Novel aryl ether rearrangement catalyzed by lignin peroxidase of *Phanerochaete chrysosporium*

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Received 4 June 1986

Lignin peroxidase catalyzed the rearrangement of 2-(2′-methoxy-4′-propylphenoxy)-1,3-dihydroxypropane **I** to 3-(2′-methoxy-4′-propylphenoxy)-1,2-dihydroxypropane **VI** with a maximum product yield of 64%. The reaction was strongly inhibited by oxygen. The enzyme also catalyzed the rearrangement of 2-(2′-methoxy-4′-propylphenoxy)-1-hydroxypropane **IV** to form 1-(2′-methoxy-4′-propylphenoxy)-2-hydroxypropane **VIII**. When **IV** was labeled with ^{18}O in the 1-hydroxyl group, all of the ^{18}O was retained in the ether oxygen of the product **VIII**. A mechanism explaining these results involves: (i) one electron oxidation of the substrate to form an aryl cation radical; (ii) nucleophilic attack by the hydroxyl oxygen on the cation radical with release of a proton, resulting in a dioxolane radical intermediate; (iii) cleavage of the latter and subsequent hydrogen abstraction to form the rearranged product.

Lignin degradation *β-Aryl ether* *Peroxidase* *(Basidiomycete)* *Aryl cation radical* *Hydrogen peroxide*

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]. Earlier studies demonstrated that cultures of *P. chrysosporium* metabolized dimers containing the most prevalent linkage in lignin, the β-aryl ether bond, yielding α,β bond and β-ether bond cleavage fragments, as well as possible rearranged products [6–10]. LiP has been identified as the enzyme that is responsible for several of these transformations [1–3]. In this report, we show that the β-aryl ether monomer 2-(2′-methoxy-4′-propylphenoxy)-1,3-dihydroxypropane is oxidized by LiP to yield a rearranged product. A mechanism explaining this enzyme-catalyzed rearrangement is proposed.

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

2. MATERIALS AND METHODS

2.1. *Enzyme and reactions*

LiP **II** was purified to homogeneity from acetate-buffered agitated cultures of *P. chrysosporium* as in [1,3]. Model compound oxidations were carried out at 37°C in 1 ml Na succinate (pH 5.5) containing substrate (0.02%) and enzyme (5 μg). Reaction mixtures were evacuated and flushed with argon. Reactions were started with addition of H_2O_2 (100 μM) and run for 15 min. NaCl was added to saturation and mixtures were extracted, dried, and silylated (BSTFA/pyridine, 2:1, v/v) [1,7]. Capillary GCMS was performed on a VG Analytical 7070E instrument.

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00145793/86/$3.50

2.2. *Preparation of compounds*

2-(2′-Methoxy-4′-propylphenoxy)-1,3-dihydroxypropane **I**: (i) 2-Methoxy-4-propylphenol and diethylbromomalonate, K_2CO_3, in DMF, 20°C; (ii) $NaBH_4$ in ethanol, 0°C. MS(TMS-ether) **I** exhibited *m/z* (%): 384 (M^+, 19.8). 2-(4′-Propylphenoxy)-1,3-dihydroxypropane **II** and 2-(4′-methoxyphenoxy)-1,3-dihydroxypropane **III** were prepared by the same procedure. MS(TMS-ether) **II**, *m/z* (%): 354 (M^+, 28.2). MS(TMS-ether) **III**, *m/z* (%): 342 (M^+, 15.3). 2-(2′-Methoxy-4′-propylphenoxy)-1-hydroxypropane **IV**: (i) 2-Methoxy-4-propylphenol, bromoethylacetate, K_2CO_3 in DMF, 25°C; (ii) CH_3I, NaH in DMF, 25°C; (iii) $NaBH_4$ in ethanol, 0°C. MS(TMS-ether) **IV**, *m/z* (%): 296 (M^+, 28.4).

