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Monitoring extracellular K^+ flux with a valinomycin-coated silicon nanowire field-effect transistor

Ko-Shing Chang[a,b], Chih-Jung Sun[a], Pei-Ling Chiang[a,b], Ai-Chuan Chou[c], Ming-Chou Lin[d], Chieh Liang[a], Hui-Hsing Hung[c], Yu-Hsiu Yeh[a], Chii-Dong Chen[d,e], Chien-Yuan Pan[c,f,**], Yit-Tsong Chen[a,b,*]

[a] Department of Chemistry, National Taiwan University, Taipei 106, Taiwan
[b] Institute of Atomic and Molecular Sciences, Academia Sinica, P.O. Box 23-166, Taipei 106, Taiwan
[c] Institute of Zoology, National Taiwan University, Taipei 106, Taiwan
[d] Institute of Physics, Academia Sinica, Taipei, Nankang 11529, Taiwan
[e] Department of Physics, National Cheng Kung University, Tainan 701, Taiwan
[f] Department of Life Science, National Taiwan University, Taipei 106, Taiwan

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ABSTRACT

A silicon nanowire field-effect transistor (SiNW-FET) coated with a polyvinyl chloride (PVC) membrane containing valinomycin (VAL) was employed as a biosensor (referred to as VAL-PVC/SiNW-FET) to detect the K^+-efflux from live chromaffin cells. The detection sensitivity of K^+ with the VAL-PVC/SiNW-FET covers a broad range of concentrations from 10^{-6} to 10^{-2} M. The apparent association constants between VAL and Li^+, Na^+, K^+, and Cs^+ in Tris buffer solution were determined to be 67 ± 42, 120 ± 23, 5974 ± 115, and $4121 \pm 140\ M^{-1}$, respectively. By culturing chromaffin cells on the VAL-PVC/SiNW-FET, the conductance was significantly increased by nicotine stimulation in a bath buffer without Na^+. The K^+ concentration at the cell surface was determined to be ~20 μM under the stimulation of 5 mM nicotine. These results demonstrate that the VAL-PVC/SiNW-FET is sensitive and selective to detect the released K^+ from cells and is suitable for applications in cellular recording investigations.

1. Introduction

The asymmetric distribution of various ions across the plasma membrane is an important physiological property of live cells. The two main mechanisms responsible for the differences in ionic concentrations across the plasma membrane are the impermeability of the plasma membrane to charged molecules and the Na^+/K^+-ATPases residing within the plasma membrane. The Na^+/K^+-ATPase transports three Na^+ ions out of and two K^+ ions into the cell with the consumption of one equivalent of ATP, resulting in a concentration gradient of K^+ and Na^+ across the membrane (Glitsch, 2001). A slight efflux of K^+ is essential for the resting membrane potential. When cells are excited, the quick consecutive openings of voltage-gated Na^+ and K^+ channels constitute an action potential for signal transduction. Abnormalities in K^+ permeability have been suggested to be involved in diseases like epilepsy, diabetes mellitus, and neural degeneration (Jentsch, 2000; MacDonald et al., 2001; Singh et al., 1998). Accordingly, the importance of K^+ flux across the plasma membrane of a cell to regulate its normal physiological activities cannot be underestimated. Therefore, the measurements of cellular K^+ flux to monitor the ions' effect in modulating the membrane potential are of fundamental significance to the understanding of neural excitability.

In addition to the voltage-gated ion channels, the excitatory ionotropic neurotransmitter receptors at postsynaptic synapses can respond to the released neurotransmitters and allow the flux of Na^+, K^+, and Ca^{2+} through their conjugated channels. According to the electrochemical gradient, K^+ moves outward, while Na^+ and Ca^{2+} move inward to depolarize the membrane potential, which then propagates to the soma for signal integration to determine the initiation of an action potential (Barnett and Larkman, 2007). Adrenal medulla cells, differentiated from the sympathetic postganglionic neurons, have nicotinic-type acetylcholine receptors

Abbreviations: ACh, acetylcholine; BOX, buried oxide; C_{K^+}, K^+ concentration; C_{M^+}, metal ion concentration; $C_{nicotine}$, nicotine concentration; G, electrical conductance; ΔG, conductance change; HB, hexamethonium bromide; I_{sd}, source-drain current; K_a, association constant; M^+, alkali metal ion; nAChR, nicotinic-type acetylcholine receptor; NMG, N-methyl-D-glucamine; PDMS, polydimethylsiloxane; PLL, poly-L-lysine; PVC, polyvinyl chloride; SEM, scanning electron microscopy; SiNW-FET, silicon nanowire field-effect transistor; SOI, silicon-on-insulator; Tris, tris(hydroxymethyl)aminomethane; VAL, valinomycin; V_g, backgate voltage; V_{sd}, source-drain voltage.

