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단 신

염화메틸렌 용매에서 페놀정량을 위한 식물조직-바이오센서

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Plant Tissue-Based Amperometric Sensor for Determination of Phenols in Methylene Chloride

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The plant tissues with electrochemical transducers has received considerable attention in recent years.[1,2] The resulting tissue bioelectrodes possess several advantages over their enzymatic counterparts, including improved stability, higher biocatalytic activity and lower cost. Early tissue electrodes have thus been successfully employed in numerous aqueous media. But the ability of enzymes to operate in nonaqueous media[3,4] offers a great potential for new and novel applications of biocatalytic sensors. Operating an enzyme electrode in an organic solvent enables a simpler and faster enzyme immobilization procedure to be used. Because the enzyme is insoluble in the organic phase, it remains in a thin aqueous film retained on a hydrophilic support. Enzyme loadings can be achieved with advanced enzyme immobiliztion than the covalent immobilization techniques required for aqueous systems.[5] Such unique possibilities and advantages have been reviewed recently.[6] These opportunities have already been documented in connection with enzyme electrodes incorporating tyrosinase,[7,8] horseradish peroxidase[9-10] and cholesterol oxidase.[11]

This paper demonstrates that tissue bioelectrodes can be effectively used for enzymatic assays in methylene chloride. While organic phase enzymatic assays have performed in chiefly chloroform solvent,[12-14] this study represents an example of using methylene chloride solvent.

EXPERIMENTAL

A 10-mL electrochemical cell (Model VC-2 Bioanalytical System, W. Lafayette, IN) was joined to the working electrode, reference electrode(Ag/AgCl, Model RE-1, Bioanalytical Systems), and platinum wire auxiliary electrode through holes in its Teflon cover. A magnetic stirrer provided convective stirring transport (300 rpm) during amperometric measurements. The three electrodes were connected to an voltammetric analyzer(EG&G PAR Model 264A. Princeton, NJ) and the output of which was displayed on a Houston Omniscribe strip-chart recorder.

The tissue-graphite electrodes were prepared in the following processes. The surfaces of the 6-mm diameter graphite disk (U50-2, Ultra Carbon Co.) were polished on a silicon carbide paper of 150 grit for 10s. Such pretreatment formed the roughness desired for subsequent adhesion of the tissue layer. The electrode was then modified with the biocomponent by placing it (face down) in an intact portion of the tissue and moving it in a clockwise motion for 2 min. A mild pressure was maintained during this polishing-like procedure. The excess tissue was removed from the surrounding with a soft paper.

The resulting bioelectrodes were allowed to dry for 15 min prior to use.

The enzyme electrode was prepared by placing 10 L of a phosphate buffer solution of pH 7.4 containing 0.5 mg tyrosinase over the inverted graphite disk and allowing to dry for 15 min.

Phenol, *p*-cresol, 2,4-dichlorophenol and methylene chloride (Aldrich) were used without further purification. Stock solutions of the phenolic substrates (0.1 M in methylene chloride) were prepared daily. Experiments were performed in a methylene chloride solution containing 0.1 M tetraethylammonium *p*-toluenesulfonate (TEATS, Aldrich). All used methylene chloride had been previously saturated with a phosphate buffer (50 mM, pH 7.4) as was suggested by Turner and co-workers.[4,7] Tyrosinase (EC 1.14.18.1, 2400 U mg^{-1}) was purchased from Sigma. The plants used throughout this study were purchased from a local supermarket and stored until use at 5. Amperometric detection was performed at the −0.25 V.

RESULTS AND DISCUSSION

The rich activity of tyrosinase in the banana and mushroom tissues was employed for the organic-phase enzymatic assays.[15]

Typical amperometric responses for several substrates (in a methylene chloride solution), as obtained with unmodified (a) and tissue-modified (b) electrodes were

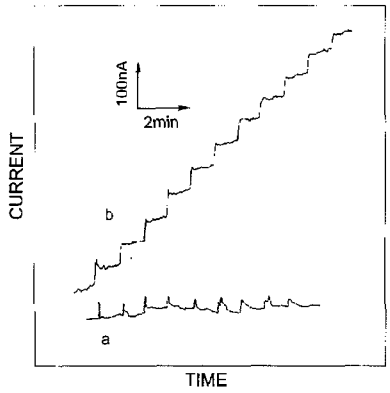

Fig. 1. Current-time recording obtained at (a) the unmodified and (b) banana tissue-modified graphite electrodes on increasing the phenol substrate concentration. Successive increments of 1×10^{-3} M phenol. Operating potential, −0.25 V. Methylene chloride solution containing 0.10 M TEATS. Stirred 300 rpm.

