View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Volume 210, number 2, 199–203 FEB 04346 January 1987

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

Keiji Miki*, V. Renganathan+, Mary B. Mayfield and Michael H. Gold

Department of Chemical and Biological Sciences, Oregon Graduate Center, Beaverton, OR 97006-1999, USA

Received 29 October 1986

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 $^{18}O_2$ 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.

Correspondence address: M.H. Gold, Department of Chemical and Biological Sciences, Oregon Graduate Center, Beaverton, OR 97006-1999, USA

* Present address: National Research Institute for Pollution and Resources, Yatabe, Tsukuba, Ibaraki 305, Japan

\+ Present address: Smith Kline & French Laboratories, 709 Swedeland Road, Swedeland, PA 19479, USA

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

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 $^{18}O_2$ 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-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50 © 1987 Federation of European Biochemical Societies

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-oxo-ethane, 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-dihydroxypropane-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**

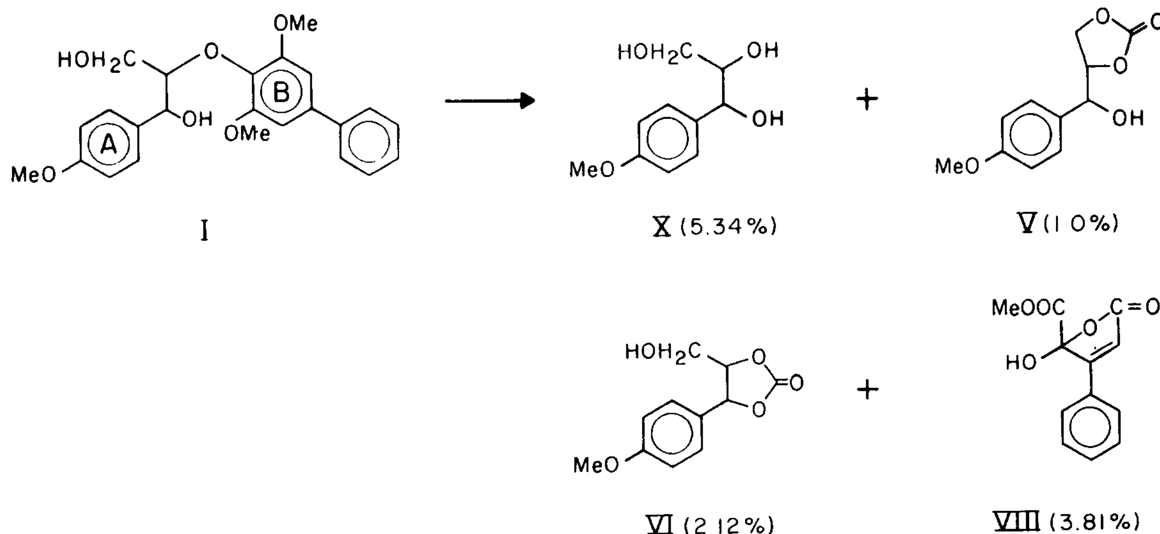

Fig.1. Oxidative cleavage of the β-aryl ether dimer **I** by homogeneous lignin peroxidase. % = (mol of product formed/mol of initial substrate) × 100.

