A Study on the Electrochemical Synthesis of L-DOPA Using Oxidoreductase Enzymes: Optimization of an Electrochemical Process

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Levodopa or L-3,4-dihydroxyphenylalanine (L-DOPA) is the precursor of the neurotransmitter dopamine. L-DOPA is a famous treatment for Parkinson’s disease symptoms. In this study, electroenzymatic synthesis of L-DOPA was performed in a three-electrode cell, comprising a Ag/AgCl reference electrode, a platinum wire auxiliary electrode, and a glassy carbon working electrode. L-DOPA had an oxidation peak at 376 mV and a reduction peak at -550 mV. The optimum conditions of pH, temperature, and amount of free tyrosinase enzyme were pH 7, 30°C, and 250 IU, respectively. The kinetic constant of the free tyrosinase enzyme was found for both cresolase and catacholase activity to be 0.25 and 0.4 mM, respectively. A cyclic voltammogram was used to investigate the electron transfer rate constant. The mean heterogeneous electron transfer rate (kₑ) was 5.8 × 10⁻⁴ cm/s. The results suggest that the electroenzymatic method could be an alternative way to produce L-DOPA without the use of a reducing agent such as ascorbic acid.

Keywords: L-DOPA, electrochemical synthesis, tyrosinase, Parkinson’s disease

The Monsanto Process was the first commercialized catalytic asymmetric hydrogenation synthesis employing a chiral transition metal complex, and it has been in operation since 1974 [6, 12]. However, this method suffers from disadvantages such as low overall yields, the need for separation of diastereomers, and the need for expensive chiral catalysts [20].

Many studies have been conducted to limit production costs, improve the conversion rate, and improve enantiomeric purity for producing L-DOPA. Microbial production of L-DOPA from Erwinia herbicola, Stizolobium hassjoo, and Escherichia coli has also been investigated. However, microbial production of L-DOPA can be expensive owing to the need to remove proteins and hormones produced by microbial cells, which is time consuming and results in a low conversion rate [10, 13, 16].

Another alternative is enzymatic production of L-DOPA using tyrosinase, which would yield the desired product and is also easy to separate the L-DOPA from the reaction medium, making it more economical [9]. Tyrosinase (monophenol, o-diphenol: oxygen oxidoreductase, E.C. 1.14.18.1) is a copper enzyme found in microorganisms, plants, and animals. This enzyme has two activities, namely the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) [19]. However, some disadvantages of the enzymatic method are high cost of the enzyme and the reducing agent, a difficulty separating L-DOPA from the reaction media, and low productivity [3, 15, 17].

The electrochemical method has been widely used for the detection, electrocatalytic reduction, and electrocatalytic oxidation of compounds [2, 4, 8, 15, 18]. The electrochemical method...
behavior of l-DOPA has been investigated using glassy carbon and modifies glassy carbon electrode as the working electrode [14]. Synthesis of l-DOPA using the electrochemical method as reducing power has also been investigated [15].

In this study, we synthesized l-DOPA by reducing DOPAquinone using electrons supplied from an electrode instead of using a reducing agent such as ascorbic acid, as shown in Fig. 1 [15].

**MATERIALS AND METHODS**

**Materials**

We used dehydrated sodium dihydrogen phosphate (Kanto Chemical Co., Inc., Tokyo, Japan), disodium hydrogen phosphate 12H₂O (Yakuri Pure Chemical Co. Ltd., Osaka, Japan), and sodium nitrite (Duksan Pure Chemical Co. Ltd., Kyungkido, Korea). Tyrosinase enzyme (E.C. 1.14.18.1, 3,610 units/mg), tyrosine, l-DOPA, and all remaining chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the highest grade available and used without further purification.

**Equipment**

The electrochemical batch reactor had a 50 ml volume and was composed of glass. The electroenzymatic synthesis was performed in a three-electrode cell that comprised a Ag/AgCl reference electrode, a platinum wire auxiliary electrode, and a glassy carbon working electrode. The reaction was controlled by a potentiostat, AUTOLAB (Metrohm Autolab B.V., Utrech, The Netherlands), to identify and maintain the cathode potential. Experiments were carried out on an electrochemical batch reactor with a three-electrode system. A 30 ml aliquot of 1 mM DOPAquinone using electrons supplied from an electrode was used as the substrate. The reaction was conducted under the reduction potential of DOPAquinone.

**Electroenzymatic Synthesis of l-DOPA with Free Tyrosinase**

l-DOPA was electroenzymatically synthesized in a 50 ml batch reactor with a three-electrode system. A 30 ml aliquot of 1 mM l-tyrosine (in 50 mM phosphate buffer) was used as the substrate. The reaction was conducted under the reduction potential of DOPAquinone.

**Kinetic Parameters of the Enzyme and Electrode**

The effect of substrate concentration on both cresolase and catacholase activities was determined using Michaelis–Menten kinetics. The Michaelis–Menten constant (Kₘ) and maximum velocity (Vₘₐₓ) were calculated using Lineweaver–Burk plots [1].