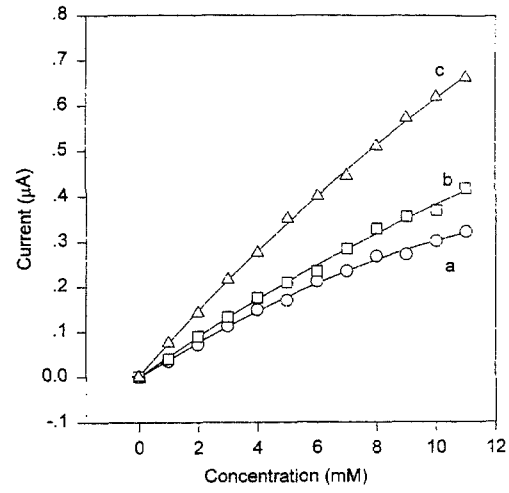

Fig. 2. Dependence of the current response of the banana tissue modified electrodes on the concentration of (a) phenol, (b) *p*-cresol and (c) 2,4-dinitrophenol. Other conditions were same as in *Fig.* 1.

showed in *Fig.* 1. Notice the absence of response at the unmodified surface. In contrast, the tissue electrodes respond very rapidly to the change in the substrate concentration, producing steady-state currents within a few seconds. The very fast response is attributed to the intimate contact between the biocatalytic and graphite sensing sites and to the negligible diffusion barriers of the relative thinnest tissue layer. *Fig.* 2 and 3 show the dependence of the amperometric response of the banana and mushroom electrodes on the concentration of phenolic compounds in methylene chloride. The bioelectrodes readily respond to all the compounds. Only small differences in the sensitivity are observed (with the exception of the larger response for 2,4-dichlorophenol). The slightly different trends in the sensitivity (*Fig.* 2 vs. *Fig.* 3) are attributed to differences in the substrate specificity of the banana and mushroom tyrosinases. Note also that the substrate specificity may be altered upon exposure to the nonaqueous environment.[2] For the three phenolic compounds, especially, 2,4-dichlorophenol, the linearity showed to 4 and 5 mM (at the mushroom and banana surfaces, respectively; correlation coefficients for the linear portions, 0.996-1.000). The response for 2,4-dichlorophenol was linear up to 7 mM (correlation coefficient, 1.000). A detection limit of 6.5×10^{-5}M *p*-chlorophenol was estimated from the signal-to-noise

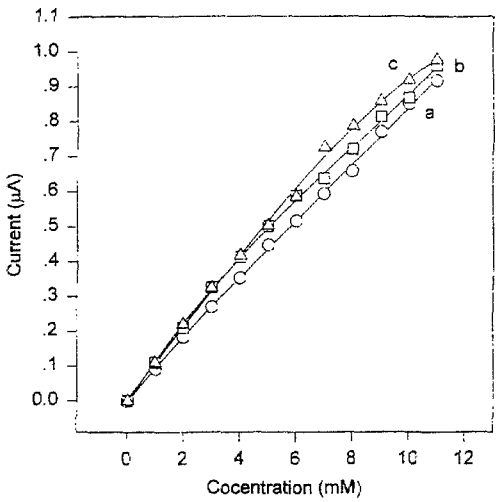

Fig. 3. Dependence of the current response of the mushroom tissue modified electrodes on the concentration of (a) phenol, (b) *p*-cresol and (c) 2,4-dinitrophenol. Other conditions were same as in *Fig.* 1.

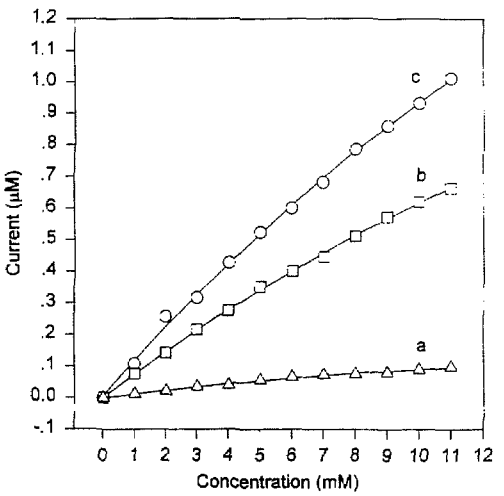

Fig. 4. Calibration plots for 2,4-dichlorophenol in methylene chloride at (a) tyrosinase and (b, c) banana tissue electrodes, using (a, b) 0.010M and (c) 0.10M TEATS. Other conditions were same as in *Fig.* 1.

characteristics (S/N=3) of response to 5×10^{-4} M *p*-chlorophenol in methylene chloride at the banana electrode (not shown).

Fifteen repetitive measurements of 1 mM *p*-2,4-dichlorophenol, with a fresh solvent aliquot each time over a 12 h period, were performed to estimate the reproducibility and stability of the mushroom electrode. A stable response with no specific trend was observed during this prolonged operation, with a mean peak current of 110 nA, a range of 101-119 nA, and a relative standard deviation (R.S.D.) of 5.3%. The electrode design permits easy and fast replacement of the tissue layer. Alternatively, because of its extremely low cost, it could be used as a disposable device. Methylene chloride-phase assays performed with tyrosinase (immobilized isolated enzyme of 1,200 unit) and banana modified electrodes (a vs. b) were compared at *Fig.* 4. The banana tissue electrode yielded an extended linear range and a slightly more increased sensitivity indicating a very high enzyme activity compared to the tyrosinase electrode. In addition, the banana surface offers a faster response and more favorable signal-to-background characteristics. Also shown in *Fig.* 4 is the corresponding calibration plot at the tissue electrode in the presence of a higher (0.10 M) electrolyte content (a). The resulting large increase in the sensitivity illustrates the necessity of sufficient electrolyte to minimize potential losses in organic solvent-phase assays.

In conclusion, we have been demonstrated that tissue bioelectrodes can be effectively used for biocatalytic assays in methylene chloride. Although the concept is presented here in terms of phenolic substrates in a methylene chloride media, it could be extended to other substrates and solvent utilizing different cellular materials.

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