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Amperometric Biosensor Based on Mushroom Tissue Tyrosinase for the Determination of Phenolic Compounds

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Amperometric Biosensor Based on Mushroom Tissue Tyrosinase for the Determination of Phenolic Compounds

Summary : *An amperometric biosensor based on carbon paste electrode modified with mushroom tyrosinase is defined as a device for quantitative and qualitative detection of phenolic compounds. Potassium ferrocyanide is used as the mediator in the reaction between the mushroom tyrosinase and phenolic compounds. This paper demonstrates the advantage of using mushroom tissues as the biocatalyst in vitro detection of phenolic compounds. Experimental variables such as pH, operating potential and temperature, change in the percentage of the mushroom tissue are discussed. The stability of the mushroom tissue modified electrode is also demonstrated.*

Key words: *Amperometry, Tyrosinase, Biosensor, Carbon-paste electrode, Phenolic Compounds*

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Fenolik Bileşiklerin Tayini için Mantar Dokusu Tirozinazına Dayalı Amperometrik Biyosensör

Özet : *Mantar tirozinaz enzimi ile modifiye edilmiş karbon pasta elektroduna dayalı, amperometrik bir biyosensör; fenolik bileşiklerin kantitatif ve kalitatif tayini için bir aygıt olarak tanımlanmıştır. Fenolik bileşikler ve mantar tirozinaz enzimi arasındaki reaksiyonda potasyum ferrosiyanür mediyatör olarak kullanıldı. Bu çalışma mantar dokularının fenolik bileşiklerin in vitro tayinlerinde biyokatalizör olarak kullanılmasının yararlarını göstermektedir. Çalışma potansiyeli, pH, sıcaklık ve mantar dokusu yüzdesindeki değişim gibi deneysel değişkenler incelendi. Mantar dokusuyla modifiye edilmiş elektrodun stabilitesi de ayrıca gösterildi.*

Anahtar kelimeler: *Amperometri, Tirozinaz, Biyosensör, Karbon pasta elektrodu, Fenolik Bileşikler*

INTRODUCTION

There has been considerable interest in recent years in replacing isolated enzymes with tissue materials as the biological entity of biocatalytic sensors[1-7]. The major advantages derived from the use of such materials are the high stability, activity and low cost. Early electrodes suffered from long response times because of the long diffusion path between the test solution and the inner detection surface usually formed by the additional support membranes. After the additional membranes were eliminated the response time decreased to a few minutes from the 10-20 minutes range[8]. With the use of polymeric coatings more rapid and effective responses are obtained[9-11]. Another promising sensing approach for dynamic systems is to incorporate the tissue directly into a carbon paste matrix. This paper describes mixed plant tissue-carbon paste amperometric bioelectrode. The construction is made simply by mixing the desired quantity of mushroom (Agaricus campestris) tissue with the initially prepared carbon paste. In our previous study[12], we used cobalt phthalocyanine (CoPC) as a mediator, whereas in this study we tested $K_4Fe(CN)_6$ as the mediator in the solution, which is simpler and cheaper. The biocatalytic activity of the mushroom-based amperometric probe for phenolic compounds arises

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from the fact that mushroom tissue contains tyrosinase[13] (Also known as polyphenol oxidase, EC 1.14.18.1). This enzyme is responsible for the conversion of mono and di phenols to their corresponding quinones.

MATERIALS and METHODS

Apparatus

Batch experiments were all carried on with a Metrohm 626 polarecord at room temperature. The daily prepared carbon paste mushroom electrode, reference electrode (Ag/AgCl - Model RE-1, BAS) and platinum wire auxiliary electrode were all settled in a 10-mL electrochemical cell (Model VC-2, BAS) through holes in its PTFE cover. Potassium ferrocyanide solution and analyte solutions were prepared daily.

Reagents

Dopamine hydrochloride (3-hydroxytyramine), catechol and phenol were obtained from Sigma. Potassium ferrocyanide was obtained from Aldrich. Phosphate buffer 0.05 M at pH 7.4 served as the supporting electrolyte. Stock solutions of phenol (10^{-2}M), catechol(10^{-2}M) and dopamine (10^{-2}M) were prepared daily using distilled water. Fresh mushrooms, obtained daily from the producer, were used in the fabrication of the tissue-modified working electrodes.

