On the interaction of lignin peroxidase with lignin

Schoemaker, H.E.; Piontek, K.

DOI
10.1351/pac199668112089
Publication date
1996

Published in
Pure and Applied Chemistry

Pure & Appl. Chem., Vol. 68, No. 11, pp. 2089–2096, 1996.
Printed in Great Britain.

On the interaction of lignin peroxidase with lignin

Hans E. Schoemaker[1] and Klaus Piontek[2]

[1]University of Amsterdam, Laboratory of Organic Chemistry, Nieuwe Achtergracht 129, 1018 WS, Amsterdam, The Netherlands
[2]ETH-Zürich, Laboratory of Biochemistry I, Universitätstrasse 16, CH-8092 Zürich, Switzerland

Abstract
The mechanism by which lignin peroxidase (LiP) interacts with the lignin polymer is discussed. Veratryl alcohol (Valc), a secondary metabolite of white rot fungi, acts as a cofactor for the enzyme. The LiP-redox cycle is discussed in terms of Marcus theory of electron transfer. It is proposed that reaction of a nucleophile in the active site channel with the incipient $Valc^{+\cdot}$ is an essential event in the cycle. Subsequently, outside the channel the $Valc^{+\cdot}$ is regenerated and will oxidize the lignin polymer. Further it is postulated that a carbohydrate residue from an O-glycosylation site at Ser-334 may act as the nucleophile in this process.

INTRODUCTION

Lignin is the most abundant natural aromatic polymer in the biosphere. It comprises 20-30% of woody plant cell walls and by forming a matrix surrounding the cellulose and hemicelluloses, it provides strength and protection, e.g. against biodegradation. Lignin is a highly branched and heterogeneous three-dimensional structure made up of phenylpropanoid units which are interlinked through a variety of different bonds. Lignin is biosynthesized via a plant-peroxidase catalyzed oxidation of (methoxy-) substituted *para*-hydroxycinnamyl alcohols. The benzylic hydroxy-groups are introduced via quinone methide intermediates.
Lignin is highly resistant to biodegradation and only higher fungi are capable of degrading the polymer via an oxidative process. This process has been studied extensively in the past twenty years, but the actual mechanism has not yet been fully elucidated. The complicated structure of the lignin polymer and major difficulties in analysis are responsible for the relatively slow progress (1).
The breakthrough in our understanding of the mechanism of lignin biodegradation came in 1983 with the discovery of ligninase, a ligninolytic enzyme isolated from *Phanerochaete chrysosporium* (2,3). In 1985 it was proposed that ligninase in fact is a peroxidase and that lignin biodegradation probably involves one-electron oxidation of lignin-substructures to form radical cations (4-6). This rationale was mostly based on studies with simple model compounds (dimers and monomers). One of the best studied compounds is veratryl alcohol (3,4-dimethoxybenzyl alcohol), a secondary metabolite of *Phanerochaete chrysosporium* and other white-rot fungi. In 1986 a hypothetical and initially highly controversial role for veratryl alcohol as a redox mediator was proposed (7). Ever since, there has been much debate on the mechanism by which lignin peroxidase (LiP) interacts with the lignin polymer. Although non-phenolic lignin was depolymerized by the enzyme (3), subsequent studies with phenolic lignin preparations -under non-natural conditions- indicated that with crude enzyme preparations polymerization occurred, a process stimulated by veratryl alcohol (8). Depolymerization studies with fungal cultures, however, indicated that both the presence of LiP and veratryl alcohol stimulated the degradation of lignin (9). Very recently, it was found that LiP indeed depolymerized lignin under the proper conditions, simulating the natural process (10). Interestingly, purified LiP, not

containing veratryl alcohol, did not react with the polymer. Only if veratryl alcohol was added, depolymerization was observed (11). These data point to a very special role of veratryl alcohol in the interaction with the polymer. This role is even more remarkable considering the fact that veratryl alcohol is very efficiently oxidized by LiP to form veratraldehyde at the expense of one equivalent of H_2O_2. Moreover, from the crystal structure of LiP it can be inferred that only veratryl alcohol can enter the active site. It is not possible for the lignin polymer to interact directly with the heme-group of the enzyme (12,13).

A further interesting feature of the LiP-crystal structure is the presence of ordered carbohydrate residues at the entrance to the active site (12). It is not known whether this is an artefact or that those residues do play a role in the mechanism.

