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The role of peroxidases, radical cations and oxygen in the degradation of lignin

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Ligninase is an extracellular peroxidase produced by several species of white-rot fungi. It is able to oxidize methoxylated substrates to radical cation intermediates that can undergo C–H or C–C bond cleavage, thereby providing the basis for the oxidation of veratryl alcohol or degradation of lignin model compounds respectively. In some cases, the radical cation intermediate can act as an oxidant, accepting an electron from a suitable donor. It can thus function as a mediator, causing oxidation in a polymer not immediately accessible to the enzyme. This could be important in the degradation of natural lignocellulose substrates. However, the removal of a single electron by a mediator would leave a radical in the polymer. We propose that oxygen will bind to this radical to generate active oxygen species. This provides a potential mechanism for the auto-oxidation of lignin at a distance from the enzyme. A scheme is presented to account for the observation that ligninase can open the ring of veratryl alcohol.

1. Introduction

Isolation and characterization of the enzyme ligninase from the white-rot fungus *Phanerochaete chrysosporium* (Tien & Kirk 1983) has greatly stimulated research towards elucidating the mechanisms involved in the biodegradation of lignin by wood-degrading basidiomycetes.

General properties of the ligninase

The ligninase is an extracellular oxidase produced by *P. chrysosporium* maintained under secondary metabolic conditions by lack of either a nitrogen or carbon source (Tien & Kirk 1983; Leisola *et al.* 1985*a*). Originally it was necessary to grow the hyphae in unstirred culture, but in recent work methods have been developed to obtain the enzyme from stirred cultures (Kirk *et al.* 1986; Leisola *et al.* 1985*a*). The activity of the enzyme can be improved by gassing the culture vessels with pure oxygen and by adding veratryl alcohol as an inducing agent (Leisola *et al.* 1985*a*).

The properties of the ligninases obtained from *P. chrysosporium* are summarized in table 1. The protein exhibits heterogeneity with respect to both molecular weight (Renganathan *et al.* 1985) and isoelectric point (Kirk *et al.* 1986; Leisola *et al.* 1985*a*). Spectral analyses show that the enzyme contains a single iron protoporphyrin IX as the prosthetic group (Tien & Kirk 1984). From the study of the resonance Raman spectra it is suggested that the high-spin ferric iron is in a five coordinate state with a histidine forming an axial ligand; this is characteristic of peroxidase enzymes (Kuila *et al.* 1985). The sixth coordination position on the iron appears

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Table 1. Properties of ligninases from *Phanerochaete chrysosporium*

	composition
size	39–43 kDa
iso-electric point	5.0–3.0
polysaccharide content	10–15% (by dry mass)
prosthetic group	single iron protoporphyrin IX (*a*) electrostatically attached (*b*) histidine as an axial ligand
	catalytic properties
pH optimum	*ca.* 2.5
oxidants	H_2O_2 and *t*-BuOOH
reductants	methoxylated aromatics phenolics, aromatic amines, $K_4Fe(CN)_6$, KI
inhibitors	CN^-, F^-, N_3^-, CO

to be available to interact with peroxides, or the inhibitors listed in table 1. Once oxidized, the enzyme is able to accept electrons from a wide variety of donors, some as simple as potassium iodide and others as complex as veratryl alcohol or dimeric lignin-model compounds.

Reactions catalysed by ligninase

Tien & Kirk (1983) showed that the ligninase would cleave C_α–C_β bonds in the propyl side chains of lignin-model dimers and depolymerize methoxylated lignin. In a later paper they reported that the enzyme was able to catalyse a wide variety of reactions, one of the most significant being the oxidation of veratryl alcohol to the aldehyde, and thereby provided the basis for a very convenient enzyme assay (Tien & Kirk 1984). A current list of reactions attributable to the enzyme is presented in table 2. The reactivity of this enzyme can account for the reactions associated with the degradation of lignin by *P. chrysosporium*; this includes the cleavage of ether bonds and the opening of methoxylated aromatic rings.