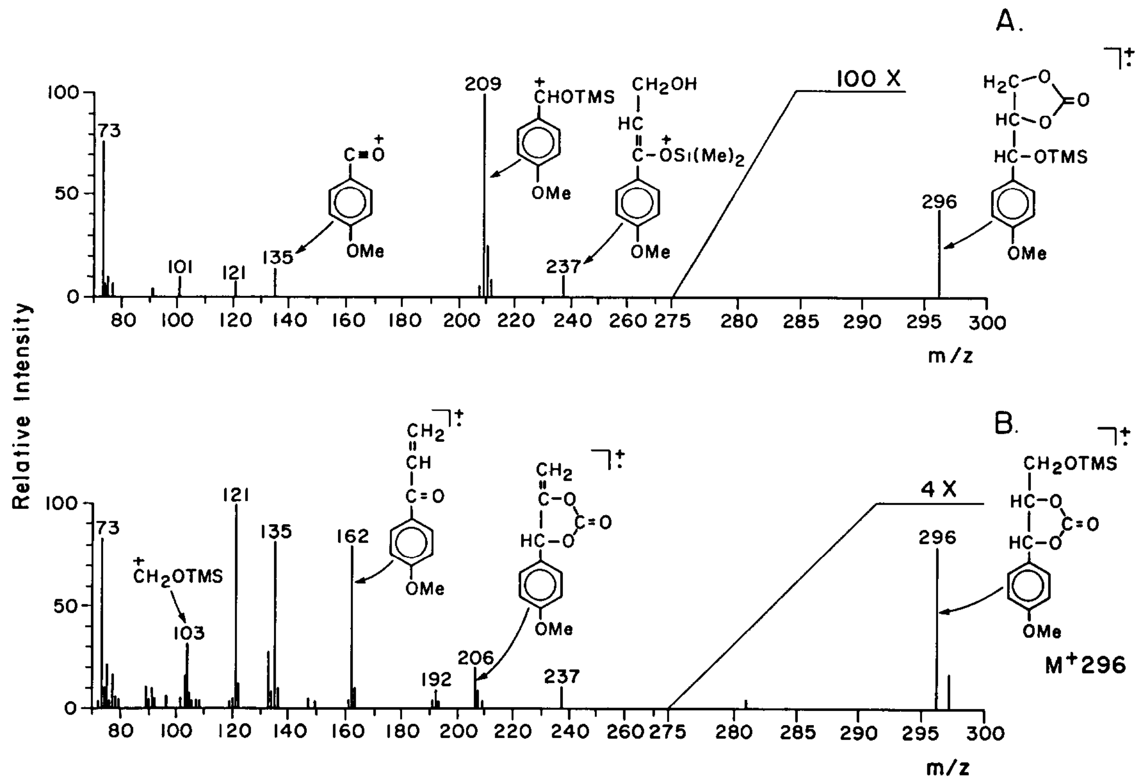

Fig.2. (A) Mass spectra of the TMSi derivatized **V** formed under $^{16}O_2$. (B) Mass spectra of the TMSi derivatized **VI** formed under $^{16}O_2$.

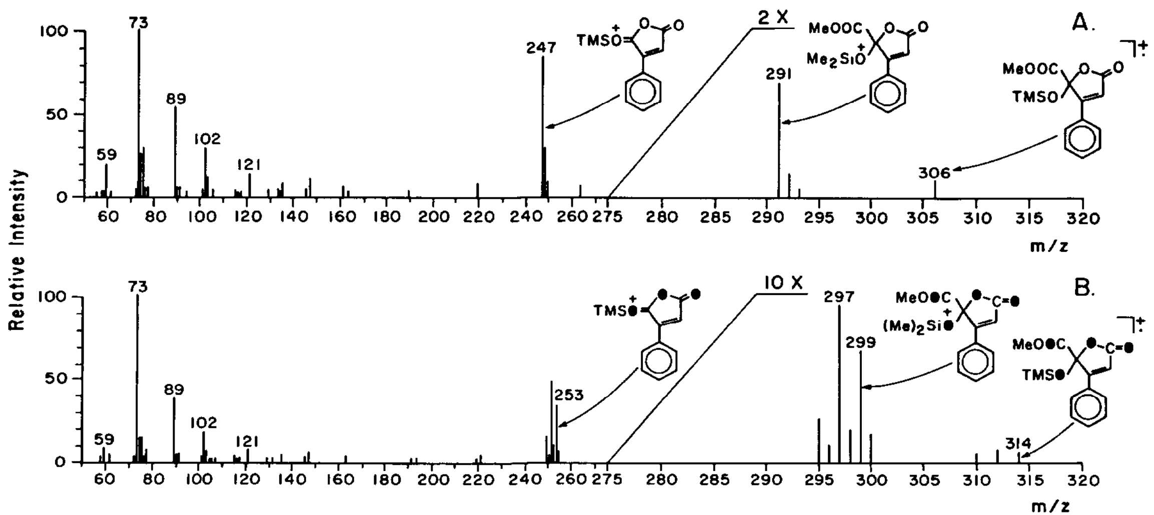

Fig.3. Mass spectra of the TMSi derivatized lactol **VIII**. (A) Reaction conducted under $^{16}O_2$. (B) Reaction conducted under $^{18}O_2$.

