Biodegradative mechanism of the brown rot basidiomycete *Gloeophyllum trabeum*: evidence for an extracellular hydroquinone-driven fenton reaction

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Abstract **We have identified key components of the extracellular oxidative system that the brown rot fungus *Gloeophyllum trabeum* uses to degrade a recalcitrant polymer, polyethylene glycol, via hydrogen abstraction reactions. *G. trabeum* produced an extracellular metabolite, 2,5-dimethoxy-1,4-benzoquinone, and reduced it to 2,5-dimethoxyhydroquinone. In the presence of 2,5-dimethoxy-1,4-benzoquinone, the fungus also reduced extracellular Fe^{3+} to Fe^{2+} and produced extracellular H_2O_2. Fe^{3+} reduction and H_2O_2 formation both resulted from a direct, non-enzymatic reaction between 2,5-dimethoxyhydroquinone and Fe^{3+}. polyethylene glycol depolymerization by *G. trabeum* required both 2,5-dimethoxy-1,4-benzoquinone and Fe^{3+} and was completely inhibited by catalase. These results provide evidence that *G. trabeum* uses a hydroquinone-driven Fenton reaction to cleave polyethylene glycol. We propose that similar reactions account for the ability of *G. trabeum* to attack lignocellulose.**

Key words: Brown rot; Wood decay; Quinone redox cycling; Fenton reaction; Hydroxyl radical; Polyethylene glycol

1. Introduction

Brown rot fungi are important contributors to the biomass recycling and soil fertility in forest ecosystems [1,2]. They also cause the most destructive type of decay in wooden structures [3]. These basidiomycetes are unusual in that they rapidly depolymerize the cellulose in wood without removing the surrounding lignin that normally prevents microbial attack. It is unlikely that the early steps in this process are enzymatic, because no known enzyme is small enough to penetrate sound wood [4]. Instead, brown rot fungi probably employ low molecular weight (M_r) degradative agents.

These agents have not been identified, but it has often been proposed that brown rot fungi use an extracellular Fenton system ($Fe^{2+}+H_2O_2$) to generate hydroxyl radical ($^{\bullet}OH$) or a similarly powerful oxidant that degrades wood [5–9]. Consistent with this proposal are observations that brown rot fungi (i) introduce carboxyl and carbonyl groups into cellulose [10,11], (ii) dealkylate lignin [12,13], (iii) generate methanesulfinic acid from dimethylsulfoxide [9], (iv) hydroxylate phthalic hydrazide [5] and (v) oxidize 5,5-dimethyl-1-pyrroline-*N*-oxide to a paramagnetic species that was attributed to reaction with $^{\bullet}OH$ [14]. However, it remains unclear what oxidants are involved in these reactions or how they are produced.

Recently, we showed that the brown rotter *Gloeophyllum trabeum* depolymerizes high M_r polyethylene glycol (PEG) by abstracting hydrogens from internal methylenes of the polymer. These reactions include carbon-carbon bond scission and are consistent with an attack by a strong oxidant such as Fenton reagent [15]. PEG depolymerization provides a convenient assay for extracellular one electron oxidant production by *G. trabeum*. We have identified the individual components that *G. trabeum* requires to degrade PEG and now report evidence that hydroquinone driven Fenton chemistry is an important component of this basidiomycete's extracellular biodegradative system.

2. Materials and methods

2.1. Reagents

2,5-Dimethoxy-1,4-benzoquinone (DMBQ) was obtained from TCI America. 2,5-Dimethoxyhydroquinone (DMHQ) was prepared by shaking a chloroform solution of DMBQ with aqueous $Na_2S_2O_4$. The hydroquinone was recrystallized twice from chloroform. 4,5-Dimethoxy-1,2-benzoquinone was obtained from Apin Chemicals. A sample of 4,5-dimethoxycatechol was prepared by reducing 4,5-dimethoxy-1,2-benzoquinone with $NaBH_4$ in ethanol/H_2O. The crude catechol was used in chromatographic experiments without further purification. Lyophilized *Aspergillus niger* catalase was purchased from Calbiochem. All other reagents were obtained from Sigma or Aldrich.

