AN OVERVIEW ON CATECHOL CLEAVAGE

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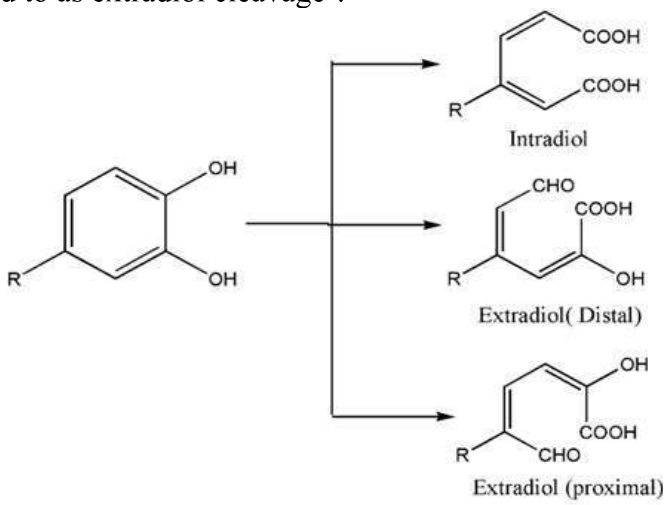
Abstract: ***Aromatic compounds present in soil can be degraded by catechol dioxygenases. They degrade these aromatic compounds present in soil mainly by two processes, intradiol and extradiol process. A brief account of the processes along with developments in this area are presented in this article.***

Keywords: ***Catechol Dioxygenase, Schiff Base, Intradiol Cleavege, Extradiol Cleavage***

1. INTRODUCTION

Catechol dioxygenases catalyze C−C bond cleavage and ring opening of catecholates, assisting in the degradation of aromatic compounds in soils[1]. The aromatic ring of the substrate can be opened either between the two hydroxyl groups, as shown in Scheme 1, which is known as intradiol cleavage, or to one side, which is referred to as extradiol cleavage[2].



SCHEME 1

The catechol dioxygenases are a non-heme enzyme that catalyzes the oxidative cleavage of catechols in the final step for the degradation of natural aromatic molecules into aliphatic products[3a-d]. Methyl Cleavage of the C-C bond adjacent to the enediol unit (extradiol) represents the more common oxidative cleavage pathway in nature, utilizing enzymes that have Fe(II) or Mn(II) in the active site. The crystal structures of the active sites of these enzymes reveal a square pyramidal geometry at the metal center that consists of three endogenous ligands (two histidines and one glutamate) in a facial array and two solvent molecules[4]. In addition, the structures of the enzyme-substrate (E-S) complexes of 2,3-dihydroxybiphenyl-1,2-dioxygenase (1,2-BphC) and protocatechuate-4,5-dioxygenase (4,5-PCD) reveal that the iron active site adopts a 5-coordinate structure with the three endogenous ligands and a chelated catecholate[5].

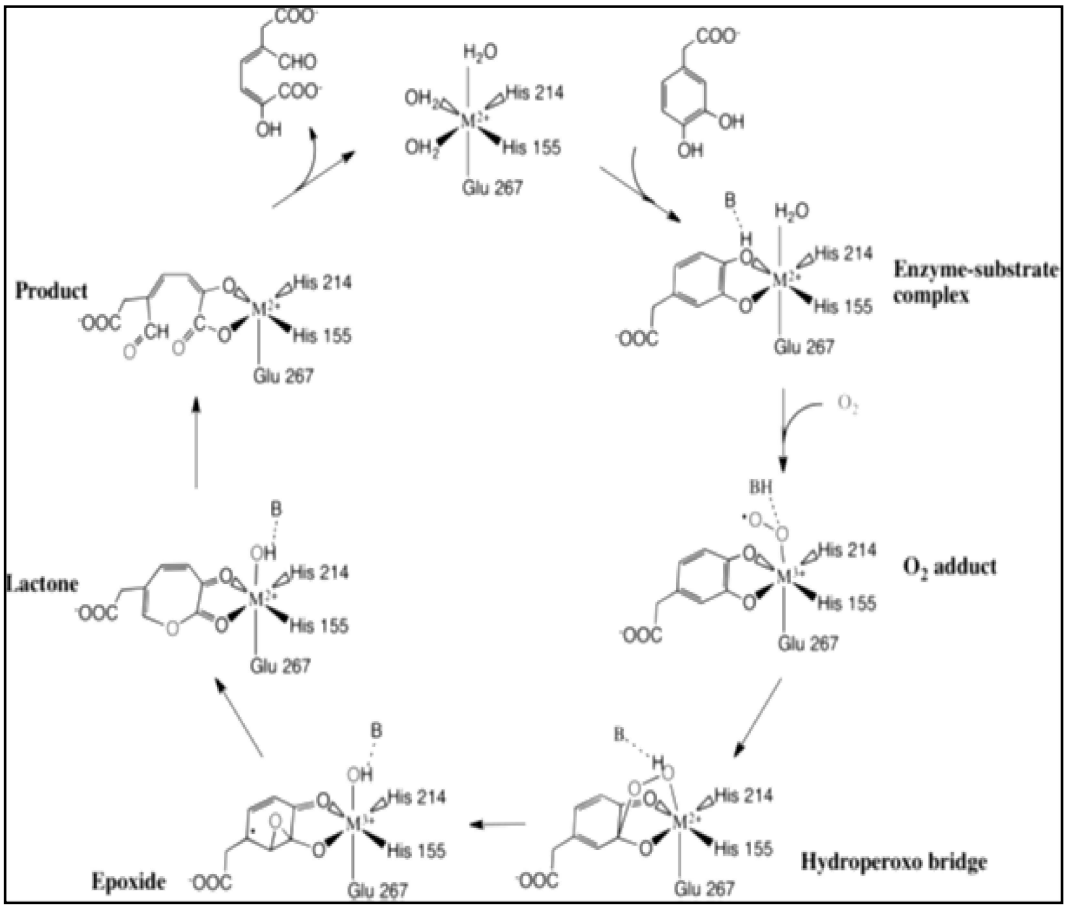
While the catecholate coordinates in a bidentate fashion, it does so asymmetrically with one Fe-O catecholate bond 0.2 Å shorter than the other. A similar bond asymmetry has also been observed in EXAFS studies of the catechol 2,3-dioxygenase (2,3-CTD)-catechol[6] complex and ascribed to a monoanionic substrate. The remaining coordination site is presumably reserved for O_2, as suggested by the binding of nitric oxide (NO) as a dioxygen surrogate[7] which converts the normally EPR-silent mononuclear high-spin Fe(II) center(s) into an EPR active 3/2 species.

