Enhanced Enzymatic Reaction of Tyrosinase-immobilized Polyacrylamide–(γ-Cyclodextrin) Membrane Coated on a Platinum Disk Electrode in Acetonitrile

Toshio Nakamura,* Xueping Ji, Kazuyoshi Endo, and Daisuke Takano
Department of Chemistry, Faculty of Science, Shinshu University, Matsumoto 390-8621

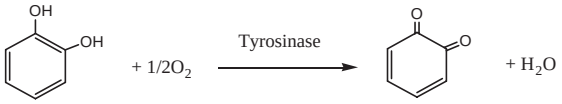
(Received December 20, 2006; CL-061487; E-mail: toshion@shinshu-u.ac.jp)

A functionalized stable film, polyacrylamide–(γ-cyclodextrin) (PAA–γ-CD), synthesized from polyacrylamide (PAA) and γ-cyclodextrin (γ-CD), was used to immobilize enzyme. The membrane containing γ-CD provided an excellent environment for enzymatic kinetics between tyrosinase in the membrane and the substrates in acetonitrile.

Previous studies have confirmed that enzymes remain active in organic solvents containing little or no water.[1–3] The use of enzymes in nonaqueous solvents has a number of advantages, such as high solubility of hydrophobic substrates, enhanced thermostability, the suppression of various side reactions, altered enzymatic selectivity, and the comparative simplicity of immobilization procedures. PAA has been used previously for enzyme immobilization.[4] Hydrophilic non-cross-linked and non-plasticized PAA has been used in our laboratory as a matrix for biofunctional material immobilization.[5] It has been confirmed that the PAA-membrane-modified electrode yields microelectrode ensembles.[6] The kinetic properties of a tyrosinase-immobilized PAA electrode in acetonitrile (AN)[7] and in *N*,*N*-dimethylacetamide[8] have been reported. These studies have shown that the PAA membrane offers a matrix for biofunctional material immobilization. However, the sensitivity of the reaction between enzymes and substrates are expected to improve further with respect to response time and sensitivity. Since cyclodextrin (CD) possesses a hydrophobic internal cavity and a hydrophilic exterior, it seems promising as an electrode modifier for enzymatic kinetics. It has been widely studied due to its ability to form inclusion complexes with a large variety of organic molecules.[9] Its well-known ability to form supramolecular complexes with suitable substances has resulted in its use to enhance electrode selectivity.[10] It appears likely that the combination of PAA and γ-CD might provide an excellent means of improving the function of enzyme electrode. In the present study, a stable film, PAA–γ-CD was used in the immobilization of enzyme, tyrosinase (Tyro), on a platinum electrode. It was expected that the electrode modification would construct enzymatic reaction sites on the PAA membrane between the substrates, catechol and 4-methylcatechol in dipolar aprotic solvents and Tyro in the membrane. PAA–γ-CD was synthesized by an esterification of carboxyl-modified PAA and γ-CD using lipase as a catalyst in *n*-heptane at 50 °C during three days as that in similar studies.[11] The mass ratio between PAA and γ-CD was 7.5:1 at the present study.

The reactivity of the Pt/(PAA–γ-CD + Tyro) electrode with catechol and 4-methylcatechol was compared with similar systems without γ-CD.

The voltammetric responses of the Pt/(PAA–γ-CD + Tyro), Pt/PAA–γ-CD, Pt/(PAA + Tyro), and Pt/PAA electrodes were measured for two substrates mentioned above in AN solution. In the absence of the substrates, no detectable signal was observed for any of the electrodes. Nor was any response observed at the Tyro-free electrodes. On addition of substrates, however, reduction peaks were observed at the Tyro-containing electrode, which resulted from the reduction of *o*-quinone produced by the enzymatic reaction.[7,8]



catechol 1,2-benzoquinone

As can be seen in Figure 1, the peak currents were much larger at Pt/(PAA–γ-CD + Tyro) than at Pt/(PAA + Tyro), which shows that γ-CD played an important role in enhancing enzymatic activity. The typical cyclic voltammograms for 4-methylcatechol are shown in Figure 1. The voltammogram at the Pt/(PAA–γ-CD + Tyro) electrode showed a more positive reduction peak potential (E_{pc}) than that obtained at the Pt/(PAA + Tyro) electrode, as can be seen in Table 1.

Figure 2 shows the response behavior of the γ-CD-modified electrodes obtained by an amperometric method. On adding substrates, no response was observed for the Tyro; Pt/PAA–γ-CD (Figure 2a) and Pt/PAA (Figure 2b), however the enzyme-containing electrode responded rapidly and reached a steady-state current. The response time and sensitivity for the Pt/(PAA–γ-CD + Tyro) electrode (Figure 2d) improved remarkably compared with the γ-CD-free enzyme electrodes. These results must be due to the effect of γ-CD; hydrophobic internal cavity and hydrophilic exterior, at the PAA membrane on the enzymatic reaction between Tyro and substrates.

