Electrocatalytic Oxidation of Vitamin B6 on a Chemically Modified Electrode

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Abstract

The electrocatalytic oxidation of vitamin B6 (pyridoxine hydrochloride) was demonstrated on a Nafion/lead ruthenate pyrochlore modified electrode (designated as NCME) by cyclic voltammetry. The catalytic activity of vitamin B6 was explored in terms of the higher oxidation state of ruthenium species, i.e., \( \text{Py}^-\text{Ru}^{IV}/\text{Ru}^{VI} \) in the pyrochlore network (\( \text{Py} \)). The mediated mechanism was derived by Michaelis-Menten kinetics. The calculated kinetics values by direct Michaelis-Menten method are Michaelis-Menten rate constant (\( K_m \)) = 4.05 mmol dm\(^{-3} \), catalytic rate constant (\( k_c \)) = 2.05\times10^{-1} s\(^{-1} \), and heterogeneous electro-chemical rate constant (\( k'_{ME} \)) = 4.09\times10^{-2} cm s\(^{-1} \).

Key Words: Vitamin B6, Electrocatalysis, Chemically Modified Electrode

1. Introduction

Vitamin B6 (pyridoxine) has been paid special attention in clinical and pharmaceutical lines because of its extensive and essential applications in bio-metabolisms. Recently, it was reported that vitamin B6 and its derivatives have major influence in gene modulation, sexual behavior, cancer research, and immune modulation in HIV-1 infection [1-4]. In biological system, pyridoxine (PYX) is oxidized to pyridoxal derivative (PYO) to act as a coenzyme for the amino group transformation [5,6]. The oxidation kinetics and mechanism of vitamin B6 are thus important to the process in vitro. We report here the electrocatalytic oxidation of pyridoxine on the Nafion/lead ruthenate pyrochlore (\( \text{Pb}_2\text{Ru}_{2-x}\text{Pb}_x\text{O}_{7-y} \)) chemically modified electrodes (designated as NCME). To the best of our knowledge, no electrocatalytic and electrochemical studies were reported on vitamin B6 so far.

The preparation of the NCME was described by our group recently for analytical applications [7-11]. In this work, the electrocatalytic oxidation studies of pyridoxine on the NCME were evaluated by cyclic voltammetric technique. Michaelis-Menten type kinetics was adopted to estimate the mechanistic parameters. The Michaelis-Menten rate constant (\( K_m, \text{mmol dm}^{-3} \)), catalytic rate constant (\( k_c, \text{s}^{-1} \)) and heterogeneous electro-chemical rate constant (\( k'_{ME}, \text{cm s}^{-1} \)) were determined by three methods of Michaelis-Menten (MM) analysis, Lineweaver-Burk (LB) plot, and Eadie-Hofstee (EH) plot.

2. Experimental

Nafion perfluorinated ion-exchange powder, 5 wt% solution in a mixture of lower aliphatic alcohols and 10% water, was purchased from Aldrich (Milwaukee, WI, U.S.A.). Lead nitrate (\( \text{Pb(NO}_3)_2 \)) and ruthenium chloride (\( \text{RuCl}_3\times\text{H}_2\text{O} \)) were also obtained from Aldrich. Pyridoxine hydrochloride (Sigma) and all other compounds (ACS-certified reagent grade) were used without
further purification. Aqueous solutions were prepared with doubly distilled deionized water.

The electrochemical measurements were carried out using a BAS 50W electrochemical analyzer (West Lafayette, IN, U.S.A.). The three-electrode system consisted of the NCME, a Ag/AgCl reference electrode (Model RE-5, BAS), and a platinum wire auxiliary electrode. A glassy carbon electrode (GCE) with a geometrical electrode area of 0.07 cm² was used for the preparation of the NCME. The GCE was first polished on a BAS polishing kit to a shiny surface. Then, it was further cleaned ultrasonically in 1:1 nitric acid. The preparation and physico-chemical characterization of the NCME has been described elsewhere [7]. Since dissolved oxygen did not interfere with the voltammetry analysis; no deaeration was performed for the kinetic analysis.

