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A Peptide-mediated Fenton Reaction in Wood-degrading Fungi

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Abstract: Gt factor is a low-molecular-weight peptide isolated from the extracellular culture of wood-degrading fungus *Gloeophyllum trabeum*. It is capable of enhancing degradation of cellulose. Its action mechanism was investigated and it was found that Gt factor could reduce Fe^{3+} to Fe^{2+}. Electron paramagnetic resonance (EPR) spectroscopy revealed in the presence of O_2, Gt factor could drive the formation of H_2O_2 *via* a superoxide anion $O_2^{\cdot-}$ intermediate and mediate the generation of hydroxyl radical $HO^{\bullet}$ in a Fenton-type reaction. All these provided evidence for the formation of $HO^{\bullet}$ in some wood-degrading fungi.

Keywords: Cellulose degradation, hydroxyl radical $HO^{\bullet}$, Gt factor, electron paramagnetic resonance.

Cellulose is the most abundant resource of regenerative biomass on earth. But due to the complexity of its structure, its degradation mechanism was failed to fully understand. It makes a great limit to its full utility. In nature, wood decay is mainly accomplished by some wood-degrading fungi such as *G.trabeum et al.*. It is well-known that cellulases are the most important components of degrading cellulose. But because the cellulase activity is low, the complete utility of cellulose by enzymatic method is unsatisfactory. In 1965, Halliwell found that the hydroxyl radical $HO^{\bullet}$ produced by Fenton's reagent, is quite destructive to cellulose[1].

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^- + Fe^{3+}$$

Thus, $HO^{\bullet}$ has been proposed to be involved in cellulose degradation[2-6] and oxidative degradation has become a potential way in utility of wood sources. According to this proposition, low molecular-weight biochemical agent was believed to be able to penetrate plant cell wall, mediate the formation of $HO^{\bullet}$ which caused cellulose decomposition[7-8]. So far lots of study involved $HO^{\bullet}$ in cellulose degradation, but the methods for detecting $HO^{\bullet}$ was not accurate; besides, the generation of $HO^{\bullet}$ mediated by Gt factor is still not clear. In this paper, we have applied EPR spin trapping to study $HO^{\bullet}$ generation mediated by Gt factor (purified peptide isolated from extracellular culture of *G.trabeum*)[9-11] and elucidated the pathway of $HO^{\bullet}$ formation.

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Methods

The EPR spin trapping studies were performed using 5,5-dimethylpyrroline-1-oxide (DMPO) at a final concentration of 1 mmol/L. EPR spectra were recorded at room temperature using Bruker Esp 300E spectrometer. The spectrometer settings were as follows: modulation frequency 100 kHz; modulation amplitude 2.024 G; microwave power 10 MW; microwave frequency 9.8 GHz; center field 3483 G; sweeping time 1 min.

Results and Discussion

Ferrozine can react with Fe^{2+}, forming stable red compound which has characteristic absorbance at 562 nm[12]. When ferrozine was added to a solution containing Gt factor and Fe^{3+}, the absorbance of the solution at 562 nm continually intensified, indicating Gt factor strongly reduced Fe^{3+} to Fe^{2+}.

