잔류농약 측정을 위한 금 나노입자를 이용한 Tyrosinase 효소 전극의 제조

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Preparation of tyrosinase enzyme electrode using gold nanoparticles for detection of a pesticide

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Introduction

Pesticides are widely used to control the growth of broadleaf plants. It was known that the association with the occurrence of cancer in humans [Blair *et al*., 1985], endocrine-disrupting activities [Sheiner *et al*., 2003], and degenerative changes in central nerve system [Matsumara, 1994]. The typical monitoring method is HPLC and GC-MS, which require extensive sample pretreatments and highly trained technical personnel. Enzyme electrodes have been shown to be a very simple and convenient tool for detecting of environmental pollutants. But the sensitivity of the enzyme electrode are not satisfying to prepare pesticide-detecting sensor.

The use of nanoparticles of difference metal has been suggested for many electrochemical applications [El-Deab *et al*., 2003; Chen *et al*., 2000]. Gold has been receving increase attention as a novel catalyst material. Especially, the use of gold nanoparticle (AuNP) is playing an increasing role for the preparation of biosensor [Carralero *et al*., 2000] due to large surface area and high conductivity. Thus it is expected that the enhancement of sensitivity and detection limit and further the application ofr the measurement of pestcidie of low concnetratio by the use of AuNP.

In this study, the AuNPs were deposited on glassy carbon (GC) electrode by electrodeposition for preparing enzyme electrode. The applying potential and time was optimized and the coverage was controlled. Tyrosinase (TYR) was immobilized on the AuNP deposited electrode by the formation of a self-assembled monolayer (SAM). The prepared TYR-AuNP-GC electrode was compared with base, AuNP-GC electrode by the measurement of cyclic voltammetry. In addition, the electrode was tested for 2,4-D (2,4-dichlorophenoxyacetic acid) as a target compound.

Experimental methods

Potassium tetrachloroaurate (III), H_2SO_4 (0.5 M), tyrosinase (TYR, from mushrooms EC 1.14.18.1), 3-mercaptopropionic acid (MPA), EDC, NHS, catechol, phenol, and 2,4-D were all used. The electrochemical experiments were performed in a Teflon cell (5 mL) using a

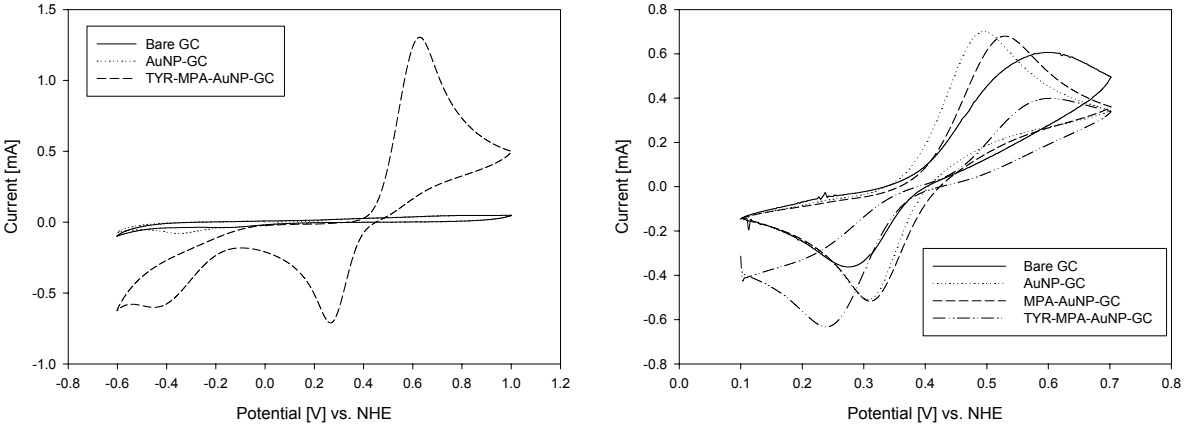
three-electrode. The cell was connected to with a PGSTAT30/GPES system (Autolab, Netherlands). A GC electrode was used as WE. A BAS MF 2030 Ag/AgCl RE and a Pt wire CE were also employed. The deposition of AuNP was performed onto the surface of pretreated GC electrode by applying potential for a specific time period using double-pulse technique (E_1 = 1.2 V vs. NHE, t_1 = 0.1 sec, E_2 = -0.2 V vs. NHE, t_2 = 60 sec). The most particle size was in the range of 25 ~ 30 nm and some particles were smaller than 10 nm. The AuNP-GC electrode was pretreated, immersed in MPA solution, and then rinsed. For enzyme immobilization, mixture of EDC and NHS was added and incubated. After 1 hr, the enzyme solution was dropped and stayed.