This ternary enzyme-substrate-NO complex has been proposed to be a model for the iron-dioxygen intermediate in the enzymatic reaction mechanism[8a-b]. Mono- and binuclear non-heme iron centers are frequently present in a variety of protein systems that perform important biological functions involving dioxygen[9a,9b]. The mononuclear non-heme iron proteins that catalyze the oxidative cleavage[10] of catechol or its derivatives with the incorporation of molecular oxygen are exemplified by catechol dioxygenases. The oxidative cleavage of catechol and other dihydroxy aromatics is a key step in the biodegradation by soil bacteria of naturally occurring aromatic molecules and many aromatic environmental pollutants[11]. Several iron (III) complexes have been synthesized and studied[12] as models for the intradiol cleaving enzymes. However, models that mimic both the catalytic activity and spectral behavior of the iron site in these enzymes are scarce. From the point of view of models for dioxygenases, a fundamental work by Funabiki *et al.* on the catalytic intra and extradiol oxygenations of 3,5-di-*tert*-butylcatechol by a py/bipy/$FeCl_3$ complex appeared[13].

In early studies Que and co-workers[14] synthesized a series of nitrogen-, carboxylate-, and phenolate-containing iron (III) complexes, the catalytic properties of which have been explored. Indeed they found a clear relationship between the reactivity of the adducts and Lewis acidity of the iron(III) centers as modulated by the tripodal ligand, which plays an important role in dictating the catecholate-to-iron(III) charge transfer absorptions occurring in the visible region. Also knowledge of the rich chemistry of the iron-phenolato complexes[15] facilitated the elucidation of the novel substrate activation mechanism of the dioxygenase enzymes. Krebs et al.[16a-d] have used a variety of nitrogen-containing tripodal ligands to obtain iron (III) model complexes. However, to date the iron (III)-salen catecholate model complex [H_2salen = *N*,*N*-bis(salicylidene)ethane-1,2-diamine] reported by Que etal.[17 a-c] is the only structurally known example containing two coordinated phenolate groups. Systematic research on iron (III) complexes with tris(2-pyridylmethyl)amine (TPA) tripodal tetradentate ligands has been done by Que.et al.[18a-b]. The spectroscopic and kinetic studies on the catechol degradation promoted by the model complexes showed the relationship between the Lewis acidity of the iron center and its catechol dioxygenase activity. Among these model complexes, $[Fe(TPA)(DBC)]^+$ (H_2DBC = 3,5-di-*tert*-butylcatechol) was found so far to be the most active adduct in the intradiol-cleaving oxidation[19].