(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 $^{18}O_2$, 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 $^{18}O_2$, 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 $^{16}O_2$ and under $^{18}O_2$. The MS of the lactol obtained under $^{16}O_2$ has a molecular ion at 306 and diagnostic ions at 291, 247, and 89. The MS of the lactol obtained under $^{18}O_2$ 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.

ACKNOWLEDGEMENTS

Supported by grant DE-FG06-86ER13550 from the USDOE and by grant DMB 8311441 from the NSF.

REFERENCES

[1] Gold, M.H., Kuwahara, M., Chiu, A.A. and Glenn, J.K. (1984) Arch. Biochem. Biophys. 234, 353–362.

[2] Tien, M. and Kirk, T.K. (1984) Proc. Natl. Acad. Sci. USA 81, 2280–2284.

[3] Renganathan, V., Miki, K. and Gold, M.H. (1985) Arch. Biochem. Biophys. 241, 304–314.
[4] Renganathan, V. and Gold, M.H. (1986) Biochemistry 25, 1626–1631.
[5] Dunford, H.B. (1982) in: Inorganic Biochemistry (Eichorn, G.L. and Marzilli, L.G. eds) vol. 4, pp. 4–68, Elsevier, Amsterdam, New York.
[6] Miki, K., Renganathan, V. and Gold, M.H. (1986) FEBS Lett. 203, 235–238.
[7] Miki, K., Renganathan, V. and Gold, M.H. (1986) Biochemistry 25, 4790–4796.
[8] Kirk, T.K., Tien, M., Kersten, P.J., Mozuch, M.D. and Kalyanaram, B. (1986) Biochem. J. 236, 279–287.
[9] Chen, C.L., Chang, H.M. and Kirk, T.K. (1983) J. Wood Chem. Technol. 3, 35–57.
[10] Umezawa, T. and Higuchi, T. (1985) FEBS Lett. 182, 257–259.
[11] Renganathan, V., Miki, K. and Gold, M.H. (1986) Arch. Biochem. Biophys. 246, 155–161.
[12] Enoki, A., Goldsby, G.P. and Gold, M.H. (1980) Arch. Microbiol. 125, 227–232.
[13] Apparao, S., Datta, A. and Junjappa, H. (1985) Synthesis 2, 169–171.
[14] Potts, K.T., Cipullo, M.J., Ralli, P. and Theodiridis, G. (1982) J. Org. Chem. 47, 3027–3038.
[15] Enoki, A., Goldsby, G.P. and Gold, M.H. (1981) Arch. Microbiol. 129, 141–145.
[16] Leisola, M.S.A., Schmidt, B., Thanei-Klyss, U. and Fiechter, A. (1985) FEBS Lett. 189, 267–270.
[17] Umezawa, T., Shimada, M., Higuchi, T. and Kusai, K. (1986) FEBS Lett. 205, 287–292.
[18] Umezawa, T. and Higuchi, T. (1986) FEBS Lett. 205, 293–298.
[19] Arciero, D.M. and Lipscomb, J.D. (1986) J. Biol. Chem. 261, 2170–2178.
[20] Kersten, P.J., Tien, M., Kalyanaraman, B. and Kirk, T.K. (1985) J. Biol. Chem. 260, 2609–2619.
[21] Shoemaker, H.E., Harvey, P.J., Bowen, R.M. and Palmer, J.M. (1985) FEBS Lett. 183, 7–12.
[22] Atkinson, R. (1985) Chem. Rev. 85, 69–201.