also monitored by quartz crystal microbalance measurements without an electric field. Fig. 5 shows the effect of the immobilization of a poly-A sequence and its hybridization with poly-T. Upon immobilization, a frequency change of Δf_{ss} = −305 Hz is observed after 45 min (Fig. 5a). A frequency decrease of 1 Hz corresponds to a mass increase on the gold substrate of 30 pg for our experimental set-up. Taking into account the molecular weight of the modified ssDNA and the spacer molecules, the observed frequency variation corresponds to the immobilization of 1.7×10^{13} ssDNA molecules per cm^2, assuming that the ssDNA and the spacer molecules are immobilized in identical proportions. Hybridization with the complementary poly-T sequence is very fast and after 3 min a frequency change of only Δf_{ds} = −140 Hz is observed (Fig. 5b), which corresponds to a partial hybridization of only circa 45% of the immobilized ssDNA. This result is consistent with the fact that, with no applied field, low intensity fluorescence from the target DNA is observed, albeit evenly spread throughout the sample. The high intensity fluorescence observed in the case where an electric field is applied, clearly indicates that under 3 min the efficiency of hybridization is highly enhanced. As a control, non-complementary DNA strands with Cy3 fluorescent tags (λ_e = 570 nm) were placed in contact with the samples modified with ssDNA under the same conditions as before. No fluorescence of the target molecules was observed in this case, which indicates that applying an electric field does not promote any non-specific interaction with a non-complementary strand.

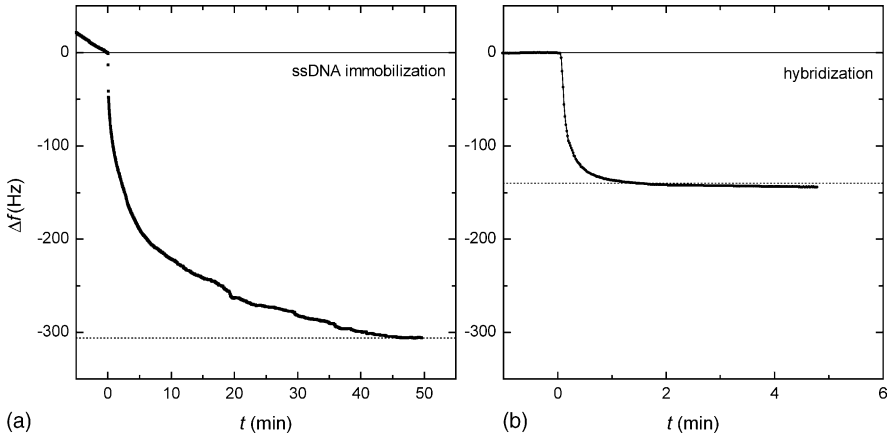

Fig. 5. Relative quartz crystal microbalance frequency change upon injection of: (a) single-stranded DNA consisting of 20 bases of Adenine, together with spacer molecules and (b) complementary strand consisting of 20 bases of Thymine.

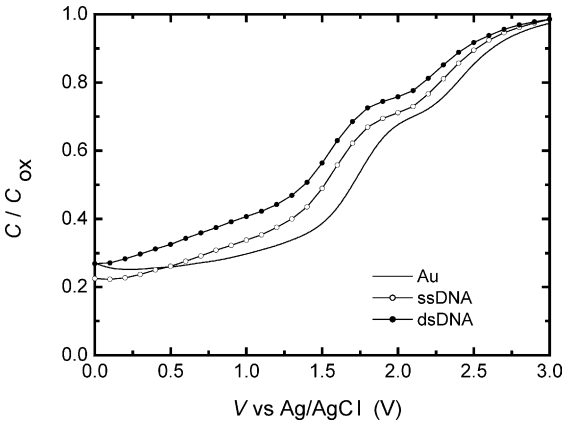

Fig. 6. Capacitance vs. voltage curves of a MIS diode for bare gold (full line), after field assisted immobilization of single-stranded DNA (hollow circles) and after field assisted hybridization with a complementary strand (full circles). The capacitance values have been normalized to the capacitance of the oxide layer.

Fig. 6 shows the capacitance versus voltage characteristics of a MIS diode where both the immobilization of ssDNA and its hybridization with a complementary strand were assisted with an electric field applied between a Pt electrode immersed in the solutions containing the DNA and the gold gate of the MIS diode. A potential of −1 V was applied for 15 min during the simultaneous immobilization of 1 μM of 20-mer poly-A thioled DNA and 1 μM of mercaptohexanol. The figure shows the *C–V* curves obtained before and after the ssDNA immobilization. A shift of −190 mV is observed. Hybridization with 1 μM of a complementary strand was then performed for 5 min under a potential of −1 V. After hybridization a further shift of −130 mV is observed on the *C–V* characteristics. These results confirm that under the influence of electric fields, both the immobilization and hybridization processes are strongly promoted.

4. Conclusions

We have demonstrated label-free electrical detection of biomolecular interactions using a metal–insulator–semiconductor diode. The technique has primarily been developed for the detection of DNA hybridization but can be extended to other biological systems. The presence of chemical species immobilized on the metal gate results in a change of the interfacial dipole affecting the potential drop across the electrochemical double layer. This modulates the voltage applied to the gate of the MIS device, resulting in a change of the capacitance versus voltage characteristics. Upon hybridization of DNA on the gold gate of a MIS capacitor, the capacitance versus voltage characteristics show a significant shift in the direction of negative voltages as expected from the immobilization of negative charges on the gate. The dominant impedance of the system is the capacitance of the MIS diode itself, due to the much lower capacitance of the SiO_2 insulator compared with the SAM, DNA and electrochemical double layer, which explains why rigid shifts are observed upon DNA immobilization and hybridization. Microarrays can be constructed where each individual MIS diode is immobilized with a known DNA probe. The shifts observed are quite substantial (∼150 mV), which makes the technique extremely promising for the development of low cost portable systems.

The immobilization of ssDNA is clearly promoted by an external electric field. Voltages of −1 V are sufficient to create a dense ssDNA layer in 10 min, instead of the usual 1 h normally required. More importantly, hybridization of DNA is strongly promoted with an electric field and high DNA hybridization efficiency can be obtained in just 3 min or less. Reports in the literature (Fixe et al., 2003) suggest that fast electric pulses can also be used to reduce the hybridization time to the seconds range.

Work is presently being carried out in order to optimize the conditions for the detection mechanism and determine the sensitivity of the technique. Ideally, single nucleotide polymorphisms could be detected with the technique here described. As the shifts observed depend mainly on the change of charge density at the metal gate, the technique can also prove useful for the detection of protein and cell interactions.

The same detection principle is presently being extended to MIS field effect transistors using polycrystalline silicon thin film transistor technology. This technology, very well developed for flat screen displays and available on plastic substrates, is very promising in view of producing multichannel, low cost, disposable biosensors and has already been shown to be applicable to pH and enzyme-based sensors (Yan et al., 2004).

Acknowledgements

The authors would like to thank Dr. B.M. McGregor, Dr. S. Li and Mr. P. Butterfield of Cambridge Research Laboratory of Epson for assistance in sample preparation; Mr. H. Maeda, Dr. H. Hiroshima and Dr. S. Inoue of Epson Technology Platform Research Center for valuable discussions; Dr. F. Yan and Dr. A.G. Stewart of Cambridge University Engineering Department for help and suggestions in the experiments and theoretical understanding. Financial support from the UK Engineering and Physical Sciences Research Council (EPSRC) and the Isaac Newton Trust of Cambridge University is acknowledged.

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