PEG labelled with ^{14}C in its terminal hydroxyethyl groups (15.3 mCi/g, M_r stated as 4000) was obtained from Amersham. Gel permeation chromatography (GPC, see Section 2.7) gave a number average M_r (M_n) of 4600 and a weight average M_r (M_w) of 5700. For biodegradation experiments, the [^{14}C]PEG was diluted with unlabelled PEG (M_r = 4000) to specific activities that ranged from 7.7×10^{-2} to 9.8×10^{-2} mCi/g. In experiments that required multiple sampling, a higher specific activity of 3.0×10^{-1} mCi/g was used.

2.2. Organism

G. trabeum (ATCC 11539) was grown at 30°C under air in stationary 125 ml Erlenmeyer flasks that contained 5 ml of basal growth medium (pH 4.5) with 10 g/l glucose as the carbon source [16]. The cultures were inoculated at a rate of 1% with homogenized potato dextrose agar plates of the fungus. Cultures were harvested 7 days after inoculation, at which time the average dry weight of a mycelial mat was 10 mg and the pH of the extracellular medium was 4.1.

2.3. Identification and purification of extracellular metabolites

Analytical high performance liquid chromatography (HPLC) of *G. trabeum* metabolites was done on a phenylhexyl column (Phenomenex Luna, 5 μm particle size, 150×4.6 mm). The column was operated at 1.0 ml/min and ambient temperature, and was eluted with linear gradients as follows. 0 min: H_2O/methanol/formic acid, 95:5:0.1. 40 min: H_2O/methanol/formic acid, 65:35:0.1. 50 min: methanol/formic acid, 100:0.1. The absorbance of the eluate was monitored at 280 nm. External standards were used for preliminary product identification

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and for quantitation. They had the following elution times: 4,5-dimethoxycatechol, 7 min; 4,5-dimethoxy-1,2-benzoquinone, 17 min; DMHQ, 18 min; DMBQ, 27 min.

For metabolite purification, fungal extracellular fluid (500 ml) was filtered through a 0.45 μm pore size membrane and applied to a 3.6 cm diameter column packed with 15 g of solid phase extraction resin (Varian Bondesil phenyl, 40 μm particle size) that had been equilibrated beforehand with H_2O. The column was eluted with 50 ml each of 25% and 50% aqueous methanol. A major metabolite eluted in 50% methanol and was approximately 95% pure by HPLC. The eluate was concentrated to dryness by rotary vacuum evaporation and the yellow metabolite was recrystallized twice from a few ml of methanol.

Gas chromatography/electron impact mass spectrometry (GC/MS) of the purified metabolite was done on a non-polar silicone polymer column in a Hewlett-Packard instrument that was operated at 70 eV. Fourier transform infrared spectrometry (FTIR) was done in KBr pellets on a Mattson Galaxy Series spectrometer.

2.4. PEG depolymerization with G. trabeum extracellular fluid

The mycelial mats were removed from 50 cultures and shaken gently for 1 h in 1 l of distilled, deionized H_2O at ambient temperature. The mats were then transferred to fresh H_2O and the procedure was repeated. The culture fluid (200 ml) was filtered through a 0.45 μM pore size membrane to remove mycelial fragments. The fluid was then subjected to ultrafiltration through a 1 kDa cutoff membrane (YM-1, Amicon). The passthrough fraction was demetaled over 10 g of Chelex 100 resin that had been equilibrated beforehand with sodium phosphate buffer (50 mM, pH 4.1: hereafter called 'buffer'). Samples of fluid from each step after the initial filtration were analyzed for the Fe content by atomic absorption spectrometry, and were retained for use in depolymerization assays.

