Electrical Biosensor Of Kinase Assay on the MWCNT Nanoelectrode

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Abstract

We have demonstrated the use of MWCNT as a nanoscale probe to monitor the activity of enzyme kinase. To immobilize the substrate peptide using carbodiimide chemistry, plasma or strong acid treatments were used to induce carboxyl groups on the sidewall of MWCNTs. After the susbtrate peptide immobilization, increase of conductance from MWCNT devices was observed. When peptide modified MWCNTs react with enzyme kinase, conductance decreases by several orders of magnitude, and this conductance change can be explained by the phosphorylation reaction of enzyme kinase. When the sample was incubated with phosphatase to dephosphorylate the substrate peptide, nearly complete recovery of the conductance signal has been observed. 4 for 6 devices appeared the same trends. So, we can confirm that we have monitored the kinase activity on the MWCNT surface by electrical detection.

1. Introduction

Carbon nanotubes offer a range of extraordinary properties that make them ideal for chemical and biological sensing applications[1]. For example, significant conductance change of single wall carbon nanotubes (SWCNTs) in response to the adsorption of gas molecules and biomolecules demonstrate the possibility of extremely sensitive sensor systems based on them. Recently many researches using MWCNTs have started also in various sensor fields. They could be utilized for the development of carbon-based field-effect transistor(FET) through a simple deformation[2]. MWCNTs also have the potential for the biological sensors since their surfaces can be functionalized by the biomolecules or chemicals for an ultrasensitive electrical detection, which are caused by a binding and adsorption of a charged biological macromolecule or chemicals on the surface[3]. The subtle electronic behaviors of CNT reveal that they have the ability to promote electron-transfer reactions of important molecules, including proteins, DNA, polymers, chemicals and gases, showing a promising system as chemical and biological sensors. In this research, we study a model system of MWCNT-based sensor for kinase A. The schematic procedure of this study is shown in Fig. 1.

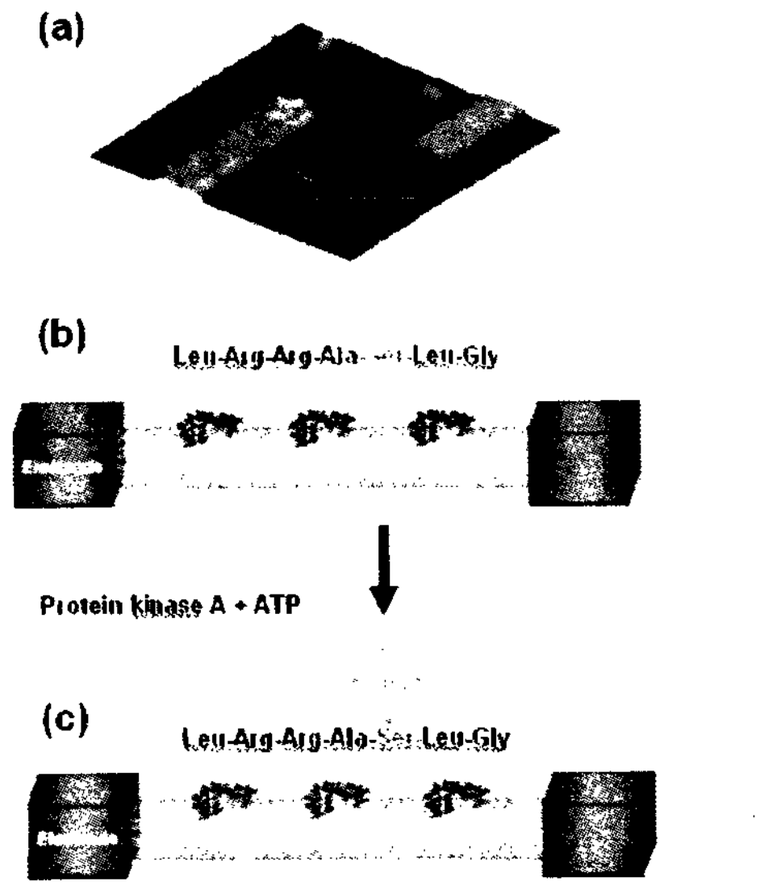

Figure 1 (a) Tapping mode AFM image of a MWCNT device. The nanotube is contacted by two Ti/Au electrodes. Scale bar represents 1 μm. (b) Scheme for peptide substrates immobilization on a metallic MWCNT and electrical measurement. (c) Scheme for phosphorylation of the peptide substrate on a metallic MWCNT by protein kinase A.