Table 2. Reactions catalysed by ligninase

cleavage of C_α–C_β bonds in lignin-model dimers
oxidation of benzylic alcohols
oxidation of benzylic methylene groups
hydroxylation of benzylic methylene groups
hydroxylation of olefinic bonds in styrenes
decarboxylation of phenylacetic acids
cleavage of ether bonds
aromatic ring-opening
polymerization of phenols

2. Ligninase as a peroxidase

Tien & Kirk (1984) reported that the activity of the enzyme was dependent on hydrogen peroxide and that when it catalysed C_α–C_β cleavage or hydroxylation of benzylic methylene groups it oxygenated the substrate by using dioxygen rather than hydrogen peroxide. On this basis, the enzyme was initially considered to be an oxygenase. However, the study of plant peroxidases has shown that oxygen uptake often accompanies the classical peroxidase activity;

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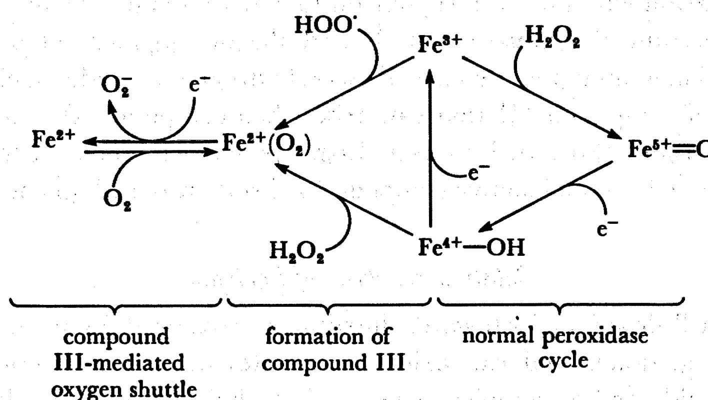
the consumption of oxygen occurs mainly because oxygen binds to a free radical produced as an intermediate in the peroxidase reaction (Yamazaki & Yokota 1973) or via an oxygen shuttle associated with the peroxidase cycling between compound III and the ferrous form (Smith *et al.* 1982). Therefore, all the data so far available concerning the catalytic activity of ligninase is consistent with the view that the enzyme is a peroxidase (Harvey *et al.* 1985*a*) rather than a unique form of an oxygenase.

Redox states of peroxidases and ligninase

Horseradish peroxidase is a 'classical' peroxidase, and its redox states have been extensively studied (Yamazaki 1974). The interrelations between the different redox states of horseradish peroxidase are outlined in scheme 1. In the normal catalytic cycle, the ferric form of the enzyme



SCHEME 1. Interrelations between the redox states of horseradish peroxidase.

is oxidized by hydrogen peroxide to a two-electron deficient oxy-ferryl state known as compound I. Compound I is then reduced by two consecutive single-electron transfer steps to the ground state via compound II. Phenols or aromatic amines are the typical electron donors for horseradish peroxidase and they are oxidized to the free radical, which subsequently reacts to yield stable products. Peroxidases are often sensitive to excessive amounts of hydrogen peroxide that can, under appropriate conditions, convert the enzyme into a less catalytically active form known as compound III. This state can also be achieved by reacting the ferric form of the enzyme with perhydroxy radicals. The role of compound III in the catalytic cycle is complex; it can set up a shuttle with the ferrous form that results in the oxidation of substrates and reduction of dioxygen to superoxide, as shown in figure 1, or can be converted back to the ferric ground state (Yamazaki & Yokota 1973; Manthey & Hager 1985).

Spectral evidence has been obtained to show that the ligninase can achieve the five different redox states characteristic of a classical peroxidase. The spectral properties of the different redox states are presented in table 3. Little kinetic data concerning the stability of the different redox states are available; however, it seems that compound I is rather unstable. Renganathan *et al.* (1985) found that titrating the enzyme with a molar equivalent of hydrogen peroxide rapidly converted the enzyme to the one-electron deficient compound II form. They considered that the instability of compound I may be caused by its reduction by a group on the protein itself. Tien *et al.* (1986) found that, in the presence of excess hydrogen peroxide, compound I was