PEG depolymerization assays were done in foil-capped 125 ml erlenmeyer flasks that contained one *G. trabeum* mycelial mat, 5 ml of fluid and 1 ml of [^{14}C]PEG solution (330 mg/l final). In one experiment with Chelex-treated fluid, $FeCl_3$ (25 μM final) was included with the PEG. For comparison, an additional reaction was conducted in which extracellular fluid was replaced by DMBQ (50 μM final) and $FeCl_3$ (15 μM final) in buffer. The reactions were rotary shaken at 100 rpm and 30°C. Samples (750 μl) were taken from duplicate reactions after 48 h and were combined for analysis by GPC.

2.5. PEG depolymerization with G. trabeum extracellular metabolites

Culture fluid was harvested, filtered and subjected to ultrafiltration as described above. A portion (100 ml) of the filtrate was applied to a 3.6 cm diameter column packed with 10 g of phenyl solid phase extraction resin (see Section 2.3) that had been equilibrated beforehand with H_2O. The passthrough fraction was retained for analysis and the column was eluted with 25 ml each of 10%, 25% and 50% aqueous methanol, followed by 25 ml of 100% methanol. The methanol and some of the H_2O were removed from the eluates by rotary vacuum evaporation, and the volumes were all adjusted to 10.0 ml with H_2O.

PEG depolymerization assays were done as described above, except that they contained one mycelial mat, 0.60 ml of column eluate, 5.4 ml of buffer with $FeCl_3$ (100 μM final) and [^{14}C]PEG (330 mg/l final). An additional reaction was done with 5.0 ml of passthrough fraction and 1.0 ml of H_2O that contained the [^{14}C]PEG and $FeCl_3$.

2.6. PEG depolymerization with DMBQ

The glassware for these reactions was soaked for several h in 1 N HCl and rinsed with distilled, deionized H_2O. Assays were done as described above, except that they contained one mycelial mat, DMBQ (200 μM), $FeCl_3$ (100 μM) and [^{14}C]PEG (330 mg/l) in 6.0 ml of Chelex-treated buffer. In reactions that included catalase, 0.5 mg (3500 U) was added. An additional reaction was done with DMHQ (1.0 mM) and $FeCl_3$ (50 μM) in the absence of mycelium. In experiments that required multiple sampling, the assays were done with 10 mycelial mats and the volume was increased 10-fold. At the conclusion of each experiment, the mycelium was removed and the remaining liquid was assayed for Fe by atomic absorption spectrometry.

2.7. GPC analyses of degraded PEG

A portion (600 μl) of each combined sample was filtered through a 0.45 μm pore size membrane. Of the filtrate, which contained >99% of the ^{14}C, 500 μl was subjected to GPC on a 1.0×31 cm column of Superdex 30 (Pharmacia). The eluent was 100 mM sodium acetate, pH 5.0, that contained 0.01% w/v thimersol. The column was run on a Pharmacia FPLC system at 0.5 ml/min and ambient temperature. Fractions (0.5 ml) were collected and assayed for ^{14}C by scintillation counting. PEG standards with M_r values of 7500, 4000, 1540, 600, 400, 194, 150, 106 and 62 (ethylene glycol) were used to calibrate the column and were detected in the eluate by refractometry. M_n values and M_w values were calculated with the standard equations [17].

2.8. Reduction of DMBQ by G. trabeum

DMBQ (175 μM) in buffer (10 ml) was placed in a 20 ml glass vial and the solution was recirculated through a 1 cm path length quartz flow cell at 6 ml/min and ambient temperature. After 1 min, the reaction was started by adding two mycelial mats to the vial. The UV/visible absorption spectrum of the sample was recorded at 0 min and 60 min. Samples (100 μl) were removed from the vial at 0, 10, 35 and 60 min for HPLC analysis. $FeCl_3$ (1 mM final) was added to the recirculating solution at 61 min and an additional sample was taken for HPLC analysis at 66 min.

HPLC to quantitate DMHQ and DMBQ in the samples was done on the phenylhexyl column described in Section 2.3. The column was eluted with H_2O/methanol/formic acid, 80:20:0.1 at 1.0 ml/min and ambient temperature. DMHQ eluted at 9 min and DMBQ at 16 min. Authentic solutions of the two compounds were used as external standards for quantitation.

