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H_2O_2 and Glutamate Imaging with Improved Sensitivity Based on Charge-Transfer-Type Potentiometric Redox Sensor Arrays

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Abstract:
Toward imaging the extrasynaptic distribution of glutamate (Glu) in brains, label-free potentiometric redox sensor arrays with 128 × 128 pixels have been developed. The Glu is detected through H_2O_2 production by an enzyme reaction and the oxidation of a mediator by H_2O_2. In this study, an adhesion layer of titanium was employed for the deposition of a gold layer, which is used to detect redox potential. The sensitivity to redox potential greatly increased, reaching near the theoretical value compared with the previous device. Due to a small variation in the sensitivity among the pixels, the distributions of H_2O_2 and Glu were successfully visualized using a poly-ion complex membrane entrapping enzymes and a mediator of ferrocenyl methanol. Furthermore, the limit of detection was achieved to be 10^{-6} M for both H_2O_2 and Glu.

Key words: bioimaging, H_2O_2, glutamate, redox sensor array, potentiometry

Introduction

Neurotransmitters such as adenosine-triphosphate (ATP) and glutamate (Glu) play an important role in signal transmission in nerve systems. In particular, the extrasynaptic distribution of Glu is considered to be closely related to excitatory actions in brains [1], and thus, spatiotemporal imaging of its distribution is highly demanded.

Recently, we reported label-free Glu image sensors based on charge-transfer-type potentiometric sensor arrays which sensing areas are covered with a gold (Au) film. Combing H_2O_2-generating enzymatic reaction with redox reaction involving H_2O_2, Glu was detected as the interfacial potential at the Au and the solution [2]. However, the sensitivity was still lower than the theoretical value, and the quality of the imaging needs to be improved.

In this study, we report the increase in the sensitivity of the image sensors and the improved imaging of H_2O_2 and Glu by improving the deposition condition of the Au layer.

Sensing Principle

The sensor has 128 × 128 pixels, and the pixel pitch is 23.5 μm. An Au film is deposited on the sensor surface to detect redox potential. The sensor output (V_{Out}) changes with the interfacial potential (E) between the sensor surface and the solution containing a redox mediator according to the Nernst equation [3]:

$$E = E^{o} + \frac{RT}{nF}\log\frac{[\mathrm{Ox}]}{[\mathrm{Red}]}. \quad (1)$$

For H_2O_2 and Glu detection, ferrocenium ions (Fe(II) and Fe(III)) in the form of ferrocenyl methanol (FcMeOH) were employed as a redox mediator. Glu is broken down to produce H_2O_2, which oxidizes Fe(II) to Fe(III) by the enzyme reactions as [2]:

$$\mathrm{Glu} + \mathrm{O_2} + \mathrm{H_2O} \xrightarrow{\mathrm{GluOx}} \alpha\text{-ketoglutarate} + \mathrm{NH_3} + \mathrm{H_2O}, \quad (2)$$

$$\mathrm{H_2O_2} + 2\mathrm{H^+} + 2\mathrm{FcMeOH} \xrightarrow{\mathrm{HRP}} 2\mathrm{H_2O} + 2\mathrm{FcMeOH^+}, \quad (3)$$

where GluOx and HRP are glutamate oxidase and horse radish peroxidase, respectively.

Experimental Procedure

A 50-nm-thick Au layer was deposited using an evaporation with an insersion layer of titanium (Ti) to strengthen adhesion to the sensor surface, while Au was directly depoisted in the previous study [2]. First, sensitivity to the redox potential was measured by changing the concentration ratio of Fe(III) to Fe(II) using hexacyanoferrate as a redox reactant (Fig. 1(a)). Responses to

H_2O_2 and Glu were measured in the samples on which a poly-ion complex membrane entrapping the enzymes were deposited (Fig. 1(b)) . Recording medium (RM) containing 10-mM HEPES buffer and FcMeOH [2] was used as sample solutions. A solution containing H_2O_2 or Glu was dropped into the RM, and then the output changes were measured.