Results and discussion

Cyclic voltammetric measurements of TYR-AuNP-GC electrode for phenol and catechol

A detection of a pesticide at an enzyme electrode is based on the inhibition mechanism. It is measured by using a current change with respect to the addition of inhibitor (pesticide). Prior to the measurement of 2,4-D, a detection of phenol, which is typically used as a substrate, at the prepared TYR electrode was examined by the measurements of cyclic voltammograms. As shown in Fig. 1 (a), there were no significant redox peak at the bare and AuNP-GC electrode. However, in case of TYR-AuNP-GC electrode, an anodic and a cathodic peak were observed near 0.6 and 0.2 V vs. NHE, respectively. These are coincided with the redox peak potential of catechol. That is, the phenol was oxidized by tyrosinase and change to catechol and it was further oxidized by enzymatic reaction as well as electrochemical reaction with generating quinone. So the anodic peak was due to the electrochemical oxidation of catechol, which is the oxidation product of phenol by tyrosinase. But, the cathodic peak was due to the reduction of quinone, which was the oxidation product of catechol (or phenol) not only by the electrochemical reaction but also by enzymatic reaction. Also those two peaks were linearly correlated with (scan rate)$^{1/2}$ implying diffusion limited (data was not shown). Another cathodic peak at -0.4 V vs. NHE was due to the reduction of dissolved oxygen, which was found from the voltammograms in the absence of phenol, too (data was not shown).

Fig. 1 (b) shows a comparison of cyclic voltammograms for catechol using the bare, AuNP-, MPA-AuNP- and TYR-AuNP-GC electrode. The coverage of deposited AuNPs was about 13 %. In the presence of catechol, it was observed that increasing of the peak current and decreasing of the peak separation ($\triangle E_p$) after AuNP deposition. When MPA layer was modified, there were no significant changes in peak currents but $\triangle E_p$ increased. The MPA can acts as layer for an enzyme immobilization but has an insulating effect. It was reported that decreasing and increasing of $\triangle E_p$ for catechol after the Au deposition and SAM modification, respectively [Finot *et al*., 2000]. After enzyme immobilization, I_{pa} decreased but I_{pc} increased and $\triangle E_p$ increased. The change of peak current was due to the enzymatic oxidation of catechol. In the presence of tyrosinase, catechol is oxidized to quinone by enzymatic reaction so the amount of catechol, which is able to be oxidized by electrochemical reaction, decreased. For quinone, but the reaction product of catechol, its amount increased because quinone is generated by the enzymatic as well as electrochemical reaction so the significant increasing of I_{pc} was investigated.

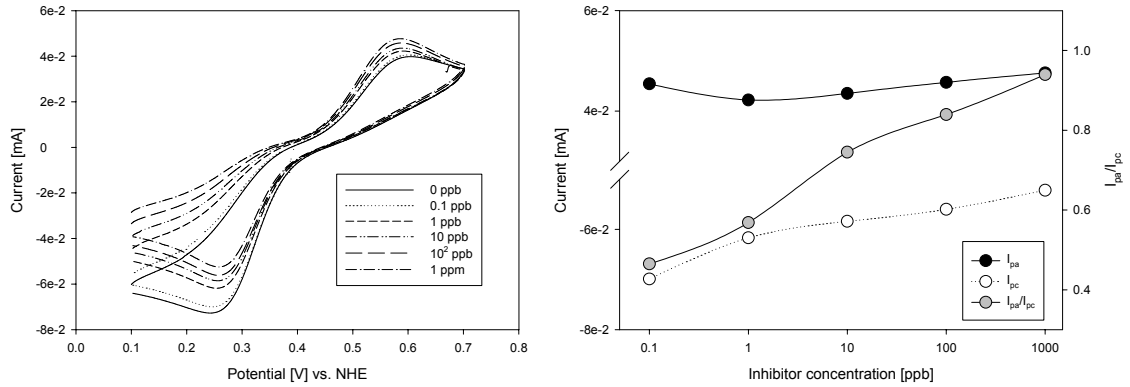


(a) (b)

Fig. 1. Cyclic voltammograms of bare, AuNP-, and TYR-AuNP-GC electrode for (a) 1 mM of phenol and (b) 1 mM of catechol in O_2-saturated PBS (0.1 M, pH 7) at 25 mV/s.