In this paper we will review the most recent experimental results and discuss the role of veratryl alcohol in the interaction of lignin peroxidase with the polymeric lignin structure. The mechanism of veratryl alcohol oxidation plays a crucial role in this process. Therefore, this mechanism will be discussed in detail, both from experimental and theoretical points of view. However, before doing so, we will briefly review the lignin biodegradation process *in vivo*.

GENERAL SCHEME FOR LIGNIN BIODEGRADATION *IN VIVO*

The lignified cell wall is progressively degraded from the inside outwards. In this process fungal hyphae grow within the lumen of the woody cells, excreting both ligninolytic and (hemi-)cellulolytic enzymes. Also, during ligninolysis the fungus produces an extracellular mucilaginous sheath, closely associated both with the hyphae and with the decaying wood. At the early stages of decay the ligninolytic enzymes, like LiP, are found at the surface of the lignified wall, unable to penetrate it. At later stages, LiP is found within the degraded regions. It is unclear if small, diffusible agents are necessary for the initial degradation.

Lignin is insoluble in water, its degradation *in vivo* takes place in a polysaccharide gel. H_2O_2, the oxidant for LiP, is present in low concentrations in fungal cultures. It is to be expected that oxygen gas, although absolutely required for ligninolysis, will also suffer from diffusional limitations in the polysaccharide gel. In general, diffusion is limited in this system, especially for large molecules like enzymes and the cell wall constituents. Moreover, lignin is (in part) covalently bound to hemicelluloses. Furthermore, an important practical observation is the fact that right from the start of the depolymerization initial breakdown products of lignin are assimilated by the fungus and oxidized to CO_2 (1).

Fungal uptake of the initial breakdown products and further assimilation of the small fragments is important since it provides one of the basic mechanisms of lignin degradation (14). In the oxidative depolymerization process, phenolic compounds are formed and are prone to polymerize again. Manganese peroxidase (MnP) or - in other classes of white-rot fungi - laccases, will convert the phenolic LiP breakdown products to form quinones, which are further metabolized in a process that most probably involves reduction to the corresponding hydroquinones. Other small fragments may have a ringopened structure. Also, in the degradation process, glycolaldehyde is formed from the Cγ-fragment, which is a substrate for glyoxal oxidase, one of the H_2O_2-producing enzymes. Other H_2O_2-producing enzymes are part of the polysaccharide-degrading system of the fungus (1,14,15).

LIGNIN PEROXIDASE CATALYZED VERATRYL ALCOHOL OXIDATION

Ever since the discovery of lignin peroxidase, veratryl alcohol has played a pivotal role in the study of the lignin biodegradation process. Veratryl alcohol is used as an assay for enzyme activity due to the easily detectable absorbance of the product veratraldehyde at 310 nm. The optimum pH of the enzyme is rather low (pH = 2.5) and the fact that lignin peroxidase is capable of oxidizing non-phenolic substrates has attracted considerable attention (14,15). The fact that lignin peroxidase will oxidize (non-phenolic) compounds with a relatively high redox potential has been interpreted by many authors as the result of an unusually high redox potential (16,17) of the oxidized enzyme intermediates, lignin peroxidase Compound I (LiPI) or Compound II (LiPII). Alternatively, it has been argued that the enzyme is capable of stabilizing the initial product of the veratryl alcohol oxidation (18,19), the veratryl alcohol radical cation ($Valc^{+\cdot}$). The redox cycle of the enzyme is schematically depicted in scheme 1.

$$LiP + H_2O_2 \longrightarrow H_2O + LiPI \quad \text{(Compound I)}$$

$$LiPI + Valc \longrightarrow Valc^{+\cdot} + LiPII \quad \text{(Compound II)}$$

$$LiPII + Valc + 2H^+ \longrightarrow Valc^{+\cdot} + H_2O + LiP \quad \text{(native enzyme)}$$

