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Potentiometric responses of ion-selective microelectrode with bovine serum albumin adsorption

Tatsuro Goda*, Eriko Yamada, Yurika Katayama, Miyuki Tabata, Akira Matsumoto, Yuji Miyahara*

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda, Tokyo 101-0062, Japan

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ABSTRACT

There is a growing demand for an *in situ* measurement of local pH and ion concentrations in biological milieu to monitor ongoing process of bioreaction and bioresponse in real time. An ion-selective microelectrode can meet the requirements. However, the contact of the electrode with biological fluids induces biofouling by protein adsorption to result in a noise signal. Therefore, we investigated the relationship between the amount of nonspecific protein adsorption and the electrical signals in potentiometry by using ion-selective microelectrodes, namely silver/silver chloride (Ag/AgCl), iridium/iridium oxides (Ir/IrOx), and platinum/iridium oxides (Pt/IrOx). The microelectrodes reduced a potential change following the adsorption of bovine serum albumin (BSA) by comparison with the original metal microelectrodes without oxide layers. Suppression in the noise signal was attributed to the increased capacitance at the electrode/solution interface due to the formation of granulated metal oxide layer rather than a decrease in the amount of protein adsorbed. Ion sensitivity was maintained for Ir/IrOx against proton, but it was not for Ag/AgCl against chloride ion (Cl^-), because of the interference of the equilibrium reaction by adsorbed BSA molecules on the electrode surface at $< 10^{-2}$ M [Cl^-] in the solution. The results open up the application of the Ir/IrOx microelectrode for measuring local pH in realistic dirty samples with a limited influence of electrode pollution by protein adsorption.

1. Introduction

Ionic homeostasis differs in conditions of pathogen, cancer, infection, and so on due to alternation of metabolic activity and biological signaling at local cells and tissues at any time *in vivo*. For example, extracellular pH in the cancer tissue microenvironment is lowered by enhanced glycolysis, which is so-called the Warburg effect (Gatenby and Gillies, 2004). Hypoxia in tumor tissue modulates metabolic profiles to acidify extracellular pH (Svastova et al., 2004). Activation of the complement system and innate immunity also leads to acidification and hypercalcemia at the foci of inflammation and infection (Lardner, 2001; Rossol et al., 2012). In the field of dental science, an *in situ* measurement of pH in the cavity of tooth caries serves as an indicator for the activities of bacteria and the severity of destruction (Murakami et al., 2006). Therefore, time-lapse monitoring of pH and ion concentrations in confined biological space may elucidate the effect of ionic microenvironments on pathogen progression and/or immune responses. Moreover, proton (H^+) is a product in many biochemical and biocatalytic reactions by enzymes *in vitro* (Schoning and Poghossian, 2002, 2006). For example, Toumazou et al. used proton as a reporter molecule for electrically monitoring DNA amplification without using a fluorescent probe (Toumazou et al., 2013). Ion sensing is applied for fundamental understanding of transporter machinery on cell membranes or supported lipid bilayers (Schaffhauser et al., 2012; Watanabe et al., 2014).

Ion sensors are categorized into optical and electrical systems. The former measures either change in intensity or peak shift in a fluorescence spectrum as a function of ion concentrations using an optical setting (Domaille et al., 2008; Wouters et al., 2001). So far, a wide variety of fluorescent probes has been developed in terms of affinity (dissociation constant: K_d) and specificity to target ion species as well as the excitation/emission wavelength characteristics. One advantage for optical ion imaging is to allow time-lapse monitoring of two-dimensional distribution with a minimally invasive manner to biological systems. However, in general, the determination of absolute concentration is not possible for conventional optical acquisition modes. Moreover, optical sensing is difficult at the high concentrations greater than millimolar levels due to the absence of proper fluorophore with a low binding affinity and high specificity to a target ion species. On the other

* Corresponding authors.
E-mail addresses: goda.bsr@tmd.ac.jp (T. Goda), miyahara.bsr@tmd.ac.jp (Y. Miyahara).

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hand, the electrical method is based on potentiometry using an ion-selective electrode (ISE) system (Ammann et al., 1983; Buhlmann et al., 1998). The difference in ion concentration (activity) between the internal and external solutions across the semi-permeable ion-selective membrane is transduced into potentiometric signal as described by the Nernst equation so that this is a fluorescent probe-free technique. Normally, a standard solution with a known ion concentration is used as an internal solution for ISE to quantify an ion concentration of interest in the external solution. The sensitivity and specificity for target ion depend on the performance of ionophore as a main component of ion-selective membrane. So far, a series of ionophores has been synthesized to cover various ion species and dynamic ranges (Bakker et al., 1999, 2000; Buhlmann et al., 1998). Alternatively, ion-sensitive field-effect transistor (ISFET) has been used for measuring the concentration of proton or other ions in potentiometry (Bergveld, 2003; Matsuo and Esashi, 1981). The pH sensitivity is generated by the equilibrium reaction of self-dissociation of water molecules in the solution with abundant hydroxide groups on the surface of metal oxide such as silicon oxide, tantalum oxide, and iridium oxide as a gate insulator (vanHal et al., 1996). An ISFET modified with an organic ion-selective membrane on the gate surface exhibits sensitivity and specificity to target ions (Izquierdo and Decastro, 1995). Therefore, ISE and ISFET share the mechanism in determining free ionic concentration by the Nernst response at the electrode/solution interface in potentiometry. Advantageously, the ISFET system can be downsized and integrated into a high-throughput device with low cost because of the compatibility to the complementary metal oxide semiconductor (CMOS) fabrication process. These features have realized massively parallel analysis of biochemical reactions such as base extension of DNA by polymerase through electrical proton sensing (Rothberg et al., 2011). An integrated ISFET chip enabled real-time acquisition of two-dimensional ion distribution of biological samples with lateral resolution of tens micrometers and time resolution of seconds (Nakazawa et al., 2013; Takenaga et al., 2011).

