

DEVELOPMENT OF PROGRAMMABLE BIOSENSOR BASED ON THE ELECTROCHEMICAL DETECTION OF METAL ION

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ABSTRACT

A label free programmable biosensor for electrochemical detection of metal ions using on-chip synthesized probe was developed. This programmable biosensor system is capable of detecting multiple metal ions by synthesizing specific peptide probe on the chip. A copper ion specific peptide probe, Gly-Gly-His (GGH) was synthesized on chemically modified gold electrode using solid phase peptide synthesis (SPPS) on microchip. After probe synthesis, copper ion solution was added, and forms a complex with GGH probe. The differential pulse voltammetry method was used for determination of Cu²⁺. Developed biosensor was found to be highly selective to Cu²⁺ in the range of μM.

KEYWORDS: Programmable biosensor, Label free, peptide synthesis, peptide probe, copper ion

INTRODUCTION

Environmental contamination by trace metals is a serious problem not only for ecosystems, but also for human health. Current electrochemical methods for heavy metals detection have particular advantages of high sensitivity, inherent simplicity, miniaturization and low cost. These conventional sensors, however, are manufactured for fixed target ions, and do not have a flexibility to change the target on-site and on-demand. In MicroTAS 2012, we proposed the concept of programmable biosensor which can flexibly change the target by synthesizing different probes on-site and on-demand [1]. Recently, a metal ion specific oligopeptide probes were reported [2]. By using these peptide probes, the programmable biosensor for metallic ion also can be developed.

In this paper, we developed a programmable biosensor to detect the metal ions by flexibly changing the probe sequence directly synthesized on the chip. A new design of microfluidic chip was proposed to synthesize the metal ion specific probe sequence on the chip. The conventional setup for electrochemical analysis of metal ions was also integrated with the microfluidic probe synthesizer. The oligopeptide probe was synthesized over chemically modified gold working electrode. After probe synthesis, metal ion solution was added to chip to be preconcentrate on the electrode. The captured metal ion was detected using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and Differential pulse voltammetry (DPV) techniques.

EXPERIMENTAL

Figure 1 shows the schematic and photograph of the biosensing device. The device is made of a glass chip, having three Au electrodes as auxiliary, working and reference electrodes, and a PDMS chip having micro-channels and inlet/outlets. Tripeptide probe, GGH is synthesized on Au working electrode modified with 20 mM cysteamine (CA), by on-chip SPPS [2]. EIS and CV measurements were performed to characterize the modified electrode in presence of 1 mM and 5 mM [Fe(CN)₆]^{4-/3-} in 0.1 M KCl as redox solution respectively.

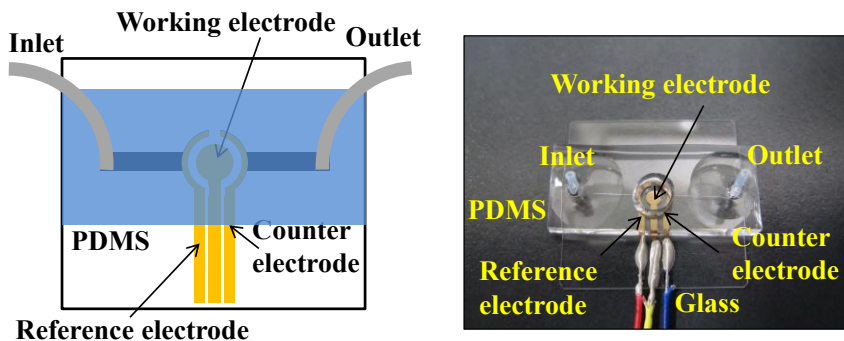


Figure 1: Schematic and the photograph of developed microchip.

For accumulation of Cu(II) on the modified electrode, Cu(II) was first chemically preconcentrated by dropping 60 μl of the 1×10^{-6} M copper solution in 0.1M PBS (pH 5.8) for 10 min in an open circuit. The Au electrode was then carefully washed with miliQ water and the chamber was again filled with 30 μl of supporting electrolyte solution (0.1 M Cu^{2+} free PBS, pH 5.8). A negative potential of -1.2V was applied to electrode immediately for 60s to reduce Cu(II) into elemental metallic Cu(0). Subsequently, DPV measurement was performed in presence of 0.1 M phosphate buffer solution (PBS, pH 5.8). After each measurements, electrodes were “electrochemically cleaned” by a solution containing 0.1M EDTA at +0.5V for 60s.

RESULTS AND DISCUSSION

The Au working electrode was characterized at each modification steps using EIS and CV measurement. The CVs of modified electrodes obtained in the presence of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe in acidic solutions (pH 3.8) are presented in Fig. 2A. Formation of CA monolayer on gold electrode led to an increase in the faradic currents due to electrostatic attraction between positively charged CA monolayer and negatively charged probe in test solution (Fig. 2A, compare curves a and b). The synthesized GGH layer, i.e., formation of Au/CA/GGH, imparted a neutral charge to the electrode surface at pH 3.8, therefore, the faradic currents were decreased (Fig. 2A, curve c). The complex plane plots obtained on modified electrodes in the presence of 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in acidic solutions, pH 3.8 are displayed in Fig. 2B. When the CA SAM assembled on the bare Au surface, the semicircle diameter of the bare Au (curve a) was further decreased to almost a straight line (curve b), which owes to the electrostatic active CA assembled on the electrode surface and enhanced the electron transfer on the electrode surface.