The heterogeneous rate constant for the electrical reduction of DOPAquinone to l-DOPA was determined using Eq. (1) [5, 8].

\[ i_c = -nF \alpha A C_0^{\frac{1}{n}} \alpha^{-1} \]  

where \( A \) = surface area of electrode (0.07 cm²)  
\( C_0 \) = concentration (mM)  
\( F \) = Faraday constant (Coulomb/mol)  
\( \alpha \) = cathode charge transfer coefficient, dimensionless  
\( \alpha_c \) = cathode peak current (µA)  
\( n \) = total number of electrons.

The cyclic voltammogram was conducted with an Ag/AgCl system to determine the DOPAquinone reduction potential.

**RESULTS AND DISCUSSION**

**Electrochemical Synthesis of l-DOPA Using Oxidoreductase Enzymes**

[1] Fig. 1. Schematic diagram of electroenzymatic synthesis of l-3,4-dihydroxyphenylalanine (l-DOPA).

[2] \[ E^0_{pc} = \frac{E^0_{C}}{2} + \frac{R T}{n F} \ln \left( \frac{D n_0}{R T} \right) \]  

where \( D \) = diffusion coefficient (cm²/s)  
\( n_0 \) = number of electrons involved in the rate-determining step  
\( R \) = gas constant (J/Kmol)  
\( T \) = temperature (K)  
\( v \) = scan rate (mV/s).

Plots of current vs. root mean scan rate were drawn to obtain the l-DOPA diffusion coefficient.

**Quantitative Analysis**

The l-DOPA content of the samples was determined in the following manner: 1 ml of 2 M hydrochloric acid, 1 ml of sodium hydroxide, and 1 ml of a solution containing 15% (w/v) sodium molybdate and 15% (w/v) sodium nitrite were added to each sample (1 ml) in that sequence. The hydrochloric acid inactivates free residual tyrosinase, stopping the formation of l-DOPA, and prevents the subsequent conversion of l-DOPA to melanin. Sodium hydroxide was added to neutralize the medium. Sodium nitrite reacts with l-DOPA to yield a yellow solution, which was detected spectrophotometrically at 460 nm. Sodium molybdate was added to prevent sample decomposition. The sample was then shaken vigorously on a vortex mixer. As formation of the l-DOPA complex is time dependent, the l-DOPA concentration was determined after 1 h at 460 nm [3, 15, 17, 21].

**Cyclic Voltammogram of l-DOPA**

Fig. 2 shows a cyclic voltammogram of 1 mM l-DOPA in 50 mM (pH 7) phosphate buffer in the three-electrode system to determine the DOPAquinone reduction potential. The cyclic voltammogram was conducted with an Ag/AgCl...
reference electrode, a platinum wire auxiliary electrode, and a glassy carbon working electrode. As shown in Fig. 2, the L-DOPA cyclic voltammogram had an oxidation peak at 376 mV and one reduction peak at -550 mV. The oxidation peak indicates that L-DOPA was oxidized to DOPAquinone, and the reduction peak indicates that the DOPAquinone was reduced to L-DOPA again. From the cyclic voltammogram, the tyrosine substrate cannot be affected by the reduction potential of DOPAquinone, and the working potential was -550 mV for electroenzymatic L-DOPA synthesis. This result was similar to that of Min et al. [15]. They reported that L-DOPA is oxidized to DOPAquinone at 380 mV and that DOPAquinone is reduced to DOPA at -530 mV. Liu et al. [14] tested the electrochemical behavior of L-DOPA and reported the cathode and anode potential values as 576 mV and 610 mV, respectively. They carried out the experiment in an acidic pH. This might be the cause for the difference in cathode and anode potential for the electrochemical reaction.

Effects of pH on the Electroenzymatic Reaction
Fig. 3 shows the effect of varying pH on electroenzymatic synthesis. Synthesis of L-DOPA using the electroenzymatic system was observed. The enzyme was added to the L-tyrosine solution in the electrochemical system. As shown in Fig. 3, the optimum pH for L-DOPA synthesis using tyrosinase in the electrochemical system was pH 7. Tuncagil et al. [22] also reported an optimum pH of 7 for the free tyrosinase enzyme system with a sharp drop with acidic or basic pH values. Fig. 4 shows the electrochemical behavior of L-DOPA with respect to pH. The highest current for cathode potential was found at pH 7. It was necessary to identify the optimum electrochemical behavior condition for efficient reducing power. The cyclic voltammogram of 1 mM of L-DOPA in 0.05 mM phosphate buffer solution at different pHs (6, 6.5, 7, and 8) at a 50 mV scan rate was recorded to determine the optimum pH for the electrochemical system.

Effects of Temperature on the Electroenzymatic Reaction
Fig. 5 shows the effect of varying temperature on electroenzymatic synthesis. As shown in Fig. 5, the optimum temperature condition for L-DOPA synthesis using tyrosinase in the electrochemical system was 30°C. Tuncagil et al. [22] and Chuang et al. [7] also demonstrated that the optimum temperature for free tyrosinase was 30°C. As shown on Fig. 6, the electrochemical behavior of the reduction peak of DOPAquinone to L-DOPA with respect to temperature was observed. The highest current for cathode potential was found at 30°C. It was necessary to identify the optimum electrochemical behavior condition for efficient reducing power. The cyclic voltammogram of 1 mM of L-DOPA in 0.05 mM phosphate buffer solution at different temperatures (20°C, 30°C, 40°C, and 50°C) at a 50 mV scan rate was recorded to determine the optimum temperature for the electrochemical system.
Effect of temperature achieved the highest current at 30°C. As a reduced peak current change occurred with respect to temperature, we can say that temperature affected how much current will be supplied to the reaction. In warm temperatures, electron motion will be faster. Thus, it will increase electrical resistance and affect decreasing current.

