Potentiometric Responses of Ion-Selective Microelectrode with Bovine Serum Albumin Adsorption

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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 $[\text{Cl}^-]$ 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.

KEYWORDS. Ion-selective electrode; potentiometry, protein adsorption, X-ray photoelectron spectroscopy, cyclic voltammetry

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 also modulate 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 Poghosian 2002, 2006). [ENREF 7](#) 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 changes 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 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; Bakker et al. 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

Materials.

Ag (99.99 %, $\phi = 0.4$ mm), Pt (99.98 %, $\phi = 0.4$ mm), and Ir (99.9 %, $\phi = 0.4$ 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.

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, respectively, 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 $[\text{Ir}(\text{COO})_2(\text{OH})_4]^{2-}$ 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.

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 IrO₂ 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.

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-K α 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.

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-piperazineethanesulfonic 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 μ L 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, Walltham, MA, USA) using a microplate reader (Smith et al. 1985).

Potentiometry for BSA adsorption

The electrodes were connected to a high input impedance electrometer (6517B, Keithley Instruments, Cleveland, OH, USA) with a low current, 10-channel scanner card (Model 6522, Keithley) (Figure S1). The Ag/AgCl (in 3.3 M KCl solution) were used as a reference electrode via a salt bridge. The electrodes were incubated in the HEPES buffer solution (1 mM HEPES, 100 mM KCl; pH 7.4) for 100 min at 25°C. The baseline potential was obtained from an average for the last 15 min before replacing the buffer into the BSA solution. Then, the buffer solution was replaced into 0.01 mg/mL BSA solution for 15 min, and exchanged into the buffer solution for 15 min. The process of solution exchange was repeated two times for measuring the potential under the conditions of 0.1 and 1 mg/mL BSA solutions. An average of potentials at the period of the last one minute during the rinse step after incubation in the different concentrations of BSA solutions was used to determine the degree of potential shift from the baseline as a signal.

Cyclic voltammetry (CV)

CV scans were performed in the HEPES buffer solution (1 mM HEPES, 100 mM KCl; pH 7.4) at the potential window from -50 to 50 mV at the scan rate of 100 mV/s by using a potentiostat (PGSTAT302, Metrohm Autolab, Utrecht, the Netherlands) with a counter electrode of Pt and a reference electrode of Ag/AgCl (in 3.3 M KCl solution).

The interface capacitance at no DC bias potential to the reference electrode was determined using the following equation;

$$C = \Delta I / A\nu \quad (1)$$

where ΔI , A , and ν represent the difference between the forward and reverse currents at 0 V, the geographical surface area of electrode, and the scan rate, respectively.

Potentiometry for measuring the Nernst responses

The potentiometric response of the Ag/AgCl electrode as a function of $[Cl^-]$ in the HEPES buffer solution (1 mM HEPES; pH 7.4) was determined by the electrometer. The potential changes of the Pt/IrOx and Ir/IrOx electrodes were determined in the standard solutions of pH 4, 7, and 9. The Nernst response were also measured for the electrodes after in contact with 1 mg/mL BSA solution for 15 min at 25 °C, followed by rinse in the buffer. The Ag/AgCl (in 3.3 M KCl solution) was used as a reference electrode via a salt bridge.

3. Results and Discussion

Surface characterization was conducted by XPS (Figure 1, Figure S2 and Table S1 in supporting information). A pristine Ag electrode showed two known peaks corresponding to Ag₂O and AgO both at Ag $3d_{3/2}$ and Ag $3d_{5/2}$, respectively (Figure 1a). For an Ag/AgCl electrode, there were single peaks at Ag $3d_{3/2}$ and Ag $3d_{5/2}$ together with a distinct Cl $2p$ peak ($2p_{1/2}$ for red line and $2p_{3/2}$ for orange line), indicating the formation of AgCl layer on the outermost surface. For an Ir/IrOx electrode, the peaks corresponding to IrOx increased at Ir $4f$ (green and blue lines) and O $1s$ (orange line) by comparison with a pristine Ir electrode (Figure 1b). A strong O $1s$ peak for the pristine Ir electrode suggests intrinsically formed passivation oxide and/or hydroxide layers on the outermost surface. The Pt $4f$ peak disappeared while the Ir $4f$ and O $1s$ peaks increased for a Pt/IrOx electrode compared with a pristine Pt electrode due to the formation of a thick IrOx layer (Figure 1c). Again, the strong O $1s$ peak for the pristine Pt electrode was observed due to the presence of hydroxide groups on the surface.

