The Mechanism of Intradiol Catechol Dioxygenases


One of the first examples of dioxygen fixation in biological systems was the discovery by Hayaishi et al. (1955) [1], of the enzymatic cleavage of catechol to cis,cis-muconic acid, resulting in the incorporation of the elements of dioxygen. In general, these enzymes are bacterial non-heme iron dioxygenases which are involved in the metabolism of many aromatic compounds [2]. Protocatechuate 3,4-dioxygenase (PCD) and catechol 1,2-dioxygenase (CTD) are the most widely studied of these types of intradiol cleavage enzymes.

The coordination environment of the iron in these enzymes consists of two tyrosines and two histidines as determined with resonance Raman spectroscopy [3] and EXAFS [4]. Furthermore, the intradiol dioxygenases have a characteristic visible absorption spectrum, with a maximum at 460 nm and a large extinction coefficient of 3000-4000 M⁻¹ cm⁻¹, as a result of the ligand-to-metal charge transfer from the phenolate to the iron center. Upon the coordination of catechol, an increase in absorption is observed in the 600-800 nm range, also indicating a ligand-to-metal charge transition from the substrate to the iron center in the enzyme-substrate complex [5].

Due to the tyrosinate ligands the iron has a low reduction potential i.e., the iron is difficult to reduce. Upon coordination of catechol, the potential is lowered even more. This lowered reduction potential makes it difficult to reduce the iron center under biological conditions [6]. It is therefore believed that the metal stays in a high spin ferric state throughout the reaction with catechol. This is confirmed by using EPR and Mössbauer studies on the enzyme, enzyme-substrate complex, and two oxygenated intermediates. All of these species exhibit magnetic hyperfine splitting at liquid-helium temperatures, indicative of high-spin ferric iron [7].

With the above evidence, it appears that the reaction does not occur through oxygen activation by the transfer of an electron from the iron center as was originally believed [8]. Que et al. (1977) proposed a novel mechanism for the reaction involving substrate activation, as outlined in Figure 1 [9].

![Figure 1](image_url)
this scheme the monodentate bound catechol loses both of its protons upon coor-
dination to the iron center. Charge-delocalization subsequently occurs on the
substrate from the ligand-to-metal-charge transfer interaction. The catechol
then becomes susceptible to reaction with dioxygen to yield a peroxide interme-
diate. Finally, this intermediate rearranges to form an anhydride which is
hydrolyzed to form the final product, cis,cis-muconic acid.

Since the formulation of this reaction mechanism, there have been many
physical studies on the enzyme to illustrate various aspects of the proposed
mechanism [6,7]. For example, NMR spectroscopic studies on several enzyme
complexes have shown that the catechol substrate does indeed bind in a mono-
dentate fashion. Also, from the contact shifts, charge delocalization seems
evident on the catechol [10]. Furthermore visible absorption spectra supply
tentative evidence for an intermediate with a basic ligand, which would be con-
sistent with a peroxide species as outlined in the above mechanism [11].

Several model systems have been developed to mimic the reactivity of CTD
and PCD [12]. The simplest system developed by Funabiki et al. consists of a
mixture of FeCl₃, bipyridine, and pyridine which converts 3,5-di-tert-butyl-
catechol into intradiol cleavage products. However, in this system extradiol
products are also formed [12e]. Recently, Que et al. synthesized a series of
triiodal, tetradentate ligands with varying degrees of electron withdrawing
abilities (Figure 2) [12f]. The variation in cleavage yield and side products
obtained from these complexes indicates the importance of the coordination
environment and the Lewis acidity of the iron center in these enzymes. It is
concluded from these studies that the mechanism proposed has proven to be the
most consistent model describing the reactivity of these enzymes.

References

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