**Effect of Enzyme Concentration on the Electrochemical Reaction**

Fig. 7 shows the effect of enzyme concentration on L-DOPA production. The optimum amount of enzyme for this reaction with a working volume of 30 ml and 1 mM L-tyrosine was 250 IU. To determine the optimum enzyme concentration, the reaction was carried out with 100, 150, 250, and 500 IU enzyme concentrations. As the reducing power supplied to the reaction was insufficient to reduce DopAquinone to L-DOPA, enhancing the enzyme concentration from the optimum value would decrease L-DOPA concentration.

**Comparison Between Electroenzymatic and Enzymatic Synthesis of L-DOPA**

Fig. 8 was obtained as a result of a comparison between the electroenzymatic and enzymatic synthesis of L-DOPA with 1 mM ascorbic acid as the reducing agent. This experiment was carried out using 250 IU of enzyme at pH 7 and 30°C. The result showed that electroenzymatic synthesis of L-DOPA produced a higher L-DOPA concentration than that of enzymatic L-DOPA synthesis using ascorbic acid as the reducing agent. As shown in Fig. 8, the maximum concentration of L-DOPA for electroenzymatic synthesis was higher than that of the enzymatic synthesis.
synthesis was 0.4 mM and that for the enzymatic system with ascorbic acid was 0.3 mM after 4 h.

Kinetic Constant of the Enzyme and Electron Transfer Rate

The kinetic parameters ($K_m$ and $V_{max}$) for both cresolase and catacholase activity of tyrosinase are shown in Table 1. The $K_m$ value of catacholase activity was higher than that of cresolase activity.

The experimental cyclic voltammetry result showed well-defined and reproducible anode and cathode peak currents. As can be seen in Fig. 2, the peak separation potential was $>59$ mV, which was expected for a reversible system. This result suggests that the redox coupling in L-DOPA shows a quasireversible behavior in aqueous medium with a glassy carbon electrode [2].

Fig. 9 shows the effect of scan rate by cyclic voltammography. Fig. 10 shows that the plot of cathode peak currents were linearly dependent on the square root of scan rate ($v^{1/2}$) at various scan rates (25–500 mV/s) and suggests that the reaction is controlled by diffusion [4, 18].

According to Eq. (2), the value of D can be obtained from the slope of the current vs. the root mean of scan rate plot. The heterogeneous electron transfer rate of electrochemical reduction of DOPAquinone to L-DOPA was determined from Eq. (1). The value of D and the heterogeneous electron transfer rate are shown in Table 2. The mean electron transfer rate of the electrode was $5.8 \times 10^{-4}$ cm/s.

In conclusion, L-DOPA was synthesized using L-tyrosine as a substrate and tyrosinase as the enzyme. The electrochemical method was used as reducing power to convert DOPAquinone to L-DOPA. The reduction peak of

<table>
<thead>
<tr>
<th>L-DOPA concentration (mM)</th>
<th>Plot equation</th>
<th>$D \times 10^4$ (cm$^2$/s)</th>
<th>$k_e \times 10^4$ (cm/s)</th>
</tr>
</thead>
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<tr>
<td>0.05</td>
<td>$y = -0.9888x - 1.0138$</td>
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<td>0.10</td>
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<td>4.311428</td>
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<td>0.25</td>
<td>$y = -1.0473x - 1.3493$</td>
<td>1.3</td>
<td>5.357667</td>
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<tr>
<td>0.50</td>
<td>$y = -1.2723x - 0.4877$</td>
<td>0.72</td>
<td>5.769918</td>
</tr>
<tr>
<td>0.75</td>
<td>$y = -1.3963x - 0.4186$</td>
<td>0.52</td>
<td>6.956354</td>
</tr>
<tr>
<td>1.00</td>
<td>$y = -1.3962x - 1.7075$</td>
<td>0.378</td>
<td>8.432246</td>
</tr>
</tbody>
</table>
1-L-DOPA was -550 mV from a cyclic voltammogram. The maximum pH and temperature were pH 7 and 20°C, respectively, for electrochemical enzymatic synthesis of 1-L-DOPA. The optimum amount of enzyme with a working volume of 30 ml of 1 mM 1-tyrosine was found when enzyme activity was 250 IU. The kinetic constants of the enzyme for cresolase and catalacholase activity were 0.25 mM and 0.4 mM, respectively. The electroenzymatic method could be an alternative for producing 1-DOPA without the use of a reducing agent such as ascorbic acid.

The mean electron transfer rate of the electrode was 5.8 × 10⁻⁴ cm/s.

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References