Scheme 1. The lignin peroxidase redox cycle

Thus, LiPI will oxidize the first molecule of veratryl alcohol to the corresponding radical cation ($Valc^{+\cdot}$), which is liberated from the active site. Subsequently, the second substrate molecule is oxidized by LiPII to form the second $Valc^{+\cdot}$. In the process LiPII is converted to native enzyme.
In this paper we will argue that an alternative explanation may rationalize the experimental data. In our rationale the redox potentials of the oxidized enzyme states donot necessarily have to be higher than that of the substrate (15). We envisage a process in which veratryl alcohol binds in the active site channel close to the heme. This binding is followed by an electron transfer process in the endergonic region of the driving force, which is rendered essentially irreversible due to a concomitant reaction with a nucleophile, which can be either solvent or a part of the enzyme (protein or carbohydrate, *vide infra*). In this way the highly reactive $Valc^{+\cdot}$ is temporarily neutralized both in charge and in activity and as such transported away from the active site (20). Outside the active site channel protonation of the enzyme-product or the solvent-product complex will result in the regeneration of the very strong oxidant, $Valc^{+\cdot}$, which subsequently might oxidize any recalcitrant chemical present, like the lignin polymer.

EVIDENCE FOR THE FORMATION OF THE VERATRYL ALCOHOL RADICAL CATION AND ITS ROLE AS A MEDIATOR

The intermediacy of the $Valc^{+\cdot}$ in LiP catalyzed oxidation of veratryl alcohol was first postulated in 1985 (5). However, only circumstantial evidence was available at that time and the actual existence of $Valc^{+\cdot}$ as an intermediate in LiP catalyzed reactions has been questioned (21). Only very recently, two groups have reported on the formation of the $Valc^{+\cdot}$, providing direct spectroscopic evidence for its formation. Harvey *et al* (18), using pulse radiolysis techniques reported on the UV spectrum of the radical cation and estimated a lifetime in water of 59 ms. Aust and coworkers (19) have recorded the ESR-spectrum of $Valc^{+\cdot}$ via oxidation with cerium ammonium nitrate in a stopped flow apparatus at very low pH-values. More importantly, these authors also found an analogous ESR-spectrum in the LiP catalyzed reaction. However, line broadening had occurred and the concentration of the radical cation was less than the enzyme concentration. It was suggested that an enzyme-stabilized radical cation is formed.
The notion that veratryl alcohol may function as a redox mediator between the enzyme and the lignin polymer emerged in 1986 (7). It was suggested that the initially formed $Valc^{+\cdot}$ is the actual oxidant of the lignin polymer. This notion is now widely accepted since the concept rationalizes a wide variety of experimental data such as the veratryl alcohol stimulated oxidation of e.g. benz[a]pyrenes, oxalic acid, EDTA, (polymeric) dyes, synthetic lignin, etc which have been reviewed recently (15,22,23). More recently it was shown that veratryl alcohol also stimulated the oxidation of chlorpromazine (24), ferrocytochrome c (25) and natural polymeric lignin (10). Remarkably, veratryl alcohol will also stimulate the oxidation of compounds with a redox potential higher than veratryl alcohol itself, like 4-methoxymandelic acid and 4-methoxybenzyl alcohol (anisyl alcohol). Several explanations for this phenomenon have been put forward, including redox mediation by the veratryl alcohol radical cation (7,15,23), and recently also completion of the redox cycle of the enzyme via reduction of LiPII by veratryl alcohol (26). Alternatively, it has been suggested that LiPII might react with H_2O_2 to form $LiPIII^*$ which subsequently is converted to native enzyme by veratryl alcohol via a ligand displacement reaction (27). Although the latter possibility could be ruled out on kinetic grounds (26), the two other alternatives could not be distinguished so far. It may well be that both mechanisms can be operative, depending on the specific substrates. Thus, in the co-oxidation of veratryl alcohol and anisyl alcohol both anisaldehyde and veratraldehyde are observed as products (26,27). In this case the

most important role for veratryl alcohol could be the reduction of LiPII to complete the redox cycle. Still, veratryl alcohol is by far the preferred substrate for LiPI, so mediation by $Valc^{+\cdot}$ cannot be excluded. In the co-oxidation of veratryl alcohol and 4-methoxymandelic acid however, only anisaldehyde is formed as the product (7,23), indicating that any $Valc^{+\cdot}$ formed (e.g. via reduction of LiPII by veratryl alcohol) is reduced again by another species, for example by 4-methoxymandelic acid (MMA).

