Exhaustive laccase-catalysed oxidation of a lignin model compound (vanillyl glycol) produces methanol and polymeric quinoid products

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(Received 20 March 1985/3 May 1985; accepted 13 May 1985)

Laccase-catalysed oxidation of the lignin-related phenol vanillyl glycol results in the initial formation of dimers and subsequent polymerization. The polymerization is accompanied by a liberation of methanol corresponding to 15–20% demethylation. Visible spectra together with reduction experiments suggest the simultaneous formation of *o*-quinones. The participation of quinone formation in the polymerization process and the possible role of such intermediates in lignin biodegradation is discussed.

We have reported previously a study of the oxidation of the lignin-related compound vanillyl glycol (compound I, Scheme 1, 4-hydroxy-3-methoxyphenylpropane-1,2-diol) by laccase, peroxidase and tyrosinase (Bergbom *et al.*, 1981). Oxidation catalysed by fungal laccase resulted in dimerization (biphenyl, Compound II, Scheme 1, was the primary product) and subsequent polymerization. Dimer formation is the expected outcome of oxidative phenol coupling proceeding via initial formation of phenoxyl radicals. To gain more information about the reactions involved in the transformation of vanillyl glycol to polymers, we have now investigated reaction mixtures from exhaustive oxidation by laccase. Knowledge about

such reactions is of interest in connection with the role of laccase and other phenol-oxidizing enzymes in lignin biodegradation (Kirk, 1984; Kirk & Shimada, 1985).

Vanillyl glycol was subjected to exhaustive laccase-catalysed oxidation in an aqueous medium (pH 5). The reaction mixture acquired a strong wine-red colour. No precipitation of reaction products was observed, in all likelihood a consequence of the strongly hydrophilic character of vanillyl glycol.

Gas-chromatographic analysis showed that the oxidation had resulted in a liberation of methanol that corresponded to 15–20% demethylation. The red colour of the reaction mixture indicates that the

Scheme 1. Conceivable reaction route for the polymerization of vanillyl glycol (I) during enzymic oxidation

demethylation is accompanied by quinone formation. This is supported by the fact that the reaction mixtures were largely decolorized on reduction with SO_2 or ascorbic acid (Figs. 1 and 2). *o*-Quinones of type (III) (Scheme 1), which are expected to be formed in connection with the demethylation, exhibit a visible absorption maximum around, or slightly above, 400 nm (ϵ_{max} values are roughly of the order of $1500 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) (Rieker *et al.*, 1969). The decrease in absorbance at 410 nm on reduction corresponds to the presence of about 7% quinone groups of type (III) (Scheme 1). The structural changes on oxidation also cause an

Fig. 1. Decrease in molar absorptivity ($\Delta\epsilon_r$) on reduction of enzyme-oxidized vanillyl glycol by ascorbic acid (—) or SO_2 (----)

Results were calculated from the spectra of solutions A, B and C (see the Experimental section).

Fig. 2. U.v. spectra of vanillyl glycol(III, Scheme 1) in water (—) (λ_{max} , 279 nm, $\epsilon = 2759 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) and its reaction product from extensive laccase-catalysed oxidation [----; solvent dimethyl sulphoxide/water (1:1, v/v)]

increase of the absorbance in the u.v. region (Fig. 2). [The increase in absorbance at 280 nm is slight, and most of the increase in absorbance at this wavelength found in previous gel-permeation-chromatographic work (Bergbom *et al.*, 1981) is an artefact, an effect related to the bandwidth of the filter used in the spectrometer.]

Our previous study showed that laccase-catalysed oxidation of vanillyl glycol results initially in the formation of dimers. Quinone structures like *o*-quinones of type (III) (Scheme 1) are known to be reactive and are probably involved in the transformation of the dimers to polymeric material (Scheme 1; cf. Adler & Berggren, 1960; Flaig *et al.*, 1963; Musso *et al.*, 1964).

Demethylation, quinone formation and oligomerization have been reported in recent studies of the oxidation, by phenol-oxidizing enzymes, of vanillic acid (Ishihara & Ishihara, 1975; Bollag *et al.*, 1982; Ander *et al.*, 1983) and syringic acid (Ishihara & Ishihara, 1975, 1976; Liu *et al.*, 1981; Ishihara, 1983). Oxidative elimination of the carboxy group is characteristic in the reactions with these compounds. Thus a direct comparison with the vanillyl glycol results is difficult. Our studies with vanillyl glycol show, however, that a carboxy substituent is not a requirement for the reactions observed with vanillic acid and syringic acid (besides decarboxylation).

Demethylation in connection with phenol oxidation probably involves an acid-catalysed liberation of methanol. Initially formed dimers of vanillyl glycol lack free *ortho* and *para* positions, and enzymic oxidation can therefore be expected to result in the formation of products of the cyclohexadienone type (for examples, see Lundquist & Ericsson, 1971). The susceptibilities of methoxy groups in such compounds to acid-catalysed hydrolysis has been studied (Lundquist & Ericsson, 1971; Hemrá & Lundquist, 1973). Such an acid-catalysed mechanism provides an explanation for the pH-dependence of the demethylation reaction (Ishihara, 1983).

In conclusion, our results show, for the first time, that extensive oxidation of phenolic lignin-related phenylpropane structures by fungal laccase leads to significant demethylation and quinone formation. During biodegradation, quinones may undergo addition of water with formation of phenolic structures susceptible to ring cleavage; both methoxy loss and ring cleavage occur in the lignin polymer during fungal degradation (Kirk, 1984). A contributory role for extracellular laccase in such reactions is supported by our results. It should, however, be kept in mind that oxidases other than laccases operate during fungal lignin decay; one of them is even capable of oxidizing non-phenolic phenylpropane units (Kirk & Shimada, 1985).

Experimental

Determination of methanol by gas chromatography

The column (20 cm $\times$ 0.3 cm outer diam.) was made from stainless-steel tubing. The solid support was Chromosorb W, and the stationary phase was 5% 20 M Carbowax terminated with terphthalic acid. The column temperature was 100°C and the carrier gas was N₂ (flow 25 ml/min). Butanol was used as the internal reference.

Enzymic oxidation and spectral examinations

Vanillyl glycol (I, Scheme 1) (198 mg, 1 mmol) was dissolved in 45 ml (20 ml of 0.1 M-acetate buffer, pH 5, and 25 ml of water). After the addition of fungal laccase (2 mg), the solution was stirred magnetically for 100 h (cf. Bergbom *et al.*, 1981). The spectrum of the reaction mixture diluted with dimethyl sulphoxide/water (1:1, v/v) is given in Fig. 2. Dimethyl sulphoxide was added to secure complete dissolution of the reaction products.

Solutions used for the examinations of spectral properties in the visible region were prepared as follows from a stock solution prepared by acidification (42 μ l of constant-boiling HCl) of 15 g of the reaction mixture from enzymic oxidation. Solution A: water (3 g) and dimethyl sulphoxide (5 g) were added to the stock solution (2 g). Solution B: ascorbic acid (18 mg) in water (0.1 ml) was added to the stock solution (1 g). After 20 min the mixture was diluted with water (1.5 g) and dimethyl sulphoxide (2.5 g). Solution C: 5% (w/v) SO₂ solution (120 μ l) was added to the stock solution

(1 g). After 20 min, water (1.5 g) and dimethyl sulphoxide (2.5 g) was added.

We thank Dr. T. K. Kirk for valuable discussions.

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