2.9. Reduction of Fe^{3+} by G. trabeum

DMBQ (200 μM), $FeCl_3$ (200 μM) and ferrozine (1.0 mM) in buffer (15 ml) were recirculated in the flow system described above. After 2 min the reaction was started by adding 2 mycelial mats to the vial and the increase in absorbance at 562 nm was recorded for 20 min. In reactions that employed DMHQ instead of DMBQ, the mycelial mats were omitted or were added before the assay and a freshly prepared solution of the hydroquinone was used to start the reduction. An extinction coefficient of 27.9/mM/cm at 562 nm was used to quantitate the ferrozine-Fe^{2+} complex [18].

2.10. Production of H_2O_2 by G. trabeum

A modification of the procedure in [19] was used. Samples (0.1–0.5 ml) were taken from PEG depolymerization assays or from *G. trabeum* cultures and were adjusted to a volume of 1.0 ml with H_2O. Phenol red (sodium salt, 0.1 ml of a 1.0 g/l solution) and horseradish peroxidase (Sigma Type II, 0.01 ml of a 1 g/l solution) were added and the mixture was incubated at ambient temperature for 2 min. NaOH (0.05 ml of a 2 N solution) was then added and 2 min later the absorbance of the sample at 610 nm was recorded. H_2O_2 standards from a stock that had been titrated beforehand with $KMnO_4$ were subjected to the same treatment and were used to quantitate the H_2O_2 present in experimental samples.

Table 1
PEG cleaving activity of *G. trabeum* extracellular fluid after fractionation by reversed phase chromatography[a]

Fraction added	M_n of PEG	M_w of PEG	DMBQ detected
Buffer	1310	4616	−
Column passthrough	1192	4080	−
10% Methanol eluate	703	3154	−
25% Methanol eluate	1409	4724	−
50% Methanol eluate	401	2036	+
100% Methanol eluate	1660	4702	−

[a]See Sections 2.5 and 2.7.

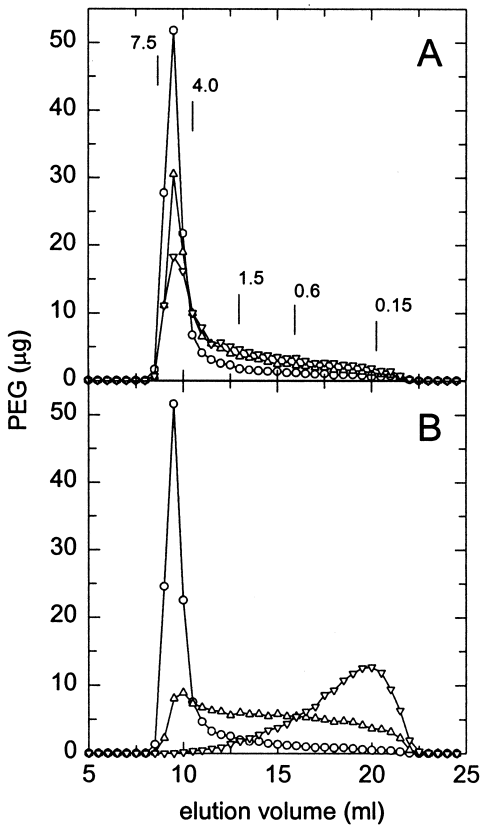

Fig. 1. GPC analyses of PEG that was treated with *G. trabeum* mycelium and fractionated extracellular fluid. (A) 48 h reactions with mycelium plus extracellular fluid (△, $M_n = 929$, $M_w = 3563$), with mycelium plus the low M_r fraction of extracellular fluid (▽, $M_n = 681$, $M_w = 2993$) and with mycelium plus buffer (○, $M_n = 1470$, $M_w = 4710$). Extracellular fluid without mycelium did not depolymerize PEG (data not shown). The elution positions of PEG standards with the indicated M_r values (kDa) are shown. (B) 48 h reactions with mycelium plus the low M_r fraction of extracellular fluid after Chelex treatment (○, $M_n = 1799$, $M_w = 4681$), with mycelium plus the Chelex-treated fraction after addition of 25 μM $FeCl_3$ (△, $M_n = 380$, $M_w = 1754$) and with mycelium plus DMBQ (50 μM) and $FeCl_3$ (15 μM) in place of extracellular fluid (▽, $M_n = 158$, $M_w = 377$). See Sections 2.4 and 2.7.