$$Valc^{+\cdot} + MMA \rightleftharpoons Valc + MMA^{+\cdot}$$

Scheme 2. Equilibrium between $Valc^{+\cdot}$ and $MMA^{+\cdot}$

In view of the respective lifetimes of the $Valc^{+\cdot}$ and $MMA^{+\cdot}$ species, it may well be that the equilibrium (Scheme 2) is shifted to the right via a very fast and irreversible degradation of $MMA^{+\cdot}$, according to the Curtin-Hammett principle (15). In this way no veratraldehyde is formed, in concord with the experimental results. It should be noted that with the enzyme system hardly any $MMA^{+\cdot}$ will be generated, only $Valc^{+\cdot}$ is formed. Recently, Candeias and Harvey (18) have investigated this equilibrium using pulse radiolysis as a potential mimic of the enzyme system. Based on the experimental results they concluded that in this artificial system the $Valc^{+\cdot}$ did not oxidize MMA, both veratraldehyde and anisaldehyde were observed as the products. They postulated that in the enzyme system the $Valc^{+\cdot}$ might exist as an enzyme-bound species that has either a longer life time or a higher reduction potential than the free radical cation in bulk solution. However, their results may also be rationalized in another way. The difference between the enzyme system and the pulse radiolysis experiment is the fact that in the latter substantial amounts of $MMA^{+\cdot}$ are formed, unlike the enzyme system. Therefore, in the equilibrium in Scheme 2 no oxidation of MMA by the $Valc^{+\cdot}$ will occur (endergonic process) and veratraldehyde is formed as the product. In the enzyme system, initially $Valc^{+\cdot}$ is formed and subsequently the equilibrium of Scheme 2 will be established.
Whatever the mechanism of this intriguing reaction might be, all results presented above point to a special role of veratryl alcohol in the LiP redox cycle. We therefore embarked on a theoretical analysis of the electron transfer reactions using Marcus theory.

THE LiP REDOX CYCLE IN TERMS OF MARCUS THEORY

Formulation of the LiP redox cycle as depicted in Scheme 1, in which electron transfer from veratryl alcohol to LiPI and LiPII, respectively, affords two $Valc^{+\cdot}$ species, makes the process amenable to a simple theoretical treatment in terms of Marcus theory of electron transfer (28). In addition, the oxidation of the lignin polymer by $Valc^{+\cdot}$ can also be treated similarly. Also, long-range electron transfer processes in proteins have recently been studied extensively (29,30) and basic principles are emerging rapidly. Thus, also long-range electron transfer processes in LiP should be considered. Interestingly, the closely packed aromatic lignin polymer can also be considered as an ideal matrix for long-range electron transfer processes. It is beyond the scope of this review to discuss the Marcus theory in detail. We have recently discussed the application of the theory to the LiP redox cycle. In the present review, we will focus on the experimental results - including some very recent ones -, which prompted us to initiate these studies and we will summarize our conclusions.
First, the fact that LiP will oxidize non-phenolic compounds with a relatively high redox potential has stimulated considerable research efforts. Most studies have been based on the implicit assumption that the oxidized forms of LiP (LiPI and LiPII) must have a - for peroxidase enzymes - unusually high redox potential (16,17), in order to oxidize the substrates in a thermodynamically favorable manner. At the same time, redox mediation by veratryl alcohol in the oxidation of anisyl alcohol or 4-methoxy mandelic acid was questioned because it was considered to be thermodynamically unfavourable (26, 27). However, in the literature there are numerous examples of oxidations in the endergonic region of the driving force, both with regard to stoichiometric and catalytic conversions. Also redox mediators have been used, especially in electrochemistry. Relevant examples for the present discussion include

the oxidation of methyl substituted benzenes or veratryl alcohol with tris-iron(III)phenanthroline complexes (5,31) and the electrochemical oxidation of methylbenzenes mediated by ruthenium(III)polypyridine complexes as catalysts (32). Thus, the fact that LiP will oxidize non-phenolic compounds does not necessarily mean that the redox potential of the oxidized enzyme species is higher than the substrate.