Electrode preparation

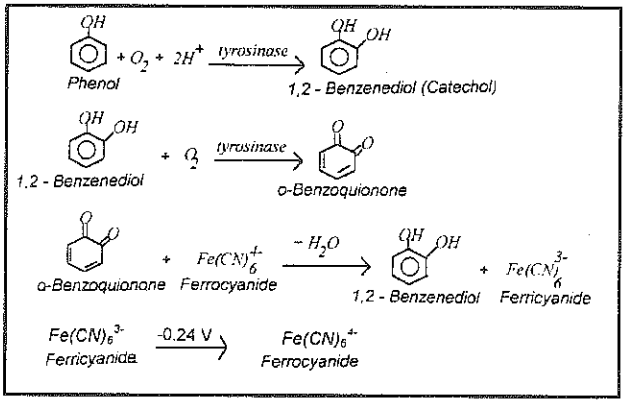
The carbon paste was prepared by mixing graphite powder with mineral oil (Ficher, Acheson38) (40/60 % w/w carbon/oil). 1-2 mm tissues were cut as slices from the surface of the fresh mushrooms (Agaricus campestris) which were obtained daily from the producer and the tissues cut, crushed in a mortar. An optimum slice thickness (1-2 mm) must be obtained to reach good mechanical stability and response time. Carbon paste was then immediately added to the desired amount of mushroom tissue. The mixing was then carried out for 15 min. Subsequently a portion of the resulting paste was packed firmly into a glass tube (3-mm diameter), with a copper wire inserted from the other end for electrical connection. The surfaces were smoothed with a weighing paper.

Procedure

All measurements were performed at room temperature (20°C). Amperometric biosensing proceeded under batch conditions. The phosphate buffer solution contains 10 mM ferrocyanide and the operating potential was applied (Eappl= -0.24 V vs. Ag/AgCl) with 400 rpm stirring. Transient currents were allowed to rise to a steady-state value before the injections of the substrate (spiked of 3µL of the substrate solution). At the same time the amperometric monitoring was carried out.

RESULTS AND DISCUSSION

Figure 1 compares the amperometric response of the plain (A) and 5% mushroom tissue (w/w) containing (B) carbon paste electrodes (CPEs) to successive increments of $3x10^{-5}$ M catechol, by using 10 mM $K_4Fe(CN_6$ at an applied potential -0.24V. The electrochemical redox reactions taking place with phenolic compounds can be summarized in the scheme below:



A plain electrode gives no response to the addition of catechol. In contrast, the mushroom electrode responds rapidly to the change in the substrate concentration, approaching a steady state response within one minute. $K_4Fe(CN)_6$ was used as the electron mediator because it is electrochemically reversible(6), water soluble and needs lower applied potential. As observed above in the scheme of redox reaction, $K_4Fe(CN)_6$ reduces the oxidized analyte and permits the analyte cycling. Then $K_3Fe(CN)_6$ is reduced back to $K_4Fe(CN)_6$ under the operating potential of -0.24 V. The signal-to-noise (S/N = 3) characteristics indicate a detection limit of $1.6x10^{-6}$ M catechol (not shown). Also shown (inset) is the resulting calibration plot(Fig1).

