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Protein adsorption to graphene surfaces controlled by chemical modification of the substrate surfaces

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ABSTRACT

We have investigated effects of the support substrate surfaces on properties of the attached graphene flakes by observing protein adsorption to the graphene surfaces on SiO_2/Si substrates that are modified with self-assembled monolayers to control their hydrophilicity. Using atomic force microscopy operated in aqueous environment, we found that high-density clusters of agglomerated avidin molecules form on the graphene flakes in the areas supported by a hydrophobic substrate surface, whereas very low density of large avidin clusters form at the edge of graphene flakes in the area supported by a hydrophilic surface. These results demonstrate that hydrophilicity of the support surface affects hydrophilicity of the graphene surface also in aqueous environment and that surface modification of the support substrate is a useful technique to control protein adsorption phenomena on graphene surfaces for realization of high sensitive graphene biosensors.

1. Introduction

Graphene, a 2-dimensional sheet of carbon atoms, has many remarkable properties such as high electron mobility, high mechanical strength and ultimately thin thickness [1], and is expected to be a next-generation material for many device technologies. In device applications, graphene films should be supported with a substrate. Effects of the support substrate surfaces on properties of the attached graphene films have been investigated in air [2,3]. One of the remarkable phenomena related to the substrate is permeable wettability through graphene films that appears as substrate-material dependence of contact angles on the attached single layer graphene (SLG) [4]. It has also been reported that the electrostatic potential of graphene films can be modulated by chemical properties of the support surfaces [5,6]. In spite of intensive research on substrate effects on graphene properties in air, there have been very few reports on them in liquid. Biosensors, including label-free devices [7,8], are one of the promising applications of graphene, and interaction between graphene surfaces and biological molecules in aqueous environment is more important in this target than that in air. Since protein adsorption is strongly influenced by surface characteristics [9,10–12], its dependence on the substrates for graphene support also should be revealed for higher performance of biosensing techniques [13].

In this paper, we investigated protein adsorption to graphene films in aqueous environment focusing on influence of surface modification of the support substrates. We used self-assembled monolayers (SAMs) to modify the SiO_2/Si substrate surfaces because a flat, densely packed monolayer is spontaneously formed on the substrate surface owing to stable chemical bonds to the substrate [14]. In addition, we can easily control the chemical properties of the substrate surfaces, such as their wettability and surface charge, by choosing the functional head groups of SAM molecules. We observed protein adsorption behavior on the graphene films attached to SAM-modified substrates in aqueous environment.

2. Experimental procedures

We used silicon (100) substrates with a 300 nm SiO_2 layer. The substrates were cleaned by a mixture of sulfuric acid and hydrogen peroxide (H_2SO_4: H_2O_2 = 3: 1) at 90 °C for 10 min and then sonicated for 5 min in deionized water. SAMs were patterned by the latex beads projection method [15,16]. Polystyrene beads of 2 μm in diameter were arrayed on the SiO_2 substrates, and a gold layer was deposited by vacuum evaporation. After removal of the polystyrene beads by sonication in deionized water, Au patterns were obtained. Then, a SAM monolayer was formed on the substrates by the vapor phase deposition method. We used two kind of SAMs, octadecyltrichlorosilane (OTS) and 3-aminopropyltrieth-oxysilane (APTES), both of which were purchased from Sigma–Aldrich. The OTS SAM was formed by heating an OTS solution of

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0.5 ml at 100 °C for 20 h in a sealed vessel with the substrate. An APTES monolayer was formed by leaving the substrate with an APTES solution of 0.5 ml in a sealed vessel at room temperature for 20 h. The OTS SAM exhibits hydrophobic nature [17] and the APTES SAM hydrophilic one [18]. The used SAMs were bound to the SiO_2 surface by stable chemical bonds (silane coupling) in the area where the gold deposition was masked by the polystyrene beads. Since the gold layer was weakly bound to the SiO_2 surface, it could be removed by mechanical wiping of the substrates with a paper soaked in acetone. Finally, the substrates were cleaned by sonication in an acetone, ethanol and deionized water for 5 min, respectively.

Graphene flakes were exfoliated from highly oriented pyrolytic graphite (HOPG) using the standard mechanical process [19] and attached to the SAM-modified SiO_2 substrates without any chemical treatment. We used avidin from egg white purchased from Nacalai Tesque as the model protein to investigate adsorption behaviors to graphene surfaces. Avidin molecules were dissolved in a buffer solution of N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid solution (HEPES, 10 mM, pH 7.0) and its concentration was regulated to be 0.5 μg/ml. The substrates were soaked in the avidin solution at room temperature for 30 min. Finally, the avidin solution was replaced by a fresh HEPES solution without containing the avidin molecules to stop further adsorption of avidin molecule.

We observed surface topography and frictional force images of the SAM-modified and graphene-attached substrates by the contact mode of atomic force microscopy (AFM) in air. Graphene properties were characterized by Raman spectroscopy. Avidin molecules adsorbed to the substrates were observed by the dynamic force mode of AFM in the buffer solution to prevent conformational change of avidin molecules. Finally, we removed the buffer solution and observed the avidin molecules that remained on the substrate in air using the dynamic force mode.