Second, recent stopped flow spectroscopic studies (26,33) of LiP catalyzed oxidations of veratryl alcohol and related species indicate that the oxidation of the substrate by LiPI is relatively fast and shows second-order kinetics, whereas the oxidation of the substrate by LiPII is slow and shows saturation kinetics. Interestingly, LiP will also oxidize manganese(II) oxalate complexes (34) showing a similar kinetic behaviour. Moreover, the reduction of LiPI by the substrates appears to be almost irreversible. In literature the saturation kinetics observed with the LiPII reduction are associated with a binding of the substrate prior to electron transfer. However, such a binding is not invoked in the reduction of LiPI by substrate. These results puzzled us, since there is *a priori* no reason why the binding properties of LiPI and LiPII would be so much different, whereas in related peroxidases (35,36) also the redox potentials of both Compound I and Compound II are similar (around 1 V vs NHE). For that matter, the redox potential of peroxidase Compound II is even somewhat higher than that of Compound I. Already in 1984 (37) it was pointed out that the difference in reactivity of horse radish peroxidase Compound I and II could be the consequence of a difference in reorganization energy λ as defined by Marcus. Analogously, in the reduction of LiPI a low spin oxyferryl porphyrin radical cation species is reduced to the corresponding low spin oxyferryl porphyrin species, whereas in the reduction of LiPII the low spin oxyferyl complex is converted to a high spin iron (III) porphyrin species via protonation of the oxygen to form water and a concomitant change in the protein conformation as a consequence of the change in spin state of the iron, a process requiring much more reorganizational energy than the reduction of LiPI to LiPII. Using realistic values for the reorganizational energy in this type of protein, we were able to show that the observed saturation kinetics in the reduction of LiPII may be the consequence of a rate-limiting electron transfer step (20). However, in order to match the calculated values for the intra-complex electron transfer step with the experimentally observed values, and at the same time have physically relevant values for the reorganization energy (λ= 1-1.7 eV), the calculations indicated a reaction in the endergonic region of the driving force (ΔG°= 0.2-0.3 eV). In this way the rate of electron transfer becomes rate limiting, but will still be very fast for the reduction of LiPI - in fact too fast to be measured -, whereas the reduction of LiPII to native enzyme will be slow enough to account for the observed saturation kinetics (20). This type of theoretical treatment not only rationalizes the difference in the kinetics of the reduction of LiPI and LiPII by veratryl alcohol (26,33), it also indicates that LiPI is able to oxidize anisyl alcohol, methoxymandelic acid (7,23,26,27) and manganese (II) oxalate (34), but that LiPII will oxidize these substrates either not at all or only very slowly. Thus, most results recently obtained in stopped flow studies can be rationalized using the assumptions mentioned above; i.e. a difference in reorganizational energy in the reduction of LiPI and LiPII of about 0.5 eV and a reaction in the endergonic region of the driving force (ΔG°= 0.2-0.3 eV). Although at first sight this theoretical rationalization of the LiP redox cycle seems to be very satisfactory, the simple representation of the redox cycle in Scheme 1 cannot be the full description of the mechanism, since it is in conflict with one other important experimental observation, *viz.* the reduction of LiPI by veratryl alcohol is essentially irreversible, which at first sight appears to be contradictory to a reaction in the endergonic region of the driving force. However, since in our opinion this rationalization of the difference in reactivity between LiPI and LiPII using Marcus theory is highly appealing, we have tried to reconcile the observed irreversibility of the reduction of LiPI with an endergonic electron transfer. Therefore we have postulated (15,20) that concomitant with the formation of the incipient $Valc^{+\cdot}$, an irreversible chemical reaction will occur which will make the overall process irreversible, as advanced among others by Perrin (38). One possibility for such an irreversible chemical step could be proton loss. However, since proton loss from the radical cation will result in veratraldehyde formation and no veratryl alcohol radical cations will be available for redox mediation, we discounted proton loss as the subsequent step (15, 20). Instead, we envisage a reaction of a nucleophile with the incipient radical cation in the enzyme active site as the best alternative, a reaction well known in radical cation chemistry. In this way this highly oxidative species is neutralized while in the neighbourhood of the protein residues - which otherwise could be oxidized -, whilst at the same time rendering the endergonic electron transfer step essentially irreversible. Outside the enzyme active site

the reversed reaction takes place under the acidic reaction conditions to regenerate the $Valc^{+\cdot}$ again, which subsequently may act as a strong oxidant to oxidize the lignin polymer. This nucleophile in the active site could be water or a protein residue.