3. Results and Discussion

Figure 1 shows a typical CV response of the NCME in the absence (a) and presence (b) of 1.5 mM pyridoxine in pH 6.0 phosphate buffer solution at a scan rate of 50 mV s⁻¹. A clear catalytic anodic peak corresponding to the oxidation of pyridoxine was noticed at ~0.8 V (vs. Ag/AgCl). The electrocatalytic oxidation on the NCME is controlled by a certain oxidation state of ruthenium in the catalyst. It is proposed that the ruthenium in the Ru₂O₆ octahedral sites of the pyrochlore network participates in the reaction mechanism. The most proper oxidation state of Ru species in pH 6.0 is Ru⁴⁺/⁶⁺ based on previous studies on RuO₂ [12-14]. The fact that the anodic current (Py-Ru⁴⁺ → Py-Ru⁶⁺ + 2e, where Py denotes to the pyrochlore network) increases upon the addition of vitamin B6 indicates the oxidation is mediated by the surface confined Ru⁶⁺/⁴⁺ redox sites through surface redox catalytic path. Possible mediated oxidation mechanism of pyridoxine to pyridoxal derivative through Py-Ru⁴⁺/⁶⁺ is show in Figure 2. As can be seen, the pre-catalyst Py-Ru⁴⁺ can be regenerated by pyridoxine (PYX) to pyridoxal (PYO) transformation. Note that the cathodic current decreases when pyridoxine is present in solution is also a typical characteristic feature of a redox process assisted by a mediator [15].

Figure 1. Cyclic voltammograms of the NCME in pH 6.0 phosphate buffer solution with (a) and without (b) 1.5 mM vitamin B6. Scan rate = 10 mV s⁻¹

Figure 2. Proposed mediated reaction mechanism for the oxidation of pyridoxine to pyridoxal derivative at the NCME. An octahedral site of Ru present at the pyrochlore network is expected to participate in the oxidation kinetics.

We then performed CV analysis to assess its mechanistic aspects. The catalytic current (i₉cat) is calculated as the difference between the baseline-corrected limiting currents with and without pyridoxine in aqueous solution. Figure 3(a) is the concentration (Cbulk) vs. anodic peak current (i₉pa) plot for the vitamin B6 oxidation reaction obtained by CV at a slow scan rate of 10 mV s⁻¹. As can be seen, the i₉cat linearly increases up to 2 mM of vitamin B6 and tends to level off beyond that. It is an indication of Michaelis-Menten type kinetics, i.e., in lower concentrations; the reaction follows first order kinetics and zero order kinetics at higher concentrations. If [PYX—Py-Ru⁶⁺] is the substrate-
catalyst high-energy intermediate and PYO is the oxidized product, i.e., pyridoxal derivative, the electrochemical equivalent of the Michaelis-Menten equation in terms of current density (I) can be presented as [16,17]:

\[
I = nFk_c \Gamma_{\text{t}} C_{\text{bulk}}/(K_m + C_{\text{bulk}}) = I_m C_{\text{bulk}}/(K_m + C_{\text{bulk}})
\]

(1)