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Table 3. Redox states of ligninase

redox species	absorption maxima/nm		
ferric peroxidase ($Fe^{III}p$)	407	500	630
compound I ($Fe^{IV}(O)p^{+\cdot}$)	410		
compound II ($Fe^{IV}(O)p$)	418	526	553
ferrous peroxidase ($Fe^{II}p$)	435	556	
compound III ($Fe^{II}(O_2)p$)	414	543	578

rapidly converted to another redox state, which they suggested could have been compound II. Renganathan *et al.* (1985) observed that their compound II could be instantaneously converted back to the ferric ground state by adding veratryl alcohol as an electron donor, while Tien *et al.* (1986) reported that veratryl alcohol would not convert their compound II to the ground state at a meaningful rate. It is significant to note that Harvey *et al.* (1985*a*) published an absorption spectrum of ligninase that showed the development of marked absorption maxima at 540 and 580 nm in the presence of excess hydrogen peroxide. Such spectral features are characteristic of compound III that can arise when compound II reacts with peroxide. Because Tien *et al.* (1986) also added excess hydrogen peroxide it is possible that they may have converted the enzyme into the inactive compound III state rather than compound II as they assumed.

Substrate specificity of ligninase

Peroxidases show little specificity towards the substrates oxidized. Ligninase is also unspecific towards the electron donor and can oxidize substrates as simple as potassium iodide or potassium ferrocyanide and as complex as veratryl alcohol and lignin model dimers. Stoichiometric measurements show that under anaerobic conditions two electrons are removed from the donor for every mole of hydrogen peroxide used. Ligninase oxidizes all the substrates oxidized by horseradish peroxidase; in addition, it will oxidize a variety of methoxylated aromatic compounds that are not substrates for horseradish peroxidase. The basic differences between ligninases and horseradish peroxidase responsible for such differences in substrate specificity are not known. It may be possible that the redox potential of the compound I in ligninase is a better oxidant than that in horseradish peroxidase, or alternatively it may be associated with the very acidic pH conditions required by this enzyme that would facilitate the formation of radical cations.

The enzyme has recently been shown to decarboxylate the dimethoxylated phenylacetic acid, homoveratric acid, while it is unable to sustain the oxidation of monomethoxymandelic acid without the involvement of a suitable mediator (Harvey *et al.* 1986). This decarboxylation reaction is consistent with the view that the enzyme is a peroxidase inducing radical cations into the methoxylated aromatic nucleus of the substrates.

The redox cycle of ligninase

The view that ligninase was a peroxidase was first clearly stated by Harvey *et al.* (1985*a*) when they showed that single-electron transfer reagents, such as iron phenanthroline or ceric ammonium nitrate, would oxidize lignin-model dimers or veratryl alcohol to the same products as the isolated enzyme.

The simplest model consistent with the role of the enzyme as a peroxidase is presented in

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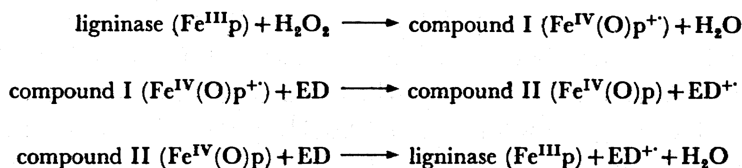

$$\text{ligninase } (Fe^{III}p) + H_2O_2 \longrightarrow \text{compound I } (Fe^{IV}(O)p^{+\cdot}) + H_2O$$

$$\text{compound I } (Fe^{IV}(O)p^{+\cdot}) + ED \longrightarrow \text{compound II } (Fe^{IV}(O)p) + ED^{+\cdot}$$

$$\text{compound II } (Fe^{IV}(O)p) + ED \longrightarrow \text{ligninase } (Fe^{III}p) + ED^{+\cdot} + H_2O$$

Scheme 2. The redox cycle in ligninase. $Fe^{III}p$ represents the haem group of the ferric form of the enzyme. ED represents an electron donor such as dimethoxybenzene or lignin model dimer. $ED^{+\cdot}$ is the radical cation formed by the removal of a single electron from the donor.

scheme 2, where it is suggested that both the single-electron donation steps leading to the conversion of compound I to compound II, and compound II to the ferric enzyme result in the production of radical cations. Such a model is consistent with the data of Renganathan *et al.* (1985), but does not fit with the conclusions reached by Tien *et al.* (1986). Harvey *et al.* (1986) have recently presented evidence that the radical cations of dimethoxybenzene and veratryl alcohol, produced by the enzyme, are good oxidants and can act as mediators to bring about the oxidation of substrates not readily accessible to the active site of the enzyme. Such observations show beyond doubt that the radical cations, produced from a single-electron donation step, are released from the active site of the enzyme and suggest that the ligninase can complete its redox cycle without oxidizing the radical cation further to the aldehyde.

