Development of a Bioelectrode for Detecting Creatinine in Urine

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Abstract

Creatinine measurement is essential for standardizing urinary components and evaluating renal and muscle function. We developed a bioelectrode for creatinine detection using three enzymes, : creatininase (CRN), creatinase (CN), and sarcosine oxidase (SOx), with ferro-cyanide ion ($Fe(CN)_6^{4-}$) as a mediator. The mediator was effectively immobilized using its adsorption ability in solution. A stable configuration of both the mediator and enzymes resulted in reproducible catalytic currents.

1. Introduction

Creatinine, a metabolite of creatine, is mostly excreted in urine. Urinary creatinine is used not only to evaluate renal and muscular function, but also as a standard substance for correcting the concentrations of other components in urine. Therefore a convenient measure is required. We are currently developing an amperometric creatinine biosensor that uses the three-step enzyme reaction of CRN, CN, and SOx to observe the oxidation reaction current of SOx, which is the final step (Fig. 1). Because it is considered to be difficult for SOx to directly exchange electrons with the electrode, we are using a mediator. Our previous work showed successful enzyme immobilization using a polyion complex (PIC) of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA). In this study, $Fe(CN)_6^{4-}$ was used as a mediator due to its adsorption properties to PLL. Both enzymes and the mediator were immobilized on a glassy carbon electrode (GCE) using a PIC. Finally, the electrode was coated with Nafion to prevent leaching them and interference reaction.

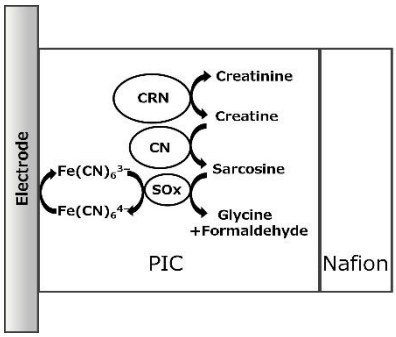

Fig. 1 Reaction scheme of creatinine biosensor.

2. Experiment

The SOx-immobilized electrode was prepared by casting a mixture of equal volumes of 10 mg/mL SOx, PGA (25 mM as monomer unit, average Mw 70,000-150,000) and PLL (100 mM as monomer unit, average Mw 12,000) onto GCE. The electrode was then dried in ambient air for 1 hour. Three-enzyme-immobilized electrodes were similarly prepared using enzyme solutions at an activity ratio of CRN:CN:SOx = 1:1:1. For the mediator immobilization, these enzyme-containing PLL/PGA membranes were immersed in 50 mM phosphate buffer solution (PBS) containing 20 μM $K_4[Fe(CN)_6]$ for approximately 30 min, followed by Nafion coating.

3. Results and discussion

3.1 Immobilization of the mediator

Immersion of PGA-SOx-PLL/GCE in $K_4[Fe(CN)_6]$ solution resulted in adsorption of $Fe(CN)_6^{4-}$ on the PLL/PGA membrane. This adsorption was more enhanced on cation-rich membranes with higher PLL ratios. Although the immobilized $Fe(CN)_6^{4-}$ were eluted in PBS, application of a Nafion membrane effectively suppressed the elution. Higher Nafion concentrations provided better retention (Fig. 2). These results suggest that $Fe(CN)_6^{4-}$ was stabilized within the PIC membrane through electrostatic repulsion between the negatively charged Nafion membrane and $Fe(CN)_6^{4-}$.

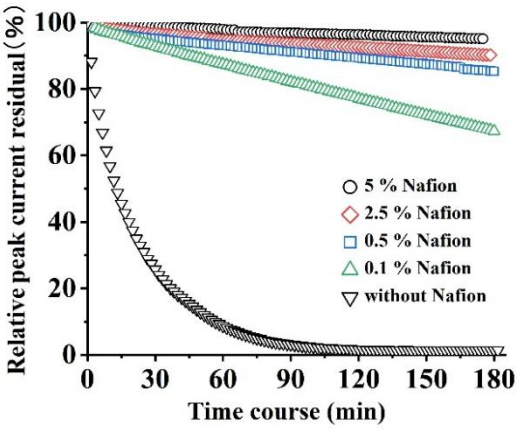

Fig. 2 Effect of Nafion coating concentration on $Fe(CN)_6^{4-}$ retention at PGA-SOx-PLL/GCE monitored by CV measurements in PBS over 3 h. Peak potentials initially shifted with Nafion concentration but stabilized at 0.25 V. The initial peak currents were set as 100%.

3.2 SOx-immobilized electrodes

Regarding Nafion/PGA-SOx-PLL/GCE, it showed catalytic currents in sarcosine solution in Nafion concentrations region where $Fe(CN)_6^{4-}$ was stably immobilized. The catalytic current decreased with increasing Nafion concentrations, suggesting reduced substrate permeability at higher Nafion concentrations.

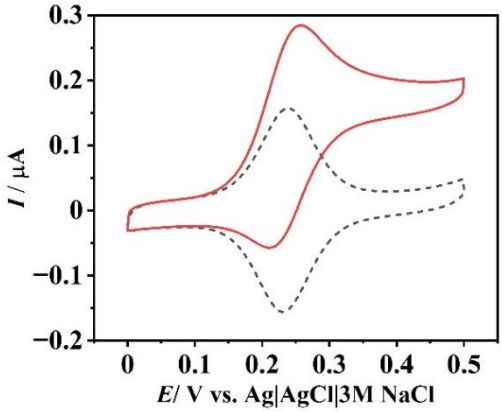

Fig. 3 CVs for Nafion/$Fe(CN)_6^{4-}$/PGA-CRN-CN-SOx-PLL/GCE with 2.5 % Nafion in the absence (dashed line) and presence of 100 mM creatinine (solid line). v=2 mVs^{-1}

3.3 Three-enzyme cascade electrode

The three-enzyme immobilizing electrodes generated a little reproducible catalytic current in the presence of creatinine, demonstrating successful sequential enzyme reactions (Fig. 3). Further optimization of the three-enzyme activity ratios may enhance the catalytic current response. Detailed sensor characteristics of this bioelectrode are currently under investigation.

4. Conclusion

In this study, creatinine was successfully measured using a bioelectrode for creatinine measurement in which three enzymes and a mediator were immobilized by using a PIC to immobilize the enzymes and the adsorption of $Fe(CN)_6^{4-}$ on a cation-rich membrane. By attaching a Nafion membrane, the mediator and enzymes can be stably immobilized, enabling the three enzymes to react successively, and a catalytic current can be stably obtained. We plan to improve further sensor functionality.