* Corresponding author at: Department of Chemistry, National Taiwan University, Taipei 106, Taiwan. Tel.: +886 2 33668198; fax: +886 2 23636359.
** Corresponding author at: Institute of Zoology, National Taiwan University, Taipei 106, Taiwan. Tel.: +886 2 33662452; fax: +886 2 23636837.
E-mail addresses: cypan@ntu.edu.tw (C.-Y. Pan), ytcchem@ntu.edu.tw (Y.-T. Chen).

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(nAChRs) at the plasma membrane that can respond to the acetylcholine (ACh) released from sympathetic neurons. Upon agonist binding, the nAChR nonselective ion channels allow the net flow of positively charged ions inward to depolarize the membrane potential (Sala et al., 2008). Subsequently, the depolarization will open the voltage-gated Na^+ channels to further depolarize the membrane potential and then activate the voltage-gated Ca^{2+} channels to elevate the intracellular Ca^{2+} concentration for catecholamine release (Barrantes et al., 1995; Dani and Bertrand, 2007). However, if there is no extracellular Na^+, the membrane potential would not be elevated to a level high enough for the activation of other voltage-gated ion channels; meanwhile, only K^+ and Ca^{2+} will move across the nAChR.

To characterize the ion flux across the plasma membrane, electrophysiological recording that can provide the detailed kinetics of ion currents is the most direct technique to monitor the activities of ion channels. However, recording is time-consuming, needs specialized equipment, and requires special skills. In addition, electrophysiological recording measures the net flux of all ions, rather than just one particular ion. Specific ion-sensitive fluorescence dyes have been developed to monitor the concentration of ions like Na^+ and Ca^{2+} in the cytosol (Xu et al., 2001). However, the efflux of K^+ from a cell and its subsequent effect on neighboring cells has not yet been well characterized.

Effluxed K^+ ions diffuse quickly away from the cultured cell; in contrast, effluxed K^+ ions will accumulate at the cell–cell interface in tissue and elevate the local concentration of K^+ (denoted by C_{K^+}). Such elevation in C_{K^+} may affect the membrane potential and excitability of nearby cells (Bostock and Grafe, 1985). Therefore, the K^+ efflux from a cell not only repolarizes its own membrane potential but also regulates the excitability of neighboring cells. When a certain number of neurons fire together, as in epilepsy, the effect is amplified despite this effect being distance-limited. As a consequence, the C_{K^+} on the membrane surface is an important issue in neurotransmission and needs to be investigated.

Over the past two decades, nanomaterials such as quantum dots (Raymo and Yildiz, 2007), nanoparticles (Rosi and Mirkin, 2005; Tansil and Gao, 2006), nanowires (Cui et al., 2001), nanotubes (Star et al., 2003; Tsai et al., 2008; Wang et al., 2007), nanogaps (Liang and Chou, 2008), and nanoscale films (Shan et al., 2009) have received enormous attention because of their suitable properties for designing novel nanoscale sensors. For instance, the dimension of nanomaterials at 1–100 nm provides a perfect feature to study most biological entities (Curreli et al., 2008), such as nucleic acids, proteins, viruses, and cells. In addition, the high surface-to-volume ratios of nanomaterials allow a huge proportion of the constituent atoms to be located at or near the material surface. This characteristic makes the surface atoms play an extremely important role in determining the physical, chemical, and electronic properties of nanomaterials. Compared with devices made of micrometer-sized or bulk materials, nanomaterial-configured sensors commonly possess much better sensitivity, which is closely related to the reduced dimensionality and large surface-to-volume ratio. In general, nanoscale sensors have the advantages of high sensitivity, specificity, high-speed sample delivery, fast response time, and the requirement of only trace amounts of analyte (Chen et al., 2011). To date, nanoscale sensors have found a plethora of applications in many areas of the life sciences, clinical tests, and small molecule analysis (Chen et al., 2011).

