Short Communication

New Mechanism for Oxygenative Ring Cleavage of 3,4-Dimethoxybenzyl Alcohol Catalyzed by the Ligninase Model

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Recently, biotechnology with white-rot fungi and their enzyme systems has been receiving much attention in the research and development of a new energy-saving and non-polluting delignification process for converting agricultural and forest lignocellulose materials.¹⁾ To this end, the biomimetic system²⁾ is very helpful not only for understanding the mechanisms of complex lignin biodegradation, but also in the search for a better catalyst that can be applied to industrial delignification.

We report here the first example of the oxygenative ring cleavage of 3,4-dimethoxybenzyl alcohol as a non-phenolic lignin model substrate (**1**),³⁾ by the use of hemin (protoporphyrin IX iron(III) chloride).

By analogy with the lignin peroxidase,³⁾ veratryl alcohol (**1**, 20 μ mol) in water (1.8 ml) was oxidized with *tert*-butyl hydroperoxide (Bu'OOH, 10 μ mol) in the presence of hemin (2 μ mol) in air at 30°C for 30 min. The ring cleavage products formed were identified by GC-mass- and NMR-spectrometries as the lactone isomers (**2**, **3** and **4**; Scheme 1), although veratraldehyde was the major product (90% yield based on the catalyst).

Tracer experiments with H₂¹⁸O (80 atom%) and ¹⁸O₂ (99 atom%) were carried out in a similar way. The results obtained in each case

FIG. 1. Mass-chromatograms of Fragment Ions for m/z 124 and 126 of the *cis*-Lactone **2** and *trans*-Lactone **3**.

a, the non-labelled lactones; **b**, the γ -lactones formed in the reaction system with H₂¹⁸O; **c**, the γ -lactones obtained from the substrate (**1**) labelled with ¹⁸O at the benzylic oxygen; **d**, the γ -lactones formed in the reaction system with ¹⁸O₂.

clearly indicate that only one ¹⁸O atom each from ¹⁸O-enriched water and dioxygen was incorporated into all the lactone products.

However, the striking evidence obtained is that, like ligninase,⁴⁾ the present enzyme model system also exhibited regiospecific oxygenation with water at the original C₃ position of **1** during the formation of the *cis*-isomer (**2**, 100% ¹⁸O incorporation), because both the non-labeled lactone (**2a**) and the one (**2b**) labeled with ¹⁸O-water gave common daughter ions at m/z 124, but no significant ion at m/z 126 (Fig. 1). The results suggest that the ¹⁸O atom from water was incorporated into the carbonyl oxygen of the lactone ring of **2b**, from which the labeled CO₂ was completely lost during the fragmentation process (**b** in Fig. 1). In order to substantiate this interpretation, the lactones (**2c**, **3c** and **4**) labeled with ¹⁸O at the etheric oxygen of the lactone moiety (96 atom% for all) were similarly prepared from the substrate (**1**) labeled with ¹⁸O at the benzylic oxygen, veratraldehyde being incubated with H₂¹⁸O and followed by NaBH₄

SCHEME 1. Mechanisms for the Oxygenative Ring Cleavage of **1** to form the Products (**2**, **3** and **4**) in the Hemin-catalyzed Reaction.

reduction. The mass-chromatograms of **2c** (Fig. 1) were almost the same as those of **2a** and **2b**. Thus, these fragmentation analyses conclusively indicate that the ¹⁸O of **2b** was lost, together with the CO₂ removed from the lactone ring, which had been formed by cyclization of the benzyl alcohol with C₃ of **1**. On the other hand, the product (**2d**) labeled with ¹⁸O-dioxygen yielded the significant daughter ion peak at *m/z* 126, despite the same fragmentation losing the CO₂. This indicates, in turn, that the oxygen (13 atom%) derived from ¹⁸O₂ was incorporated into the carbonyl oxygen of the methyl ester moiety of **2d** or the original C₄ position of **1** during the ring cleavage process.

No such regiospecific oxygenation was recognized for the formation of **4**, whose carbonyl oxygens were found to be differently labeled with ¹⁸O at either the lactone or methylester moiety. The reason for this random labeling is not clear at present, and elucidation of an alternative route (**Zb**; Scheme 1) is still under way.

In conclusion, the present enzyme model system simulates the regiospecificity of ligninase in the ring cleavage of 3,4-dimethoxybenzyl alcohol.⁴⁾ The result also indicates that regiospecific oxygenation is not necessarily

governed by the protein moiety of ligninase. The ring cleavage mechanism involving the oxo-iron(IV) porphyrin cation radical species (**X**) in a high-valent form to produce a possible aryl cation radical intermediate (**Za**) of **1** is shown in Scheme 1, which is in good accord with the one-electron oxidation mechanism previously proposed.⁵⁾

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