THREE PARAMETRIC BIOSENSOR MEASUREMENT SYSTEM

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ABSTRACT
A biosensor measurement system containing as biological element tissue from edible mushroom Agaricus bisporus for the determination of dopamine, epinephrine and norepinephrine was investigated. The measurements based on oxygen consumption in relation to analyte oxidation. The calibration graphs are linear from 2-100 µM dopamine, 5-80 µM epinephrine and 6-60 µM norepinephrine. The results obtained by the proposed enzymatic method are in close agreement with those obtained using a HPLC.

Introduction
Catecholamines play an important role in the central nervous systems as neurotransmitters. The main sites of catecholamine production are the brain, chromaffin cells of the adrenal medulla and the sympathetic neurons (1). Their concentration in different body fluids serves as a prognostic marker for several diseases (2, 3).

Various types of sensing systems for catecholamines have been reported in the literature. The highly sensitive assay of catecholamines has been carried out using HPLC with an electrochemical detector (4), spectrophotometry and fluorimetry (5, 6), a radio-enzyme method (7), which required expensive equipments, special reagents and complicated procedure.

In the recent years, there has been considerable interest in biosensors (8), which use biological materials such as enzyme, tissue slices and bacterial cells. One of the most popular trends in the field of biosensors is developing of biosensor information&measurement systems (9). They are used for simultaneous, consecutive and simultaneous-consecutive detection of group of analytes. Biosensor information and measurement systems have found promising application in various fields, such as medicine, biotechnology and pollution monitoring (10, 11, 12).

In addition, biosensor systems having similar analytical properties to HPLC, but with faster response times and improved ease-to-use, would therefore be a perspective alternative.

The edible mushroom Agaricus bisporus, has been found to produce tyrosinase [E.C. 1.14.18.1], a copper-containing enzyme (13). This enzyme catalyzes the ortho - hydroxylation of monophenols (monophenolase activity) and the oxidation of o - diphenols to o - quinone (diphenolase activity) (14). The monophenolase activity is coupled to its diphenolase activity and the non-enzymatic reactions from the corresponding o - quinines. Consequently, tyrosinase from Agaricus bisporus is good candidate for investigation, as it has large potential for biosensor applications (15).

In this paper, a biosensor information&measurement system based on oxygen electrode, modified with homogenized tissue obtained of the mushroom Agaricus bisporus, is developed for the determination of the catecholamines dopamine, epinephrine and norepinephrine. The aim of the present is to evaluate the transfer function, linear range and sensitivity of biosensor systems.
Constructive model

The biosensor was obtained by clamping on the surface of the Clark electrode three different membranes: an inner oxygen gas-permeable membrane (thickness 25 µm), an intermediate compartment consisting of homogenized tissue of *Agaricus bisporus* (thickness 80 µm), and outer dialysis membrane (thickness 25 µm). The dialysis membrane prevents the loss of biological material to the sample and avoids contamination of the biocatalytic layer. A schematic diagram of biosensor system is given in Fig. 1.

Principle of Measurements

The proposed biosensor system was used for the consecutive assay of dopamine, epinephrine and norepinephrine. Fig. 2 shows scheme of the enzymatic reactions between catecholamines and tyrosinase of mushroom tissue. The enzyme catalyzes oxidation of dopamine, epinephrine and norepinephrine to dopamine-quinone, adreno-quinone and noradreno-quinone, respectively. The oxygen consumption was measured with Clark oxygen electrode. The consumed oxygen correlates directly with the concentration of each catecholamine in the sample solutions. The current on the platinum electrode was measured with oxygen meter APK 101.

Results and Discussion

Biosensor preparation

Mushroom tissue from *Agaricus bisporus* was used as a biological component for the biosensor and was prepared as follows: firstly, tissues (30 mg) were broken into pieces and then were homogenized in 1 ml of acetate buffer (0.1M). The homogenate has been spread on the tip of the oxygen electrode. A rubber O-ring was used to fix the dialysis membrane and mushroom tissue on the electrode. Freshly prepared tissue samples were used for each day.

