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Monitoring Post-Translational Protein Sulfation on Silicon Nanowire Field Effect Transistor

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Abstract

We combine an *in situ* protein sulfation system with an ultra-high sensitive nanowire sensor, poly silicon nanowire field effect transistor (Poly-Si NWFET), to observe enzyme catalyzed protein sulfation in real-time.

1. Introduction

Protein sulfation is a vital post-translational modification that facilitates protein-protein interaction, which in turn regulates many physiological responses and pathological diseases. One of the prominent examples involves protein sulfation is the interaction between sulfated P-selectin glycoprotein ligand-1 (PSGL-1) and the capsid protein VP1 of enterovirus 71 (EV71), which enables virus infection [1]. Monitoring protein sulfation has been difficult due to the instability of sulfated protein and the lack of suitable detection method in real-time. In this study, we combine an *in situ* protein sulfation system with an ultra-high sensitive nanowire sensor (poly silicon nanowire field-effect transistor, poly-Si NWFET) to observe protein sulfation label-free and in real-time. A peptide containing sulfation site of PSGL-1 was immobilized on the nanowire surface of poly-Si NWFET by 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde as linker molecules. The immobilization process was confirmed by X-ray photoelectron spectrometer (XPS) analysis. A coupled-enzyme sulfation system including tyrosylprotein sulfotransferase (TPST) and phenol sulfotransferase (PST) was used to supplement the sulfuryl groups onto the immobilized PSGL-1 [2]. The protein sulfation on PSGL-1 was confirmed by ELISA and SPR. The I_D–V_G curves of poly-Si NWFET were obtained and a significant difference before and after protein sulfation was observed. Further electric variation of the poly-Si NWFET was observed following incubation with anti-tyrosylsulfo antibody. This is the first report to describe *in situ* protein sulfation and monitoring on semiconductor devices. Our next step is to observe the protein-protein interaction between sulfated PSGL-1 and VP1 of EV71 and develop the biosensing system in early EV71 diagnosis.

2. Material and methods

Device Fabrication

Poly-Si NW FETs were fabricated at the National Nano Device Laboratories (Hsinchu, Taiwan) according to a side-wall spacer technique previously reported [3]. The n-type poly crystalline silicon (poly-Si) NWs serving as 1D conducting channels were fabricated using fully complementary metal–oxide–semiconductor (CMOS) compatible processes [4] (Fig. 1a and 1b).

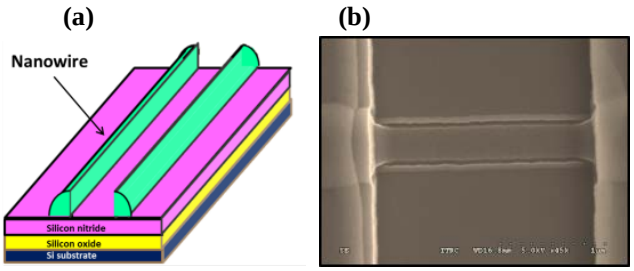

Fig. 1 (a) Schematic of poly-Si NWFET (b) Scanning electron microscope (SEM) image of poly-Si NWFET.

Electric measurements

All of the measurements were carried out at room temperature. NW Sensors were characterized in terms of their drain current-gate voltage (I_D-V_G) response measured using commercial semiconductor analyzer (Keithley 2636).

Immobilization of PSGL-1 on the NW surface

As-fabricated device was cleaned with acetone and ethanol for 5 min, respectively, and then treated with oxygen plasma for 5 min. After Oxygen plasma treatment the device was immersed in 2% APTES (3-Amino-propyltriethoxysilane)/ethanol solution for 30 min. The device was then heated at 120 ^{o}C for 10 min to produce amine groups on Si nanowires. The nanowire was then incubated with 12.5% glutaraldehyde/10mM pH7 phosphate buffer solution for 1 hr. PSGL-1 peptide (ATEYEYLDYDFL) whose concentration was 10 μm in PB solution attach to the surface of device for 12hr. Finally, the device was immersed in 10mM Tris-HCl (pH 7.0) with 4 mM sodium cyanoborohydride for 10 min and the

un-reacted aldehyde groups were blocked (Fig. 2).

Sulfation of PSGL-1

PST-TPST coupled-enzyme system was used to catalyze the protein sulfation of PSGL-1 [2]. The reaction mixture contained 50 mM MES buffer (pH 6.5), 30 μM PAPS, 2 mM MUS, 17 mU (28 μg) K65ER68G PST and NusA-DmTPST (5-30 μg) in 200 μl. The total volume of reaction mixture was increased ten times (2 ml) and added into microfluidic channel to avoid the gas bubble on the surface of the biosensor. The reaction was pre-incubated at 37°C for 5 minutes before adding TPST. The reaction solution was injecting into the microfluidic channel and the protein sulfation took place on the surface of the nanowire immobilized with PSGL-1 at RT for 10 minutes.