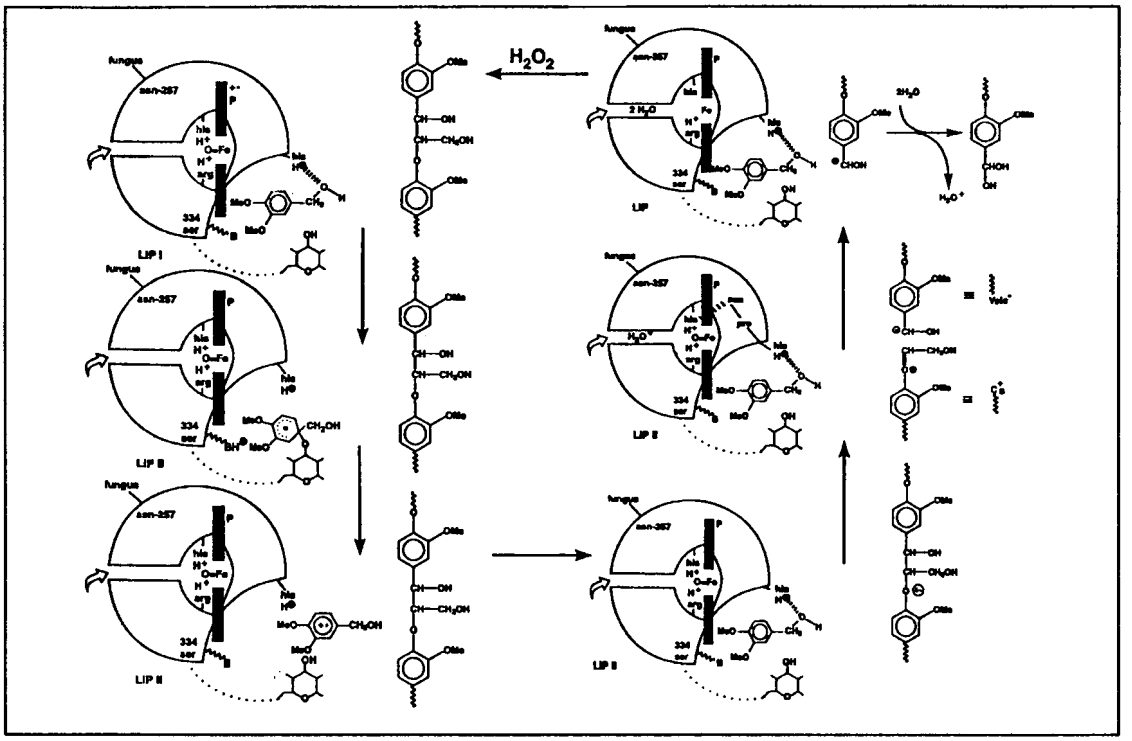

Figure 1: Hypothetical lignin peroxidase redox cycle

Careful inspection of the crystal structure of LiP, in which a molecule of veratryl alcohol was modeled, however, revealed a third possibility. In the LiP-crystal structure solved at the ETH (12), ordered carbohydrate residues - from a O-glycosylation site at Ser-334 -, are more or less blocking the entrance to the active site channel. In fact, after modeling veratryl alcohol in the active site channel (20) one of the hydroxyl groups in this particular modeling study is 3.5 Å away from the C1 atom of veratryl alcohol. Therefore we have postulated that the electron transfer steps in the LiP redox cycle might occur in the endergonic region of the driving force, provided that the incipient $Valc^{+\cdot}$ is removed from the heme via a reaction with a nucleophile such as a carbohydrate residue (20). In this way, the $Valc^{+\cdot}$ can be transported over a considerable distance as a cyclohexadienyl radical-carbohydrate complex. Protonation of this complex will regenerate the one-electron oxidant $Valc^{+\cdot}$ at the oxidation site of the lignin polymer.

This process is schematically represented in the left part of Fig. 1. Then, after oxidation of the lignin polymer veratryl alcohol is regenerated again close to LiPII. An analogous process can now occur in which veratryl alcohol will reduce LiPII -possibly with assistance of the carbohydrate residue - to form a second $Valc^{+\cdot}$ species together with native enzyme. This is the LiP redox cycle according to Scheme 1. However, in the *in vivo* process an alternative cycle can be envisaged, which is depicted in the right part of Fig. 1. Most probably, the LiP initiated degradation process will occur near the fungal hyphae in the extracellular mucilaginous sheath (15). This carbohydrate gel will cause severe diffusional limitation to the large protein and the polymer, in fact the enzyme is more or less immobilized on the lignin polymer. Also, most probably, the reaction will occur under almost anaerobic conditions due to diffusional limitations for oxygen gas as well.