3. Results

3.1. PEG cleavage requires Fe and a low M_r metabolite

Washed mycelial mats of *G. trabeum* depolymerized PEG when they were shaken in their own growth medium. When the medium was ultrafiltered through a membrane with a 1 kDa cutoff, the low M_r filtrate supported a higher cleavage activity than the original medium did (Fig. 1A). The high M_r retentate fraction did not support PEG cleavage (data not shown).

Ultrafiltered medium lost the ability to support PEG depolymerization when it was demetaled on a column of Chelex 100 resin (Fig. 1B). Atomic absorption spectrometry of the medium showed that it contained approximately 15 μM Fe before Chelex treatment and less than 0.1 μM Fe after treatment. Addition of 25 μM $FeCl_3$ to the Chelex-treated medium completely restored its ability to support PEG cleavage (Fig. 1B).

3.2. The metabolite required for PEG cleavage is DMBQ

HPLC analyses showed that ultrafiltered medium from *G. trabeum* cultures contained two major UV-absorbing compounds that eluted at 18 min and 27 min. The more polar metabolite was unstable in air and we were unable to purify it on a preparative scale. We purified the less polar metabolite to approximately 95% homogeneity by preparative reversed phase chromatography and tested its ability to support PEG cleavage in the presence of Fe^{3+} and *G. trabeum* mycelium. Most of the depolymerizing activity and all of the metabolite eluted together (Table 1).

The less polar metabolite was recrystallized after chromatography and was subjected to spectrometric analysis. GC/MS: *m/z* (relative intensity): 168 (M^+, 2), 153 (14), 139 (16), 125 (10), 122 (6), 111 (6), 95 (31), 69 (100). FTIR: ν (cm^{-1}): 1665, 1599, 1228, 1206, 997, 877. The spectrometric data and chromatographic retention times of the metabolite were identical to those exhibited by authentic DMBQ. The more polar, unstable metabolite had an HPLC retention time that was identical to that of authentic DMHQ.

HPLC analyses showed that the concentration of DMBQ in 7 day *G. trabeum* cultures was 40–60 μM. The concentration of DMHQ was more variable, at 10–50 μM. The extracellular fluid did not contain detectable levels of another metabolite previously reported in *G. trabeum* cultures, 4,5-dimethoxycatechol [20]. The oxidized form of this metabolite, 4,5-dimethoxy-1,2-benzoquinone, was also absent.

DMBQ and Fe^{3+} supported PEG cleavage better than ultrafiltered growth medium did when they were added to a *G. trabeum* mycelium in buffer at concentrations similar to those found in intact cultures (Fig. 1B). At higher concentrations of the quinone and Fe^{3+}, depolymerization was considerably faster (Table 2). Experiments done under the latter conditions confirmed that mycelium, DMBQ and Fe^{3+} were all required in the reaction. Several other quinones, including 2,6-dimethoxy-1,4-benzoquinone, 4,5-dimethoxy-1,2-benzoquinone and coenzyme Q_0, supported PEG cleavage as well as DMBQ did (data not shown).

Table 2
M_r values for PEG after treatment with the reconstituted *G. trabeum* system[a]

Reaction conditions	M_n of PEG	M_w of PEG
Complete with DMBQ, Fe^{3+} and mycelium (3 h reaction)	280	1277
Minus DMBQ	2856	5363
Minus Fe^{3+}	2901	5213
Minus mycelium	3801	5370
Plus catalase	3627	5358
Plus boiled catalase	359	1691
Complete with DMHQ and Fe^{3+} (5 h reaction)	783	3412

[a]See Sections 2.6 and 2.7.