DMPO was used to measure $HO^{\bullet}$ generation by Gt factor in EPR study. Gt factor(5 μg) was placed in 50 mmol/L acetate buffer(pH 4.5) containing 2 mmol/L cellobiose and 0.1mmol/L Fe^{3+}. After addition of DMPO for 30 s, EPR spectrum showed a 1: 2: 2: 1 quartet (**Figure 1)** originating from the hydroxyl radical adduct of DMPO- OH($\alpha_N = \alpha_M$ =14.9 G). This verified the generation of $HO^{\bullet}$. Addition of EDTA (100 mmol/L) diminished $HO^{\bullet}$ generation, indicating the presence of Fe^{3+} is necessary in $HO^{\bullet}$ generation. When catalase was added, $HO^{\bullet}$generation decreased, indicating $HO^{\bullet}$ was generated from H_2O_2. It was further verified by the fact that after 1 mmol/L H_2O_2 was added, EPR signal of DMPO-OH significantly intensified (**Figure 2**) . Thus, we can deduce that $HO^{\bullet}$ formation may occur in a Fenton-type reaction. But the question is that where H_2O_2 comes from? In **Figure 3**, EPR signal of DMPO-OH was firstly produced in the presence of cellobiose, Gt factor , Fe^{3+} and O_2. Then additional Gt factor was added, DMPO-OH signal was intensified, this is because Gt factor reduced Fe^{3+} to Fe^{2+}, which subsequently made $HO^{\bullet}$ generation cycling possible. Besides, a characteristic DMPO- $O_2^{\cdot-}$ signal appearred after addition of Gt factor, suggesting the existence of superoxide anion $O_2^{\cdot-}$ in reaction system and $O_2^{\cdot-}$ formation may be driven by reduction of Fe^{3+} by Gt factor as $Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\cdot-}$. This might somewhat enlighten on the mechanism of H_2O_2 generation. As we know H_2O_2 can potentially form by a one-electron transfer to O_2 to generate superoxide anion $O_2^{\cdot-}$. **Figure 3** showed the $O_2^{\cdot-}$ signal. We can conclude that H_2O_2 was generated by Gt factor *via* a superoxide anion intermediate- $O_2^{\cdot-}$. In this reaction, Gt factor acted as a reductant of Fe^{3+}, Fe^{2+} drove the formation of $O_2^{\cdot-}$ and further lead to the H_2O_2 generation.

In summary, Gt factor, a peptide from the extracellular culture of wood-degrading fungus- *G.trabeum*, could reduce Fe^{3+} to Fe^{2+}, mediate H_2O_2 formation *via* a superoxide anion intermediate - $O_2^{\cdot-}$, and lead to $HO^{\bullet}$ production in a Fenton-type reaction. $HO^{\bullet}$ is strongly oxidative, it could abstract electron from hydroxyl group on glucose monomer of cellulose, left a carbonyl in place of hydroxyl group, thus causing disturbance of

interchain and intrachain hydrogen bonds and then cleavage of a polysaccharide chain of cellulose[13]. FT-IR spectroscopy and X-ray diffraction analysis of dewaxed cotton fiber proved that Gt factor really could disrupt hydrogen bonds and decrease the cellulose crystallinity. The above EPR study provided evidence for $HO^{\bullet}$ formation in some wood-degrading fungi; Furthermore, the free radical generating system described above has potential application in variety of industrial processing and environment control.

Figure 1 EPR spectrum of DMPO-OH adduct formed in the presence of Gt factor, cellobiose, Fe^{3+} and O_2.

Figure 2 EPR spectra of DMPO-OH adduct obtained from Gt factor, cellobiose, Fe^{3+} and O_2. (A) without H_2O_2; (B) 1 mmol/L H_2O_2 was added.

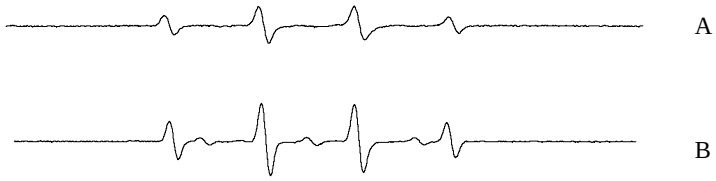
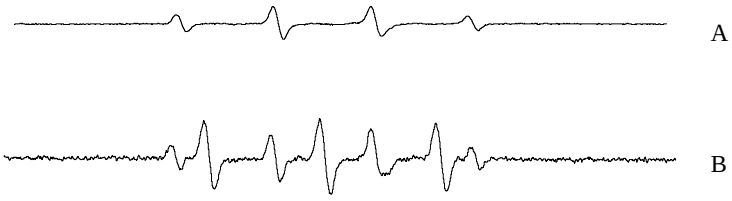


Figure 3 EPR spectra of DMPO-OH adduct obtained from Gt factor, cellobiose, Fe^{-} and O_2. (A)spectrum from Gt factor, cellobiose, Fe^{3+} and O_2.; (B)After addition of additional Gt factor.



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