There were small differences in the amount of BSA adsorption between the six electrodes (Figure 2), in agreement with previous studies (Selvakumaran et al. 2008). The BSA amounts per geographical surface area were about 0.1 $\mu\text{g}/\text{mm}^2$. A modest increase in the BSA adsorption by increasing the surrounding BSA concentration indicates the process to reach full monolayer coverage of BSA on the electrode surfaces (Mao et al. 2001). Generally, adsorption of proteins on a material surface occurs in a time-dependent irreversible manner which is composed of an initial adsorption of proteins driven by a synergetic effect by electrostatic forces, hydrogen bonding, van der

Waals forces, and hydrophobic interaction, followed by a conformational change and denaturation of the adsorbed protein. In addition, protein adsorption depends on a surrounding microenvironment such as ionic strength and pH of the solution, surface morphology and composition of the material, and higher order structures of the protein (Fraaije et al. 1991; Xie et al. 2003). Interaction of protein with metal or metal oxide has been extensively studied on a material with various dimensions from nano to macro scales (Hughes et al. 2013; Hulander et al. 2009; Selvakumaran et al. 2008; Williams et al. 1985; Wilson and Rauh 2004). [ENREF 8](#) Proteins containing disulfide (S-S) group can bind firmly to silver via disulfide bridges that makes it irreversibly adsorbed with respect to dilution (Podstawka et al. 2004). Moreover, the monolayer coverage by protein molecules reduces the amount of released ions at the interface (Wang et al. 2014).

We performed simultaneous potentiometry for the six types of electrode during BSA adsorption (Figure 3a). The baseline potential during the initial conditioning process in the buffer solution was stable and the potential shift following BSA adsorptions was suppressed for the Ag/AgCl, Pt/IrOx, and Ir/IrOx electrodes (Figure 3b). In contrast, the baseline potential was unstable for the pristine Ag, Pt, and Ir electrodes. The potential was changed dramatically by incubating with BSA because of the adsorption of BSA on the Ag, Pt, and Ir electrodes. The potential shift (ΔV) after rinse process compared with the baseline was pronounced by increasing bulk BSA concentration for the Ag, Pt, and Ir electrodes (Figure 3c). The commonly observed potential recovery during the rinse process indicates partial detachment of loosely bound BSA from the electrodes. Based on the MicroBCA™ assay in Figure 2, the amount of BSA adsorption was about $0.1 \mu\text{g}/\text{mm}^2$ among the six electrodes with the limited influence on the bulk BSA concentrations. Therefore, it is reasonable to understand that the apparent inconsistency between the potential change and the amount of adsorbed BSA is attributed to differences in the electrical signal transduction at the interface among the pristine and modified electrodes. Generally, the potential shift indicates a change in the microenvironment at the electrode/solution interface caused by protein adsorption (Goda and Miyahara 2010a, b). The charge density change can be described by the Poisson–Boltzmann theory in either linearized approximation or in a full solution. The charge effect depends on the type of chemical groups in the protein, pH, conformation of the molecules, distance to the surface, surface roughness, ionic strength of the solution, cation type, temperature, and so on (Poghossian et al. 2005). By assuming the constant value of the capacitance of the electrical double layer at the interface (C) during protein adsorption event, the interface potential change (ΔV) can be

described by using the overall charge density change at the interface (ΔQ) as follows;

$$\Delta V = \Delta Q/C \quad (2)$$

Since ΔQ is the sum of the charge effect from individual protein adsorbed on the electrode (Goda et al. 2012; Goda and Miyahara 2010a), the negative potential shifts was derived from the net-negative charges of BSA molecule (isoelectric point: 4.6) at pH 7.4. In our previous work, ΔQ was proportional to the amount of BSA adsorbed on an electrode because of the globular conformation, isotropic orientation of adsorption, and evenly distributed surface charges of BSA molecules (Goda and Miyahara 2012). From eq. (2), the small changes in the potential after BSA adsorption on the Ag/AgCl, Pt/IrOx, and Ir/IrOx electrodes are attributed to relatively high capacitances at the interface due to the presence of granular oxides with many boundaries on the metal electrodes as visualized by scanning electron microscopy (SEM) (Figure S3). However, the positive potential change following an initial contact with 0.01 mg/mL BSA solution on the Ag and Pt electrodes cannot be explained by the charge effect as a result of BSA adsorption. One reason is the sulfuration of the noble metals due to the formation of disulfide bridges between the cysteine or cysteine residues in adsorbed BSA and the bare Ag surface (Podstawka et al. 2004). Indeed, the presence of disulfide bridge was confirmed exclusively on the pristine Ag electrode after in contact with BSA (Figure S4). Kamahori *et al.* reported the chemisorption of a thiol compound to form a self-assembled monolayer onto a gold electrode generated a dramatic positive change in the interface potential (ΔV) in potentiometry (Kamahori et al. 2007).

