

Lignin Peroxidase-Catalyzed Depolymerization
of Water Soluble Polymer
Derived from Subbituminous Coal and Lignite

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In view of reports that enzymes can depolymerize or otherwise alter lignin, it is to be anticipated that enzymes will also attack some of the linkages in coal. However, the solid and vitreous nature of coal precludes a direct test of this possibility. For this reason, it has been necessary to prepare a coal substrate with minimal chemical alteration which is water soluble in the pH range where enzymes are active.

This report describes the preparation and characterization of a coal liquid substrate suitable for enzymes and the ability of lignin peroxidase to alter this material.

Preparation of Coal Polymer

Use was made of the well-known ability of nitric acid to oxidize low ranked coals and lignite to yield an alkali soluble material. Ground North Dakota lignite or German subbituminous coal was treated with 20% to 40% nitric acid at less than 70°C for 2 to 6 hours. The washed and dried material was suspended in 1N NaOH at room temperature for 30 minutes and then centrifuged. HCl was added to the brown-black supernatant solution to pH 3.5, and the copious brown-black precipitate, which salted out with the 1N NaCl produced on acidification, recovered by centrifugation. In an alternative procedure, the nitric acid-treated subbituminous coal was suspended in water and the pH raised to 10 to 11 with NaOH. After acidification to pH 3.5 with HCl and centrifugation, NaCl was added to the brown-black supernatant to 1M concentration and the precipitate recovered by centrifugation.

The salted out precipitate obtained in either procedure was extracted with water at room temperature for 20 minutes to 12 hours to obtain a dark-brown soluble material which was dia-lyzed and retained in 12,000-14,000 dalton cut off membranes.

The yield of soluble organic carbon by an acid dichromate colorimetric method was 35 to 61% based on the organic carbon content of the nitric acid-treated material.

The water soluble material was also soluble in dimethyl formamide, dimethyl sulfoxide, tetrahydrofuran, sparingly soluble in methanol, and insoluble in acetonitrile, ethyl acetate, hexane and benzene.

Gel filtration chromatography of the soluble material from North Dakota lignite on Sephadex G-75 and G-200 with effluent monitoring at 254 nm revealed a single relatively narrow peak migrating with Blue Dextran on G-75 and slightly slower than Blue Dextran on G-200. Similar results were obtained with the soluble material from German subbituminous coal by high performance gel filtration chromatography with the exception that in addition to the major peak of about 125,000 daltons, there were minor components of about 50,000 and 32,000 daltons. No low molecular weight components were present in either preparation. In dialysis experiments at 0, 1, and 3M KCl and at acidic and basic pH values, virtually all of the material was retained. From these results it is concluded that the bulk of the pH 3.5 water soluble material had a molecular weight of greater than 100,000 daltons, and as such is a satisfactory substrate for enzyme studies.

Lignin Peroxidase

Lignin peroxidase of Phanerochaete chrysosporium is unique among enzymes: (a) in its ability to catalyze reactions without regard for stereoconfiguration of the substrate; and (b) in the seemingly large number of different reactions it catalyzes. In this regard both lignin and coal are appropriate substrates being stereoirregular polymers with a multiplicity of intercomponent linkages.

The partially purified lignin peroxidase used in these experiments was recovered from Phanerochaete chrysosporium cultures by adsorption to and specific elution from DEAE-Sephadex coated porous silica (Macrosorb Kax.DEAE, Sterling Organics US, New York, NY) and concentration on an American YM-10 membrane (American Corporation, Danvers, MA). Manganese peroxidase activity was virtually absent from these preparations. Except for this recovery procedure, growth of the organisms, enzyme assay, etc., followed published procedures.

Incubation of Coal and Lignite Preparations with Lignin Peroxidase

Soluble coal and lignite preparations were incubated at 37°C with lignin peroxidase in tartrate buffer, pH 3.0, and H₂O₂ for 4 to 24 hours. The incubations were oxygenated periodically. Aliquots of reaction mixtures were either directly applied to conventional gel permeation columns of Sephadex G-200 or to high performance gel permeation chromatographic columns. In some analyses samples were first treated with acetone-dimethyl formamide (1:1). Following removal of denatured protein by centrifugation, the supernatant solution was recovered, dried, and dissolved in buffer for gel permeation chromatography (0.02 M KPO₄, pH 7 - 0.5% Tween 80).

The elution diagrams for soluble material from North Dakota lignite obtained by monitoring the effluent at 254 nm showed a substantial loss of the major peak for the polymer and formation of a mixture lower molecular weight components in low yield; no production of "monomeric components" was not observed. With more peroxidase and incubation at pH 4.5, the accumulation of lower molecular fragments was enhanced.

Similar results were obtained with soluble polymer from German subbituminous coal. There was a substantial disappearance of the major peak and an appearance of material of both

higher and lower molecular weight. The addition of veratryl alcohol caused the nearly complete disappearance of the major peak without the appearance of fragments absorbing at 254 nm. This indicates extensive alteration of aromatic rings in the starting material had occurred.

Discussion

The results clearly show that lignin peroxidase can modify soluble coal polymers derived from North Dakota lignite and German subbituminous coal, probably by several reactions involving depolymerization and polymerization. This process probably should be considered as distinct from the liquification of powdered low ranked coals and lignite on mats of growing fungi as has been reported in numerous publications. The nature of the chemical modifications are unknown. Based on the current concepts of the mechanism of lignin peroxidase, it is to be expected that a donation of electrons from oxidizable groups to the Fe(IV)-oxo form of the peroxidase will form a cation radical. This electron transfer may occur either directly or via the veratryl alcohol cation radical. Non-enzymatic rearrangement of the cation radical could follow many paths depending on the substructure involved and these could occur with or without the introduction of oxygen. It is, therefore, to be expected that a large number of products of both higher and lower molecular weight will be formed. Hence, it will be necessary in future research to separate the products and to determine their structure so as to establish the nature of the lignin peroxidase attack on soluble coal polymer.