Real-time Detection of Neurotransmitter using Enzyme-immobilized CNT Network Transistors in 11 Vessel Occlusion Rat Model

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ABSTRACT

Glutamate is the principal excitatory neurotransmitter in the brain, and its excessive release plays a key role in neuronal death associated with a wide range of neuronal disorders. We immobilized glutamate oxidase (GLOD) on carbon nanotube (CNT) network junctions by non-covalent functionalized method to detect L-glutamate. After immobilizing GLOD on CNT network, the excess reactive groups of linker molecule remaining on the CNT surface was deactivated and blocked by ethanolamine. The real time electronic response of CNT network transistors immobilized by GLOD was conducted with glutamate standard solution in vitro. For real-time in-vivo glutamate measurements, we prepared the 11vessel occlusion rat model. The ultrahigh sensitivity, selectivity, and fast response time of GLOD-immobilized CNT network transistor could provide great potential for real time electronic detection in the extracellular glutamate level of the brain.

Keywords: Neurotransmitter, CNT network, 11 vessel occlusion rat model, real-time detection, transistor

1 INTRODUCTION

The FET made of carbon nanotube(CNT) can be used as a sensor due to its extreme sensitivity to local chemical environments [1]. The high sensitivity of CNT originates from its atoms which are located at the surface. Proteins such as enzymes and antibodies which are specific to each target have been used as recognition elements. It is essential to immobilize biological molecules on CNTs by a reliable method to preserve their electronic characteristics. In 2003, Besteman et al. found that immobilization of glucose oxidase (GOD) on the sidewall of a semiconducting SWNT (single-walled carbon nanotube) decreased the conductance of the tube. As a result, they suggested that an enzyme-activity sensor could be constructed at the single molecule level of an individual SWNT [2].

It is generally accepted that glutamate is the principal excitatory neurotransmitter in the brain, and that its excessive release might play a key role in neuronal death associated with a wide range of neural disorders [3]. A better understanding of these excitotoxic processes requires accurate assessment of the time course of changes in extracellular glutamate levels, during and after the various insults suspected of initiating this type of damage.

In this article, we report the results of real-time glutamate detection by the GLOD-coated CNT network transistors in 11 vessel occlusion rat model.

2 METHODS

2.1 Materials

The CNT network transistors by surface programmed assembly method were supplied from the Nano Lab. in the School of Physics & Astronomy, Seoul National University. L-glutamate oxidase (GLOD) was obtained from Yamasa Corp.(Tokyo, Japan). 1-Pyrenebutanoic acid succinimidyl ester was purchased from Molecular Probes, Inc.(USA).

2.2 Procedure

The CNT network junctions were incubated with 1mM 1-pyrenebutanoic acid succinimidyl ester in methanol for 2h while stirring, then washed with methanol [4]. Next, the junctions were exposed to the GLOD solutions in 10mM PBS (pH 7.4) overnight. After rinsing with clean PBS, in order to deactivate and block the excess reactive groups remaining on the surface, the 100mM ethanolamine was added onto the CNT junctions and incubated for 30min.

After washing with PBS, 40 μL of fresh-PBS on the GLOD-immobilized CNT junctions was injected for electrical measurements at room temperature. We measured the change of current between source and drain electrode (I_{sd}) when L-glutamic acid was added to the junctions with and without GLOD. A drain-source voltage (V_{sd}) of 10mV was applied by DC power supply (Agilent E3646A) and the conductance change was measured with picoammeter (Keithley 6485).

For real-time in-vivo glutamate measurements, we prepared the 11VO rat model. Changes in cerebral cortical blood flow were monitored by laser-Doppler flowmetry with cortical glutamate levels by the probe-type CNT

transistor with and without GLOD. The CNT probes were inserted into the motor cortex at coordinates A 1; L 4; V 2mm (from the bregma and the dura). A ten minute 11VO cerebral ischemia was initiated by pulling the snares on the common carotid arteries (CCAs) and the external carotid arteries (ECAs). The snares were released and withdrawn after 10 min.

3 RESULTS AND DISCUSSION

GLOD had net negative charge in PBS (pH 7.4) solution because of their isoelectric point that was about 6.2. From the FET experiment by liquid-gating, we confirmed that GLOD immobilization would decrease the conductance of CNT network junctions, like other proteins, regardless of the sign of the net charge [5]. Therefore, the result suggested that the decreased conductance resulted from rather the change in the capacitance of the tube than the simple electrostatic gating effect that occurred when GLOD was immobilized on it.

In order to confirm the sensitivity to the glutamate, we measured real-time changes in the conductance of CNT network junctions. As shown in Figure 1(A), when glutamate was injected to the buffer solution on CNT network junctions without GLOD, no significant change in conductance was observed. However, in case of immobilizing GLOD, the conductance of the junctions was changed by the repetitive addition of a standard glutamate solution, as shown in Figure 1(B). In the catalytic reaction where glutamate was converted to the 2-oxoglutarate by glutamate oxidase, hydrogen peroxide and ammonia molecule were produced. We utilized ethanolamine which deactivated and blocked the excess reactive groups remaining on the CNT surface to reduce the adsorption of ammonia [6] and nonspecific binding of proteins existing in-vivo. Therefore, it seemed that the conductance increased by the L-glutamate addition was attributed to the charge transfer from carbon nanotubes to hydrogen peroxide.

