

A MECHANISM FOR SULFUR-SPECIFIC BACTERIAL DESULFURIZATION

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ABSTRACT

The desulfurization of dibenzothiophene by *Rhodococcus rhodochrous* IGTS8 was recently demonstrated to result in formation of 2-hydroxybiphenyl or of 2,2'-dihydroxybiphenyl, depending on whether growth or nongrowth conditions are used in the desulfurization experiments. The key intermediate in the bacterial degradation under nongrowth conditions is 2'-hydroxybiphenyl-2-sulfinic acid (or the corresponding cyclic sultine), which is the immediate precursor of 2-hydroxybiphenyl. Addition of either dibenzothiophene or the sulfinic acid (sultine) to pregrown cells that are using ethanol- d_6 resulted in the formation of only unlabeled 2-hydroxybiphenyl. Therefore, formation of the carbon-hydrogen bond that accompanies cleavage of the carbon-sulfur bond of the sulfinic acid was demonstrated not to require hydrogen transfer from a reducing cofactor. A mechanism consistent with proton incorporation at the ipso carbon is proposed.

Key Words: bacterial desulfurization, sulfur-specific degradation

INTRODUCTION

Because of their potential commercial use in removing sulfur from high-sulfur coals and sour crude oil, microbial systems that desulfurize organosulfur compounds have been intensively studied for several years. Some organisms utilize a catabolic pathway that results in excision of the sulfur from dibenzothiophene (DBT). Intermediates corresponding to this thiophenic-ring scission (4S) pathway have been isolated and characterized (1). Recent results obtained in feeding the various intermediates to *Rhodococcus* IGTS8 elucidated two pathways for the thiophenic scission of DBT (2). Under nongrowth conditions, the DBT is oxidized to 2'-hydroxybiphenyl-2-sulfinate **1** via the DBT 5-oxide (or the corresponding cyclic sultine ester form), and the sulfinate is subsequently cleaved to 2-hydroxybiphenyl **2** (Scheme 1). Under growth conditions, very little of the 2'-hydroxybiphenyl-2-sulfinate is converted to 2-hydroxybiphenyl, and instead most is oxidized to 2'-hydroxybiphenyl-2-sulfonate **3** (or the corresponding sultone), and 2,2'-dihydroxybiphenyl **4** is the major product. The oxidation of the sulfinate to the sulfonate occurs spontaneously (nonenzymatically) in aqueous buffer exposed to air. Further understanding of the details of the enzymes and mechanisms of the various steps in the pathways is needed.

The desulfurization of 2'-hydroxybiphenyl-2-sulfinate to 2-hydroxybiphenyl, which occurs in the last step of the *Rhodococcus* degradation of DBT under nongrowth conditions, involves replacement of the carbon-sulfur bond by a carbon-hydrogen bond. Thus the carbon appears in a reduced state in the final product, but the oxidation state of the sulfur resulting from the last step is not known with certainty. Only sulfate was found in the culture medium (2), but initially formed sulfite could have been converted to sulfate in a subsequent reaction. To elucidate the mechanism for sulfur cleavage of the sulfinate intermediate, a labeling experiment was needed to determine whether the hydrogen that is added to the carbon is

derived from a reducing cofactor, such as NADH or NADPH, or instead from a hydrogen ion derived from water.

Since ethanol is an excellent energy source for the *Rhodococcus* bacterium, deuterium-enriched ethanol was used to generate in vivo the deuterated reducing cofactor (NADH-d) that might be postulated to be involved in the carbon reduction. The 2-hydroxybiphenyl produced in this reaction was examined by mass spectrometry (MS) to determine whether a significant amount of 2-hydroxybiphenyl-2'-d was formed via a mechanism involving an addition of deuterium from the reducing cofactor. The results of the experiment can then be used to infer what kind of redox reactions occur during the last reaction of the desulfurization.

RESULTS AND DISCUSSION

Two sulfur-containing substrates (DBT and the intermediate sultine) were utilized in the desulfurization experiments with ethanol-d₆ as the energy (or potential reduction equivalent) source as well as carbon source for the cells. As expected, both substrates were converted by the bacterium *Rhodococcus rhodocrous* (ATCC #53968) to 2-hydroxybiphenyl. Gas chromatography/mass spectrometry (GC/MS) analysis of the products showed that the 2-hydroxybiphenyl produced from both substrates contained no deuterated product.

Since 2-hydroxybiphenyl-2'-d was not formed in the *Rhodococcus* desulfurization reaction of 2'-hydroxybiphenyl-2-sulfonic acid in the presence of ethanol-d₆, where reducing cofactors would have been enriched in deuterium, the mechanism of carbon-sulfur bond cleavage in the final reaction is unlikely to have occurred via transfer of a hydrogen from a reducing cofactor (as a hydride equivalent) to the ipso carbon. Thus a mechanism involving simple displacement of hydride for the sulfonyl group or a mechanism involving addition of hydride to a positive carbon intermediate cannot be used in modeling this reaction.