2. Theory

MWCNT can have the metallic and semiconducting characteristics shell by shell[4]. In the measurement of electricity, sensitivity depends highly on the state of the surface. The defect of MWCNTs are due to metallity or semiconductivity in semiconductor or metal surface. In addition, when the carboxylic group on the MWCNT make the amide bonding with amine group the protonation is caused[5]. The protonation increases the conductance in semiconductor. Other CNT surface without the carboxylic group adsorbs the protein by nonspecific binding. The metallic CNTs induce the change of workfunction through the immobilization of biomolecules[1].

Phosphorylation of protein occurs on the hydroxyl group of serine, threonine and tyrosine residues[6]. It catalyzes the transfer of a phosphate group, which is an electron-rich functional group, from ATP (adenosine 5'-triphosphate) to the serine residue of the heptapeptide Leu-Arg-Arg-Ala-Ser-Leu-Gly (LRRASLG) called as kemptide and immobilized on MWCNT electrode. The catalytic transfer of a phosphate group originated from the enzymatic reaction of protein kinase A(PKA) on the MWCNT samples leads to the change of electric properties of the MWCNT for the efficient detection of kinase assay.

3. Experimental

A simple CNT sensor platform is fabricated. In this platform, MWCNTs were deposited on the Au/Ti grid-electrodes using a solution casting process. The MWCNTs were then sonicated in the acidic solution or treated by plasma to produce defects on which carboxyl end-groups are formed. In the next step, suspension was formed by the dispersion of MWCNT in dichloroethane(DCE). A CNT-DCE solution was drop-deposited onto the Au/Ti grid area of the electrodes. Electrodes are deposited on an ending upper part of the MWCNTs with the e-beam lithography. We confirm the carboxylic group by X-ray photoelectron spectroscopy. The XPS measurements were carried out on a ESCAR 2000. After the MWCNT sample was incubated in the peptide solution, the two-probe conductance of MWCNT was measured. All detections were performed using a probe stationCASCADE microtech(HP4156A). Then, we explored PKA reaction on the kemptide immobilized on the MWCNT nanoelectrode device. The sample was incubated with kinase solutions for 1 hour at 37℃. The two-probe conductance of MWCNT was measured following the incubation. Finally, the dephosphorylation experiment was carried out. We repeated the same experiment for 6 devices to obtain more exact results.

4. Results and discussion

We can find the XPS binding energy at 287-289 eV as shown in Fig. 2. Immobilization of kemptide increased the conductance. We postulated the MWCNTs were modified with substrate peptides (kemptide) on the partial region of carboxylic group *via* carbodiimide chemistry or kemptides were simply adsorbed on the other empty side of MWCNTs. The carbodiimide chemistry was to form the amide linkages between their amine residues on the kemptide and carboxylic acid groups on the MWCNTs, while the latter was the simple adsorbtion. From the fact that MWCNT surface had both the metallic and semiconducting property[4], we can guess the changed electricity is caused by the protonation on the semiconductor and the change of workfunction on the metal[1]. Through the phosphorylation, a phosphate group has two negative ionic active sites, so they interact with the surrounding ions of the MWCNTs. The reaction of PKA with the substrate peptides coated on the sidewall of MWCNTs significantly decreases the tube conductance up to several times as shown in fig. 3b.