2-(2′-Methoxy-4′-propylphenoxy)-1-[^{18}O]hydroxypropane **IVa**: (i) Ethyl-2-(2′-methoxy-4′-propylphenoxy)propionate, $LiAlH_4$ in THF, −70°C to generate aldehyde; (ii) 5% HCl from Cl_2 gas and $H_2{}^{18}O$ (96%); (iii) $NaBH_4$ in $H_2{}^{18}O$, 0°C. MS(TMS-ether) **IVa**, *m/z* (%): 298 (M^+) (75 atom% excess).

2-(2′-Methoxy-4′-propylphenoxy)-1-[^{18}O]hydroxyethane **V** was prepared by the above procedure using 1,1-dihydroxy-2-(2′-methoxy-4′-propylphenoxy)ethane which was prepared as in [11]. 3-(2′-Methoxy-4′-propylphenoxy)-1,2-dihydroxypropane **VI** and 3-(4′-methoxyphenoxy)-1,2-dihydroxypropane **VII** were prepared from 3-chloro-1,2-dihydroxypropane and either 2-methoxy-4-propylphenol or 4-methoxyphenol using NaH, KI in DMF, 100°C. MS(TMS-ether) **VI**, *m/z* (%): 384 (M^+, 26.5). MS(TMS-ether) **VII**, *m/z* (%): 342 (M^+, 18.5). 1-(2′-Methoxy-4′-propylphenoxy)-2-hydroxypropane **VIII** was prepared from propylene oxide and 2-methoxy-4-propylphenol [12]. MS(TMS-ether) **VIII**, *m/z* (%) 296 (M^+, 3.33). Chemically synthesized compounds were also identified by ^{1}H NMR.

3. RESULTS

As shown in fig.1, the 2-phenoxy ether **I** was converted readily to the 3-phenoxy ether **VI**. A small amount of the α-hydroxypropyl product **IX** was also obtained. The percent yield of **VI** at various pH values was as follows: 3.0(12.0%), 3.5(20%), 4.0(25%), 4.5(35%), 5.0(50%), 5.5(64%), 6.0(1%). Under air at pH 5.5, the yield

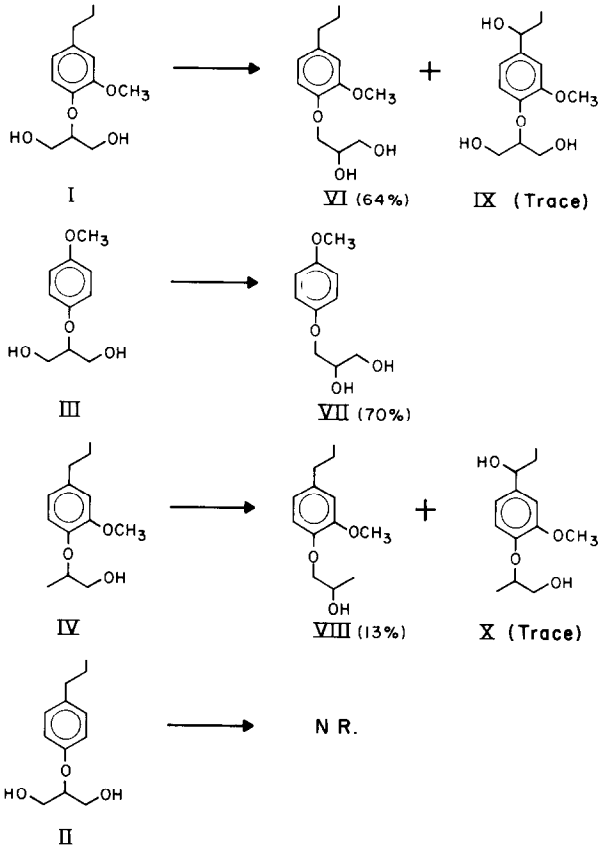

Fig.1. Aryl ether rearrangements catalyzed by homogeneous lignin peroxidase in the presence of H_2O_2 under argon. N.R. = no reaction.