Generally, ion-selective biosensors are used in a direct contact with biological solution, which arises interaction of the electrode surface with biomolecules such as proteins (Oki et al., 2001). Since biomolecules possess electrostatic charges, the irreversible adsorption of charged molecules has a risk to disturb electrical sensing. In addition, the coverage of sensor surface by adsorbed biomolecules may inhibit the equilibrium reaction of ions to lead the decreased sensitivity compared with the ideal Nernst response. Therefore, the effect of biomolecular adsorption on the generation of nonspecific signal and the passivation of ion sensitivity should be investigated for better understanding of signal transduction mechanism in potentiometry at the interface with biological solutions. Here we investigated the effect of bovine serum albumin (BSA) adsorption onto ion-selective microelectrodes such as silver coated with silver chloride (Ag/AgCl), iridium coated with iridium oxide (Ir/IrOx), and platinum coated with iridium oxide (Pt/IrOx) on the potentiometric ion-sensitivity. The Ag/AgCl electrode is sensitive to free chloride ion, while the others are sensitive to free proton. The sensing system is composed of a direct contact of BSA solution with the electrodes connected to a high input impedance electrometer. The BSA adsorption amount as a function of the potentiometric signal was determined by real-time potentiometry during BSA adsorption experiments using the electrodes with and without the oxide layers. Further, ion sensitivity was compared between the electrodes in the presence and absence of adsorbed BSA molecules on the surface.

2. Materials and method

2.1. Materials

Ag (99.99%, $\varphi = 0.4$ mm), Pt (99.98%, $\varphi = 0.4$ mm), and Ir (99.9%, $\varphi = 0.4$ mm) wires were obtained from Nilaco Co., Tokyo, Japan. All the other reagents of extra pure grade were purchased from Wako Pure Chemicals Co., Tokyo, Japan, and were used as received. Milli-Q water (EMD Millipore Co. Billerica, MA, USA) was used throughout the study.

2.2. Electrolytic process

Ag/AgCl and Pt/IrOx electrodes were prepared by electrolysis. Ag and Pt wires were washed by acetone, ethanol, and water with sonication for 10 min, followed by chemical etching in concentrated nitric acid solution for 1 min. After washing with water, Ag wires were electrolyzed in 0.1 M hydrochloric acid solution under DC bias voltage of +1.0 V versus Pt anode for 3 min to deposit an AgCl layer on the surface. The clean Pt wire was further treated by Piranha solution for 1 min at 100 °C (Caution! It is a strong oxidizing process). Pt wires were electrolyzed in the presence of $[Ir(COO)_2(OH)_4]^{2-}$ under DC bias voltage of −1.0 V versus Pt cathode for 15 min at 15 °C to deposit an IrOx layer on the surface. The electrolytic solution was prepared by dissolving 0.22 mmol iridium tetrachloride to a 50 mL water, followed by adding 0.5 mL of 30% hydrogen peroxide solution and 1.98 mmol oxalic acid dehydrate. Then, the solution was adjusted to pH 10.5 by adding potassium carbonate and were incubated for two days under dark at room temperature prior to use.

2.3. Thermal oxidation process

The long term stability for Pt/IrOx was poor due to the easy exfoliation of the IrOx layer from the Pt surface during handling. Therefore, we used thermal oxidation of an Ir wire to form the porous IrO_2 layer on the surface (Huang et al., 2013). A washed Ir wire was dipped in 5 M sodium hydroxide solution for 48 h. The wires were heated at 800 °C in air for 30 min using a muffle furnace, followed by quenching in water. The above processes were repeated for three times to obtain Ir/IrOx wires.

2.4. X-ray photoelectron spectroscopy (XPS)

The surface elemental composition of the electrodes was determined by XPS (AXIS-HSi165; Shimadzu-Kratos, Kyoto, Japan) equipped with a 15 kV Mg-Kα radiation source at the anode. The take-off angle of the photoelectrons was set at 90°. The curve fitting of the high-resolution spectra was performed by the Gaussian functions.

2.5. Quantification of BSA adsorption by the MicroBCA™ method

The clean wire electrodes of 15 mm in length were dipped in a 4-(2-Hydroxyethyl)-1-piperazineetahnesulfonic acid (HEPES) buffer solution (1 mM HEPES, 100 mM KCl; pH 7.4). The solution was then switched to 0.01, 0.1, or 1 mg/mL BSA solution to allow nonspecific adsorption of BSA onto the electrode surface for 15 min at room temperature. After that, the electrode was gently rinsed by dipping in the buffer solution to remove loosely bound BSA. The adsorbed BSA was detached by sonication of the electrodes in a 160 μL of 1% sodium dodecyl sulfate (SDS) solution. The concentration of BSA in the SDS solution was determined by the MicroBCA™ spectrophotometric assay (absorbance at 562 nm, Pierce-Thermo Scientific, Wallthan, MA, USA) using a microplate reader (Smith et al., 1985).