Thus, addition of a proton to the ipso carbon is demanded by the results, but a simple hydrolysis mechanism is not likely here. The 2'-hydroxybiphenyl-2-sulfonic acid is not hydrolytically cleaved to the aromatic compound plus sulfite in the buffered culture media. Such a cleavage of the carbon-sulfur bond would require a very strong acidic site on the enzyme that could protonate the sulfonic acid ipso ring carbon. Any very acidic site would be unstable in the buffered media. We can find no precedent for this type of enzyme activity.

The reactions of dibenzothiophenes and sulfonic acids with basic reagents have been investigated, but these studies do not appear to be applicable to the enzyme-catalyzed degradation to hydroxybiphenyl. Attack of nucleophilic oxygen has been reported to occur at the ring carbon of biphenylsulfonic acid at high temperatures in the presence of crown ether, not at the sulfur atom, resulting in the formation of 2-hydroxybiphenyl rather than biphenyl (3).

An oxidase-catalysis mechanism for the transformation of 2'-hydroxybiphenyl-2-sulfonic acid to 2-hydroxybiphenyl is the more likely alternative. The biological cleavage of aliphatic sulfoxides is known to occur via hydroxyl attack (4). Several oxidative enzymes degrade aromatics by one-electron oxidation followed by attack of hydroxide. This mechanism can produce the same intermediate, however, and the mechanism is essentially equivalent to the hydroxyl radical attack. Addition of hydroxyl radical to the arylsulfinate anion, as shown in Scheme 2, or its equivalent would result in the sulfonate radical anion 5 with substantial

anionic character in the ring. An alternative to the hydroxyl radical attack on the sulfinate sulfur is the adjacent phenol group forming the phenoxy radical 7 that adds to the sulfinate group to give the analogous cyclic sulfonate (sultone) radical anion 8. Subsequent protonation of the ipso ring carbon of either radical anion intermediate would form protonated sulfonate radical intermediates 6 or 9. In the addition reactions of the analogous sulfur ylids (sulfenes) in a hydroxylic medium, hydrogen adds to the carbon, and oxygen to the sulfur to form the sulfonate (5).

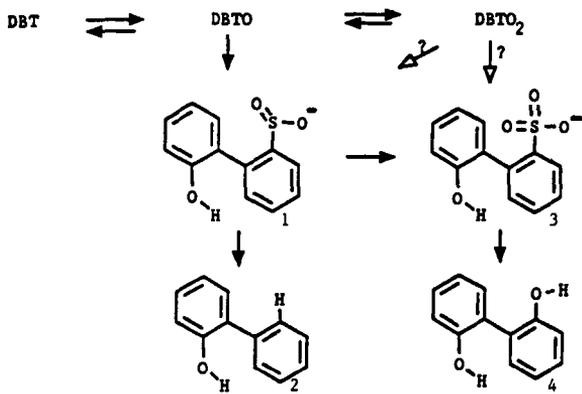
The intermediate radicals formed by this addition across the arylsulfinyl system resemble those implicated in the ipso substitution reaction investigated by Motherwell (6). In that reaction, a similar radical intermediate was formed by addition of an adjacent radical to the ipso carbon, and the sulfonyl radical was then eliminated. A similar displacement of the sulfonyl radical is proposed here in the microbial oxidase mechanism.

Depending on whether the final step involves gain or loss of an electron, either sulfite or sulfate will be formed. Or possibly disproportionation will give a mixture of the sulfite and sulfate forms. If an aryl sulfate ester forms, then an arylsulfatase enzyme may be required to complete the transformation to sulfate.

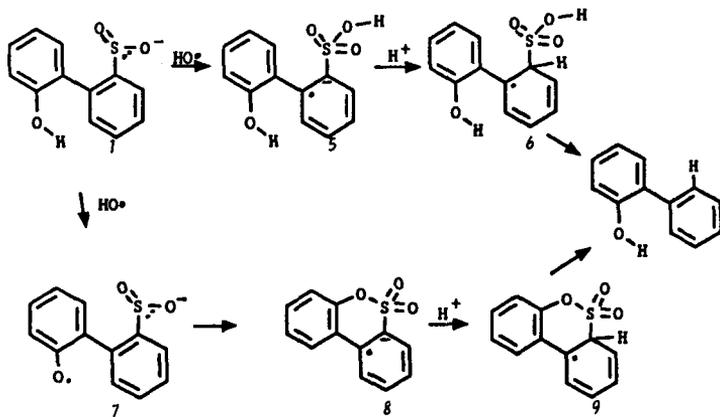
As shown in Scheme I, the reductive sulfur cleavage actually involves use of a sulfinate electron to "internally reduce" the ring carbon, the sulfinyl sulfur being converted to a sulfonate group in the process. Thus reducing cofactors are not required, as demanded by the lack of deuterium incorporation. Attack at the sulfur must be prevented in the enzyme that forms the 2-hydroxybiphenyl, or else the sulfur is simply oxidized, as it is in the formation of DBT 5-oxide and DBT 5,5-dioxide from DBT or in the formation of 2'-hydroxybiphenyl-2-sulfonic acid from 2'-hydroxybiphenyl-2-sulfinic acid.

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Scheme 1. Desulfurization Pathways in *Rhodococcus*.



Scheme 2. Mechanism of Sulfinate Desulfurization.