of **VI** was only <2%; hence, all reactions were carried out under argon. No products were obtained in the absence of H_2O_2. Fig.1 shows the 2-phenoxy ether **III** was converted to the 3-phenoxy ether **VII** with a 70% yield at pH 5.5. 2-(2′-Methoxy-4′-propylphenoxy)-1-hydroxypropane **IV** was also converted to 1-(2′-methoxy-4′-propylphenoxy)-2-hydroxypropane **VIII** with a 13% yield. However, the 2-phenoxy ether **II** was not oxidized. Under identical conditions, the reverse reactions, i.e., conversion of **VI** to **I**, **VII** to **III** and **VIII** to **IV**, did not occur.

Fig.2 shows the mass fragmentation patterns of the products obtained from the oxidation of **IV** and **IVa**. The MS of the ^{16}O-containing product **IV** has a molecular ion at 296, and diagnostic ions at 179, 166, 137, 131, and 117. When **IVa** containing ^{18}O in the 1-hydroxyl group was used as the

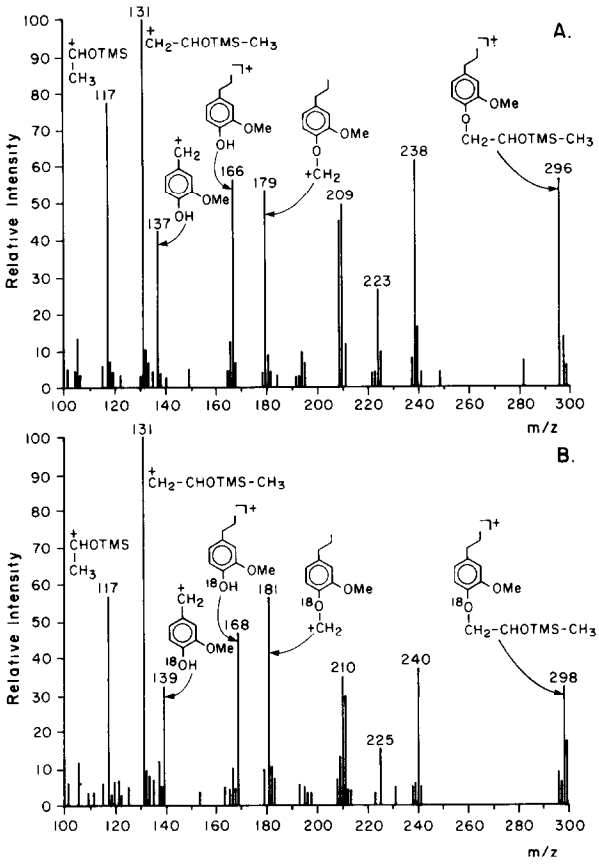

Fig.2. Mass spectra of the TMSi derivatized 1-(2′-methoxy-4′-propylphenoxy)-2-hydroxypropane. (A) Formed upon the rearrangement of 2-(2′-methoxy-4′-propylphenoxy)-1-[^{16}O]hydroxypropane **IV**. (B) Formed upon the rearrangement of 2-(2′-methoxy-4′-propylphenoxy)-1-[^{18}O]hydroxypropane **IVa**.

substrate, 100% of the ^{18}O was retained as the ether oxygen in the product (see molecular ion at 298 and diagnostic ions at 181, 168, 139, 131, and 117). These results indicate migration of the phenyl radical.

When the oxidation of **IV** was conducted under $H_2{}^{18}O$ (95 + %), no incorporation of ^{18}O was observed. Finally, when 2-(2′-methoxy-4′-propylphenoxy)-1-[^{18}O]hydroxyethane was used as a substrate, there was no evidence for ^{18}O incorporation into the ether position, indicating no analogous rearrangement.