2. Catechol Cleavage by enzymatic process: Regiospecific Aspect

Although it would be tempting to ascribe the enzyme selectivity to the differences in the active site and the metal oxidation state, a mutant of homoprotocatechuate 2,3-dioxygenase (HPCD), His200Phe, was found to perform intradiol ring opening of a substrate analogue, 2,3-dihydroxybenzoate (2,3-DHB)[20]. This was the first time that an extradiol enzyme had been induced to show intradiol behavior. Also, as early as 1975 an intradiol enzyme was found to carry out small amounts of extradiol cleavage for modified substrates[21]. This, along with other model studies[22] demonstrates that the Fe (III) oxidation state is not a prerequisite for intradiol activity. The complexity of the problem is further manifested by the reactivity of Fe (III) model complexes. While a large range of model complexes exhibit intradiol cleavage[23a-b], a far smaller



Proposed mechanism for extradiol dioxygenases : Homoprocatechuate 2,3 dioxygenases [M= Fe(II)]

The proposed mechanism (Scheme 2) is supported by the isolation and spectroscopic characterization of many of the key intermediates[25,26] as well another computational work[27]. The first step of the reaction is binding of the substrate to the Fe (II) center, displacing the solvent molecules. Spectroscopic and crystallographic studies show that as the substrate binds to the metal it is singly deprotonated and the deprotonated hydroxyl function forms a hydrogen bond with second sphere residue Tyr257 [28]. The displacement of the solvent opens a coordination site where oxygen binds and is reduced to a superoxo species. During the reaction His200 is shown to function as an acid/base catalyst to relay protons from the substrate to the oxygen species yielding the dianionic form of the substrate[29], a facile process involving negligible barriers[30].The superoxo species then attacks the substrate to generate a hydroperoxo complex bridging the metal center and the substrate. This Fe (II)-alkylperoxo complex undergoes homolytic O−O bond cleavage to give a Fe(III)-hydroxo unit bound to an epoxide, which then rearranges to a Fe(II)-lactone intermediate. Hydrolysis of the lactone leads to the final product.

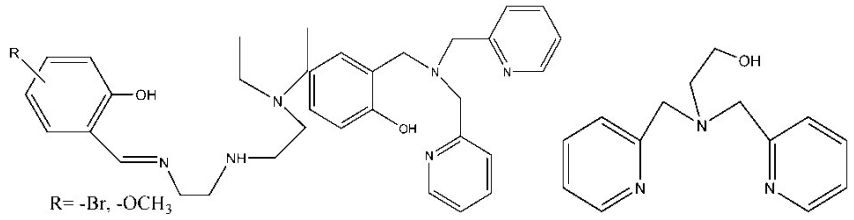
3. Non Enzymatic Catechol Cleavage

Our recent state of understanding indicates that the fine details of either the extradiol- or the intradiol-cleaving reaction are still elusive, and in addition to the numerous artificial iron model complexes new model studies can be still informative. As Xin and Bugg frames it: "...further modeling studies on these mechanisms might reveal the effects that the precise geometry of the hydroperoxide complex and the positioning of active site residues have on the O−O homolysis..."[31]. Previous model studies demonstrated the effect of Lewis acidity on reaction rates[32], but other factors, such as steric hindrance at the iron center[33], asymmetry in substrate binding[34], combined solvent/ligand effects[35],

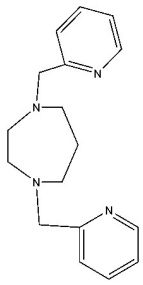
stereoelectronic effects[36], and spin transition at the metal site[37] have been also addressed as of potentially general importance in reactivity. Models applying N4 ligands were shown to produce intradiol products very selectivelye[40]. In a more recent study, however, the regioselectivity with N4 ligands was driven by the amount of added base[38]: Extradiol products were predominant when only 1 equivalent of base was added, while 2 equivalent of base leads to intradiol products. These ligands are therefore good mimics for the internal base-effect that operates in the substrate binding process. Selectivity is shifted toward intradiol cleavage when meridional N_3 geometry[39] is available (or forced by the ligand), whereas facial coordination of the supporting ligand leads to extradiol-cleavag.

Some ligands used in recent model complexes for catechol dioxygenase enzyme:

N_3O Donor Ligands



N_4 Donor ligands



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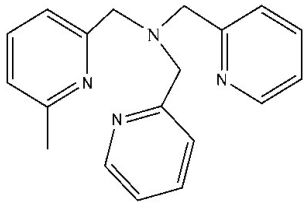

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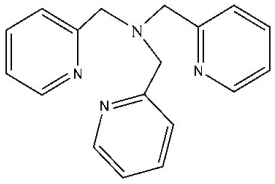

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4. IDENTIFICATION OF THE DIOXYGENATED PRODUCTS

The dioxygenase activities of complexes can be determined using known procedure. The complex (0.5 mmol), 3,5-di-*tert*-butylcatechol (0.5 mmol), and piperidine (1 mmol) were dissolved in DMF (10 mL) at 1 atm oxygen. After 48 h, the reaction was quenched by the addition of 2 M HCl (30 mL). Organic products were extracted from the aqueous DMF solution with diethyl ether (3x50mL), dried over anhydrous Na_2SO_4, and then concentrated. The products were identified by using GC-MS analysis[48].

5. UTILITY

Catechol dioxygenases catalyze C−C bond cleavage and ring opening of catecholates, assisting in the degradation of aromatic compounds in soils[1] . The oxidative cleavage of catechol and other dihydroxy aromatics is a key step in the biodegradation by soil bacteria of naturally occurring

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