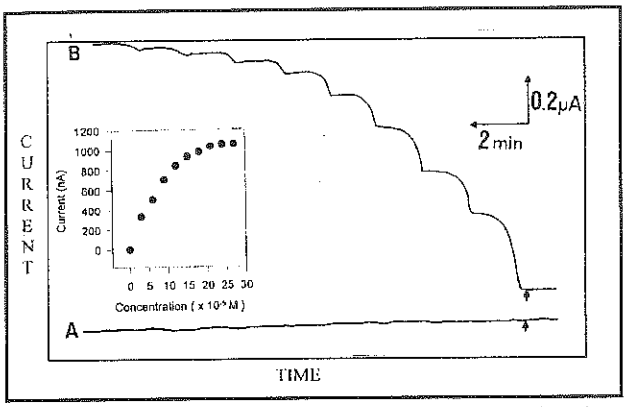


Figure 1. Current-time recording obtained at (A) the plain and (B) 5% mushroom tissue (w/w) containing carbon paste on increasing the concentration of catechol in $3x10^{-5}$ M steps. Batch experiment, stirring the solution at 400 rpm and -0.24 V operating potential. Solution 0.05M phosphate buffer (pH 7.4) containing 10 mM $K_4Fe(CN)_6$. Also shown (inset) is the resulting calibration plot for the modified electrode.

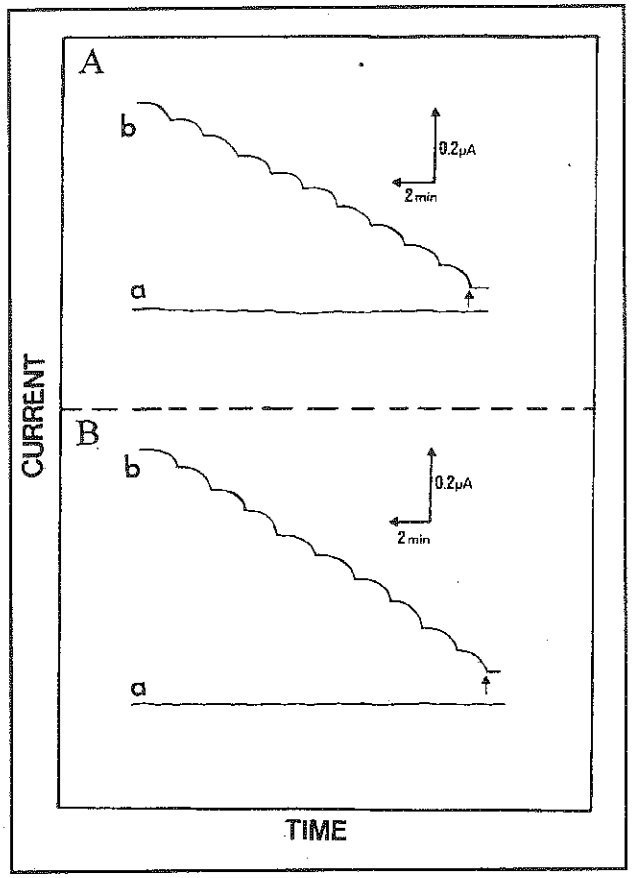


Figure 2. Amperometric response for successive additions of $3x10^{-5}$ M dopamine (A) and phenol (B) at the ordinary (a) and mushroom tissue based (b) carbon paste electrodes. Other conditions as in Fig. 1.

Figure 2 compares current-time recording at -0.24 V for successive batch additions of dopamine (A) and phenol (B), each affecting the $3x10^{-5}$ M increase in concentration as obtained at the plain (a) and mushroom tissue based (b) electrodes. Notice the absence of response at the plain carbon paste electrodes. In contrast , mushroom containing electrode responds very rapidly to the change in the substrate concentrations , producing steady-state currents within one minute. Such rapid response is attributed to the close proximity of the mushroom enzyme and the absence of external (membranous) barriers to the substrate transport.

Figure 3 shows the dependence of the $5x10^{-3}$ M phenol response on the solution pH (A) and operating potential (B), respectively. The current rapidly in-

