

Aromatic ring cleavage of a β -biphenyl ether dimer catalyzed by lignin peroxidase of *Phanerochaete chrysosporium*

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Under aerobic conditions homogeneous lignin peroxidase catalyzed the oxidation of 1-(4'-methoxyphenyl)-2-(2'',5''-dimethoxy-4''-phenylphenoxy)-1,3-dihydroxypropane (**I**) to yield four products: 1-(4'-methoxyphenyl)-1,2,3-trihydroxypropane (**X**), 4-[α -hydroxy- α -(4'-methoxyphenyl)-methyl]-1,3-dioxolane-2-one (**V**), 4-(4'-methoxyphenyl)-5-hydroxymethyl-1,3-dioxolane-2-one (**VI**) and 5-hydroxy-5-carbomethoxy-4-phenyl-oxol-3-en-2-one (**VIII**). **V**, **VI** and **VIII** are all products of ring opening reactions. When the reaction was conducted under anaerobic conditions, the substrate was oxidized but no ring-cleaved products were detected. During the oxidation of **I**, 4 atoms of ^{18}O from $^{18}\text{O}_2$ were incorporated into the lactol product **VIII**.

Aromatic ring cleavage; Lignin degradation; β -Aryl ether; Lignin peroxidase; Aryl cation radical

1. INTRODUCTION

Recently, lignin peroxidase (LiP), a heme-containing, H_2O_2 -requiring enzyme, has been purified from the extracellular medium of the fungus *Phanerochaete chrysosporium* [1–3]. The H_2O_2 -oxidized states of LiP [3,4] are similar to those of HRP [5]. The homogeneous enzyme oxidizes a variety of lignin model compounds [1–3] including β -aryl ether monomers and dimers [6–8]. Earlier studies demonstrated that cultures of *P.*

chrysosporium oxidized lignin [9] and β -aryl ether dimeric model compounds [10] via pathways involving ring opening reactions. In this report, we show that the β -aryl ether dimer 1-(4'-methoxyphenyl)-2-(2'',5''-dimethoxy-4''-phenylphenoxy)-1,3-dihydroxypropane (**I**) is oxidized by homogeneous LiP to several products derived from ring opening reactions.

2. MATERIALS AND METHODS

LiP **II** was purified to homogeneity from acetate-buffered agitated cultures of *P. chrysosporium* [1,3]. Model compound oxidations were carried out at 37°C in 1 ml Na-succinate (pH 4.5) containing substrate (0.02%) and enzyme (5 μg). Reaction mixtures were purged with O_2 or evacuated and flushed with argon as indicated. Reactions performed under $^{18}\text{O}_2$ were carried out as described [11]. Reactions were initiated by addition of H_2O_2 (100 μM) and run for 15 min. NaCl was then added to saturation and the reaction mixtures extracted, dried and silylated (BSTFA/py-

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Abbreviations: LiP, lignin peroxidase; TMS-ether, trimethylsilyl ether; MS, mass spectrum

ridine, 2:1, v/v) [1,7]. Capillary GCMS was performed on a VG analytical 7070E instrument.

2.1 Preparation of compounds

1-(4'-Methoxyphenoxy)-2-(2'',5''-dimethoxy-4''-phenylphenoxy)-1,3-dihydroxypropane (**I**): 4-Phenylphenol and bromine dioxane complex in ether at 0°C to yield 2,6-dibromo-4-phenylphenol (**II**). MS (*m/z*) (mono TMS ether) 402 (M^+ , 10%); **II** and NaOMe (28% MeOH solution), CuI (catalytic) in DMF, at 120°C for 16 h yields 2,6-dimethoxy-4-phenylphenol (**III**). MS (*m/z*) (mono TMS ether) 302 (M^+ , 42%); condensation of **III** with 1-(4'-methoxyphenyl)-2-bromo-1-oxoethane, K_2CO_3 , in acetone yields 1-(4'-methoxyphenyl)-2-(2',6'-dimethoxy-4-phenylphenoxy) ethanone (**IV**); hydroxymethylation with HCHO followed by reduction with $NaBH_4$ [12] to yield **I**. MS (*m/z*) (di TMS ether) 554 (M^+ , 0.4%), 302 (29.8%), 230 (25.9%), 222 (23.3%), 162 (31.3%), 133 (43.3%).