3. Radical cations as intermediates

If the ligninase is a peroxidase only able to generate radical cations, then all the stable products must arise as the result of non-enzymic interactions between radical cations and other components in the assay system. Schoemaker *et al.* (1985) and Harvey *et al.* (1985*b*) have discussed the properties of radical cations and have concluded that reactions characteristic of ligninase can be explained by reactions of radical cations.

Properties of radical cations

Radical cations are formed when substrates such as dimethoxybenzene or veratryl alcohol, which cannot dissociate to form anions, are oxidized by a single-electron transfer process, with reagents such as iron phenanthroline, the oxy-ferryl form of tetraphenylporphyrinatoiron (III) chloride (Shimada *et al.* 1984) or the oxy-ferryl form of ligninase (Harvey *et al.* 1985*a*).

The basic properties of radical cations are given in table 4. Firstly, they may function as one-electron oxidants (Camaioni & Franz 1984) and are able to oxidize an appropriate donor and become reduced themselves to the ground state; they are thus able to mediate in the

Table 4. Reactions characteristic of radical cations

reactions	consequence
act as one-electron oxidants	mediators
undergo side-chain reactions	C_α–C_β bond cleavage C–H bond cleavage decarboxylation
addition of solvent (water)	hydroxylation of styrenes cleavage of ether bonds phenol formation
reaction with $HOO^\cdot$	ring opening

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oxidation of monomethoxymandelic acid (Harvey *et al.* 1986) or in the release of ethylene from 2-keto-4-thiomethylbutyric acid (Renganathan *et al.* 1985). Radical cations can also undergo side-chain cleavage reactions, the products of which depend on the nature of the radical cation. If the radical cation has the general formula of $[ArCHOHR]^{+\cdot}$ then C–R cleavage will occur if the R group will yield a stable radical or neutral molecule. This occurs when C_α–C_β cleavage is the main reaction yielding a stable radical on the C_β atom. C_α–C_β cleavage also occurs during the decarboxylation of homoveratric acid and monomethoxymandelic acid (Harvey *et al.* 1986), when carbon dioxide is liberated and a radical is generated on the C_α atom. If the R group is unsuitable for the formation of a neutral product or a stable radical, then C—H bond cleavage will occur yielding a proton and free radical on the benzylic carbon; this occurs during the oxidation of veratryl alcohol. Radical cations can also add solvent, such as water, when they convert methoxybenzenes to quinones (Hammel *et al.* 1985) or when they degrade ether bonds (Habe *et al.* 1985). Finally, radical cations are able to react with free radicals. A scheme is presented later in this paper to account for the reaction between a radical cation of veratryl alcohol and the perhydroxyl radical in the opening of the aromatic nucleus (see §4).

Radical cations as redox mediators

Radical cations are good oxidants and they are therefore able to oxidize a variety of substrates. The possibility that such a mediated oxidation could play a significant role in the reactions associated with the ligninase was first proposed by Schoemaker *et al.* (1985) and the first direct experimental evidence was provided by Harvey *et al.* (1986). Renganathan *et al.* (1985) have suggested that a radical of veratryl alcohol may mediate the liberation of ethylene from 2-keto-4-thiomethylbutyric acid, but this seems unlikely because the radical formed from veratryl alcohol is a reducing agent. Recent research has shown that an oxidation product of veratryl alcohol is active in mediating the oxidation of benzopyrene (Haemmerli *et al.* 1987*a*) and the polymerization of milled-wood lignin (Haemmerli *et al.* 1987*b*) by the ligninase. It seems likely that veratryl alcohol does act as a natural mediator, because the appearance of veratryl alcohol parallels the appearance of the ligninase. If the alcohol was the primary substrate for the enzyme, accumulation of the aldehyde would have been expected. Such a low molecular-mass redox mediator is an attractive concept because it could help in the enzymic attack on a substrate that is part of an insoluble complex and thus unable to gain access directly to the active site of the enzyme.

