5.7: Oxygenases

Background

The oxygenase enzymes catalyze reactions of dioxygen with organic substrates in which oxygen atoms from dioxygen are incorporated into the final oxidized product.[2-4] These enzymes can be divided into dioxygenases, which direct both atoms of oxygen into the product (Reaction 5.53), and monooxygenases, where one atom of oxygen from dioxygen is found in the product and the other has been reduced to water (Reaction 5.54):

$$Dioxygenase:\ substrate + {}^*O_2 \rightarrow substrate({}^*O)_2 \tag{5.53}$$

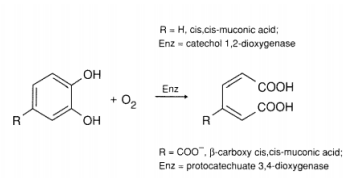
$$Monoxygenase:\ substrate + {}^*O_2 + 2H^+ + 2e^- \rightarrow substrate({}^*O) + H_2\,{}^*O \tag{5.54}$$

Dioxygenases

Dioxygenase enzymes are known that contain heme iron, nonheme iron, copper, or manganese.[66,67] The substrates whose oxygenations are catalyzed by these enzymes are very diverse, as are the metal-binding sites; so probably several, possibly unrelated, mechanisms operate in these different systems, For many of these enzymes, there is not yet much detailed mechanistic information. However, some of the intradiol catechol dioxygenases isolated from bacterial sources have been studied in great detail, and both structural and mechanistic information is available.[66,67] These are the systems that will be described here.

1. Intradiol Catechol Dioxygenases

The role of these nonheme iron-containing enzymes is to catalyze the degradation of catechol derivatives to give muconic acids (Reaction 5.55, for example). The enzymes are induced when the only carbon sources available to the bacteria are aromatic molecules. The two best-characterized members of this class are catechol 1,2-dioxygenase (CTD) and protocatechuate 3,4-dioxygenase (PCD).



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a. Characterization of the Active Sites

Even before the x-ray crystal structure of PCD was obtained, a picture of the active site had been constructed by detailed spectroscopic work using a variety of methods. The success of the spectroscopic analyses of these enzymes is a particularly good example of the importance and usefulness of such methods in the characterization of metalloproteins. The two enzymes referred to in Reaction (5.55) have different molecular weights and subunit compositions,[66] but apparently contain very similar active-site structures and function by very similar mechanisms. In both, the resting state of the enzyme contains one Fe^{III} ion bound at the active site. EPR spectra show a resonance at g = 4.3, characteristic of high-spin Fe^{III} in a so-called rhombic (low symmetry) environment,[66] and the Mössbauer parameters are also characteristic of high-spin ferric.[66-68] Reactions with substrate analogues (see below) cause spectral shifts of the iron chromophore, suggesting strongly that the substrate binds directly to the iron center in the course of the enzymatic reaction.

It is straightforward to rule out the presence of heme in these enzymes, because the heme chromophore has characteristic electronic-absorption bands in the visible and ultraviolet regions with high extinction coefficients, which are not observed for these proteins. Likewise, the spectral features characteristic of other known cofactors or iron-sulfur centers are not observed. Instead, the dominant feature in the visible absorption spectrum is a band with a maximum near 460 nm and a molar extinction coefficient of 3000 to 4000 $M^{-1}cm^{-1}$ per iron (see Figure 5.5).

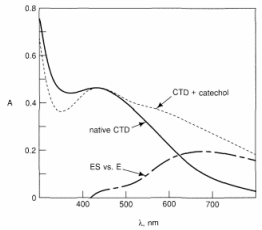

Figure 5.5 - Visible absorption spectra of catechol 1,2-dioxygenase and its substrate complex: E, native enzyme; ES, enzyme-substrate complex.[66]

This type of electronic absorption spectrum is characteristic of a class of proteins, sometimes referred to as iron-tyrosinate proteins, that contain tyrosine ligands bound to iron(III) in their active sites, and which consequently show the characteristic visible absorption spectrum due to phenolate-to-iron(III) charge-transfer transitions. This assignment can be definitively proven by examination of the resonance Raman spectrum, which shows enhancement of the characteristic tyrosine vibrational modes (typically ~1170, 1270, 1500, and 1600 cm^{-1}) when the sample is irradiated in the charge-transfer band described above. Ferric complexes of phenolate ligands may be seen to give almost identical resonance Raman spectra (see Figure 5.6).