We conducted cyclic voltammetry to prove the higher capacitance (C) in the Ag/AgCl, Pt/IrOx, and Ir/IrOx electrodes than the Ag, Pt, and Ir ones as a result of the formation of the granulated oxidized layers on the metal electrode. The cyclic voltammograms showed a large hysteresis between the forward and reverse scans for Ag/AgCl, Ir/IrOx, and Pt/IrOx electrodes compared with Ag, Ir, and Pt ones (Figure 4). The formation of oxide layer increased the capacitance by 5-fold for Ag and by 110-fold for Pt (Table 1). Considering model electrical equivalent circuit of a parallel connection of two capacitors for an electrode covered with defective metal oxide layer (Figure S5) as confirmed by SEM (Figure S3) (Sun and Wang 2014), the combined interface capacitance is described as;

$$C = C_{dl} + C_{ox} \quad (3)$$

where C_{dl} and C_{ox} represent the capacitance of the electrical double layer and the metal oxide layer, respectively. The extremely high capacitance of the Pt/IrOx electrode compared with the bare Pt one is interpreted as the presence of a high C_{ox} due to the formation of thin IrOx layer on the electrode layer during the cathodic electrochemical

treatment. The high C_{ox} may also depend on an increase in eventual surface area of the electrodes as observed by SEM images (Figure S3).

Finally, ion sensitivity was determined to investigate the effect of protein adsorption on the surface of metal oxide on the ion sensitivity (Figure 5). Generally, the Ag/AgCl electrode shows the Nernst response for $[Cl^-]$ in a solution because of the equilibrium reaction of

at the electrode/solution interface. The Nernst response (E) can be described as follows:

$$E = E_0 - 2.303 \times (RT/zF) \log_{10}([Ox]/[Red]) \quad (5)$$

where E_0 , R , T , z , F , $[Ox]$ and $[Red]$ represent the standard potential, gas constant, the absolute temperature, the number of moles of electron transferred in the reaction, the Faraday's constant, the oxidant concentration, and the reductant concentration, respectively. At room temperature of 25°C, $-2.303 \times RT/F$ is treated as a constant with the value of -59.2 mV. The clean Ag/AgCl electrode had the sensitivity of -56.6 mV/log₁₀[Cl⁻] at the dynamic range of 10⁻³ to 10⁻¹ M [Cl⁻] (Figure 5a). After performing BSA adsorption on the surface, the Ag/AgCl electrode impaired the chloride ion sensitivity at the lower [Cl⁻] concentrations (< 10⁻² M) although the sensitivity was maintained (-51.9 mV per decade) at [Cl⁻] > 10⁻² M. The loss of the ion sensitivity at the lower [Cl⁻] following BSA adsorption indicates an interference of the equilibrium reaction by adsorbed protein at the electrode/solution interface, in agreement with previous results that the monolayer coverage of protein reduces the amount of released ions at the interface (Wang et al. 2014). E_0 in eq. 5 is altered by the interface potential change (ΔV) as a result of BSA adsorption. Since the charge effect is negligible on the Ag/AgCl electrode (Figure 3c), the difference of E in the conditions with/without BSA adsorption at [Cl⁻] < 10⁻² M is caused by the impaired freedom of ionization by interfering BSA molecules covered on the Ag/AgCl surface rather than by the charge effect due to BSA adsorption. The Ir/IrOx electrode prepared by thermal oxidation showed good sensitivity and stability for pH response with minimum hysteresis in repeated measurements for three times (Figure 5b). The equilibrium reaction of the Ir/IrOx electrode for protons at the interface is

so that the Ir/IrOx electrode follows the Nernst response. The observed pH responses were close to the theoretical value at the wide range from pH 4 to pH 9. The pH sensitivity and small hysteresis was maintained after performing BSA adsorption on the electrode. Differently from the Ag/AgCl equilibrium in eq. (4), the equilibrium reaction of eq. (6) which involves self-ionization of water was not interfered by the surrounding

BSA molecules on the electrode surface. The result indicates that the Ir/IrOx electrode is suitable for measuring pH in biological fluid due to the less sensitivity to adsorption of biomolecules and relatively high stability and robustness of the IrOx layer. The Pt/IrOx electrode showed a good pH response of -79.5 mV/pH in the first cycle of the measurement at the pH range measured (Figure 5c). However, the sensitivity and linearity of the pH response was impaired in the following cycles. The loss of sensitivity was more pronounced in the acidic condition. Moreover, the measured potential was decreased by increasing the number of cycles at the same pH, indicating macroscopic degradation or exfoliation of the deposited IrOx layer on the Pt electrode. Therefore, it was not possible to measure the pH response after BSA adsorption. The Pt/IrOx is known to show the super Nernst response based on the equilibrium reaction of

at the electrode/solution interface (Steegstra and Ahlberg 2012). More than one proton participated in the pH response is believed to cause the super Nernst response so that the pH response of eq. (7) equals $-59.2 \times (1+y-x)$ mV (Burke and Whelan 1984).