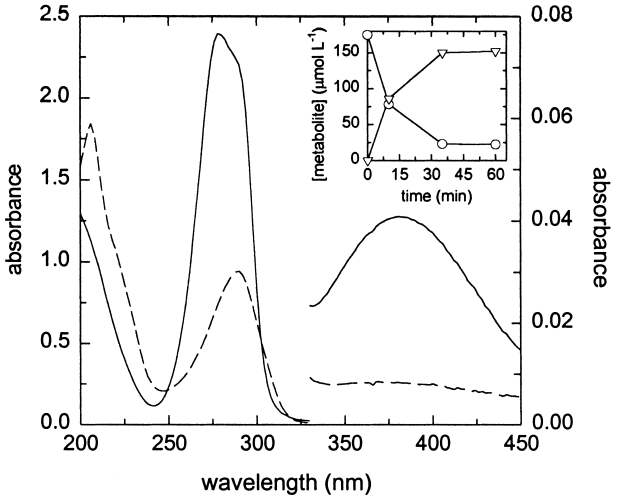

Fig. 2. Absorption spectra of DMBQ before treatment with *G. trabeum* mycelium (—) and 60 min after treatment (- - -). The inset shows concentrations of DMBQ (○) and DMHQ (▽) in the reaction. See Section 2.8.

3.3. *G. trabeum mycelium reduces DMBQ to DMHQ. DMHQ then reduces Fe^{3+} to Fe^{2+}, which is required for PEG cleavage*

When DMBQ was added to washed mycelial mats in buffer without added Fe^{3+}, the yellow color of the quinone disappeared and the UV/visible absorption spectrum of the extracellular fluid shifted from that of DMBQ (λ_{max} = 278 nm, 380 nm) to that of DMHQ (λ_{max} = 290 nm) (Fig. 2). HPLC analysis of the extracellular fluid confirmed that DMBQ was reduced almost entirely to DMHQ and showed that neither the oxidized nor the reduced form of the metabolite became bound to the mycelium (Fig. 2, inset). When Fe^{3+} was subsequently added to the extracellular fluid, the DMHQ that had accumulated was reoxidized to DMBQ (data not shown).

When DMBQ was added to washed mycelial mats in buffer that contained Fe^{3+}, Fe^{2+} accumulated after a brief lag. No Fe^{3+} reduction occurred when quinone or mycelium was omitted. When the experiment was repeated with DMHQ, Fe^{3+} reduction was faster, occurred without a lag and no longer required mycelium (Fig. 3). Therefore, the mycelium's role in Fe^{3+} reduction was simply to reduce DMBQ to DMHQ, which in turn acted as the proximal reductant of Fe^{3+}. The Fe^{2+} thus produced was essential for PEG cleavage, because depolymerization occurred in the absence of mycelium when DMHQ and Fe^{3+} were combined, but not when DMBQ and Fe^{3+} were combined (Table 2).

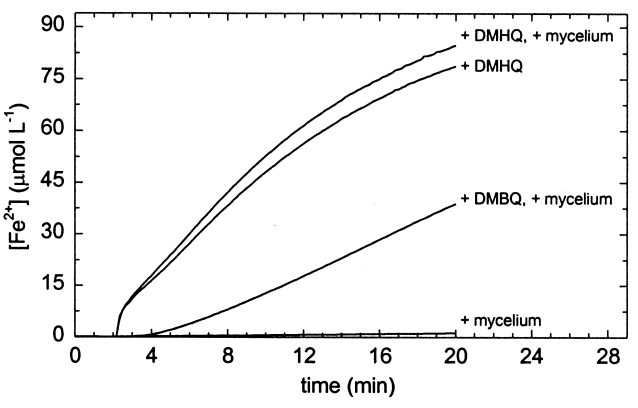

Fig. 3. Reduction of Fe^{3+} by various combinations of *G. trabeum* mycelium, DMBQ and DMHQ. No reduction occurred with DMBQ alone (data not shown). See Section 2.9.