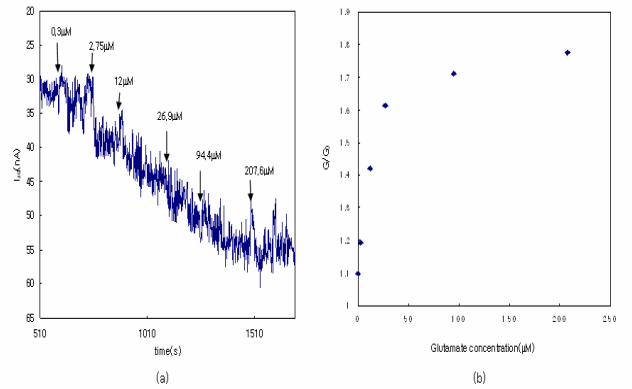

Figure 1. (a) A real-time conductance measurement in GLOD immobilized CNT network transistor in 40μl PBS (pH 7.4) solution. (V_{sd}=0.1V, V_g=0V) (b) The ratio of conductance changes (G/G_0) of CNT network transistor as a function of glutamate concentration.

Figure 2 shows the results of real-time monitoring of extracellular glutamate levels in the 11VO rat model. As shown in Figure 2(A), no significant changes of I_{sd} in the CNT probe without GLOD were observed before and after ischemia, including 10 min occlusion. However, in Figure 2(B) it was shown that the increase in the conductance of the CNT probe with GLOD was monitored as soon as ischemia was initiated, followed by two, sharp peaks and a return to its initial value during reperfusion. It was suggested that the initial increase of glutamate levels throughout the 10 min ischemic period was attributed to the reversed uptake of glutamate transporters which was due to an ionic imbalance [7]. On the other hand, it was thought that the second and third sharp peaks, which were not often shown in previous studies, were caused by a reperfusion-evoked release of glutamate. Therefore, these results suggest that the GLOD-immobilized SWNT probe has a fast response time and a high level of sensitivity both of which are essential in the real-time monitoring of glutamate transients during and after ischemia.

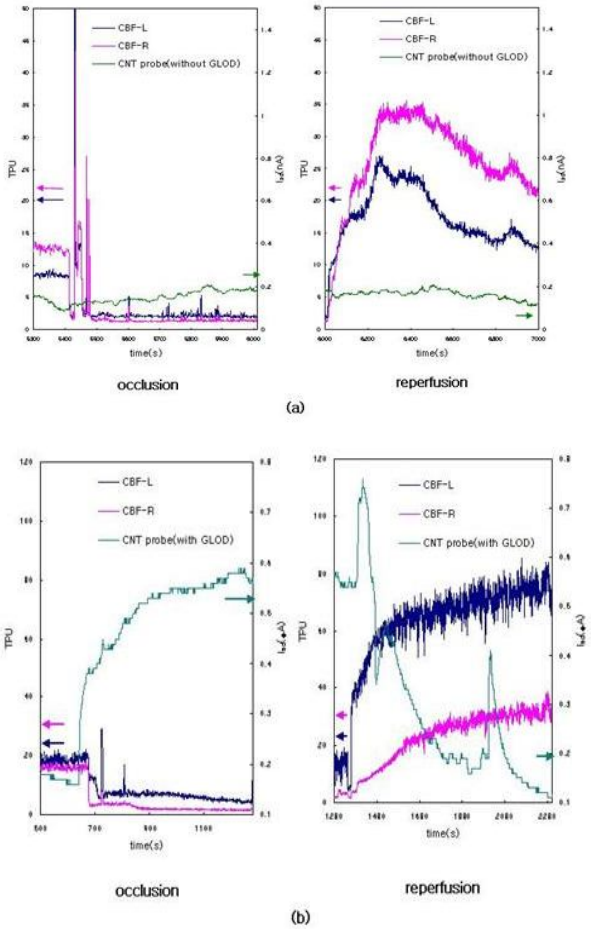

Figure 2. Real-time measurements of glutamate and CBF levels in the 11VO rat model. (A) CNT transistor without GLOD (B) CNT transistor with GLOD (deep blue line : CBF-left, pink line : CBF-right, green line : CNT probe)

4 CONCLUSIONS

Glutamate oxidase immobilized on CNT network junctions retained the enzymatic activity which was responsible for the change of conductance on L-glutamate addition. It was proposed that hydrogen peroxide worked more effectively compared to the ammonia in the conductance of CNT networks treated by the ethanolamine.

The continuous, real-time changes of glutamate release in the 11VO rat model were successfully measured with the GLOD-immobilized CNT network transistor. The initial release of glutamate by the glutamate re-uptake system was rapidly monitored in the intra-ischemic period. In addition, the CNT probe even detected the glutamate release which was temporarily evoked by reperfusion. From these results, the CNT network junctions built in the probe possess great possibilities for the real time monitoring of the neurotransmitter due to its fast response and high sensitivity.

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