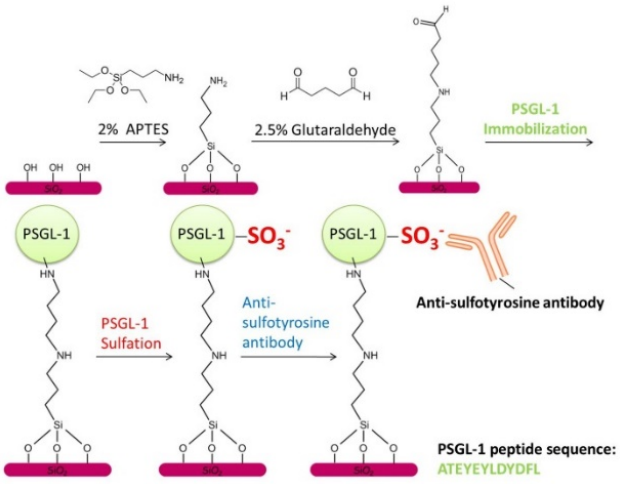

Fig. 2 Schematic illustration of the immobilization of PSGL-1 peptide on the nanowire surface.

3. Results and Discussion

Protein sulfation was first confirmed by both enzyme-linked immunosorbent assay (data not shown) and surface plasmon resonance (SPR). As shown in Fig. 3, both GST and GST-PSGL-1 were recognized by anti-GST antibody which was immobilized on the SPR chip, indicating the GST and GST-PSGL-1 were successfully immobilized on the SPR chip. Anti-sulfo antibody did not recognize either GST or GST-PSGL-1 (data not shown), indicating that there was not sulfation on either proteins. Following sulfation treatment, anti-sulfo antibody recognized only sulfated GST-PSGL-1 (Fig. 3), indicating that GST-PSGL-1 but not GST along can be sulfated catalyzed by the PST-TPST coupled-enzyme system.

Immobilization procedures on nanowire were confirmed by XPS (data not shown). Elemental analyses gave significant variations following each step (Fig. 2) of surface modification on silicon oxide wafer. This result indicated that immobilization of PSGL-1 peptide on the nanowire was successful.

Electric response of functionalized poly-Si NWFET for biosensing PSGL-1 sulfation was shown in Fig. 4. Significant I_D-V_G curve variations were observed only following protein sulfation and anti-sulfotyrosine antibody treatments (Fig. 4a). In the absence of sulfation enzyme, TPST, PSGL-1 would not be sulfated and the I_D-V_G curve remained constant (Fig. 4b). These data indicated that sulfation of PSGL-1 peptide immobilized on the nanowire surface can be sulfated and can be observed directly with the polySi NWFET device.

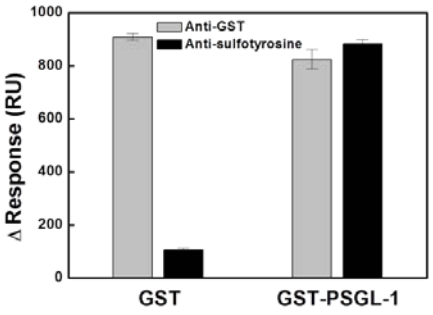

Fig. 3 SPR detections of GST-PSGL-1/sulfated GST-PSGL-1 with anti-GST antibody and anti-sulfotysosine antibody. Anti-GST antibody was immobilized on the SPR chip. 30 μg/ml (1 μM) GST-PSGL-1/sulfated GST-PSGL-1 and 30 μg/ml (1.1 μM) GST/sulfated GST were separately added, followed by 20 μg/ml (0.13 μM) anti-sulfotyrosine antibody.

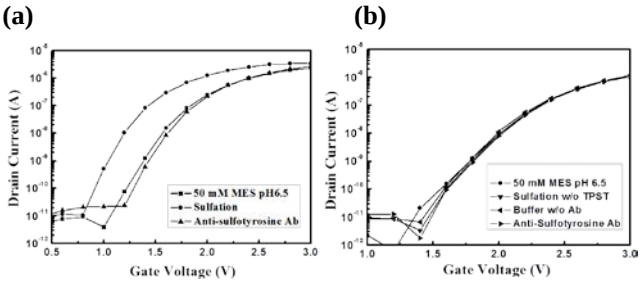

Fig. 4 Electric response of functionalized poly-Si NWFET to (a) PSGL-1 sulfation and the interaction between sulfated PSGL-1 and Anti-sulfotyrosine antibody. (b) Control experiment without PSGL-1 sulfation.

4. Conclusions

We developed a method to monitor post-translational protein sulfation by using poly-Si NWFET that can be used as a biosensor for detecting protein sulfation and its following protein-protein interaction event.

References

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