4. The role of oxygen in the ligninase reaction

In the early studies of Tien & Kirk (1984), it was observed that $^{18}O_2$ was incorporated into the products of lignin breakdown. Oxygen utilization is a characteristic of normal peroxidase action especially when oxidizing substrates such as indoleacetic acid or dihydroxyfumaric acid (Yamazaki 1974).

Oxygen can be consumed by two different types of mechanisms. Firstly, it can bind to a free radical to form an organic peroxy radical (Harman *et al.* 1986) or it can participate in a shuttle mechanism, where it reacts with the ferrous form of the peroxidase to yield compound III, which then oxidizes an electron donor to produce an oxidized product and superoxide (Smith *et al.* 1982). If the ligninase is a peroxidase then oxygen could enter organic combination by one or both of these mechanisms. We have measured the uptake of oxygen that occurs when the enzyme oxidizes a variety of substrates. We observed that oxygen utilization was only

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significant when substrates capable of bond cleavage, such as veratryl alcohol or homoveratric acid, were supplied, while oxidation of substrates such as ferrocyanide or dimethoxybenzene did not result in the significant uptake of oxygen. Therefore it would seem that the degradation of radical cations to a free radical is necessary for oxygen utilization. The free radicals are highly reactive species and can undergo a variety of reactions, which are listed in table 5.

TABLE 5. FATE OF CARBON-BASED RADICALS

oxidized by ligninase
oxidized by radical cation
bind oxygen
react with another radical (dimerization)
react with neutral molecules (polymerization)

Stoichiometry of oxygen uptake

Free radicals can undergo a variety of reactions; consequently it can be anticipated that the stoichiometry of oxygen uptake will be complex and will reflect factors such as the concentration and stability of both the radical cations and the free radicals, the amount of the enzyme and concentration of oxygen.

Data presented in figure 1 show the amounts of oxygen used and aldehyde formed when veratryl alcohol or homoveratric acid were oxidized in the presence of limited amounts of hydrogen peroxide. The ratio of oxygen consumed to peroxide supplied depended on the substrate supplied. The oxidation of veratryl alcohol resulted in the uptake of 0.36 moles of oxygen per mole of hydrogen peroxide added, while the oxidation of homoveratric acid resulted in the uptake of 2.7 moles of oxygen per mole of hydrogen peroxide supplied. It is also apparent that the oxidation of veratryl alcohol produced one mole of aldehyde per mole of hydrogen peroxide under both aerobic and anaerobic conditions (figure 1*a*), showing that the consumption of oxygen did not result in the formation of extra aldehyde. In contrast with this result, Hammel *et al.* (1985) found that oxygen uptake accompanying the oxidation of the lignin-model dimer, 1-(3,4-dimethoxyphenyl)-2-phenylethanediol (DMHB), yielded two moles of veratraldehyde per mole of oxygen consumed. When homoveratric acid was the substrate (figure 1*b*), the yield of veratraldehyde per mole of hydrogen peroxide added was six times higher in the presence of oxygen than under anaerobic conditions. Therefore, in contrast with veratryl alcohol (figure 1*a*), most of the oxygen consumed during the oxidation of homoveratric acid was used as an oxidant to produce veratraldehyde. If it is assumed that oxygen acts as a four-electron acceptor then it is possible to calculate the expected yield of veratraldehyde if all the oxygen was used to produce this product. These expected values have been plotted in figure 1*a*, and it can be seen that when veratryl alcohol was the substrate the actual yield of veratraldehyde under aerobic conditions was considerably lower than the calculated value. Therefore, although the stoichiometry of aldehyde formed per peroxide added under anaerobic conditions were precisely as predicted, the level of aldehyde formed under aerobic conditions was much less than anticipated from the combined level of hydrogen peroxide added and oxygen used. It seems that some product other than veratraldehyde must arise in the presence of oxygen. In the case of homoveratric acid, the amount of veratraldehyde obtained under aerobic conditions is only just less than that predicted by summing hydrogen peroxide supplied and oxygen used.