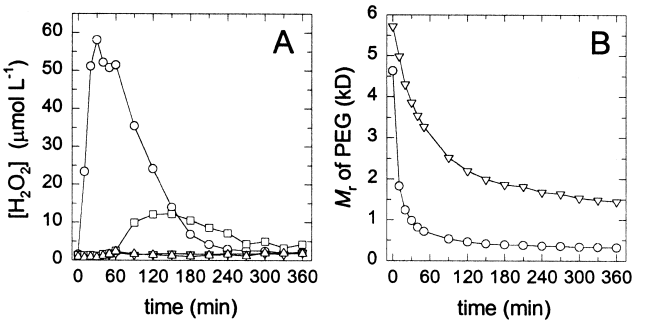

Fig. 4. Kinetics of H_2O_2 formation and PEG depolymerization by *G. trabeum* mycelium in the presence of DMBQ and Fe^{3+}. (A) H_2O_2 production in a complete reaction (○), without Fe^{3+} (□), without DMBQ (▽) and without mycelium (△). (B) M_n (○) and M_w (▽) values of PEG in the complete reaction. See Sections 2.6–2.10.

3.4. *DMHQ and Fe^{3+} support the production of H_2O_2, which is required for PEG cleavage*

7 day *G. trabeum* cultures contained an extracellular substance that enabled horseradish peroxidase to oxidize phenol red. We concluded that this substance was H_2O_2, because no phenol red oxidation occurred when the growth medium was treated with catalase before the peroxidase assay was done (data not shown). The levels of H_2O_2 in the cultures were highly variable (50–300 μM), as reported previously for other brown rot fungi [14].

H_2O_2 accumulated rapidly and then declined when *G. trabeum* mycelium, DMBQ (200 μM) and Fe^{3+} (100 μM) were combined together in buffer (Fig. 4A). The highest concentration was reached after 20–30 min and was 50–60 μM. No H_2O_2 was produced if mycelium or DMBQ was omitted. Low levels of H_2O_2 were detectable when Fe^{3+} was omitted but atomic absorption spectrometry showed that this control

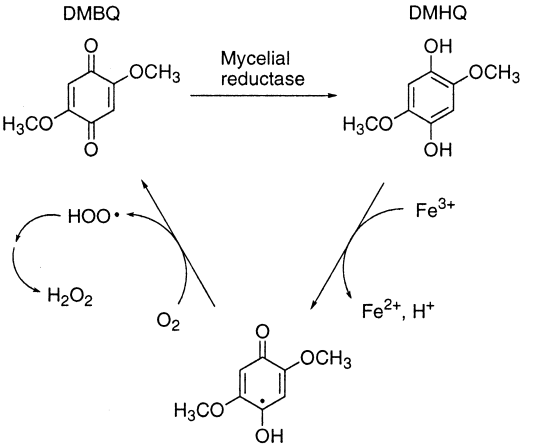

Fig. 5. Proposed pathway for extracellular Fe^{3+} reduction and H_2O_2 production by *G. trabeum*.

reaction actually contained approximately 0.5 μM soluble Fe. To model the complete reaction in the absence of mycelium, we combined DMHQ (200 μM) with Fe^{3+} (50 μM). H_2O_2 accumulated to a concentration of approximately 80 μM after 25 min in this experiment (data not shown).

Several findings linked PEG cleavage to H_2O_2 production by the complete system of mycelium, DMBQ and Fe^{3+}. First, depolymerization was completely inhibited by active catalase, whereas inactivated (boiled) catalase had no effect on the reaction (Table 2). Second, PEG was cleaved in the absence of mycelium by the combination of DMHQ and Fe^{3+} (Table 2). These two ingredients were present in complete assays and reacted together to produce H_2O_2. Finally, PEG cleavage and H_2O_2 production in complete assays exhibited similar kinetics, in that both were most rapid during the first 20 min of incubation (Fig. 4).

4. Discussion

We used PEG as a model substrate to look for extracellular agents that cause brown rot because this polyether, like lignocellulose, is unable to penetrate cell membranes [21] and resists biodegradation [22]. Moreover, the free radical chemistry of polyethers such as PEG is similar to that of polyacetals such as cellulose [15,23].