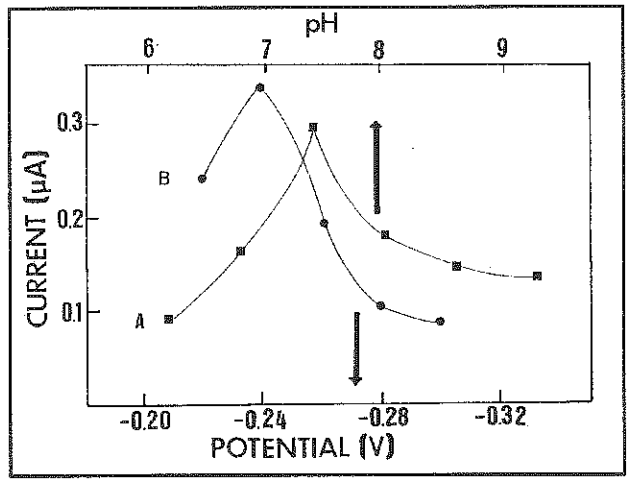


Figure 3. Dependence of the $5x10^{-3}$ M phenol response on the solution pH (A) and operating potential (B) respectively. Other conditions, as in Fig. 1.

creases and reaches a maximum at -0.24 V then sharply decreases between the potentials -0.24 V and -0.30 V. Such a profile is expected for the detection of the $K_3Fe(CN)_6$ product. The steady-state current depends strongly upon the pH of the solution. The current increases sharply over the pH range 6.2 - 7.4. Higher pH values yielded a sharp decrease in the response. The maximum sensitivity at pH 7.4 is in excellent agreement with the optimum pH reported for banana tyrosinase[8-14].

Figure 4 shows the effect of operating temperature on the $5x10^{-3}$ M phenol response. The response current has the maximum value of 120 nA (100 % Activity) at 20°C; then a sharp decrease is observed until 30°C. After 30°C activity was constant. A similar enzyme inactivation was observed to that of Wang et al[15]. As can be seen, optimum operating tem-

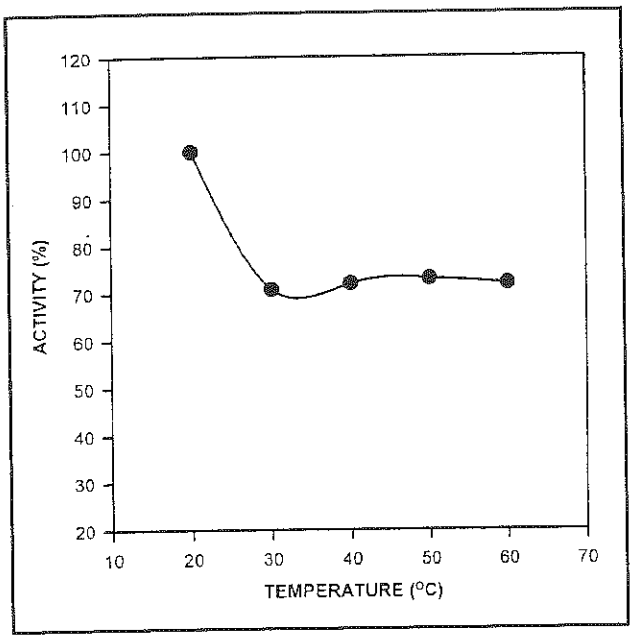


Figure 4. Dependence of the $5x10^{-3}$ M phenol response on the operating temperature. Other conditions as in Fig. 1.

perature was found to be 20°C. Therefore all subsequent work is was carried out at this temperature.

The response of the tissue-containing electrode is strongly affected by the composition. The effects of mushroom contents in carbon paste were evaluated from calibration graphs for phenol (Fig.5). As expected from the increased biocatalytic activity of the