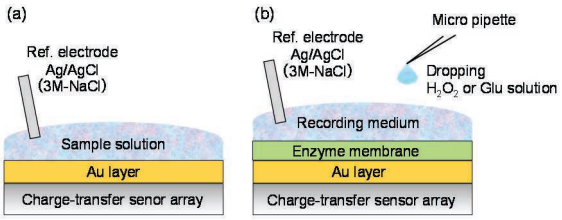

Fig. 1. Experimental setup for (a) redox potential measurements and (b) H_2O_2 and Glu imaging.

Results and Discussion

Figure 2 shows the V_{Out} change (ΔV_{Out}) for various concentration ratio of Fe(III) to Fe(II), where V_{Out} for the solution for Fe(III):Fe(II) = 1:99 is set to zero as a reference. The median values of ΔV_{Out} among the pixels were plotted. Compared with the Au sample, the Au/Ti sample shows higher sensitivity of 52.6 mV/dec., which is near the theoretical value (59.5 mV/dec. at 300 K). Furthermore, uniform sensitivity among the pixels were obtained as shown in Fig. 3.

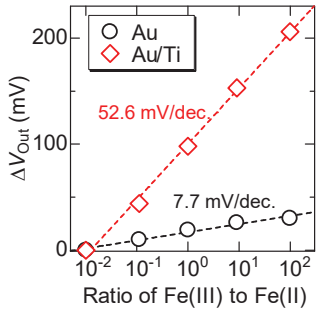

Fig. 2. Output changes for various concentration ratios of Fe(III) to Fe(II).

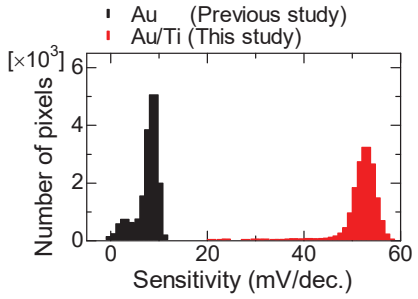

Fig. 3. Histogram of the sensitivity to redox potential among the pixels.

Then, H_2O_2 and Glu imaging was conducted in the Au/Ti sample. Figure 4 shows the imaging results when (a) 10^{-4}-M H_2O_2 and (b) 10^{-4}-M Glu solutions were dropped. In both cases, the output gradually increases and the increase spreads to the whole pixels. In this way, the distribution of the substances was clearly visualized. The output changes with the concentrations of H_2O_2 and Glu are shown in Fig. 5, where the median values among the pixels are plotted, while the standard deviations are shown as error bars. As expected from eq. (2), almost similar responses are obtained, and the limit of detection was achieved to be 10^{-6} M.

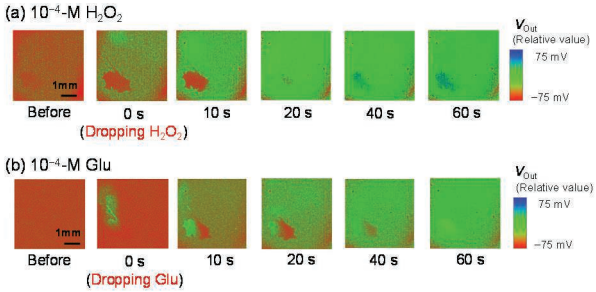

Fig.4. Imaging results for (a) 10^{-4}-M H_2O_2 and (b) 10^{-4}-M Glu solution.

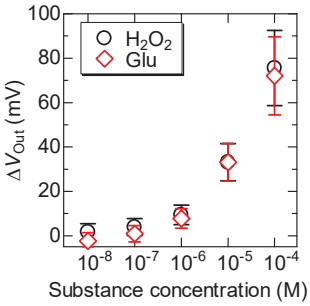

Fig. 5. ΔV_{Out} plotted against the H_2O_2 and Glu concentration.

Summary

The sensitivity to redox potential in the charge-transfer-type potentiometric sensor arrays was increased by improving the deposition condition of the metal layers on the sensor surface. The sensitivity reaches near the theoretical value and shows a small variation. Furthermore, H_2O_2 and Glu distribution is successfully visualized, and the limit of detection of 10^{-6} M is achieved.

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