4. Conclusions

We investigated the potentiometric responses of the ion-selective microelectrodes following the nonspecific adsorption of BSA. The Ag/AgCl electrode lost the Nernst response at $< 10^{-2}$ M $[\text{Cl}^-]$ due to the interference of the equilibrium reaction by the adsorbed proteins. The Ir/IrOx electrode prepared by thermal oxidation maintained the pH response even after the protein adsorption at the wide pH range because of the maintained activities of auto-dissociation of water. The Pt/IrOx electrode prepared by electrolysis showed the super-Nernst response for pH but suffered from the robustness of the IrOx layer. Our results indicate that the Ir/IrOx electrode can be directly used for a potentiometric measurement of pH in realistic samples containing biomolecules without any protection of the electrode surface.

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Figures

Figure 1. High resolution XPS spectra for Ag (top) and Ag/AgCl (bottom) (a), Ir (top) and Ir/Ox (bottom) (b), and Pt (top) and Pt/IrOx (bottom) (c) electrodes. The colored solid lines indicate curve fitting results.

Figure 2. The amount of BSA adsorbed on the electrodes at 0.01–1 mg/mL BSA concentrations determined by the MicroBCA™ method.

Figure 3. (a) Schematic representation and a photograph of a set up for the simultaneous potentiometric measurement of BSA adsorption on the working electrodes in a multi-parallel mode. The working electrodes were connected to a high input impedance electrometer (200 T Ω , Keithley 6517B) with a multi-channel scanner card for simultaneous potentiometry. (b) Time course of potentiometric signals during the alternate dipping process of the electrodes into the increasing concentrations of BSA solutions (0.01, 0.1 and 1 mg/mL) and the fresh rinse buffers. (c) Potentiometric responses of the metal electrodes after dipping in 0.01–1 mg/mL BSA solutions for 15 min at 25°C followed by dipping in the buffer solution for rinse.

Figure 4. Cyclic voltammograms on the metal electrodes before and after the formation of oxidized layer on the surfaces. Scan rate: 100 mV/s. Working electrode: Ag and Ag/Cl (top); Ir and Ir/IrOx (middle); Pt and Pt/IrOx (bottom). Counter electrode: Pt; reference electrode: Ag/AgCl in 3.3 M KCl solution. The measurement solution was 1 mM HEPES, 100 mM KCl; pH 7.4.

Figure 5. The Nernst responses of the Ag/AgCl (a), Ir/IrOx (b), and Pt/IrOx electrodes (c) before and after BSA adsorption. Three sets of pH measurements at pH 4, 7, and 9 were repeated for three times in the condition without BSA adsorption to check the potential stability of the Pt/IrOx electrode (c).

Table

Table 1. Summary of cyclic voltammetry and potentiometry on the electrodes.

Working electrode	Ag	Ag/AgCl	Ir	IrOx	Pt	Pt/IrOx
Area, cm ²	0.083	0.071	0.076	0.067	0.061	0.071
ΔI (at 0 mV), μA	0.67	2.8	0.74	6.1	0.38	52
Capacitance, $\mu F/cm^2$	81	390	97	911	63	7200
ΔV (at 1 mg/mL BSA), mV	-11	0.93	-49	-7.3	-34	0.81
Nernst response for Cl^- , mV/log[Cl^-]	—	-49.1	—	—	—	—
Nernst response for H^+ , mV/pH	—	—	—	-54.9 $\pm$ 0.9 -53.7 $\pm$ 2.8†	—	-80.2 -‡

† after BSA adsorption on the electrode surface (n = 3)

‡ not determined due to the exfoliation of IrOx layer from Pt surface

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Table
Table 1. Summary of cyclic voltammetry and potentiometry on the electrodes.

Working electrode	Ag	Ag/AgCl	Ir	IrOx	Pt	Pt/IrOx
Area, cm ²	0.083	0.071	0.076	0.067	0.061	0.071
ΔI (at 0 mV), μA	0.67	2.8	0.74	6.1	0.38	52
Capacitance, $\mu F/cm^2$	81	390	97	911	63	7200
ΔV (at 1 mg/mL BSA), mV	-11	0.93	-49	-7.3	-34	0.81
Nernst response for Cl ⁻ , mV/log[Cl ⁻]	-	-49.1	-	-	-	-
Nernst response for H ⁺ , mV/pH	-	-	-	-54.9±0.9 -53.7±2.8†	-	-80.2 -‡

† after BSA adsorption on the electrode surface (n = 3)
‡ not determined due to the exfoliation of IrOx layer from Pt surface