Where, \(I_m = nFk_c \Gamma_{\text{t}}\) is the total surface concentration of the electro-active ruthenium species (i.e., \(P_{\text{u}}\)-Ru\(^{6+}\)/\(P_{\text{u}}\)-Ru\(^{4+}\), obtained from \(q = nFA\Gamma_t\)) and other factors have their own significant values. The \(I_{\text{max}}\) value is independent of the substrate concentration and can be obtained when \(C_{\text{bulk}} \gg K_m\). The fundamental kinetic parameters \(K_m\) and \(k_c\) can then be calculated by fitting the experimental data based on the non-linear curve fitting program operating with Marquardt-Levenberg algorithm (Sigma Plot). The calculated \(I_m\) and \(K_m\) value was 22.72 \times 10^{-6} A and 4.05 \times 10^{-3} \text{ mol dm}^{-3}, respectively. The \(I_{\text{max}}\) value was estimated from \((q_t - q_{dl})\), i.e., by subtracting the \(i_{\text{cat}}\) occurs in base electrolyte when cycled in the potential between the hydrogen and oxygen evolution region \((q_t)\) with that of double layer charge \((q_{dl})\). Note that \(q_{dl}\) can be measured by small amplitude CV technique [5] at very close potential in the catalytic region (300 mV) at the same scan rate \((v = 5 \text{ mV s}^{-1})\). The calculated surface coverage \((\Gamma_t)\) was 8.13 \times 10^{-9} \text{ mol cm}^{-2}. From \(I_m = nFk_c \Gamma_{\text{t}}\), the first order catalytic rate constant, \(k_c\) was found to be 2.05 \times 10^{-1} \text{ s}^{-1}. The heterogeneous rate constant value \(k_{ME}^*\) can then be obtained by substituting \(k_c\), \(\Gamma_t\) and \(K_m\) into the equation of \(k_{ME}^* = k_c \Gamma_t/K_m\). The obtained value for \(k_{ME}^*\) was 4.09 \times 10^{-2} \text{ cm s}^{-1}.

The other simplified expressions for calculating the kinetic parameters, namely, Lineweaver-Burk (LB) and Eadie-Hofstee (EH) methods[6] are as follows.

**Lineweaver-Burke (LB) Expression:**

\[
1/I = K_m/nFk_c \Gamma_{\text{t}} C_{\text{bulk}} + 1/nFk_c \Gamma_t
\]

(2)

\[
1/I = S_{LB} C_{\text{bulk}} + I_{LB}
\]

\[
S_{LB} = K_m/nFk_c \Gamma_{\text{t}} & I_{LB} = 1/nFk_c \Gamma_t
\]

(3)

**Eadie-Hofstee (EH) Expression:**

\[
i/C_{\text{bulk}} = nFk_c \Gamma_t/K_m - i/K_m
\]

(4)

\[
i/C_{\text{bulk}} = I_{EH} - S_{EH}i
\]

\[
I_{EH} = nFk_c \Gamma_t/K_m & S_{EH} = -1/K_m
\]

(5)
In eqns. (3) and (5), $S$ and $I$ denote for the slope and intercept of the linearized equations. (2) and (4), respectively. Figures 3b and 3c are typical plots of $(i^* \text{ vs. } C_{\text{bulk}}^{-1})$ and $(i/C_{\text{bulk}} \text{ vs. } C_{\text{bulk}})$ for the LB and EH analysis, respectively. The calculated kinetics parameters, $K_m$, $k_c$ and $k'_c$, based on these two methods were listed in Table 1 together with the data from Michaelis-Menten method for comparison. The value obtained from non-linear fitting of Michaelis-Menten equation gave relatively higher values than those of LB and EH methods. Similar observation was noticed by Lyons et al. and they explained that it is due to the compression of experimental data occurred in the LB and EH type analysis [16,17].

The results obtained in the present studies clearly demonstrate that the NCME can be effectively used for the oxidation of vitamin B6. The results are very significant for the development of amperometric chemical sensor. Further work for sensor application to detect vitamin B6 in clinical and pharmaceutical samples is currently in progress.

Table 1. Kinetic Parameters for the Oxidation of Vitamin B6 on the NCME

<table>
<thead>
<tr>
<th>Analysis method</th>
<th>$K_m$ / mmol dm$^{-3}$</th>
<th>$k_c$ / s$^{-1}$</th>
<th>$k'_c$ / cm s$^{-1}$</th>
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</thead>
<tbody>
<tr>
<td>Michaelis-Menten</td>
<td>4.05</td>
<td>2.05$\times$10$^{-1}$</td>
<td>4.11$\times$10$^{-4}$</td>
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<tr>
<td>Lineweaver-Burk plot</td>
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<td>2.04$\times$10$^{-1}$</td>
<td>4.09$\times$10$^{-4}$</td>
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<td>Eadie-Hofstee plot</td>
<td>4.24</td>
<td>1.91$\times$10$^{-1}$</td>
<td>3.66$\times$10$^{-4}$</td>
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References


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