After one-electron oxidation of the lignin polymer a lignin cation radical is formed, most probably in a ß-O-4 subunit. The formation of this radical cation is rapidly followed by a Cα-Cß cleavage to form a protonated Cß-fragment and a polymer bound hydroxy-substituted benzylic radical (39). The latter species, however, is generated at the surface of the LiP protein. This benzylic radical is a very good reductant for LiPII. Indeed, calculations indicate that according to Marcus theory even long-range

electron transfer from the radical to the heme may occur in view of the large driving force of this reduction (20). Thus, as an alternative to the rather slow reduction of LiPII by veratryl alcohol in close contact, a fast reduction of LiPII via long-range electron transfer by a radical could occur, thereby completing the LiP redox cycle as depicted in Fig. 1. In this scheme veratryl alcohol acts as a cofactor for LiP whilst staying in close contact with the enzyme. Careful inspection of the crystal structure reveals (Piontek *et al*, to be published), that H_2O_2 may oxidize the heme via a separate channel, even if veratryl alcohol is bound in the active site channel.

QUANTUM-MECHANICAL PULPING OF WOOD?

In the literature there has been much debate how degradation of the lignin polymer could occur at sites not accessible to the large enzymes. Small diffusible mediators have been proposed like hydroxyl radical, chelated manganese ions or $Valc^{+\cdot}$. However, hydroxyl radical is too reactive and would immediately react with all the wood components present. The $Valc^{+\cdot}$ has a too limited lifetime to penetrate into the wood. The chelated manganese ion appears to be a good candidate, albeit that its reactivity only allows it to react with phenolic residues, thereby limiting its efficiency.
So far, one candidate to initiate a lignin degradation process at a distance from the fungal hyphae and the enzymes contained in the mucilaginous sheath has been largely overlooked, i.e. the electron. Provided that the driving force is not inhibitory, electrons may tunnel over appreciable distances with relatively high rates. If such a long-range electron transfer through the aromatic lignin polymer is followed by an irreversible C-C bond cleavage, lignin degradation is initiated and might proceed further via a number of mechanisms, either chemical or enzymatic. An attractive feature of this proposition is the fact that only very limited diffusion of the large enzymes and polymers is required for such a mechanism. The lignin polymer will bind to LiP - or rather the other way around - and subsequently $Valc^{+\cdot}$ will serve as a continuous source of oxidant, provided of course that enough H_2O_2 is generated. The electrons could tunnel through the lignin polymer from numerous sites, all with approximately the same destination, the binding site of the lignin polymer at the entrance to the active site channel, a process that aptly may be described as quantum-mechanical pulping of wood.

REFERENCES

1. K.E.L. Eriksson, R.A. Blanchette and P. Ander, *Microbial and Enzymatic Degradation of Wood and Wood Components*, Springer-Verlag, Berlin (1990).
2. J.K. Glenn, M.A. Morgan, M.B. Mayfield, M. Kuwahara and M.H. Gold, *Biochem. Biophys. Res. Comm.*, **114**, 1077 (1983).
3. M. Tien and T.K. Kirk, *Science*, **221**, 661 (1983).
4. H.E. Schoemaker, P.J. Harvey, R.M. Bowen and J.M. Palmer, *FEBS Lett.*, **183**, 7 (1985).
5. P.J. Harvey, H.E. Schoemaker, R.M. Bowen and J.M. Palmer, *FEBS Lett.*, **183**, 13 (1985).
6. P.J. Kersten, M. Tien, B. Kalyanaraman and T.K. Kirk, *J. Biol. Chem.*, **260**, 2609 (1985).
7. P.J. Harvey, H.E. Schoemaker, R.M. Bowen and J.M. Palmer, *FEBS Lett.*, **195**, 242 (1986).
8. S.D. Haemmerli, M.S.A. Leisola and A. Fiechter, *FEMS Microbiol. Lett.*, **35**, 33 (1986).
9. M.S.A. Leisola, S.D. Haemmerli, R. Waldner, H.E. Schoemaker, H.W.H. Schmidt and A. Fiechter, *Cellulose Chem. Technol.*, **22**, 267 (1988).
10. K.E. Hammel, K.A. Jensen, M.D. Mozuch, L.L. Landucci, M. Tien and E.A. Pease, *J. Biol. Chem.*, **268**, 12274 (1993).
11. K.E. Hammel and M.A. Moen, *Enzyme Microbial Technol.*, **13**, 5 (1991).
12. K. Piontek, T. Glumoff and K.H. Winterhalter, *FEBS Lett.*, **315**, 119 (1993).
13. T.L. Poulos, S.L. Edwards, H. Wariishi and M.H. Gold, *J. Biol. Chem.*, **268**, 4429 (1993).
14. H.E. Schoemaker, *Recl. Trav. Chim. Pays-Bas*, **109**, 255 (1990).
15. H.E. Schoemaker, T.K. Lundell, A.I. Hatakka and K. Piontek, *FEMS Microbiol. Rev.*, **13**, 321 (1994).
16. C.D. Millis, D. Cai, M.T. Stankovich and M. Tien, *Biochemistry*, **28**, 8484 (1989).
17. P.J. Kersten, B. Kalyanaraman, K.E. Hammel, B. Reinhammer and T.K. Kirk, *Biochem. J.*, **268**, 475 (1990).

