Incorporation of Dioxygen into the Hydroxylated Product during the C–C Single Bond Cleavage of 1,2-Bis(*p*-methoxyphenyl)propane-1,3-diol Catalysed by Hemin. A Novel Model System for the Hemoprotein Ligninase†

Tsuyoshi Habe,[a] Mikio Shimada,*[a] Tadashi Okamoto,[b] Bhinyo Panijpan,[c] and Takayoshi Higuchi[a]

[a] *Wood Research Institute, and* [b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*
[c] *Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand*

Oxidation of the lignin model compound 1,2-bis(*p*-methoxyphenyl)propane-1,3-diol (**1**) catalysed by hemin in the presence of t-butyl hydroperoxide and $^{18}O_2$ yielded *p*-methoxyphenylethane-1,2-diol (**2**) with 83% ^{18}O incorporation into the newly formed hydroxy group and *p*-anisaldehyde (**3**) as the initial Cα–Cβ bond cleavage products.

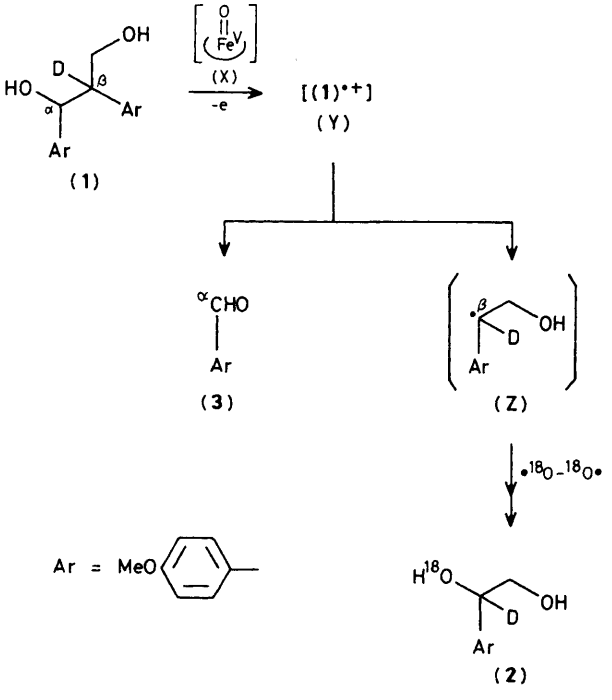
There is widespread current interest in the development of new technology for lignin conversion by use of the hemoprotein ligninase recently isolated from the white-rot fungus.[1,2] This enzyme catalyses a wide variety of oxidations including the unique oxygenative C–C bond cleavage of the lignin model compounds, diarylpropanediols, in the presence of H_2O_2 and O_2.[1,3] Since the enzymatic reaction mechanism is poorly understood, it is important to establish simple model systems[4] relevant to the complex biochemical lignin degradation. We report here new evidence for the title reaction occurring in an enzyme model system, which mimics the 'oxygenase' action of the ligninase.

By analogy with the enzyme, the Cα–Cβ bond cleavage of substrate (**1**) (5 μmol) was catalysed at 30 °C by hemin (0.5 μmol) in the presence of Bu^{t}OOH (25 μmol) in chloroform (1 ml) in the air. Complete degradation of (**1**) in 2 h yielded *p*-anisaldehyde (**3**) as the major product and the hydroxylated product (**2**) accompanied by *p*-anisic acid and the α-ketones of (**1**) and (**2**) in smaller amounts. The products were identified by g.c.–mass spectrometry, and determined by h.p.l.c. Compound (**2**) was also shown to undergo Cα–Cβ bond cleavage yielding (**3**). The reaction rates for the formation of (**3**) determined under 100 and 21% O_2 were almost equal [145% yield based on (**1**)], whereas the rate was reduced to about 1/10 in the absence of O_2 but the ultimate yield obtained after 20 h was not significantly affected.

The results of ^{18}O incorporations into the benzylic position of (**2**), *i.e.*, into the Cβ-position of (**1**), from $^{18}O_2$ or $H_2{}^{18}O$ under aerobic and anaerobic conditions are summarised in Table 1. The mass spectrum of the diacetate of (**2**) produced in the reaction system with 100% $^{18}O_2$ clearly showed 83% ^{18}O incorporation, which is comparable to the 91—95% ^{18}O incorporations reported with ligninase.[1,3] Conversely, the new observation of 16% ^{18}O incorporation from water is consistent with the above result. Interestingly, oxidation of (**1**) in the presence of $H_2{}^{18}O$ but under oxygen-free dinitrogen increased the incorporation of ^{18}O from water to 40%, which in turn indicates that at least 60% of oxygen at the benzylic position of (**2**) must be derived from Bu^{t}OOH. Thus, molecular oxygen is not a prerequisite for C–C bond cleavage nor for hydroxylation to form (**2**).

