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Effects of diamond-FET-based RNA aptamer sensing for detection of real sample of HIV-1 Tat protein

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ABSTRACT

Diamond is a promising material for merging solid-state and biological systems owing to its chemical stability, low background current, wide potential window and biocompatibility. The effects of surface charge density on human immunodeficiency virus type 1 Trans-activator transcription (HIV-1 Tat) protein binding have been investigated on a diamond field-effect transistor (FET) using ribonucleic acid (RNA) aptamers as a sensing element on a solid surface. A change in the gate potential of 91.6 mV was observed, whereby a shift in the negative direction was observed at a source-drain current of −8 μA in the presence of HIV-1 Tat protein bound to the RNA aptamers. Moreover, the reversible change in gate potential caused by the binding and regeneration cycles was very stable throughout cyclical detections. The stable immobilization is achieved via RNA aptamers covalently bonded to the carboxyl-terminated terephtalic acids on amine sites, thereby increasing the sensitivity of the HIV-1 Tat protein sensor. The reliable use of a real sample of HIV-1 Tat protein by an aptamer-FET was demonstrated for the first time, which showed the potential of diamond biointerfaces in clinical biosensor applications.

1. Introduction

Diamond is a promising material for merging solid-state electronics and bioelectronics that can be used with DNA molecules (Yang et al., 2002), proteins (Song et al., 2004; Hartl et al., 2004) and cells (Rezek et al., 2009; Dankerl et al., 2009). In recent years, many applications of biosensing using diamond as a material and electronically active devices have been developed. These include a field-effect capacitive electrolyte–diamond–insulator–semiconductor structure for the detection of penicillin (Abouzar et al., 2008), a capacitive sensor (Abouzar et al., 2009; Ingebrandt et al., 2011) and in-plane interdigitated electrodes that exhibit high sensitivity to molecules such as phosgene (Davydova et al., 2010). Capacitive sensors are generally simple to fabricate and operate and are widely applicable. However, a major drawback of capacitive sensing appears when it comes to miniaturization owing to the significant decrease in capacitance for small dimensions, causing sensing to become problematic. For miniature sensors, the electrolyte solution-gate field-effect transistor (SGFET) design is more suitable as it can provide a higher signal-to-noise ratio and good signal stability, its sensitivity can be increased and it can directly amplify signals (Kawarada et al., 2001; Kuga et al., 2008). The advantages of diamond SGFETs are that they are chemically resistant, biocompatible, can form a stable interface with biomolecules including covalent links (Song et al., 2006; Ruslinda et al., 2012) and can operate without gate oxides (Ruslinda et al., 2010). Hence, an increase in both sensitivity and selectivity is expected, resulting in new biosensing possibilities. Moreover, diamond does not require doping by impurities for device formation owing to the inherent feature of H-termination, which facilitates p-type surface conductivity when forming an interface with electrolytes (Ri et al., 1995; Maier et al., 2000; Sasaki and Kawarada, 2010).

The recent development of aptamers has led to increased interest in biosensor applications. Aptamers are single-stranded DNA or RNA molecules that have been selected from synthetic nucleic acid libraries for molecular recognition (Ellington and Szostak, 1990; Tuerk and Gold, 1990). The advantages of aptamers such as their simple synthesis, easy labeling, high stability and wide applicability have made them ideal recognition probes for protein or small-molecule detection. From the viewpoint of FET technology, if we control the pH and the concentration of buffer solution, aptamers are a superior choice because they are smaller (5–6 nm) than the Debye length (approximately 9 nm), thus providing binding inside a measurable range (Guo et al., 2005; Maehashi et al., 2007). As a result, binding between aptamers and target proteins can occur within the electrical double layer in a

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buffer solution, and therefore, an FET can easily detect changes in the charge distribution in the proximity of the diamond surface. Moreover, the density of immobilized aptamers on diamond channels can be controlled, and a high density of aptamers can easily be prepared. Therefore, these characteristics make aptamers ideal molecular recognition elements in analytical applications (Scheller et al., 2001) and versatile tools for therapeutics (White et al., 2000) and diagnostic applications (Brody and Gold, 2000). Yamamoto et al. were the first to develop an aptamer that yielded an efficient binding specificity against Tat protein but not against other cellular factors (Yamamoto et al., 2000). This RNA aptamer exhibits a binding affinity over 100-times higher than that of TAR RNA (59-mer) (Minunni et al., 2004; Tombelli et al., 2005). The aptamer has a TAR-like motif in its randomized region, with two adjacent bulging units with opposite orientations. The core element for binding to Tat is a four central base pair helix flanked by two residues on each side (Yamamoto et al., 2000).