Reagents and solutions

Stock solutions (1 x 10^{-3} M) of dopamine, epinephrine and norepinephrine (Merck) were prepared daily. The supporting electrolyte was 0.05M phosphate buffer, adjusting the respective pH by addition of 0.1M NaOH. The mushrooms used throughout this study were purchased from a local grocery store and stored in a refrigerator at 5°C until use.

Amperometric analysis

All experiments were carried out in 20 ml of 0.1M phosphate buffer in a glass cell, thermostated at 25°C. The biosensor was immersed in the measurement cell and magnetic stirring, at a constant rate, was used during the measurements. Catecholamine concentration was adjusted by stepwise addition of a concentrated solution to the filled measuring cell.

To obtain optimum response conditions
TABLE

Optimization of biosensor parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Optimal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzyme loadings</td>
<td>15-35mg</td>
<td>25</td>
</tr>
<tr>
<td>magnetic stirring</td>
<td>300-700 r.p.m.</td>
<td>600</td>
</tr>
<tr>
<td>pH</td>
<td>2-9</td>
<td>6-dopamine, 7-epinephrine, 7-norepinephrine</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of enzyme loadings on the biosensor response.

Fig. 4. Effect of pH on the biosensor response.

for the biosensor system, the effect of enzyme loadings, magnetic stirring and pH was analyzed. Table summarizes the range over which each variable was investigated and the optimal values found in this studies.

The effect of enzyme loadings from 15-35mg was investigated. The output signals increased with increasing enzyme loadings to 25mg, and it was practically constant for higher enzyme loadings.

The effect of magneting stirring in an interval from 300-700 r.p.m. on the biosensor response time was investigated. It was observed that 600 r.p.m. was the optimum for biosensor response. Increasing of stirring over 600 r.p.m. wasn't changed the time for establishing of the output signal.

The effect of pH buffer solution in the range 2-9 was also studied. The maximum output signals produced from enzyme catalyzed reactions was observed at pH 6 for 66µM dopamine, pH 7 for 40µM epinephrine and pH 7 for 30 µM norepinephrine. All experiments were performed under the optimal established experimental conditions for the three substrates. Fig. 4 shows the results obtained from pH optimisation studies of the biosensor.

**Calibration of the biosensor system**

The calibration curves obtained for dopamine, epinephrine and norepinephrine has the form characteristic of enzyme – based biosensors. Fig. 5 shows concentration dependence of the steady-state response for the three catecholamines. The linearity was obtained in concentration range between 2-100 µM dopamine, 5-80 µM epinephrine and 6-60 µM norepinephrine. At the higher concentrations the limit response value \((C_0-C_k)_{max}\) correspond to complete saturation of the enzymatically active sites.

The slopes of the calibration graphs of dopamine, epinephrine and norepinephrine were about 0.018, 0.015, 0.011 µA/µM, respectively. The low sensitivity of the sensor against epinephrine and norepinephrine compared with those of the dopamine, is considered to be due to the smallest constant \(K_M\) (16) for dopamine.

The detection limits of dopamine, epinephrine and norepinephrine were at 2 µM, 5 µM and 6 µM, respectively.

The sensor response of the biosensor system vs. various concentrations of catecholamines, it was found that the electrode
Fig. 5. Calibration graphs for dopamine, epinephrine and norepinephrine.

reaches a steady-state within 4-6 min after the addition of samples.

The lifetime of biosensor system is primarily governed by the tissue (enzyme) instability. Tissue instability is affected by solution pH, storage conditions and levels of catecholamines in the samples. After 10 days, over 100 determinations, the sensor response was 85% of the initial response, confirming the high stability of the mushroom tissue.

Conclusions

The use of extract or tissue rather than isolated enzymes represents not only alternative, but also simplicity, stability, longer lifetime and lower cost.

The described biosensor information and measurement system represents a easy-to-use device for direct catecholamine determination.

REFERENCES