Bacterial Cleavage of an Arylglycerol-β-Aryl Ether Bond

R. L. CRAWFORD, T. K. KIRK, J. M. HARKIN, AND ELIZABETH McCLOY

Department of Bacteriology, University of Wisconsin, and U.S. Department of Agriculture Forest Products Laboratory, Madison, Wisconsin 53706

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Lignin is a complex, natural polymer containing many types of chemical linkages between its phenylpropanoid units. The major intermonomer linkage in lignin is the arylglycerol-β-aryl ether bond, linking 30 to 50% of the phenylpropane units (4). Clearly, cleavage of this key type of linkage by microorganisms must be important in lignin biodegradation. Studies of microbial cleavage of arylglycerol-β-aryl ether bonds in low-molecular-weight model compounds could therefore lead to a better understanding of the mechanisms involved in the natural decomposition of lignin and lignin-related aromatic compounds.

We report here the first observation of cleavage of an arylglycerol-β-aryl ether bond by a bacterium. The bacterium was isolated from a lignin-rich environment by using 1-(3,4-dimethoxyphenyl)-2-(O-methoxyphenoxy)-1,3-propanediol (compound I) as enriching substrate. This compound is a lignin model (1, 4) containing an arylglycerol-β-aryl ether bond as its intermonomer linkage.

Compound I was prepared by the procedure of Adler et al. (1) and added to a mineral salts medium containing the following in grams per liter of distilled water: 0.1 KH_{2}PO_{4}; 0.75 K_{2}HPO_{4}; 0.25 NaCl; 1.25 (NH_{4})_{2}SO_{4}; 0.01 MgSO_{4}.7H_{2}O and 0.002 FeSO_{4}.7H_{2}O. The final pH of this medium was 7.0. Liquid medium used during this investigation contained 500 mg of compound I per liter. Solid medium containing 1.0 g of this substrate per liter was prepared by addition of 15 g of Noble special agar per liter (Difco).

A bacterium able to use compound I as its only carbon and energy source was found with some difficulty and isolated by the shake flask enrichment technique. Garden soil, leaf compost, and barnyard soil yielded no pure cultures of bacteria with the required ability to use compound I. However, isolation from decomposing wood chips obtained from a simulated pulpmill chip pile (USDA Forest Products Laboratory, Madison, Wis.) was successful. Several small chips, with some adhering soil, were added to a 500-ml flask with 100 ml of mineral salts medium containing 50 mg of compound I. The mixture was mechanically agitated at 30 C for 1 week, and 1.0 ml of the enrichment was transferred to fresh medium. After four such transfers, serial dilutions were plated onto solid medium containing 1.0 g of compound I per liter. Several colony types appeared on these plates after incubation at 30 C. These were purified by repeated streaking on homologous media. One isolate was identified as a Pseudomonas of the “acidovorans group” (6). This isolate was designated Pseudomonas E-3 and used for the remainder of this investigation. Stocks were maintained by biweekly subculture on agar slants containing 0.1% compound I.

The characteristic odor of guaiacol (compound II) soon becomes apparent in young cultures of Pseudomonas E-3 growing on compound I. As the cultures age, the odor fades. This transient production of guaiacol can be graphically demonstrated on solid medium by flooding plates with a reagent that stains phenols (7). Plates flooded with a solution of 1% FeCl_{3} plus 1% K_{3}Fe(CN)_{6} developed a blue zone around streaks or colonies of the pseudomonad.

Production of compound II from compound I by Pseudomonas E-3 was confirmed by its isolation from a culture filtrate. A 50-ml inoculum of Pseudomonas E-3 growing on compound I (500 mg/liter) was used to seed 1.0 liter of the same medium. After shaking on a rotary shaker at 30 C for 18 hr, the culture was clarified by membrane filtration, acidified to pH 2.0 with 1.0 n H_{2}SO_{4}, and extracted with three 250-ml portions of chloroform-acetone (1:1 by volume).
and once with 250 ml of chloroform. The combined solvent phases were washed once with a small volume of water, dried over anhydrous Na₂SO₄, and evaporated to dryness in a vacuum evaporator to give a residue of about 100 mg. This was dissolved in a small volume of acetone and applied as a streak to the bottom of a preparative thin-layer chromatography (TLC) plate coated with a 1.0-mm layer of silica gel (Merck HF₂₄). The plate was developed in benzene-ethyl acetate (9:1 by volume). In this system, compound I had an Rₜ of approximately 0.1, whereas a second major band had an Rₜ of about 0.8. This second band corresponded to a standard of authentic compound II spotted at the edge of the plate prior to development. Aromatic compounds on TLC plates were seen under shortwave, ultraviolet light.

The band near Rₜ 0.8 was scraped from the plate and eluted from the silica gel with acetone. Removal of the solvent by evaporation left a brown oil that smelled strongly of compound II. Both its infrared and nuclear magnetic resonance spectra were identical to those of compound II.

It is possible, although unlikely, that the compound II produced from compound I by Pseudomonas E-3 arises not by cleavage of the arylglycerol-β-aryl ether bond, but by demethylation of the 3,4-dimethoxyphenyl portion of the molecule, followed by cleavage of the C₆-C₃ carbon-carbon bond. However, several observations seem to rule out this possibility.

Washed, whole cells of Pseudomonas E-3 grown on compound I and harvested during logarithmic growth, but not cells grown on succinate, oxidize vanillyl acid (compound III) in the presence of 250 μg of chloramphenicol per ml. At this concentration, chloramphenicol effectively prevents protein synthesis by our pseudomonad. This observation implies, from the theory of simultaneous adaptation (5), that compound III is on the degradative pathway used by Pseudomonas E-3 to degrade compound I. Compound III can arise only from the C₆-C₃ portion of the molecule. Compound II is not produced from compound III.

Also, compound II can be isolated in high yield from logarithmic cultures of Pseudomonas E-3 grown on compound I, but can be isolated only in low yield, or not at all, from stationary-phase cultures. Logarithmic cells grown on compound I do not significantly oxidize compound II in the presence of chloramphenicol, whereas stationary-phase cells do. Apparently the C₆-C₃ portion of the molecule is the preferred substrate and, under culture conditions, compound II is oxidized rapidly only after the C₆-C₃ portion becomes depleted.

Cell-free extracts of compound I-grown Pseudomonas E-3 contain high titers of proteocatechuic-4,5-oxygenase (2) and of catechol oxygenase (3). Cell-free extracts of cells grown on succinate lack measurable amounts of either enzyme, whereas extracts of cells grown on compound II contain catechol oxygenase, but not proteocatechuic-4,5-oxygenase. Extracts of compound III-grown cells contain proteocatechuic-4,5-oxygenase, but not catechol oxygenase.

These observations indicate that the arylglycerol portion of the aryglycerol-β-aryl ether is metabolized via compound III and proteocatechuic acid, whereas the 2-methoxyphenyl portion is metabolized via compound II and catechol.

We have not yet elucidated the mechanism by which Pseudomonas E-3 ruptures the arylglycerol-β-aryl ether linkage of compound I. Cells grown on compound I do not cleave the β-ether bond in the absence of oxygen. This indicates that the cleavage is oxidative (for example, via a mixed function oxidation) and does not proceed by any of the possible hydrolytic mechanisms. It is possible that compound I is converted to another product from which compound II is produced. Either way, the identification of compound II as a catabolite of compound I, together with observations of whole cells and cell-free extracts, demonstrates that the β-aryl-alkyl-ether linkage is being broken. To our knowledge, this is the first demonstration of bacterial cleavage of an arylglycerol-β-aryl ether bond.

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