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Development of a vitamin-protein sensor based on carbon nanotube hybrid materials

Takamichi Hirata,[a] Shoji Amiya, and Masahiro Akiya
Department of Biomedical Engineering, Musashi Institute of Technology, Tokyo 158-8557, Japan

Osamu Takei
Rational Evolutionary Design of Advanced Biomolecules (REDS) Group/Japan Science and Technology Agency (JST), Saitama Small Enterprise Promotion Corporation, Kawaguchi 333-0844, Japan

Takafumi Sakai
Department of Regulation Biology, Faculty of Science, Saitama University, Saitama 338-8570, Japan

Rikizo Hatakeyama
Department of Electronic Engineering, Tohoku University, Sendai 980-8579, Japan

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A bionanosensor consisting of a field effect transistor chip and containing a mixture of poly(ethylene glycol)-grafted single-walled carbon nanotubes (SWCNTs) and SWCNTs modified with a protein (avidin) which binds with a specific vitamin (biotin) is developed. An increase in impedance due to biotin-avidin binding is observed when biotin is injected, while the injection of other vitamins resulted in a decrease in impedance. This bionanosensor reacts quickly (~60 s); in addition, the impedance recovers almost to its initial value when the bionanosensor is washed with distilled water; thus, the vitamins do not bind directly with the SWCNTs. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2746077]

Since their discovery in 1991, carbon nanotubes, including single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes, have proved interesting for use as molecular wires; their unique electronic properties confer them with particular potential for development in nanoelectronics.[1,2] Recent years have seen a significant increase in interest in biomedical applications for new types of inorganic nanomaterials, such as nanocrystals, nanowires, and nanotubes, and the creation new types of analytical tools for life science and biotechnology.[3–6]

We are currently carrying out research on the development of bionanosensors using nanocarbon materials such as fullerene and carbon nanotubes, with the aim of investigating the application of these new types of sensor devices in the field of biomedical engineering. In these results, we developed a taste sensor[7] based on a network of poly(ethylene glycol) (PEG, $C_{2n}H_{4n+2}O_{n+1}$)-grafted SWCNTs (PEG-SWCNTs) which can quickly distinguish between various taste materials. In particular, this sensor shows a clear distinction between bitterness and sweetness, which is difficult for the Langmuir-Blodgett film taste sensors.[8]

On the other hand, in the field of biosensing techniques, the interaction between biotin and avidin has the highest known ligand-protein affinity (1 000 000 times more than the antibody-antigen reaction), and the biotin-avidin system has become a universal tool in many biotechnological applications, such as markers for proteins and nucleic acids. Especially, the attachment of biotin to various chemical sites can be used as an important technique to study various processes including DNA transcription and replication. Avidin is a basic glycoprotein found in egg white and in tissues of birds, reptiles, and amphibians. The protein contains four identical subunits with a combined molecular mass of 67 000–68 000 daltons. Each subunit, consisting of 128 amino acids, binds to one molecule of biotin. Avidin has an isoelectric point at *p*H 10–10.5 and is very soluble in water and salt solutions. It is also stable over a wide range of *p*H and temperatures. Biotin is a vitamin from the B group, which was originally called vitamin H, and is a water-soluble compound produced in the body by certain types of intestinal bacteria.

In this letter, we demonstrated the characteristics of a vitamin-protein (biotin-avidin) bionanosensor based on mixture materials of PEG-SWCNTs and avidin-modified SWCNTs by using biotin, which binds characteristically with avidin.

The fabricated bionanosensor employs a back-gate-type field effect transistor (FET) chip with an Al–Si electrode, as shown in Fig. 1(a). A SiO_2 insulating layer (thickness: 290 nm) is formed on top of the *n*-type Si wafer by thermal

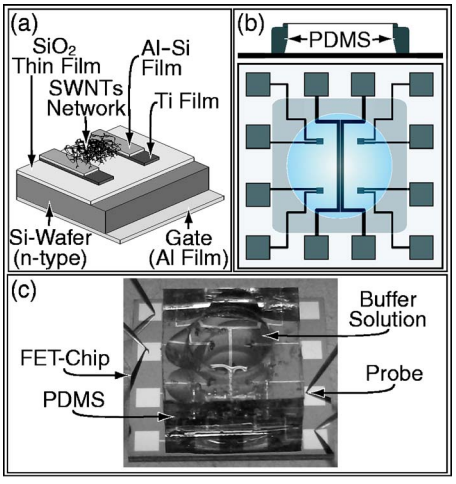

FIG. 1. Schematic view of the bionanosensor: (a) structure; (b) layout of the FET chip; (c) photograph of FET chip and PDMS rubber.