4-[α -Hydroxy- α -(4'-methoxyphenyl)-methyl]-1,3-dioxolane-2-one (**V**) and 4-(4'-methoxyphenyl)-5-hydroxymethyl-1,3-dioxolane-2-one (**VI**) were synthesized by modification of a reported procedure [10]. *p*-Methoxyacetophenone was brominated. The product was treated with K-formate, reflux, for 20 h; hydroxymethylation with HCHO to yield 1-(4'-methoxyphenyl)-2,3-dihydroxypro-

pane-1-one (**VII**). **VII** and *N,N'*-carbonyldiimidazole in benzene, reflux; reduction with $NaBH_4$ to yield a mixture of **V** and **VI** which were separated on silica gel (hexane, ethyl acetate). **V**: MS (*m/z*) (mono TMS ether) 296 (M^+ , 0.3%), 237 (3.5%), 209 (100%), 135 (17%), 121 (7.3%), 101 (7.4%). **VI**: MS (*m/z*) (mono TMS ether) 296 (M^+ , 3.1%), 206 (9.2%), 162 (64.6%), 135 (85%), 121 (100%), 103 (17.7%).

VIII: 3-Phenyl-2-pentendioic acid dimethyl ester (**IX**) was prepared by a described procedure [13,14]. **IX** MS (*m/z*) 234 (M^+ , 10.9%), 202 (54.3%), 174 (100%), 159 (32.3%), 115 (93.4%). **IX** and one equivalent SeO_2 in acetic acid were stirred at 120°C for 16 h to yield the lactol **VIII**. 1H NMR $\delta(CDCl_3)$: 3.82 (3H, s, OMe), 6.53 (1H, s, =CH), 7.3–7.6 (5H, m, Ar-H), 5.3 (1H, –OH). MS (*m/z*) (mono TMS ether) 306 (M^+ , 2.7%), 291 (14.5%), 247 (78.7%), 219 (8.2%), 102 (39.4%), 89 (39.9%), 73 (100%).

X: 1-(4'-Methoxyphenyl)-1,2,3-trihydroxypropane was prepared from 4-methoxycinnamate by a procedure described in [15].

3. RESULTS

As shown in fig.1, under oxygen **I** is oxidized by LiP to produce four identifiable products: **X** (5.34 mol%), MS (*m/z*) (414 M^+); the lactol **VIII**

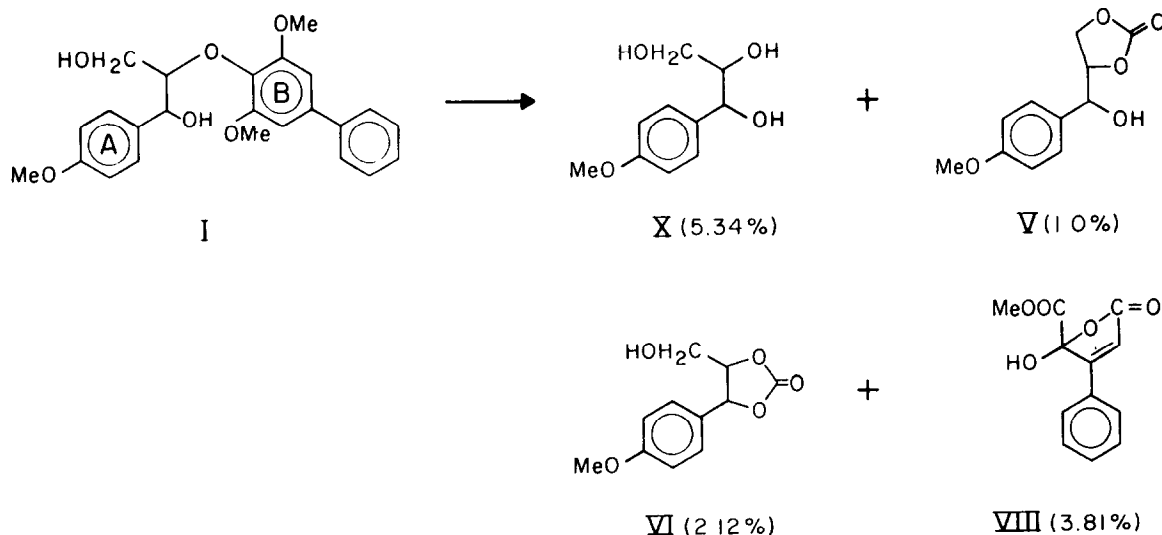


Fig.1. Oxidative cleavage of the β -aryl ether dimer **I** by homogeneous lignin peroxidase. % = (mol of product formed/mol of initial substrate) \times 100.

