Kinetic Analysis of the Noncompetitive Inhibition of Lignin Peroxidase by Cellobiose: Quinone Oxidoreductase

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Abstract—The steady-state kinetics of the redox interaction between lignin peroxidase (LiP) and cellobiose: quinone oxidoreductase (CBQ) was analyzed. To explain the unique noncompetitive inhibition of LiP by CBQ, a scheme was proposed with a novel equation derived. The inhibition constant here differs from that occurring in the equation for the inhibition of LiP by oxalic acid (OX), in that it involves more parameters.

Keywords: lignin peroxidase, cellobiose: quinone oxidoreductase, noncompetitive inhibition, kinetics, Phanerochaete chrysosporiu

1. Introduction

Since the demonstration by Kerstern et al.1 that 1,4-dimethoxybenzene was converted to p-benzoquinone and methanol via an aryl cation radical, it has been widely accepted that all the oxidative reactions of nonphenolic lignin model substrates catalyzed by lignin peroxidase (LiP) proceed via aryl cation radical intermediates. Veratryl alcohol (VA) is a secondary metabolite of Phanerochaete chrysosporium2) and is proposed to act as a mediator to induce the formation of cation radicals in remote lignin structures. However, so far no direct evidence has been obtained showing the occurrence of VA cation radical (VAt) in LiP-catalyzed systems. It was found that the oxidation of VA by LiP was noncompetitively inhibited by oxalic acid (OX)3,4).

Later soon it was reported that OX also inhibited the LiP-catalyzed oxidation of other nonphenolic substrates similarly but not that of phenolic ones5). On the other hand, Samejima and Eriksson6,7) have also reported that LiP was noncompetitively inhibited by cellobiose: quinone oxidoreductase (CBQ) in the presence of cellobiose (CB). The above mentioned noncompetitively inhibitions of LiP by OX and CBQ can be explained in the light of aryl cation radicals as mediators. By analyzing the steady-state kinetics of the noncompetitive inhibition of LiP-catalyzed Ca-Cβ bond cleavage of veratrylglycerol (VG)
by OX, Ma et al.\(^6\) derived a novel equation to explain the mechanism of the unique noncompetitive inhibitions of LiP by OX which is different from the classical ones caused by allosteric inhibitors.

Here we report another novel equation, derived for the unique noncompetitive inhibition of LiP activity by CBQ (FAD/FADH\(_2\)).

### 2. Results and Discussion

Samejima and Eriksson\(^6\) found that in the presence of cellobiose, CBQ non-competitively inhibited VA oxidation by LiP and from their results concluded that VA acts as a radical mediator of the redox interaction between LiP and CBQ (Fig. 1).

The overall reactions of LiP/CBQ system can be expressed in the following scheme (Fig. 2).

![Mechanism for the redox interaction between LiP and CBQ (FAD/FADH\(_2\)) proposed by Samejima and Eriksson.](image1)

![Scheme proposed for the mechanism of the redox interaction between LiP and CBQ. LiP, lignin peroxidase; VA, veratryl alcohol.](image2)

\[\text{LiP}^* + \text{VA} \xrightleftharpoons[k_2]{k_1} \text{LiP}^* - \text{VA} \xrightarrow[k_p]{k_3} \text{VA}^+ \xrightarrow[k_i]{k_4} \text{P} \]

\[\text{CBQ}_{\text{red}}\]

\[\text{CBQ}_{\text{ox}}\]

\[\text{CB}\]

\[\text{CBQ}_{\text{ox}}\]

\[\text{CBQ}_{\text{red}}\]

\[\text{VA}^+\]

\[\text{P}\]

LiP, lignin peroxidase; VA, veratryl alcohol. \(k_1, \ldots, k_7, k_p, k_i,\) and \(k'_p\) are the rate constants of the corresponding reactions, \(\text{VA}^+,\) cation radical of VA; \(P\) and \(P'\) are veratraldehyde and cellobiono-\(\delta\)-lactone, respectively. LiP-I and LiP-II are the compound I and II of LiP, respectively. CBQ, cellobiose: quinone oxidoreductase; CB, cellobiose; CBQ\(_{\text{red}}\) (FADH\(_2\)) and CBQ\(_{\text{ox}}\) (FAD) are the reduced and oxidized forms of CBQ, respectively.
Ma et al. showed a ping-pong mechanism for the LiP/H$_2$O$_2$/VA system. For the sake of convenience, LiP/H$_2$O$_2$/VA may be taken as a unireactant system here. LiP-I and LiP-II are the active oxidizing intermediates in this system. According to the scheme, the following equations (1)–(4) are given,

\[ v_a = k_3 [\text{LiP} - \text{VA}] \]  

\[ v_p = k_p [\text{VA}^+] \]  

\[ v_i = \frac{[\text{VA}^+] V_{i \text{max}}}{K_m + [\text{VA}^+] \left(1 + \frac{K_m}{[\text{CB}]}\right)} \text{ (ping-pong)} \]  

\[ v_o = v_p + v_i \]  

where \( v_a, v_p \) and \( v_i \) are the reaction rates of the reactions of \( \text{LiP} - \text{VA} \rightarrow \text{VA}^+, \text{VA}^+ \rightarrow \text{P} \) and \( \text{VA}^+ \rightarrow \text{CBQ/CBQ} \rightarrow \text{VA} \), respectively; \( V_{i \text{max}} \) is the maximum of \( v_i \); \( K_m \) and \( K'_m \) are the \( K_m \)s for \( \text{VA}^+ / \text{CBQ} \) and \( \text{CBQ/} \text{CBQ} \), respectively.

According to definition, the following equation (5) can be obtained.

\[ V_{i \text{max}} = \frac{k_i \cdot k_p'}{k_i + k_p'} [\text{CBQ}] \]  

By substituting Eq. 5 in Eq. 3, we can obtain the following equation (6),

\[ v_i = \frac{k_i \cdot k_p'/(k_i + k_p')} {K_m + [\text{VA}^+] \left(1 + \frac{K_m}{[\text{CB}]}\right)} [\text{CBQ}] [\text{VA}^+] \]  

When \( [\text{VA}^+] \) is small enough,

\[ v_i = \frac{k_i \cdot k_p'}{K_m \left(k_i + k_p'\right)} [\text{CBQ}] [\text{VA}^+] \]  

From Eqs. 2, 4, and 7, we obtain the following equation (8),

\[ v_p = \frac{v_o}{1 + \frac{1}{K_i} [\text{CBQ}]} \]  

where \( K_i = \frac{k_p \cdot K_m \left(k_i + k_p'\right)}{k_i \cdot k_p'} \)

Therefore

\[ \frac{1}{v_p} = \left(1 + [\text{CBQ}]/K_i\right) \left(\frac{K_m}{V_{i \text{max}}} \cdot \frac{N}{[\text{VA}]} + \frac{1}{V_{i \text{max}}}\right) \]  

Thus the noncompetitive inhibition type of Lineweaver-Burk plots can be explained.

This rationalization is consistent with that of Ma et al. in supporting the hypothesis that VA plays a mediator role in LiP-catalyzed reactions, although no direct evidence for the occurrence of \( \text{VA}^+ \) has been obtained yet. The noncompetitive inhibition of LiP by CBQ system indicates that among the three schemes Ma et al. proposed, Scheme a is most likely
to be true, since it is hardly conceivable that the LiP-VA$^+$ complex would interact directly with the CBQ enzyme.

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