Note

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 chrysosporium*[2] 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 ($VA^{+\cdot}$) 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 ones[5]. On the other hand, Samejima and Eriksson[6,7] 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 Cα-Cβ bond cleavage of veratrylglycerol (VG)

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by OX, Ma *et al.*[8] 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 noncompetitively 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).

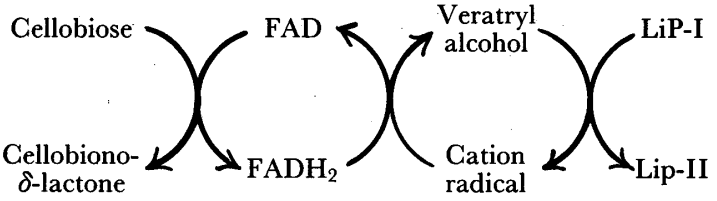

Fig. 1. Mechanism for the redox interaction between LiP and CBQ ($FAD/FADH_2$) proposed by Samejima and Eriksson.

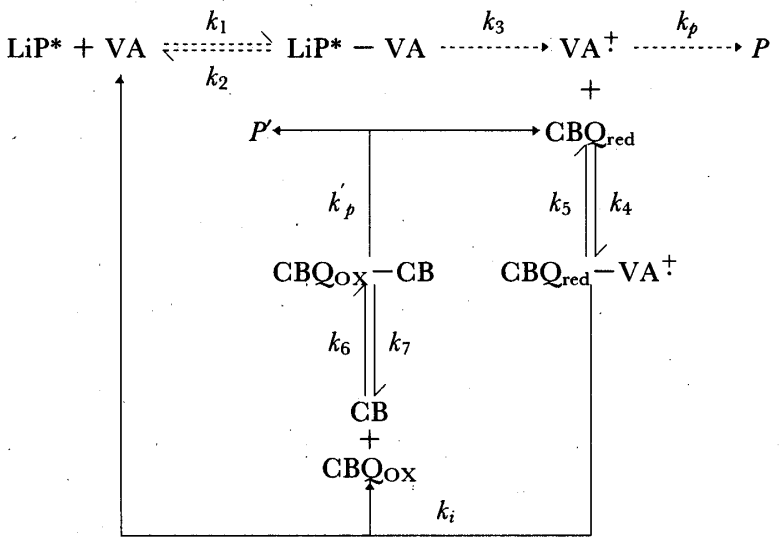

Fig. 2. Scheme proposed for the mechanism of the redox interaction between LiP and CBQ. LiP, lignin peroxidase; VA, veratryl alcohol. $k_1, \ldots, k_7$, k_p, k_i, and k'_p are the rate constants of the corresponding reactions, $VA^{+\cdot}$, cation radical of VA; P and P' are veratraldehyde and cellobiono-δ-lactone, respectively. LiP-I and LiP-II are the compound I and II of LiP, respectively. CBQ, cellobiose : quinone oxidoreductase; CB, cellobiose; CBQ_{red} ($FADH_2$) and CBQ_{OX} (FAD) are the reduced and oxidized forms of CBQ, respectively.

et al.[9] showed a ping-pong mechanism for the LiP/H_2O_2/VA system. For the sake of convenience, LiP/H_2O_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,

$$v_o=k_3\,[\mathrm{LiP-VA}] \tag{1}$$

$$v_p=k_p\,[\mathrm{VA}^{+\cdot}] \tag{2}$$

$$v_i=\frac{[\mathrm{VA}^{+\cdot}]V_{i\,\max}}{Km_i+[\mathrm{VA}^{+\cdot}]\left(1+\dfrac{K'm}{[\mathrm{CB}]}\right)}\ \text{(ping-pong)} \tag{3}$$

$$V_o=v_p+v_i \tag{4}$$

where v_o, v_p and v_i are the reaction rates of the reactions of LiP−VA$\longrightarrow$VA$^{+\cdot}$, VA$^{+\cdot}\longrightarrow P$ and VA$^{+\cdot}\xrightarrow{\text{CBQ/CB}}$VA, respectively ; $V_{i\,\max}$ is the maximum of v_i ; Km_i and $K'm$ are the Km_s for VA$^{+\cdot}$/CBQ and CB/CBQ,respectively.

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

$$V_{i\,\max}=\frac{k_i\cdot k'_p}{k_i+k'_p}\,[\mathrm{CBQ}] \tag{5}$$

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

$$v_i=\frac{k_i\cdot k'_p/(k_i+k'_p)}{Km_i+[\mathrm{VA}^{+\cdot}]\left(1+\dfrac{K'm}{[\mathrm{CB}]}\right)}\,[\mathrm{CBQ}]\,[\mathrm{VA}^{+\cdot}] \tag{6}$$

When [VA$^{+\cdot}$] is small enough,

$$v_i=\frac{k_i\cdot k'_p}{Km_i(k_i+k'_p)}\,[\mathrm{CBQ}]\,[\mathrm{VA}^{+\cdot}] \tag{7}$$

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

$$v_p=\frac{v_o}{1+\dfrac{1}{Ki}}\,[\mathrm{CBQ}] \tag{8}$$

where $Ki=\dfrac{k_p\cdot Km_i(k_i+k'_p)}{k_i\cdot k'_p}$

Therefore

$$\frac{1}{v_p}=(1+[\mathrm{CBQ}]/Ki)\left(\frac{Km}{V_{\max}}\cdot\frac{N}{[\mathrm{VA}]}+\frac{1}{V_{\max}}\right) \tag{9}$$

Thus the noncompetitive inhibition type of Lineweaver-Burk plots[6] can be explained.

This rationalization is consistent with that of Ma *et al.*[8] in supporting the hypothesis that VA plays a mediator role in LiP-catalyzed reactions, although no direct evidence for the occurrence of VA$^{+\cdot}$ has been obtained yet. The noncompetitive inhibition of LiP by CBQ system indicates that among the three schemes Ma *et al.*[8] proposed,Scheme a is most likely

to be true, since it is hardly conceivable that the LiP-VA$^{+\cdot}$ complex would interact directly with the CBQ enzyme.

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Added in prof.: Quite recently, formation of cation radical of VA has been demonstrated by Khindaria and Aust , *Biochemistry*, **34**, 6020–6025 (1995).