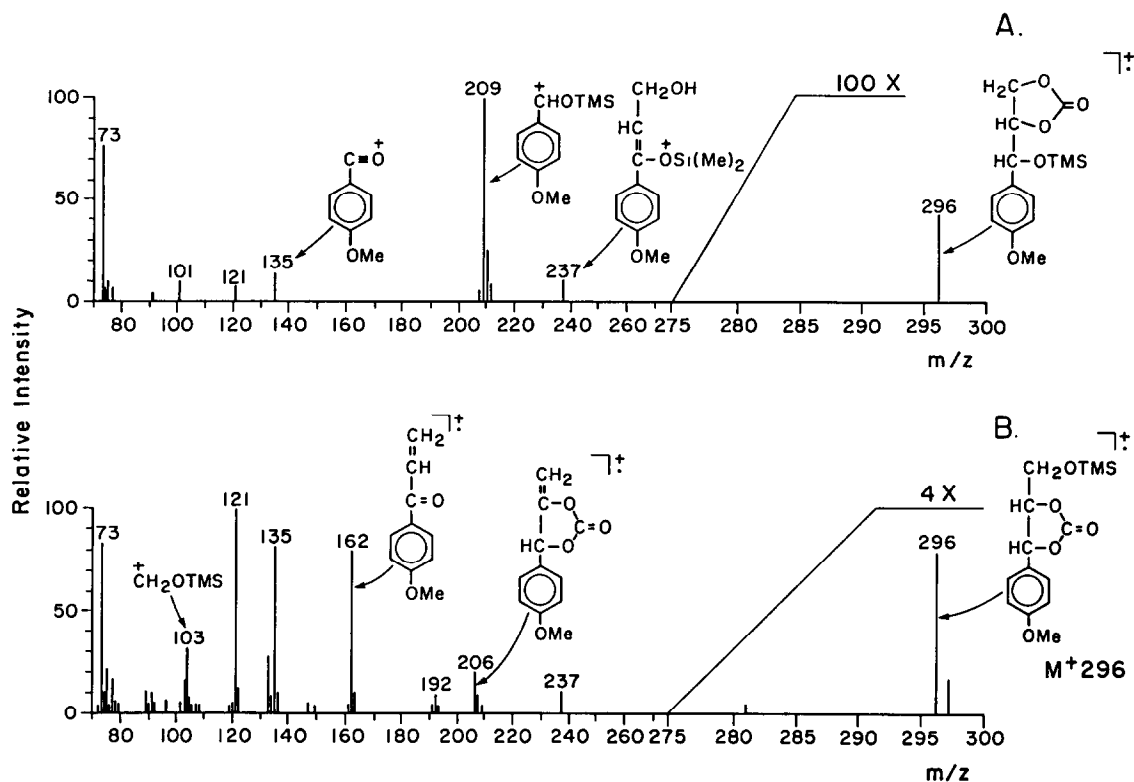


Fig.2. (A) Mass spectra of the TMSi derivatized V formed under $^{16}O_2$. (B) Mass spectra of the TMSi derivatized VI formed under $^{16}O_2$.

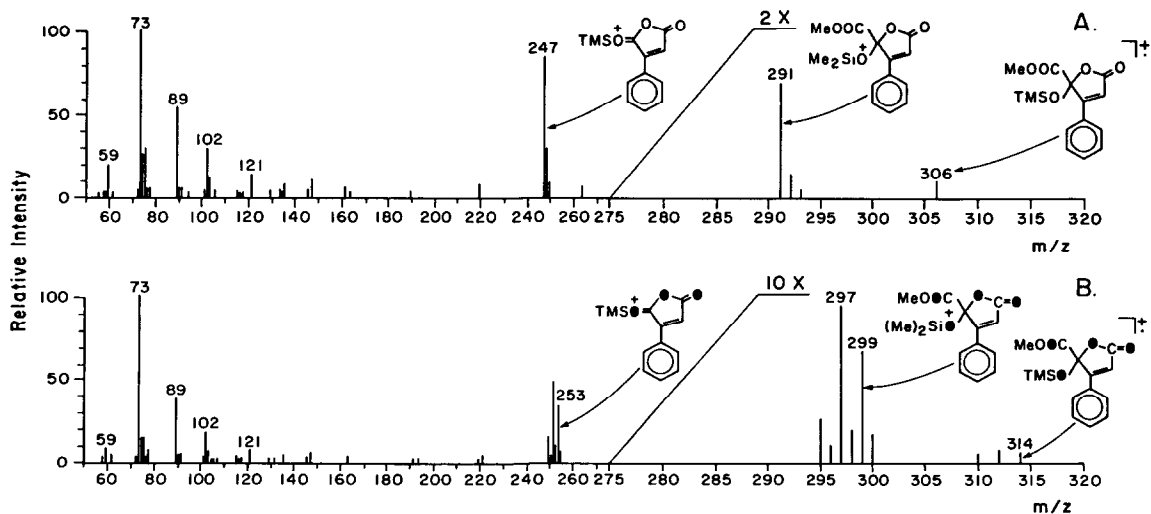


Fig.3. Mass spectra of the TMSi derivatized lactol VIII. (A) Reaction conducted under $^{16}O_2$. (B) Reaction conducted under $^{18}O_2$.