18. L. P. Candeias and P.J. Harvey, *J. Biol. Chem.*,, **270**, 16745 (1995).
19. A. Khindaria, T.A. Grover and S.D. Aust, *Biochemistry*, **34**, 6020 (1995).
20. H.E. Schoemaker, T.K. Lundell, R. Floris, T. Glumoff, K.H. Winterhalter and K. Piontek, *Bioorg. Med. Chem.*, **2**, 509 (1994).
21. M. Tien, T.K. Kirk, C. Bull and J.A. Fee, *J. Biol. Chem.*, **261**, 1687 (1986).
22. D.P. Barr and S.D. Aust, *Rev. Environ. Contam. Toxicol.*, **138**, 480 (1994).
23. P.J. Harvey, R. Floris, T. K. Lundell, J.M. Palmer, H.E. Schoemaker and R. Wever, *Biochem. Soc. Trans.*, **20**, 345 (1992).
24. D.C. Goodwin, S.D. Aust and T.A. Grover, *Biochemistry*, **34**, 5060 (1995).
25. H. Wariishi, D. Sheng and M.H. Gold, *Biochemistry*, **33**, 5545 (1994).
26. R.S. Koduri and M. Tien, *Biochemistry*, **33**, 4225 (1994).
27. K. Valli, H. Wariishi and M.H. Gold, *Biochemistry*, **29**, 8535 (1990).
28. R.A. Marcus and N. Sutin, *Biochem. Biophys. Acta*, **811**, 265 (1985).
29. C.M. Moser, J.M. Keske, K. Warncke, R.S. Farid and L. Dutton, *Nature*, **355**, 796 (1992).
30. D.N. Beratan, J.N. Onuchic, J.R. Winkler and H.B. Gray, *Science*, **258**, 1740 (1992).
31. C.J. Schlesener, C. Amatore and J.K. Kochi, *J. Am. Chem. Soc.*, **106**, 3567 (1984).
32. R.A. Reed and R.W. Murray, *J. Phys. Chem.*, **90**, 3829 (1986).
33. H. Wariishi, J. Huang, H.B. Dunford and M.H. Gold, *J. Biol. Chem.*, **266**, 464 (1991).
34. A. Khindaria, D.P. Barr and S.D. Aust, *Biochemistry*, **34**, 7773 (1995).
35. Y. Hayashi and I. Yamazaki, *J. Biol. Chem.*, **254**, 9101 (1979).
36. Z.S. Farhangrazi, B. R. Copeland, T. Nakayama, T. Amachi, I. Yamazaki and L.S. Powers, *Biochemistry*, **33**, 5647 (1994).
37. T.G. Traylor, W.A. Lee and D.V. Stynes, *Tetrahedron*, **49**, 553 (1984).
38. C.L. Perrin, *J. Phys. Chem.*, **88**, 3611 (1984).
39. T.K. Lundell, H.E. Schoemaker, A.I. Hatakka and G. Brunow, *Holzforschung*, **47**, 219 (1993).