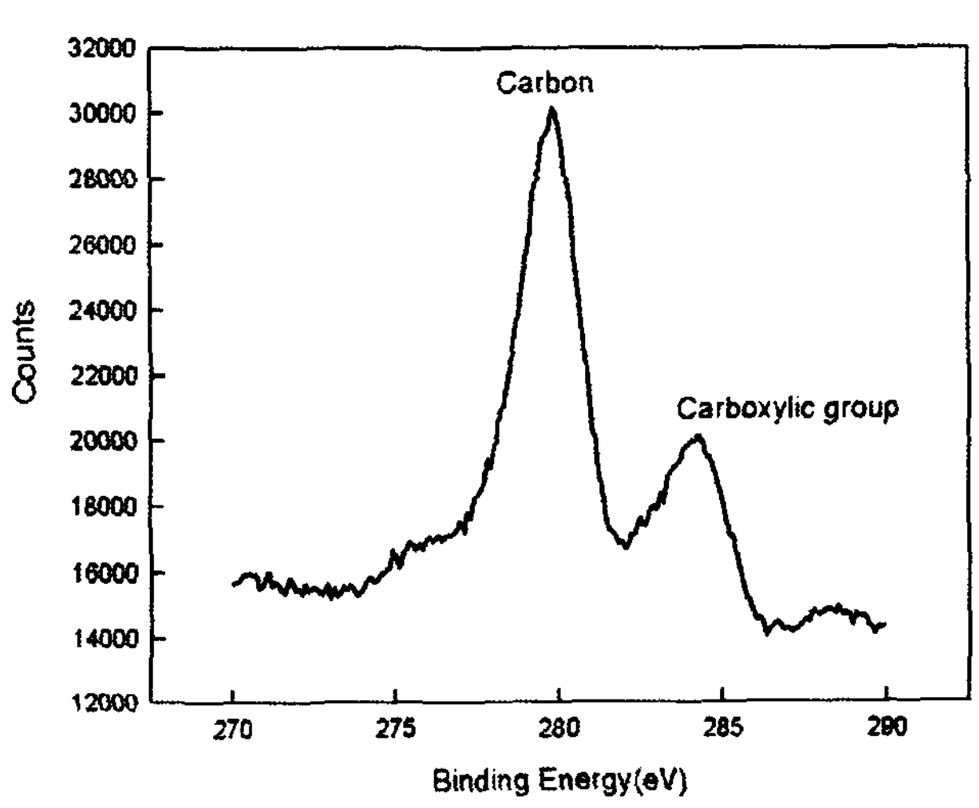

Figure 2 XPS data recorded on a carboxylic acid-functionalized SiO_2 surface by plsama discharge.

After the dephosphorylation reaction of the sample, the conductance was almost restored as shown in Figure 3c. Finally, the conductance decreases due to a phosphorylation of the peptides by kinase. Thus, we can find the recovery property by a biological reaction in the biosensor as shown in Fig.4. Furthermore, through the repetition experiments of 6 devices, we could notice the similar tendency of conductance variation for such processes as after kemptide deposition, after phosphorylation and after

dephosphorylation(recovery) devided by before reaction value. The conductance of phosphorylation devided by initial value decreases and dephosphorylation(recovery) devided increase again. This result is similar to one device's from Fig.4.