(3.81%), the dioxolane-2-one **V** (1.0%), and the dioxolane-2-one **VI** (2.12%). No products were obtained when the reactions were conducted in the absence of H_2O_2 or enzyme. Ring-opened products were not obtained when the reaction was conducted in the absence of oxygen.

Fig.2A shows the mass fragmentation pattern of the dioxolane-2-one **V**. An identical pattern was obtained when the reaction was conducted under $^{18}\text{O}_2$, indicating the ^{18}O was not incorporated into the product. Fig.2B shows the mass fragmentation pattern of the dioxolane 2-one **VI**. When the reaction was conducted under $^{18}\text{O}_2$, increased ions at 298 and 208 (not shown) indicated 30 atom% incorporation of the oxygen in the keto position.

Fig.3 shows the mass fragmentation patterns of the lactol **VIII** obtained from the oxidation of **I** under $^{16}\text{O}_2$ and under $^{18}\text{O}_2$. The MS of the lactol obtained under $^{16}\text{O}_2$ has a molecular ion at 306 and diagnostic ions at 291, 247, and 89. The MS of the lactol obtained under $^{18}\text{O}_2$ has a molecular ion at 314 and diagnostic ions at 299, 253 and 89, indicating that a maximum of 4 atoms of oxygen were incorporated into the product.

4. DISCUSSION

Earlier studies have shown that under aerobic conditions, cultures of *P. chrysosporium* oxidize both lignin [9] and β -aryl ether dimeric compounds [10] via pathways involving ring-opening reactions. Recently, it has been shown that lignin peroxidase can open the aromatic ring of veratryl alcohol [16]. Although it has just been reported that this enzyme also opens rings in β -aryl ether dimers [17,18], a mechanism has not been suggested. In this study we use a unique β -aryl ether substrate containing a biphenyl derivative of ring B which allowed the isolation, for the first time, of a B-ring opened aromatic product. Dioxygenases are the only oxygen-mediated enzymes previously known to open aromatic rings. These non-heme iron proteins, which require *o*-diphenolic substrates, activate molecular oxygen during the reaction [19]. In contrast, LiP is a peroxidase [4,11, 20,21] and is not known to activate molecular O_2 [11,20]. The results reported here, together with other currently available evidence on the mechanism of LiP, enable us to propose a mechanism for aromatic ring cleavage by this enzyme. This

mechanism involves an initial one-electron oxidation of ring B to form an aryl cation radical [11,20,21]. This intermediate undergoes one of the following reactions: (a) nucleophilic attack by either the α - or γ -hydroxyl on the ether carbon of ring B and loss of a proton to form a 1,3-dioxolane-cyclohexadiene radical structure as described [6,7]; (b) nucleophilic substitution by the α -hydroxyl on a methoxyl-bearing carbon of ring B with the elimination of methanol and formation of a benzodioxane radical intermediate [7]. The structural resemblance of the ring-cleaved products **V** and **VI** to the 1,3-dioxolane-cyclohexadiene radical suggests that this radical is an intermediate in the formation of the dioxolane-2-ones **V** and **VI**. Earlier reports have described non-enzymatic ring cleavage of cyclohexadiene radicals in the presence of O_2 [22]. By analogy the cyclohexadienyl radical produced by LiP could lead to a dioxygenase-like ring fission, producing the 1,3-dioxolane-2-ones. This is supported by our observation that ring cleavage occurs only in the presence of O_2 . The lactol **VIII** is a novel product because it retains almost all of the carbons of ring B. During its formation, a maximum of 4 atoms of oxygen are incorporated into the product **VIII**. The loss of one methoxyl group during the formation of **VIII** indicates that this product arises from a demethoxylated intermediate such as the benzodioxane described above. It is therefore possible that the lactol **VIII** may not be the counterpart product of **V** and **VI**. In contrast to dioxygenase reactions the novel ring cleavage proposed here appears to occur through the non-enzymatic reaction between an enzyme-produced radical and molecular oxygen.

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