Current change of TYR-AuNP-GC electrode to the addition of 2,4-D

Although higher enzyme specificity was shown when phenol was used as a substrate, catechol was used for the detection of 2,4-D. Because the enzymatic oxidation of catechol is a single-step, the redox reaction of catechol is a reversible, and it is less toxic. In addition, it shows a clear current ratio (I_{pa}/I_{pc}), which is represent an inhibition index.



(a) (b)

Fig. 2. Cyclic voltammograms of bare, AuNP-, and TYR-AuNP-GC electrode for (a) 1 mM of phenol and (b) 1 mM of catechol in O_2-saturated PBS (0.1 M, pH 7) at 25 mV/s.

In order to examine the applicability of TYR-Aunt-GC electrode for detecting of 2,4-D, cyclic voltammetric measurements was conducted using a catechol (0.1 mM) as a substrate before and after the addition of 2,4-D. As shown in Fig. 2 (a), the I_{pc} decreased as the concentration of 2,4-D added increased while the I_{pa} increased. As it was mentioned above, I_{pa} and I_{pc} at TYR electrode when a catechol was used as a substrate was due to the oxidation of catechol and by the reduction of quinone, respectively. The quinone is oxidation product of catechol by electrode reaction and/or enzymatic reaction. When 2,4-D is added,

tyrosianse is inhibited and the oxidation of catechol by enzymatic reaction was prevented. The amount of catechol which is oxidized by electrode reaction is relatively increased, thereby I_{pa} increased. However, as the amount of quinone decreased due to inhibition of the enzymatic reaction, I_{pc} decreased as shown in Fig. 2 (b). Initially, the ratio of I_{pa}/I_{pc} was smaller than unity ($I_{pa} = I_{pc}$ for only electrode reaction) but it approached to unity as the concetration of 2,4-D increased.

Conclusion

It was demonstrated that the deposited AuNP improved the current response of tyrosianse based enzyme electrode. By comparing the cyclic voltammograms of bare, AuNP-, and TYR-AuNP-GC electrodes, a sensitive current response of the reduction of oxidation product of catechol and phenol was shown at the tyrosinase immobilized electrode. From the results, it was confirmed that the deposited AuNPs by electrodeposition are effective to increase conductivity as well as to immobilize enzyme. In addition, the prepared TYR-AuNP-GC electrode has a strong potential to detect a pesticide, 2,4-D. Further studies on quantitative measurement of a pesticide using the electrode is going on.

References

Blair, A., Malker, H., Cantor, K.O., Bumeister, J., and Wiklud, K., "Cancer among farmers: a review," Scand. J. Work Environ. Health, 11, 397 (1985).

Carralero Sanz, V., Luz Mena, M., Gonzalez-Cortes, A., Yanez-Sedeno, P., and Pingarron, J.M., "Developoment of a tyrosinase bionsensor based on gold nanoparticles-modified glassy carbon electrodes, application to the measurement of a bioelectrochemical polyphenols index in wines," Anal. Chim. Acta., 528, 1 (2005).

Chen, C.W., Takezako, T., Yamamoto, K., Serizawa, T., and Akashi, M., "Poly(N-vinylsobutyramide)-stabilized platinum nanoparticles: synthesis and temperature-responseive behavior in aqueous solution," Colloids Surfaces A: Physicochem. Eng. Aspects, 169, 107 (2000).

El-Deab, M.S., Ohsaka, T., "Hydrodynamic voltammetric studies of the oxygen reduction at gold nanoparticles-electrodeposited gold electrodes," Electrochemical Acta, 47, 4255 (2002).

Finot, M.O. and McDermott, M.T., "Characterization of n-alkanethiolate monoalyers adsorbed to electrochemically deposited gold nanocrystlas o glassy carbon electrodes," J. Electroanal. Chem., 488, 125 (2000).

Matsumara, F., "Differential toxicities of insecticides and halogenated aromatics," International encyclopedia of Pharmacology and Therapeutics, Pergamon Press, New York (1994) P. 453 (Section 113).

Sheiner, E.K., Sheiner, E., Hammel, R.D., Potashnik, G., and Carel, R., "Effect of occupational exposured on male fertility: Literature review," Ind. Health, 41, 55 (2003).