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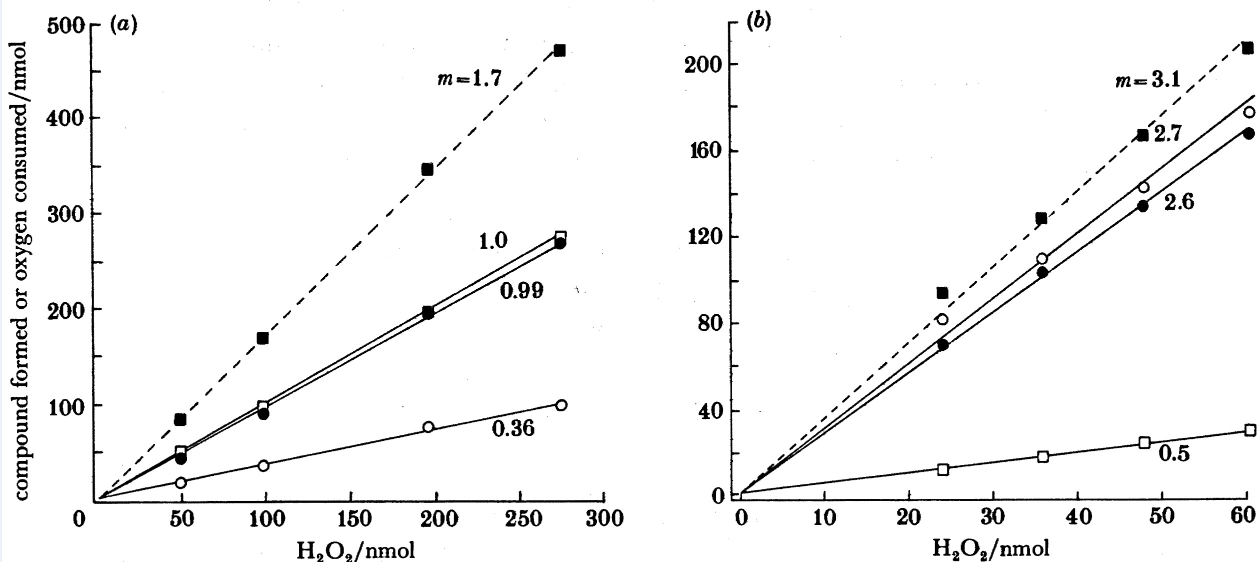

FIGURE 1. The formation of veratraldehyde and the consumption of oxygen during the oxidation of (*a*) veratryl alcohol or (*b*) homoveratric acid. The production of veratraldehyde was measured spectrophotometrically at 310 nm, oxygen uptake was measured with an oxygen electrode and the predicted level of aldehyde was calculated assuming H_2O_2 accepts two electrons and oxygen accepts four electrons. Symbols: ■, ArCHO (anaerobic, predicted); □, ArCHO (anaerobic); ●, ArCHO (aerobic); ○, O_2 used.

Theoretical role of oxygen

Hammel *et al.* (1985) suggested that oxygen bound to the carbon-centred radical resulting from bond cleavage in DMHB to produce an organic peroxy radical, which would decompose to yield product and superoxide. The superoxide would, in turn, dismutate to yield oxygen and hydrogen peroxide, which could act as an oxidant to continue the peroxidase cycle. Scheme 3 accounts for the involvement of oxygen during the oxidation of veratryl alcohol.

We propose that the radical cation is released from the enzyme and degrades to yield a free radical on the hydroxylated benzylic atom, which binds oxygen to form a peroxy radical. The peroxy radical can then undergo two possible reactions. Firstly, it can degrade to form veratraldehyde and a perhydroxy radical (scheme 3, reaction *f*), which is the protonated form of superoxide and would be the predominant species because of the acid pH used with the enzyme, because the pK of protonation is 4.5. It is generally considered that superoxide is a rather poor oxidant; however, the perhydroxy radical is a very much better oxidant (Halliwell & Gutterridge 1985) and has a redox potential 1.3 V higher than the hydrogen electrode at pH 3.0 (Hoare 1985). This is approximately the same as Fe^{3+}-phenanthroline, and should therefore oxidize lignin-model dimers to the radical cation, thus initiating an additional bond cleavage and further oxygen activation. The perhydroxy radical would then be reduced to hydrogen peroxide, which could initiate a second cycle of peroxidase activity. The second reaction the organic peroxy radical could undergo would be to react with a molecule of veratryl alcohol (scheme 3, reaction *e*) to form a new benzylic radical and an organic peroxide that eventually gives rise to the aldehyde and hydrogen peroxide that eventually gives rise to the aldehyde and hydrogen peroxide (scheme 3, reaction *g*). Either of these routes leads to the initiation of a chain reaction that could cause the extensive auto-oxidation of lignin similar to the radical-initiated chain reactions involved in the oxidation of indoleacetic acid (Yamazaki