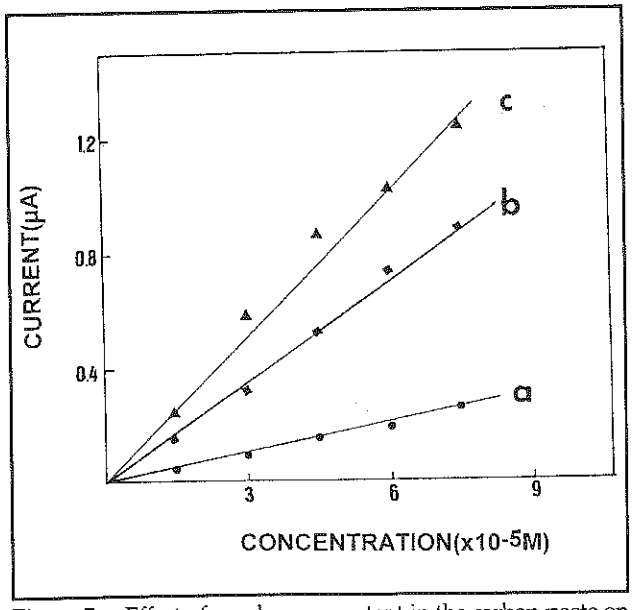


Figure 5. Effect of mushroom content in the carbon paste on the phenol response. Mushroom loadings 5 (a), 10 (b), 15 (c) % (w/w) for carbon paste with $6x10^{-5}$ M phenol increments. Other conditions as in Fig. 1.

electrode, the response increased on increasing the amount of tissue in the paste; but nonlinearly (e.g., 0.2, 0.7, and 1.0 A at 5, 10 and 15 % (w/w) mushroom for $6x10^{-5}$ M phenol). However, the increased analytic signal is accompanied by a larger amperometric background current. Overall, the 5% mushroom electrode yielded the most favorable amperometric response characteristics (minumum noise) and was used in all subsequent work.

Figure 6 demonstrates the stability of the mushroom-based electrode over a two-week period. A fast decay (with up to 70 % depression within 4 days) and then a slow decay are observed with storage of

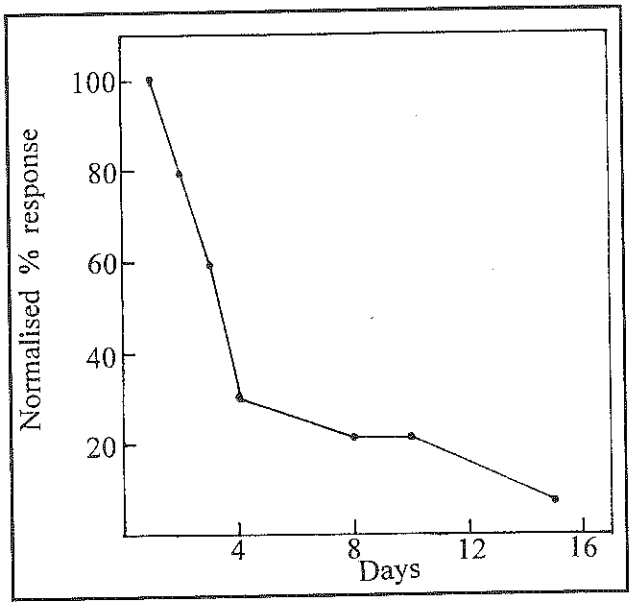


Figure 6. Stability of the response to $1.5x10^{-5}$ M for phenol. Other conditions, as in Fig. 1.

the electrode dry at 4°C in between two measurements on the same surface for $1.5x10^{-5}$ M phenol. The reason for this loss in activity is not clear at the moment. The loss of response could be because of the degradation of enzyme/mushroom tissue, or fouling of the graphite sites by electropolymerized and adsorbed product may account for this failure.

Figure 7 shows the dependence of the amperometric response on the concentration of different substrates, catechol (a), dopamine (b) and phenol (c), of mushroom tyrosinase. The increase in response is linear up to $21x10^{-5}$ M for phenol and dopamine but for catechol it is nonlinear over the 6-$30x10^{-5}$ M range examined. The following trend in

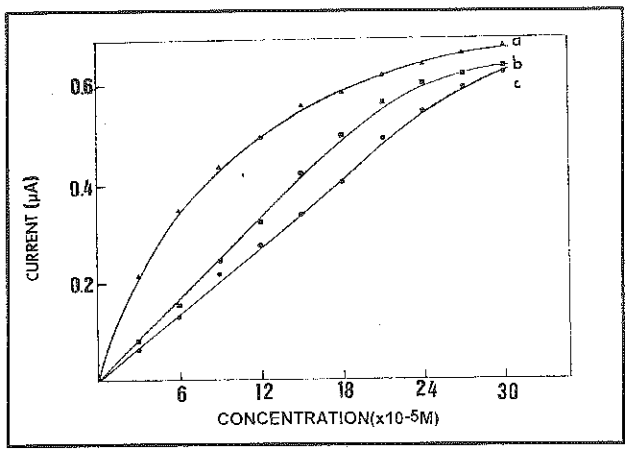


Figure 7. Dependence at the steady state current on the concentration of (a) catechol, (b) dopamine and (c) phenol, I vs. C. Other conditions as in Fig. 1.

sensitivity is observed : catechol > dopamine > phenol.