In a preliminary study, we reported HIV-1 Tat peptide detection based on an RNA aptamer using optical and potentiometric methods that only focus on an arginine-rich region (Ruslinda et al., 2011). The partial region of the Tat peptide has been successfully detected using a diamond SGFET. This peptide induced a voltage shift of −40 mV in the negative direction in the FET transfer characteristics. To used this peptide in clinical samples, it is of paramount importance to investigate the effect of a real sample of HIV-1 Tat protein on diamond-FET-based RNA aptamer sensing. HIV-1 Tat consists of 86 amino acids that encompass an acidic and proline-rich N terminus (amino acids 1–21), a cysteine-rich region (amino acids 22–37), a core (amino acids 38–47), a basic region (amino acids 48–57) and a glutamine-rich region (amino acids 58–72) (Arya et al., 1985). Among them, the basic region is important for TAR binding (Weeks et al., 1990) and nuclear localization, and the segment between amino acids 47 and 57 has been used to transport a large variety of materials, including proteins, DNA, drugs, imaging agents, liposomes and nanoparticles, across cell and nuclear membranes (Gupta et al., 2005). In addition, the Tat amino acid sequence has low overall hydrophobicity and a high net positive charge (Shojania and O'Neil, 2006), where the charge derived to HIV-1 Tat protein consists of 21 hydrophilic side chains bringing a large number of positive charges, 5 dicarboxylic amino acids that bring negative charges, and amino acids that are polar, nonpolar and aromatic, which are uncharged. This protein contributes to several pathological symptoms of HIV-1 infection and also plays a critical role in virus replication. The total number of Tat protein amino acids is 86; thus, its size is roughly estimated to be less than 5 nm in diameter.

Here, we report the contribution of HIV-1 Tat protein to the electronic response of a diamond FET-based RNA aptamer to the electronic properties of diamond with the aim of clarifying the electronic response of such biosensors. The analytical characteristics of the diamond FET biosensor in terms of sensitivity, stability and reusability have been studied in detail. The reliable use of a real sample of protein by a diamond-FET-based RNA aptamer was demonstrated for the first time, which showed the potential of diamond biointerfaces for adaption to the clinical monitoring and biological diagnosis.

2. Materials and methods

2.1. Chemicals and oligonucleotides

RNA aptamers were purchased from Sigma-Aldrich Co. The RNA aptamer sequences were 5′-UCGGUCGAUCGCUUCAUAA-3′-NH_2 (probe RNA aptamer) and 5′-GAAGCUUGAUCCCGAA-3′ (aptamer-derived second strand), where underlined residues form bulges in the duplex structure. The recombinant Tat HIV-1 (HIV-1 Tat; 86 amino acids) was purchased from Immuno Diagnostics, Inc., and stored at −75 °C. The 3′ ends of the probe RNA aptamer were terminated with an amino group. This amino group can covalently immobilize the aptamer to a carboxyl-terminated linker molecule such as terephthalic acid. All other chemicals and solvents used in this experiment were purchased from Kanto Chemical Co. Inc. The buffer used for the experiments consisted of phosphate-buffered solution (PBS) with Tween-20 (0.1% Tween-20), TE buffer solution (Tris–HCl; 4 ml and EDTA; 0.8 ml) and urea solution (8.3 M). Ultrapure water was obtained from a Milipore system.

2.2. Synthesis of partially functionalized diamond surface

Polycrystalline diamonds purchased from Element Six Co. Ltd. were used in this study. These samples are freestanding, transparent diamonds (optical grade) with a thickness of 300 μm and a large grain size (~100 μm) grown by chemical vapor deposition. Their surfaces were sufficiently flat for the fabrication of high performance FETs with a high cutoff frequency (Hirama et al., 2008). The surfaces used as substrates were H-terminated using a hydrogen plasma. At room temperature, the sheet resistance and carrier concentration of these substrates were 10–20 kΩ/square and $1–3 \times 10^{13}$ cm^{-2}, respectively, as determined by direct current Hall effect measurements (Song et al., 2006; Kuga et al., 2008). Partial surface amination of the H-terminated diamond was performed by irradiation with UV light in ammonia gas ambient at 100 sccm for 4 h. These procedures were performed at room temperature and atmospheric pressure, allowing the modification to be performed in a short time.

2.3. Diamond FET fabrication

Diamond FETs were fabricated as follows. Source and drain electrodes were deposited onto H-terminated diamond by thermal evaporation using a metal mask consisting of 150 nm-thick Au film. Then Ar ions were implanted (acceleration voltage 25 keV, ion density 2×10^{14} cm^{-2}) through another metal mask to form an insulating region outside the metal electrodes and channel/gate region. The dosed region was highly resistive and did not form graphitic defects (which can be found at ion densities greater than 10^{16} cm^{-2}) and exhibited the expected electrochemical properties. Wires were bonded to the drain and source electrodes using electroconductive paste and were covered with insulating epoxy resin to protect them from the electrolyte solution. A bulk electrode was not used in this experiment because diamond is an insulating material and the surface channel of the diamond FET was electrically isolated from the bulk like silicon on insulator (SOI). The channel was directly exposed to the electrolyte solution. The length and width of the gate channel were 500 μm and 8 mm, respectively. In each step, the static current–voltage (i_{DS}–v_{GS}) characteristics of the SGFET were measured under a fixed concentration of 1 mM PBS (pH=7) using a Ag/AgCl reference electrode as the gate electrode. i_{DS} was measured as a function of v_{GS} at a constant drain-source voltage (v_{DS}) of −0.1 V.

2.4. Covalent immobilization of RNA^{Tat} aptamer

Following the partial amination of the diamond FET, the immobilization of RNA^{Tat} aptamer via terephthalic acid linker molecules was performed on the channel surface of the diamond FET. Each sample was first treated with a 1:1 mixture of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 1 h to activate the