At the outset, we found that *G. trabeum* extracellular fluid contains substances that support the PEG degradation in the presence of *G. trabeum* mycelium and we did ultrafiltration experiments to show that these agents have M_r values less than about 1 kDa. Previous studies have suggested that soluble high M_r substances such as glycopeptide Fe chelators play a role in brown rot [9], but we found no evidence that *G. trabeum* employs any of these for PEG cleavage.

DMBQ was a prominent extracellular metabolite in fungal cultures and substituted efficiently for ultrafiltered culture fluid in assays of PEG depolymerization. Therefore, this quinone is an important component of the extracellular oxidative system that *G. trabeum* uses to cleave PEG. We did not identify some polar *G. trabeum* metabolites that supported PEG cleavage to a lesser extent (Table 1) but DMHQ and hydroxylated metabolites derived from Fenton oxidation of DMHQ are possible candidates. DMBQ was found over 20 years ago in cultures of a related species, *G. sepiarium* [24], but its biodegradative function has apparently been overlooked until now.

One of the roles of DMBQ in PEG cleavage is to operate in a redox cycle that supports Fe^{3+} reduction (Fig. 5). The following observations support this conclusion: (i) *G. trabeum* mycelium reduces DMBQ to DMHQ, (ii) DMHQ reduces Fe^{3+} non-enzymatically and is reoxidized to DMBQ, and (iii) PEG depolymerization by the mycelium requires both Fe^{3+} and DMBQ. We do not yet know what endogenous electron source the mycelium uses to reduce DMBQ, but it appears likely that the reaction is catalyzed by a quinone reductase that is either intracellular or bound to the mycelial surface.

The other main contribution of DMBQ to PEG degradation is to participate in H_2O_2 producing reactions. The following observations support this conclusion: (i) *G. trabeum* cultures contain extracellular H_2O_2, (ii) DMHQ reacts with Fe^{3+} to produce this H_2O_2 and (iii) PEG depolymerization requires H_2O_2. The extracellular H_2O_2 in *G. trabeum* cultures is probably a product of the redox cycle mentioned above: the reduction of Fe^{3+} by DMHQ yields Fe^{2+} and DMHQ semiquinone radicals. Addition of O_2 to the semiquinones then gives α-hydroxyperoxyl radicals that eliminate $^{\bullet}OOH$ to give DMBQ. Finally, $^{\bullet}OOH$ oxidizes Fe^{2+} or dismutates to generate H_2O_2 [25].

In summary, we have demonstrated that *G. trabeum* uses a quinone redox cycle to generate extracellular Fe^{2+} and H_2O_2, the two ingredients needed for Fenton chemistry. Moreover, by using PEG depolymerization to assay extracellular oxidative activity, we have shown that a redox cycle of this type is actually responsible for biodegradation by a brown rot fungus. Recently, Paszczynski et al. found another extracellular metabolite, 4,5-dimethoxycatechol, in the extracellular medium of *G. trabeum* cultures and proposed that it plays a role in brown rot via redox cycling [20]. We found neither this catechol nor the corresponding quinone, 4,5-dimethoxy-1,2-benzoquinone, in *G. trabeum* cultures, but our results show that these metabolites will support PEG cleavage if they are present.

We do not propose that the mechanism we have described is the only one brown rot fungi use to produce Fenton reagent. Wood and coworkers have shown that *Coniophora puteana* accumulates extracellular oxalic acid and produces an extracellular cellobiose oxidase that reduces Fe^{3+} to Fe^{2+}. At pH values greater than about 4, the oxalate dianion forms a strongly electron donating complex with Fe^{2+} and this chelate reduces O_2 to generate the H_2O_2 precursor $^{\bullet}OOH/O_2^{-\bullet}$ [8,26]. We do not think *G. trabeum* uses this mechanism to degrade polymers, because it does not accumulate oxalate [27]. Moreover, our cultures degraded PEG in the absence of cellobiose. Our results show, instead, that quinone redox cycling is a key component in the biodegradation by *G. trabeum*. However, more work is needed to determine whether this mechanism also applies to other brown rot fungi.

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