3. Results

Fig. 1(a) and (b) shows AFM topographic images of an OTS-modified and an APTES-modified SiO_2/Si substrates in air, respectively. The OTS and APTES monolayers are observed as flat circular patterns of approximately 2 μm in diameter, which are in good agreement with polystyrene bead diameter. Fig. 2(a) and (b) shows AFM topographic and frictional force images of graphene flakes attached to the OTS-modified SiO_2 substrate. In Fig. 2(a), a graphene flake is attached to both areas of the OTS-SAM and the SiO_2. Height of the graphene surface from the substrate is approximately 0.4 nm, which is similar to the theoretical height of a single layer graphene flake. In Fig. 2(b), frictional force on the graphene surface is smaller than that on the OTS-SAM surface. Since the frictional force in air corresponds to meniscus force between the tip and the surface, the graphene surface is more hydrophobic than that on the OTS-SAM surface though the OTS-SAM surface is more hydrophobic than the SiO_2 surface. Fig. 2(c) and (d) shows AFM topographic and frictional images of a graphene flake attached to the APTES-modified SiO_2 substrate. In Fig. 2(c), graphene film is also attached to both the APTES-SAM and the SiO_2 areas. In Fig. 2(d), the frictional force on the APTES-SAM surface is much larger than that on the graphene surface and slightly larger than that on the SiO_2 surface. It means that the APTES-SAM surface is more hydrophilic than the SiO_2 surface. Fig. 2e and f shows Raman spectra of the graphene flakes that correspond to Fig. 2(a) and (b). Layer number of the graphene flakes is important in this study because influence of the backside state of the graphene flakes is expected to appear only when the layer number is small [4]. It can be estimated by an intensity ratio of the 2D- (I_{2D}) and G- (I_G) peaks, I_{2D}/I_G, and Fig. 2(e) and (f) clearly indicates that single layer graphene flakes are attached to both the OTS-modified SiO_2 substrate and the APTES-modified one because the observed values of I_{2D}/I_G are more than 2 [20].

We investigated effects of hydrophilicity of the support substrate surfaces on protein adsorption to the graphene flakes attached to the SAM-deposited SiO_2 surfaces. Fig. 3(a) shows an AFM topographic image of the avidin-adsorbed graphene flake attached to the OTS-modified SiO_2 substrate. The SiO_2 area without an OTS-SAM is granular, indicating that isolated avidin molecules are adsorbed, but the OTS-SAM areas are apparently flat, indicating that no avidin molecule or denatured molecules are adsorbed to the OTS-SAM surface. The latter case occurs when avidin molecules change their conformation to a flat structure by tight adsorption through a hydrophobic interaction with the OTS-SAM surface. Therefore, the apparently flat OTS surface after avidin adsorption is possibly caused by a denatured avidin layer. On the graphene flake surface, avidin molecules, indicated by the black arrows, preferentially adsorb to the OTS-supported graphene areas compared with the SiO_2-supported one. The size of the adsorbed avidin grains on the OTS-supported graphene areas is much larger than that of the single avidin molecule. Agglomerated avidin molecules formed clusters in the OTS-supported graphene areas. Fig. 3(b) shows an AFM topographic image of an avidin-adsorbed graphene flake attached to the APTES-modified SiO_2 substrate. We can observe isolated avidin molecules in the SiO_2 and the APTES-SAM areas without a graphene flake. On the graphene flake surface, large agglomerated clusters indicated by the green arrows are observed only near the edges of the graphene flake. The adsorption behavior

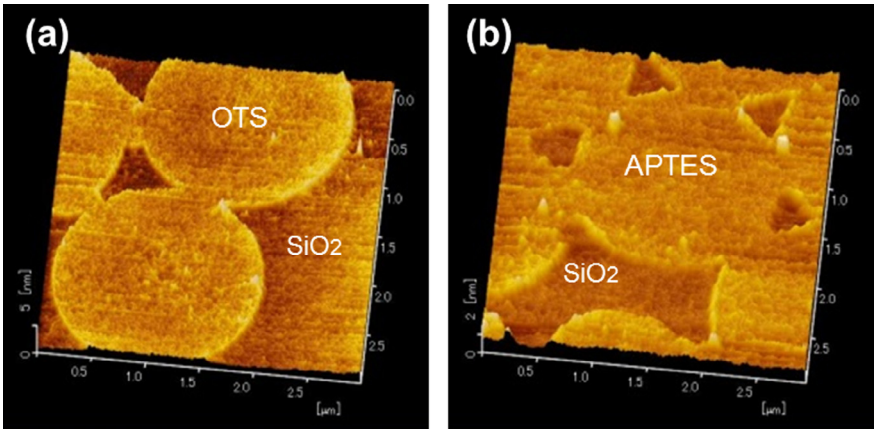

Fig. 1. AFM topographic images of (a) an OTS-modified SiO_2 substrate and (b) an APTES-modified one.