[a]Electronic mail: hirata@dev.ec.musashi-tech.ac.jp

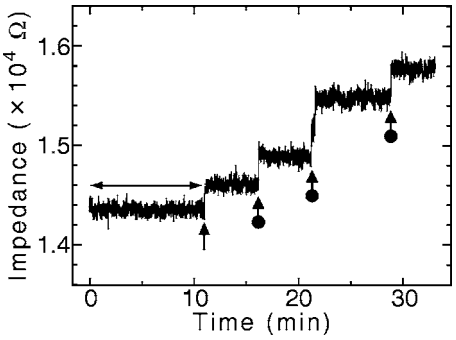

FIG. 2. Impedance vs time characteristic recorded for 1 μm pad-type electrode on the FET chip during biotin injection. The arrow indicates the initial injection time, and arrows with circles are the times at which the sample is reinjected.

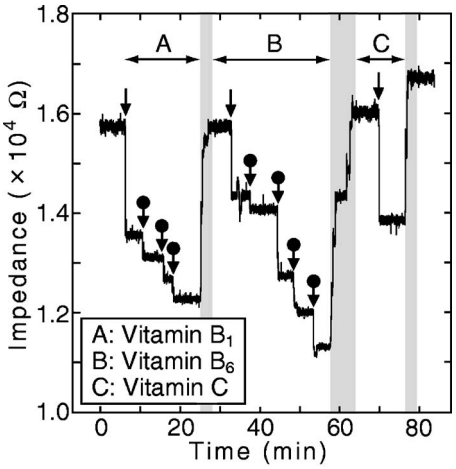

FIG. 3. Impedance vs time characteristic recorded for 1 μm pad-type electrode on the FET chip during vitamin B_1, B_6, and C injections, respectively. The arrows indicate the initial injection time, and arrows with circles indicate the times of further sample injections. The gray region represents washing with pure water.

oxidation. The source-drain electrode patterns, fabricated by photolithography, are composed of Ti/Al–Si (50/200 nm) and are of pad type (100×500 μm^2; gap: 1, 5, 10, and 50 μm, respectively) and one stripe type (100×8000 μm^2; gap: 10 μm) [Fig. 1(b)].

The SWCNT network, which is dropped between the source-drain electrodes on the FET chip, is composed of a mixture of avidin-modified SWCNTs (avidin-SWCNTs) and PEG-SWCNTs. In order to carry out chemical modification of SWCNTs, purified SWCNTs are subjected to oxidation processing to give carboxyl radical groups at the ends and sidewalls, forming carboxyl-radical-modified SWCNTs (COOH-SWCNTs). Oxidation treatment is carried out using sulfuric acid (H_2SO_4) with potassium permanganate[9] ($KMnO_4$) at 423 K for 5 h or, alternatively, using H_2SO_4 and hydrogen peroxide (H_2O_2) at 296 K (room temperature) for 15 min.

For immobilization of avidin-SWCNTs and COOH-SWCNTs, *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (a water-soluble carbodi-imide) are used. PEG-SWCNTs are synthesized by heat treatment of a toluene solution containing azo-PEG (macroazo initiators, $M\sim2000$) at 343 K for 24 h with stirring.[10,11] These materials are also made by Wako Pure Chemical Industries, Ltd.

In order to prevent solution leakage and electrical isolation, polydimethylsiloxane (PDMS) rubber, fabricated using a mold in a plastic container, was coated onto the FET chip, as shown in Fig. 1(c). PDMS elastomer (Dow Corning: Silpot 184W/C) is widely used in microfluidic applications to form components such as valves and diaphragms.

In this study, the vitamins used as measurement targets are vitamin B_1 (thiamin nitrate, $C_{12}H_{17}N_5O_4S$), vitamin B_6 (pyridoxine hydrochloride, $C_8H_{11}NO_3\cdot HCl$), and vitamin C [L-(+)-ascorbic acid, $C_6H_8O_6$]. The standard solution in the PDMS rubber is distilled water (100 μl), and biotin and other vitamins [15 μl (0.1 mg/ml)] are injected using a microsyringe. The characteristics of the bionanosensor, including impedance, are measured using an impedance analyzer (HP: 4192A) and a digital multimeter (Advantest: TR6847), and also the back-gate voltage in the FET chip is floating.