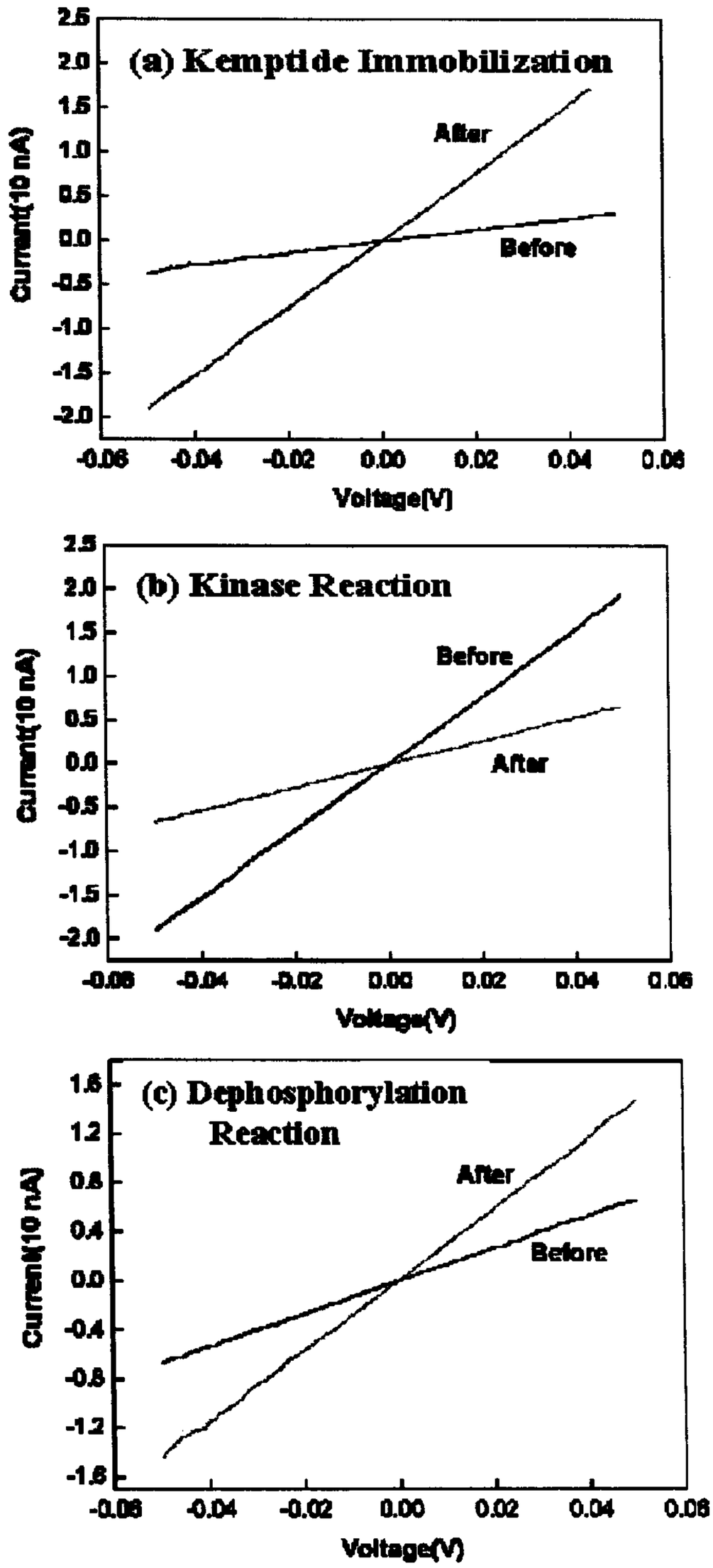

Figure 3 I-V data recorded on three different steps: before immobilization as a control, substrate peptide immobilization, PKA reaction by use of the same metallic MWCNT electrode. (a) before and after immobilization. (b) before and after kinase reaction onto its surface using the same electrode. (c) before and after dephosphorylation reaction.