Among nanoscale sensors, silicon nanowire field-effect transistors (SiNW-FETs) have been demonstrated to be a powerful molecular recognition-sensing platform for detecting proteins (Lin et al., 2009, 2010; Pui et al., 2011), DNA sequences (Bunimovich et al., 2006; Li et al., 2004), small molecules (Chang et al., 2009; Mcalpine et al., 2008), cancer biomarkers (Lee et al., 2009; Zheng et al., 2005), viruses (Patolsky et al., 2004), and cells (Huang et al., 2011; Patolsky et al., 2006; Stern et al., 2008; Tian et al., 2010). Despite the successful detections of a myriad of biomolecules by SiNW-FET, there are few examples of the employment of SiNW-FETs for probing biological metal ions. Several traditional methods, such as potentiometry and photometry (Lin et al., 2002; Meuwis et al., 1995; Nagatoishi et al., 2005; Pandey and Prakash, 1998), were reported previously for K^+ detection; however, the low sensitivity and/or poor selectivity over other metal ions (e.g., Na^+ and Li^+) have hindered the wide applications of these conventional methods for biological investigations. Notably, some micrometer-sized FETs were used previously for the investigation of the C_{K^+} released from live neurons and HEK 293 cells (Ingebrandt et al., 2005; Vassanelli and Fromherz, 1999; Wrobel et al., 2005); however, the employed FET devices were not modified with a specific receptor of high selectivity for K^+.

In this study, a nanoscale sensor for probing K^+ has been constructed by integrating valinomycin molecules ($C_{54}H_{90}N_6O_{18}$, abbreviated as VAL) with polyvinyl chloride (PVC) membrane onto a SiNW-FET surface (referred to as VAL-PVC/SiNW-FET, as illustrated schematically in Fig. 1(a)). The VAL molecule, a dodecadepsipeptide synthesized by several *Streptomyces* strains, has a high affinity for K^+ relative to other alkali metal ions like Na^+ (Lavinia et al., 1969; Eyal and Rechnitz, 1971; Rose and Henkens, 1974). The VAL-PVC/SiNW-FET responded differentially to various alkali metal ions (M^+) of Li^+, Na^+, K^+, and Cs^+ with concentrations ranging from 10 mM to the best detection limit of 1 μM. To test the effectiveness of the VAL-PVC/SiNW-FET in detecting K^+ efflux from single cells, bovine chromaffin cells were seeded on a device chip as illustrated in Fig. 1(b). When nicotine was applied to activate the nAChR and induce K^+ efflux, the electrical conductance (G, units in siemen (S)) of VAL-PVC/SiNW-FET increased as well. Our results reveal that VAL-PVC/SiNW-FET is able to discriminate various effluxed C_{K^+} from live cells, thus providing a useful platform to monitor the C_{K^+} in biological samples.

2. Materials and methods

2.1. Chemicals

Bis(1-butylpentyl) adipate, PVC, and potassium tetrakis(4-chlorophenyl) borate were purchased from Fluka. Tris(hydroxymethyl)aminomethane (abbreviated as Tris), VAL, and all other chemicals were reagent grade from Sigma–Aldrich. Dulbecco's modified Eagle's medium and all other reagents for cell culture were purchased from Invitrogen.

2.2. Fabrication of SiNW-FET devices

SiNW-FETs were fabricated from silicon-on-insulator (SOI) wafers as described in previous publications (Chen et al., 2011; Li et al., 2004; Lin et al., 2007). SOI wafers offer a layer of high quality single-crystal silicon (referred to as the "device layer") separated from the bulk substrates by a layer of buried oxide (abbreviated as the "BOX layer"). The SOI wafers are presently state-of-the-art in metal-oxide-semiconductor field-effect-transistors (MOSFETs) in the mainstream semiconductor industry. In this work, four-inch n-type SOI wafers containing a 50-nm-thick device layer and a 400-nm-thick BOX layer were used, in which the resistivity of the device layer was approximately 100 Ω cm (where the phosphor-doping concentration was 4×10^{19} cm^{-3}). After fabrication following standard electron-beam lithographic and photolithographic procedures, the SiNWs were thermally oxidized to form a SiO_2 insulating layer to prevent both the charge transfer between SiNW-FET and analyte molecules and the electrical leaking in the subsequent sensing measurements in aqueous