Figure 2 shows the impedance versus time characteristic of the 1 μm electrode gap when biotin was injected. The arrow indicates the initial injection time, and arrows with a circle are the times at which the sample is reinjected. Increases in impedance, resulting from changes of electronic state (mainly, resistance) in the SWCNT network surface due to biotin-avidin binding, are observed when biotin is injected. The impedance increases suddenly when the sample is added, because the solubility of biotin in water (0.22 mg/1 ml at 25 °C) is lower than that of other vitamins. Therefore, it is probable that the overall rise in impedance occurs because the rise in impedance due to biotin binding with avidin-SWCNTs becomes predominant in the entire impedance (Z_{total}). Here, Z_{total} is equivalent to the parallel circuit of PEG-SWCNTs (Z_{PEG}), avidin-SWCNTs (Z_{avidin}), and solution ($Z_{solution}$). Therefore, in the case of biotin injection, the observed signal is based on the change that originates from biotin binding to the surface of avidin-SWCNTs. The change in Z_{total} due to the presence of water-soluble vitamins is not easily detected when $Z_{avidin}\approx Z_{PEG}>Z_{solution}$, because the solution contains many ionized vitamins; however, when $Z_{avidin}>Z_{PEG}<Z_{solution}$, a clear change (a few kilo-ohms) can be detected. It is concluded that this sensor can identify vitamins based on the difference between diffusion in solution and biotin binding to avidin-SWCNTs.

The impedance versus characteristic for the 1 μm electrode gap when the other vitamins (B_1, B_6, and C, respectively) are injected is shown in Fig. 3. The injection of other vitamins is resulted in decreases in impedance. The solubility of vitamin B_1 in water is 2.7 g/100 ml at 20 °C, and those of vitamins B_6 and C are 19.3 g/100 ml and 33 g/100 ml at 25 °C, respectively. Vitamin C has an antioxidant effect by which the revitalization oxygen is controlled, and the oxide is formed by the following reaction:

$$C_6H_8O_6 \rightarrow C_6H_6O_6 + 2H^+ + 2e^-,$$

with a OH radical on the surface of PEG-SWCNTs. Here, $C_6H_6O_6$(dehydroascorbic acid, DHA) is an oxidized form of ascorbic acid. Therefore, it is thought that impedance increases due to this oxidation reaction, although the solubility of vitamin C is higher than that of other vitamins.

As shown in Fig. 4, when only PEG-SWCNTs are fixed on the electrodes of 10 μm electrode gap, impedance is decreased after injection of all vitamins. It is thought that the vitamins do not bind directly with PEG-SWCNTs, because

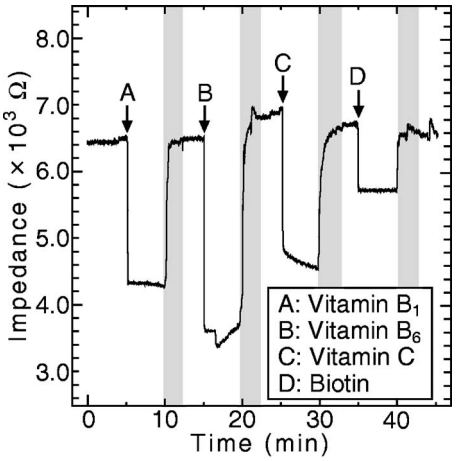

FIG. 4. Impedance vs characteristic recorded for 10 μm stripe-type electrode on the FET chip when only PEG-grafted SWCNTs are used. Samples A, B, C, and D are vitamins B_1, B_6, C, and biotin, respectively. The gray regions represent the times of washing with pure water.

the impedance recovers almost to its initial value when the electrode is washed with distilled water.

In summary, bionanosensor with a SWCNT network consisting of a mixture of avidin-SWCNTs and PEG-SWCNTs is reacted to biotin by showing an increase in impedance within a short response time ($\sim$60 sec) due to biotin-avidin binding. In contrast, the injection of other vitamins results in a decrease in impedance, and because the impedance recovers almost to its initial value when the FET chip is washed with distilled water, it is thought that the vitamins do not bind directly with the SWCNT network.

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