Figure 8 represents the corresponding reciprocal plots of $1/i_{ss}$ vs $1/C$. These plots exhibit good linearity over the entire range examined. The slopes of these Lineweaver-Burke type plots allowed calculation of the apparent Michaelis constants: 0.10 mM (catechol), 0.3 mM (dopamine) and 0.35 mM (phenol) for this study, in comparison to previously reported banana polyphenol oxidase K_M values; 2.6 mM (catechol), 0.63 mM (dopamine)[14]. In our previous study[12] the K_M values were 0.12 mM (catechol), 0.15 mM (dopamine) and 0.31mM (phenol).

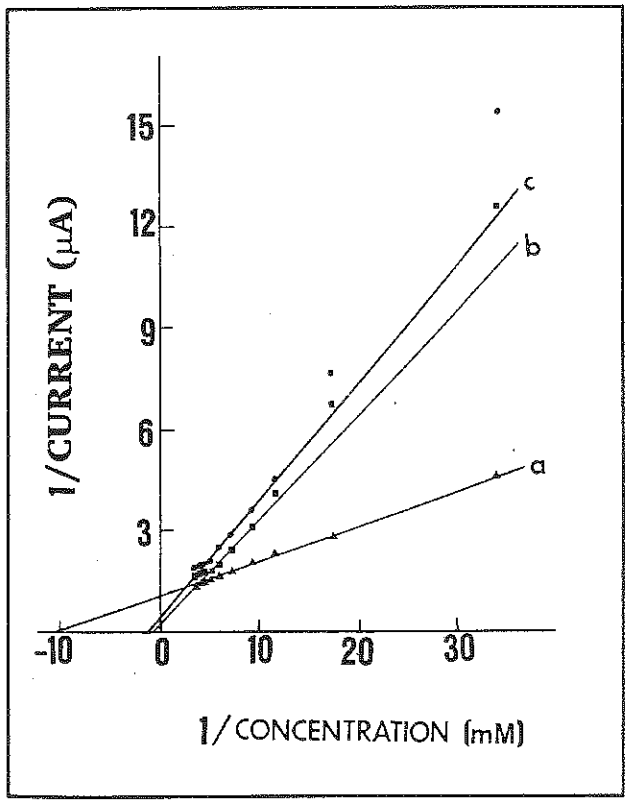


Figure 8. Dependence at the steady state current on the concentration of (a) catechol, (b) dopamine and (c) phenol, $1/I_{ss}$ vs. $1/C$. Other conditions as in Fig. 1.

The validation of the carbon paste electrode was obtained by the relative standard deviation of 3.67% (n=6) in the same surface (washing electrode only with distilled water) and 7.60% (n=6) from surface to surface, respectively for $3x10^{-5}$ M phenol. In our previous study (12) relative standard deviation from surface to surface was 8.68 % (n=8) for 1.3×10^{-2} M dopamine.

CONCLUSION

In conclusion, we have demonstrated the use of mushroom tyrosinase dispersed carbon paste electrodes in the detection of phenolic compounds. As $K_4Fe(CN)_6$ is water soluble, it is easier to obtain well-mixed mediator in solution compared to the one in carbon paste. At the same time the relative standard deviation from surface to surface observed is lower in our study when compared to CPE-CoPC. The high sensitivity and higher reproducubility, together with the simplicity and low cost, make the mushroom tissue-modified electrode very attractive for numerous biosensing applications. Further modification of the electrode configuration would significantly influence its performance. Therefore more detailed investigations on the use of some other mediators would be necessary in order to compare the effect of mediator on the response.

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