4. DISCUSSION

Earlier studies demonstrated that several important non-phenolic β-aryl ether dimers are cleaved by ligninolytic cultures of *P. chrysosporium* [6–10]. Recently, it was proposed that 2-methoxyphenoxyethanol [6] was generated via a rearrangement of a β-O-4-aryl ether dimer, followed by cleavage of the rearranged intermediate [10]. However, owing to further oxidation, the proposed γ-aryl ether intermediate has not been identified in previous studies and the yield of the final product in whole cultures is very low (<3%) [6,10]. In order to thoroughly investigate β-aryl ether rearrangement reactions, we synthesized several β-aryl ether-containing monomeric substrates and used homogeneous LiP as the catalyst. The rearrangement of **I** to **VI** is strongly inhibited by O_2 which may explain the poor yields obtained with whole cultures [6,10] where aerobic conditions are required. The H_2O_2 requirement indicates the involvement of an oxidized enzyme intermediate as recently described [3,4].

The fact that the ^{18}O label of the hydroxyl oxygen of substrate **IVa** is retained in the ether oxygen of the product 1-(2′-methoxy-4′-propylphen[^{18}O]oxy)-2-hydroxypropane **VIIIa** is consistent with the migration of the phenyl but not of the phenoxy group. Incorporation of ^{18}O from solvent $H_2{}^{18}O$ into the product is not observed, indicating that migration is not accompanied by nucleophilic

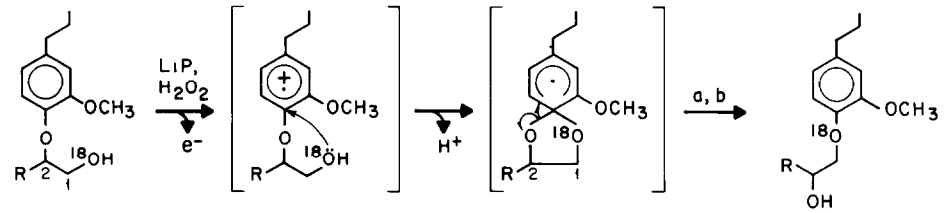

Fig.3. Proposed mechanism for the lignin peroxidase catalyzed β-aryl ether rearrangement. R, CH_2OH/CH_3; a, phenyl oxygen (C2) bond cleavage; b, hydrogen abstraction.

substitution of H_2O, i.e., the rearrangement mechanism probably does not involve a carbonium ion intermediate.

Recently, mechanisms have been proposed for several LiP-catalyzed reactions based upon the formation of an aryl cation radical [3,4,13–16]. Our recent observations indicate that the formation of an aryl cation radical by LiP requires at least two alkoxy groups on the benzene ring (in preparation). Two ring alkoxy substituents also appear to be required for aryl ether migration because unlike substrates **I** and **III**, compound **II**, which contains one alkoxy group, does not undergo aryl group migration (fig.1). Based on our present observations and on previous work [3,4,14–16], a mechanism for aryl group migration can be proposed (fig.3) involving: (i) initial one-electron oxidation of the substrate by LiP compound **I** or **II** [4] to form an aryl cation radical; (ii) nucleophilic attack by the hydroxyl oxygen on the cation radical with release of a proton [17] resulting in a cyclohexadiene radical with a bridged dioxolane structure; (iii) subsequent cleavage between the phenyl ring and C2 oxygen and abstraction of a hydrogen by the resultant C2 oxygen radical, yielding the rearranged product. The proton loss required by this mechanism may explain the higher pH optimum for this reaction as compared to other LiP-catalyzed reactions [1–3]. O_2 may inhibit the reaction by scavenging the cyclohexadiene radical (fig.3). This enzyme mechanism accounts for rearranged products isolated from whole cultures [6,10]. Furthermore, the aryl ether rearrangement reported here is unique to LiP and is not catalyzed by other known peroxidases. Studies to further elucidate this novel reaction are planned.

ACKNOWLEDGEMENT

This work was supported by Grant DMB 8311441 from the National Science Foundation.

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