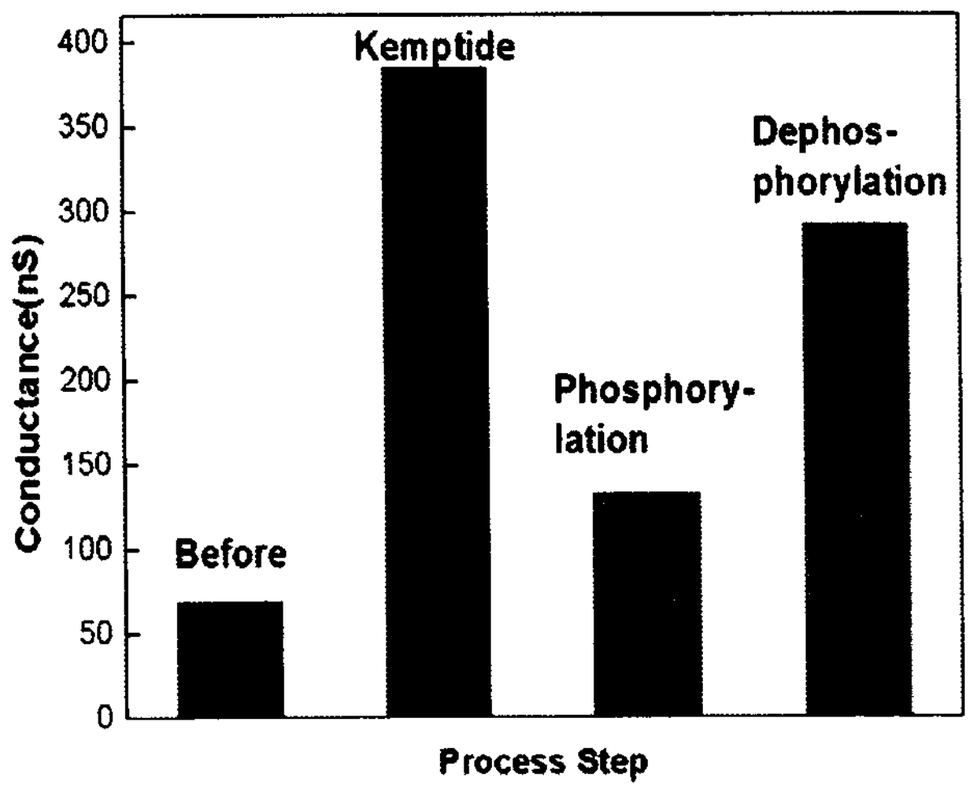

Figure 4 Histogram of conductance variation for each processes before reaction, after kemptide deposition, after phosphorylation and after dephosphorylation(recovery).

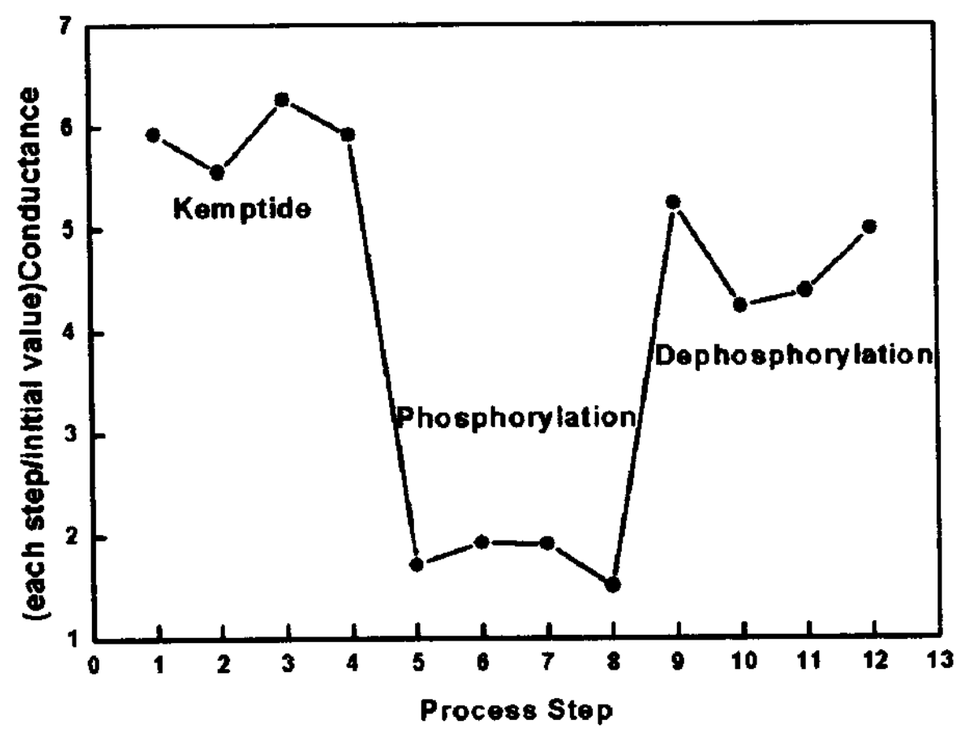

Figure 5 Conductance variation for each processes after kemptide deposition, after phosphorylation and after dephosphorylation(recovery) devided by before reaction via 4 samples.

4. Conclusion

We have shown the first biosensor based on an individual MWCNT nanoelectrode. The reaction of PKA on the MWCNT surface varified the conductance to several times. 4 for 6 devices appeared the same trends. So, we can confirm that we have monitored the kinase activity on the MWCNT surface by electrical detection.

5. References

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