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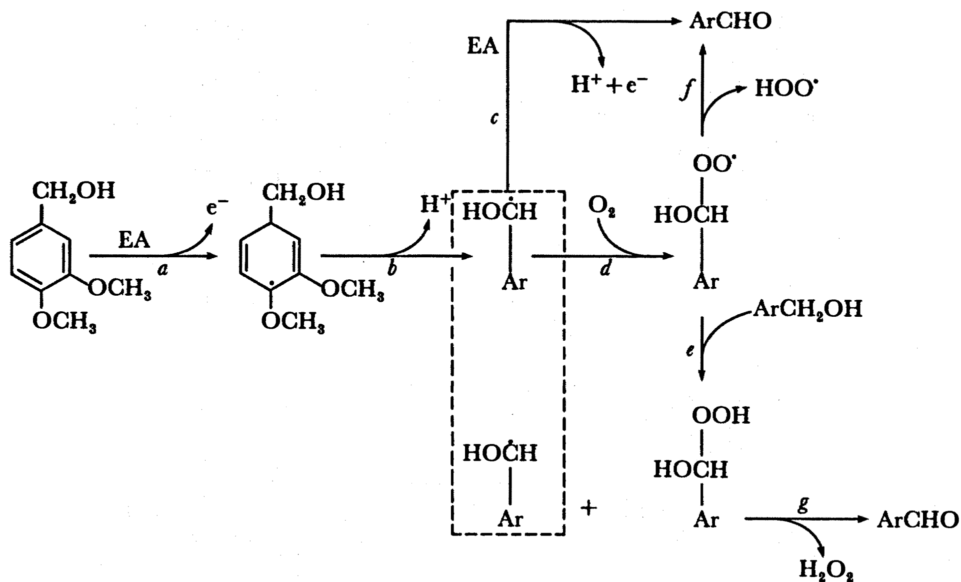

SCHEME 3. A scheme to illustrate the products resulting from the binding of oxygen to the radical formed following the C—H bond cleavage in the radical cation of veratryl alcohol. Ar presents the *o*-methoxylated aromatic ring and EA represents a suitable electron acceptor, which could be the oxidized form of a peroxidase or a chemical reagent such as iron phenanthroline.

1974). This enzyme-induced auto-oxidation could play an important role in the degradation of lignin in the insoluble lignocellulose complex in biomass. In this case, we propose that a redox mediator of low molecular mass, such as the radical cation of veratryl alcohol, initiates the degradation of lignin by the removal of a single electron; this would initiate bond cleavage and leave a radical in the degradation products. It is unlikely that a second radical cation of veratryl alcohol would remove the second electron to complete the reaction before oxygen could bind and initiate the chain reaction resulting in the production of perhydroxy radicals. If there are traces of transition metals, especially iron, then the hydrogen peroxide would be converted to hydroxy radicals via the Fenton reaction, leading to further lignin breakdown. Consequently, this process would lead to the extensive auto-oxidation of lignin taking place at significant distances from the fungal hyphae.

The possibility that radical cations and perhydroxy radicals could occur simultaneously in the same assay system leads to the possibility that they could react together (see tables 4 & 5). In scheme 4 we present a theoretical pathway to show how the enzyme may create the conditions necessary to open the aromatic ring in veratryl alcohol (Leisola 1985). It seems likely that reactions such as aromatic ring cleavage provide an explanation for the apparent lack of sufficient identifiable products during the aerobic oxidation of veratryl alcohol reported in figure 1*a*. The oxidation of veratryl alcohol forms a relatively stable radical cation under the assay conditions used, thus enabling the perhydroxy radical to interact with the radical cation and open the ring. In contrast, the lignin model DMHB used by Hammel *et al.* (1985) would form a radical cation that would immediately degrade to the radical; consequently, the perhydroxy radical would have no radical cation to bind with and thus would only be able to initiate the production of radical cations leading to stoichiometric production of veratraldehyde.