Figure 3 shows the calibration plot of the Pt/(PAA–γ-

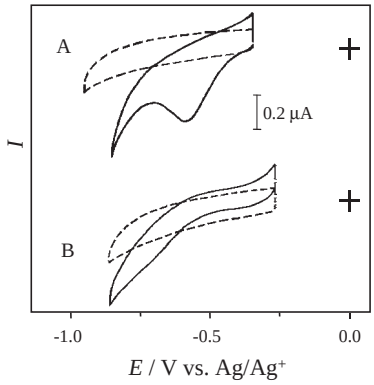


Figure 1. Cyclic voltammograms of 25 mM 4-methylcatechol in 50 mM Et_4NClO_4–AN solution at (A) Pt/(PAA–γ-CD + Tyro), and (B) Pt/(PAA + Tyro) electrodes. Dotted and solid lines are for blank and sample solutions, respectively. Scan rate: 0.10 V/s.

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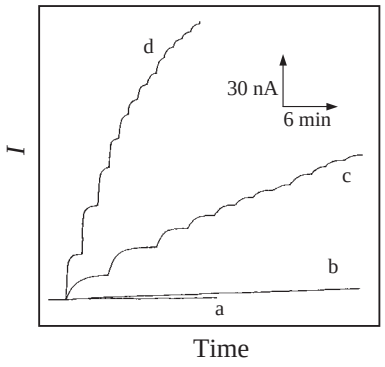

Figure 2. Typical current–time response of (a) Pt/PAA–γ-CD, (b) Pt/PAA, (c) Pt/(PAA + Tyro), and (d) Pt/(PAA–γ-CD + Tyro) electrodes on the stepwise additions of catechol from 0.90 to 13 mM in 50 mM Et_4NClO_4–AN solution at −0.70 V vs Ag/Ag^+.

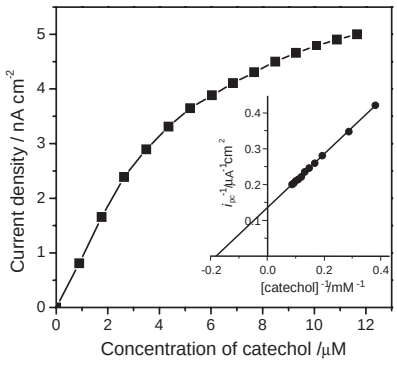

Figure 3. Calibration plot of the Pt/(PAA–γ-CD + Tyro) electrode for catechol in 50 mM Et_4NClO_4–AN solution at −0.70 V vs Ag/Ag^+ and its Lineweaver–Burk plot for the response (Inset).

CD + Tyro) electrode for catechol in AN solution. The response of the electrode exhibits characteristics of the Michaelis–Menten kinetic mechanism, and the constant, K_m, of Tyro to catechol can be calculated according to the Lineweaver–Burk equation.[12,13] $i^{-1} = i_{max}{}^{-1}K_m$, $[S]^{-1} + i_{max}{}^{-1}$, where [S] is the concentration of substrate. The inset in Figure 3 shows the Lineweaver–Burk plot for the response. The constant K_m, for the reaction of tyrosinase with catechol was calculated to be 5.6 mM. The i_{max} and K_m, for Tyro at another electrodes are listed in Table 1. It can be seen that the i_{max} at the γ-CD- containing electrode are about 1.7 times higher than that at γ-CD free electrode for cacechol and 4-methylcatechol. The K_m, which reflect enzymatic affinity, for the enzyme electrodes are somewhat variable. However, no remarkable differences in K_m, were observed for these substrates either at the γ-CD containing or the γ-CD-free electrode.

Table 1. Electrochemical parameters of the tyrosinase electrodes (Pt/(PAA–γ-CD + Tyro) (E_1) and Pt/(PAA + Tyro) (E_2)) in 50 mM Et_4NClO_4 AN solution at 35 °C

Substrate	E_{pc}/V vs Fc/Fc^+		K_m/mM		I_{max}/μA cm^{-2}	
	E_1	E_2	E_1	E_2[7]	E_1	E_2[7]
Catechol	−0.60	−0.70	5.6	6.6	7.5	4.4
4-Methylcatechol	−0.62	−0.75	6.1	5.9	15.3	9.3

In conclusion, the Tyro-immobilized PAA–γ-CD provided to be an excellent enzyme electrode. This report has described how the γ-CD containing PAA membrane provided preferable better environment for enzyme kinetics. The PAA–γ-CD with Tyro attached promoted higher enzymatic activity on reaction with catechol and 4-methylcatechol in AN. The results must be due to the CD's effect on the circumstance at the PAA Tyro-immobilized membrane.

It is very interesting to know that how to work the γ-CD to the enzymatic reaction and/or to enhancement of the electrode response. Futher investigation both from the view point of molecular dynamics using nmr and from other CDs are now in progress in our research group.

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Published on the web (Advance View) March 3, 2007; doi:10.1246/cl.2007.506