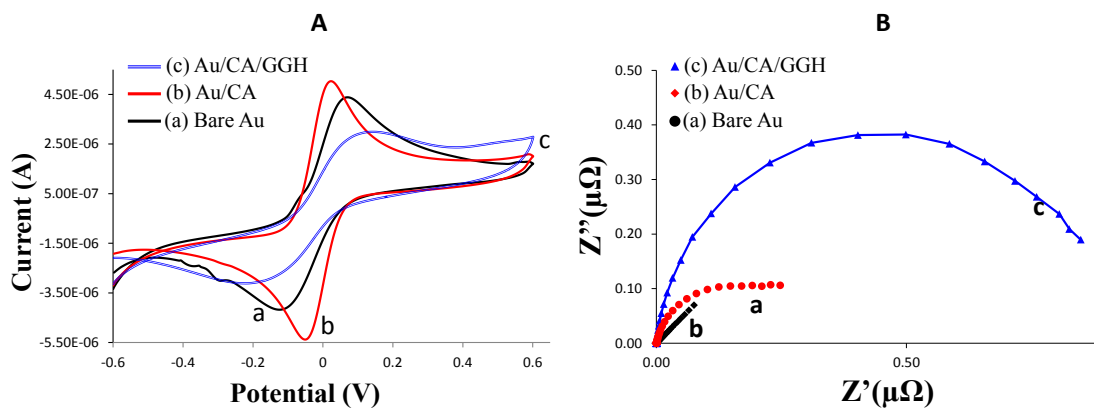


Figure 2: (A) Cyclic voltammograms obtained in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ on (a) bare Au, (b) Au/CA, and (c) Au/CA/GGH, scan rate: 50mVs^{-1} . (B) Complex plane plots obtained in 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ on (a) bare Au, (b) Au/CA, and (c) Au/CA/GGH electrodes.

After the GGH synthesis onto the electrode surface (curve c), the semicircle diameter of the Au/CA/GGH was increased. This showed that the GGH layer had a larger obstruction effect, which resulted in increasing resistance to the flow of electrons. The results are in good agreement with those obtained by CV at pH 3.0 (Fig. 2A).

In order to check the performance of Au/CA/GGH toward Cu(II), we first analyzed the DPV response in 0.1 M PBS solution after preconcentration in 1×10^{-6} M Cu(II) solution with Au/CA/GGH, Au/CA, and bare Au. As shown in Fig. 3A (curves d), the peak current of Cu(II) at the bare Au/CA/GGH indicates that the Au/CA/GGH exhibits excellent performance for Cu(II) Analysis. When Cu(II) is captured, the Au/CA/GGH interspaces provide a location and host a catalytic reaction in their structure surface. The results demonstrate that the on-chip synthesized GGH, tripeptide molecular adapters are able to capture Cu(II) in solution.

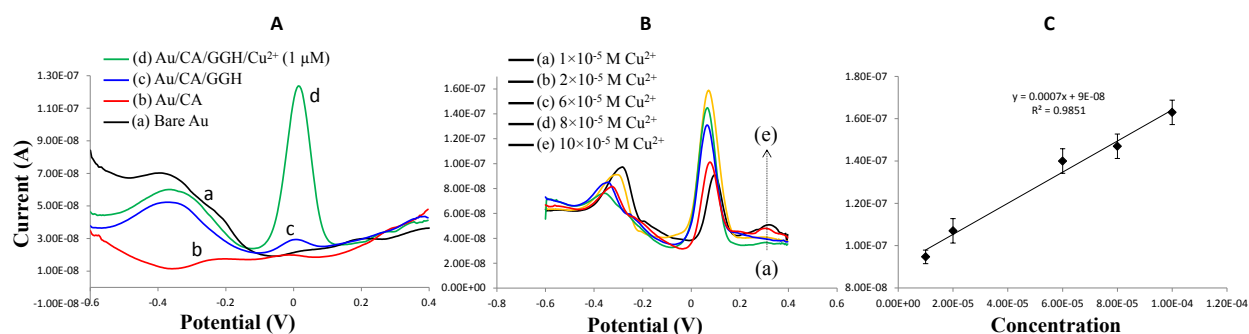


Figure 3: (A) The DPV response at (a) bare Au, (b) Au/CA, (c) Au/CA/GGH, no Cu(II) in accumulation medium, (d) Au/CA/GGH/Cu²⁺ for the determination of 1 μM Cu(II) in 0.1 M PBS. (B) DPVs at different concentrations of copper solution. (C) Linear graph between peak current and copper solution concentrations.

Under optimized conditions, the DPVs for pre-adsorbed Cu²⁺ by the Au/CA/GGH electrode at various concentrations were recorded (Fig. 3B). Figure 3 (C) shows that the DPV peak current is linear vs. pCu in the range of 1.0×10^{-5} - 1.0×10^{-4} M ($y = 0.0007x + 9 \times 10^{-8}$, $r = 0.9851$). The detection limit calculated was 8.92×10^{-6} M Cu²⁺.

CONCLUSION

We successfully developed a programmable electrochemical sensor for metal detection using on-chip probe synthesis. Peptide probe is successfully synthesized directly over Au surface on microfluidic chip. On-chip probe synthesized based biosensor reveals high sensitive to detect Cu²⁺ in μM range. This programmable bio-sensing system can applied for another metal ion analysis by synthesizing the metal ion selective probes on the chip.

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