Another result which is relevant to ligninase is the finding that deuterium at Cβ of (**1**) (91%D)[4] or the benzylic position of (**2**) (98% D) was almost quantitatively retained in the product (**2**) or (**3**), respectively, after the Cα–Cβ cleavage in the present model system, and similar results were found with the enzyme prepared from ligninolytic culture.[1]

A one electron transfer mechanism is proposed (Scheme 1) to explain the Cα–Cβ bond cleavage of (**1**) concomitant with hydroxylation to form (**2**), in which formation of a cation radical (Y) of (**1**) by one electron abstraction with the oxo-iron porphyrin complex (X), equivalent to Compound I of peroxidase, is the initial step of the C–C bond cleavage.[5] A carbon-centred radical (Z) thus formed might eventually be converted into the hydroxylated product (**2**) *via* its dioxygen adduct or the related hydroperoxide intermediate. Further, electron abstraction from the radical (Z), particularly in the absence of dioxygen, to form the benzyl cation, and subsequent hydroxylation with water or the oxo-iron complex,[6] whose oxygen atom is quickly exchanged with water,[7] could explain the incorporation of hydroxylic oxygen from water. The proposed mechanism is in good accord with the earlier findings on (a) similar types of C–C bond cleavages caused by electrochemical oxidations,[8] (b) oxygenative C–C bond cleavage of indole acetic acid by horseradish peroxidase,[9] and (c) cation radical formation from 1,4-dimethoxybenzene with ligninase.[10] Although the detailed mechanism of the reaction



Scheme 1

† This work was presented at the 29th Lignin Symposium (Tokyo), 15th October, 1984, and the 35th Annual Meeting of the Wood Research Society of Japan (Tokyo), 2nd April, 1985. After submission of this communication, we became aware of a publication proposing a very similar single electron transfer mechanism (H. E. Schoemaker, P. J. Harvey, R. M. Bowen, and J. M. Palmer, *FEBS Lett.*, 1985, **183**, 7).

Table 1. Incorporation of ^{18}O atom from $^{18}O_2$ or $H_2{}^{18}O$ into the hydroxylated product (**2**).[a]

Atmosphere	Presence of water	% Incorporation
100% $^{18}O_2$ (98.8 atom%)	Small amount of $H_2{}^{16}O$	83
21% O_2 (air)	100 μl of $H_2{}^{18}O$ (98.4 atom%)	16
N_2	100 μl of $H_2{}^{18}O$ (98.4 atom%)	40

[a] Reaction conditions are as described in the text.

requires further investigation, the present paper presents the first model system simulating ligninase and exhibiting both the oxygenative C–C bond cleavage and the D-retention patterns.

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References

1 M. Tien and T. K. Kirk, *Proc. Natl. Acad. Sci. USA,* 1984, **81**, 2280.
2 M. H. Gold, M. Kuwahara, A. A. Chiu, and J. K. Glenn, *Arch. Biochem. Biophys.,* 1984, **234**, 353.
3 M. Kuwahara, J. K. Glenn, M. A. Morgan, and M. H. Gold, *FEBS Lett.,* 1984, **169**, 247.
4 T. K. Kirk and F. Nakatsubo, *Biochim. Biophys. Acta,* 1983, **756**, 376.
5 M. Shimada, T. Habe, T. Umezawa, T. Higuchi, and T. Okamoto, *Biochem. Biophys. Res. Commun.,* 1984, **122**, 1247.
6 J. T. Groves, R. C. Haushalter, M. Nakamura, T. E. Nemo, and B. J. Evans, *J. Am. Chem. Soc.,* 1981, **103**, 2884; J. T. Groves and D. V. Subramanian, *ibid.,* 1984, **106**, 2177.
7 J. T. Groves and W. J. Kruper, Jr., *J. Am. Chem. Soc.,* 1979, **1**, 7613.
8 E. M. Maeda, *J. Am. Chem. Soc.,* 1975, **97**, 4012; M. H. Hassen and A. Rieker, *J. Chem. Res.,* 1977, (*S*) 316; (*M*) 3619.
9 S. Kobayashi, K. Sugioka, N. Nakano, M. Nakano, and S. T. Kubota, *Biochemistry,* 1984, **23**, 4589.
10 P. J. Kersten, M. Tien, B. Kalyanaraman, and T. K. Kirk, *J. Biol. Chem.,* 1985, **260**, 2609.