S6 Low molecular weight free radicals as agents of lignocellulose decay

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Objectives

Although we still do not know the detailed mechanisms for wood decay by fungi, there is increasing evidence that low molecular weight radical oxidants are important contributors. However, it remains unclear whether all decay fungi use radical oxidants, and in most cases we have little information about how these species are generated. Moreover, it is confusing that fungi that yield very different morphologies of decay- e.g. white rot and brown rot-, have both been reported to use hydroxyl radicals to attack wood.

We are attempting to determine whether various decay fungi produce significant levels of nonspecific one-electron oxidants when they grow on wood. We decided to use wood as the substrate, despite the technical problems it poses, because no other approach ensures that all of the fungi will express their complete lignocellulolytic mechanisms. To detect one-electron oxidants during decay, we infiltrate ^{14}C-labeled polyethylene glycol (PEG, 4000 mol wt) into the wood samples and then monitor the ability of the fungi to evolve $^{14}CO_2$ from it. The rationale for this approach is that high molecular weight PEG does not penetrate cell membranes and is inert to most biological cleavage mechanisms. The only way that a fungus can cleave this polymer is by generating an extracellular agent that can abstract a PEG methylene hydrogen, i.e. via one-electron oxidation (4).

Results and Discussion

So far, we know that the brown rot fungus *Gloeophyllum trabeum* mineralizes PEG to a greater extent than any other fungs we have examined. Because *G. trabeum* expresses its PEG-degrading system on defined medium as well as wood, we have been able to make some progress towards characterizing this system. *G. trabeum* uses an extracellular iron-dependent quinone redox cycle to generate Fenton reagent (Fe^{2+}+ H_2O_2), which produces the hydroxyl radical or a similarly reactive species (5). The quinones that *G. trabeum* secretes for this purpose are 2,5-dimethoxy-1,4-benzoquinone and 3,5-dimethoxy-1,2-benzoquinone (3, 5, 7). They are probably reduced in vivo by a flavoprotein NADH-quinone oxidoreductase, which we have purified to homogeneity. We have obtained partial amino acid sequences for the enzyme and are in the process of cloning the gene that encodes it.

We also find that the brown rotters *Postia placenta* and *Coniophora puteana* mineralize PEG rapidly when they are degrading wood. The mechanisms these fungi use to generate one-electron oxidants ramain unclear, but our results may support the proposal that *C. puteana* uses cellobiose dehydrogenase to initiate Fenton chemistry (2). To make further progress with these fungi, it will be necessary to find a defined culture medium on which they degrade PEG.

We find that the white rot fungi *Phanerochaete chysosporium* and *Cenporiopsis subvermispora* both degrade PEG when they grow on wood, but lack this ability on the usual defined media. Perhaps these fungi use cellobiose dehydrogenase to initiate Fenton chemistry, as proposed previously (1,6), but it is imporrant to note that Fenton reagent is not the only one-electron oxidant that can abstract hydrogens from PEG. Peroxyl radicals are also capable of this reaction, and these oxidants are produced by the action of white rotters' manganese peroxidases in the presence of unsaturated lipids. Again, an understanding of how these fungi produce small radical oxidants will depend on the development of new culture conditions.

Finally, we observe that the soft rot ascomycete *Daldinia concentrica* degrades no PEG, even on wood that it degrades extensively. These results show clearly that the hydroxyl radical is not an essential participant in all types of wood decay. The lignocellulolytic oxidants that *D. concentrica* employs, if it produces any, must be considerably less reactive than those used by basidiomycetous decayers.

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Symposium on Recent Advances in Lignin Biodegradation and Biosynthesis

Viikki Biocenter, University of Helsinki, Helsinki, Finland
June 3-4, 2001