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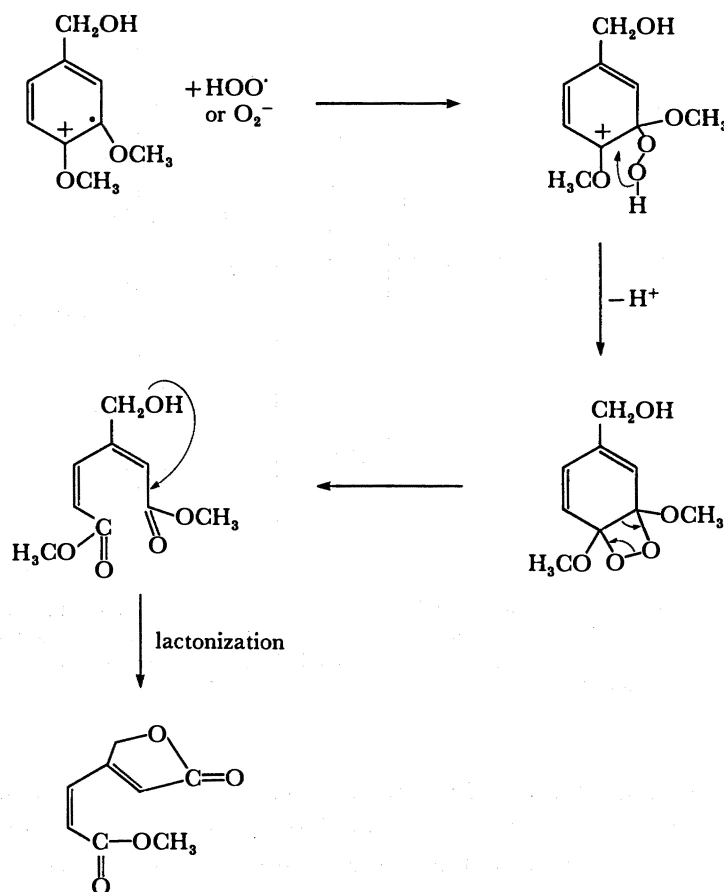

SCHEME 4. A scheme to account for the opening of the aromatic ring in veratryl alcohol by the ligninase enzyme (Leisola *et al.* 1985*b*).

From this discussion it can be seen that the reactions of the radical cations, radicals and oxygen are very complex and depend on the rate of radical cation formation, the stability of the radical cation, the nature of the radical formed and the level of oxygen present.

5. Conclusion

Data have been presented in this paper to substantiate the view that the ligninase is a unique form of a peroxidase, capable of oxidizing methoxylated aromatic electron donors to radical cations. It is proposed that the enzyme is only involved in the formation of radical cations, and that all the reactions characteristic of ligninase can be adequately explained on the basis of the way such radical cations behave in the assay systems. Radical cations can degrade to form radicals and eventually stable products. Relatively stable radical cations, such as those formed from veratryl alcohol, act as oxidants and can mediate in the oxidation of substrates unable to gain direct access to the active site of the enzyme. The radicals formed by the degradation of radical cations can undergo a variety of reactions; of particular significance in the degradative process is binding to oxygen, giving rise to the production of active species of oxygen that could lead to the non-enzymic auto-oxidation of lignin. The precise pathway of the

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non-enzymic interaction consequent on the properties of radical cations is complex and difficult to predict, and will be affected by many factors. These could include the presence of degradation products, such as free phenols, and the level of oxygen, both of which could be regulated by the activities of other enzymes such as the laccase and cellobiose–quinone oxido-reductase.

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Discussion

P. B. Tinker (*Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.*). I am puzzled how ligninase, with a pH optimum below 3, manages to function in many parts of the natural environment. For example, organic materials mixed with a soil containing calcium carbonate can hardly become highly acid, even in microenvironments. Could Dr Palmer comment on this?

J. M. Palmer. The ligninase from *P. chrysosporium* is involved in the breakdown of lignin in wood. Here, the pH is very acid because the fungus produces organic acids, possibly by the direct oxidation of polysaccharides. The enzyme needs an acid pH to be active, and will not work at pH values greater than 5.5. I agree that it is difficult to see how this enzyme could play a role in the breakdown of lignin in alkaline soils.

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