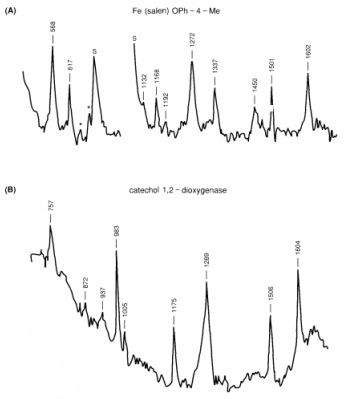

Figure 5.6 - Resonance Raman specta of (A) Fe^{III}(salen)(O - C_6H_4 -4 - CH_3) and (B) catechol 1,2-dioxygenase.[66]

These bands have been assigned as a C—H bending vibration and a C—O and two C—C stretching vibrations of the phenolate ligand.[69] In addition, NMR studies of the relaxation rates of the proton spins of water indicate that water interacts with the paramagnetic Fe^{III} center in the enzyme. This conclusion is supported by the broadening of the Fe^{III} EPR signal in the presence of $H_2{}^{17}O$, due to interaction with the I = $\frac{5}{2}$ nuclear spin of ^{17}O. Thus numerous spectroscopic studies of the catechol dioxygenases led to the prediction that the high-spin ferric ion was bound to tyrosine ligands and

water. In addition, EXAFS data, as well as the resemblance of the spectral properties to another, better characterized iron-tyrosinate protein, i.e., transferrin (see Chapter 1), suggested that histidines would also be found as ligands to iron in these proteins.[66,67]

Preliminary x-ray crystallographic results on protocatechuate 3,4-dioxygenase completely support the earlier predictions based on spectroscopic studies.[70] The Fe^{III} center is bound to two histidine and two tyrosine ligands and a water, the five ligands being arranged in a trigonal bipyramidal arrangement, with a tyrosine and a histidine located in axial positions, and with the equatorial water or hydroxide ligand facing toward a cavity assumed to be the substrate-binding cavity. The cavity also contains the positively charged guanidinium group of an arginine side chain, in the correct position to interact with the negatively charged carboxylate group on the protocatechuate substrate (see Figure 5.7).

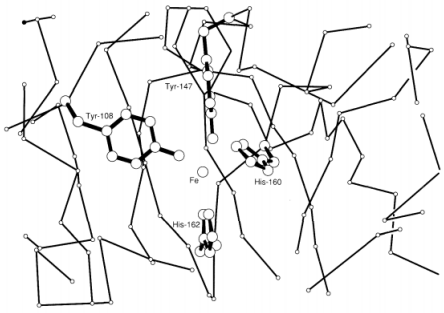

Figure 5.7 - A view of the active site of protocatechuate 3,4-dioxygenase based on the results of the x-ray crystal structure.[70] The Fe^{III} center is approximately trigonal bipyramidal, with Tyr-147 and His-162 as axial ligands, and Tyr-108 and His-160 in the equatorial plane along with a solvent molecule (not shown) in the foreground.[66]

b. Mechanistic Studies

As mentioned above, substrates and inhibitors that are substrate analogues bind to these enzymes and cause distinct changes in the spectral properties, suggesting strongly that they interact directly with the Fe^{III} center. Nevertheless, the spectra remain characteristic of the Fe^{III} oxidation state, indicating that the ferric center has not been reduced. Catecholates are excellent ligands for Fe^{III} (see, for example, the catecholate siderophores, Chapter 1) and it might therefore be assumed that the catechol substrate would bind to iron using both oxygen atoms (see 5.56).

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However, the observation that phenolic inhibitors p-X-C_6H_4-OH bind strongly to the enzymes suggested the possibility that the substrate binds to the iron center through only one oxygen atom (see 5.57).

Figure 5.8).

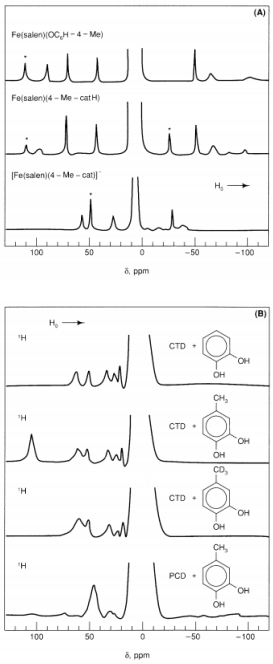

Figure 5.8 - (A) Paramagnetically shifted ^{1}H-NMR spectra of Fe(salen) complexes with 4-methylphenolate and 4-methylcatecholate ligands. Starred resonances are assigned to methyl groups. The top spectrum is of the ferric-salen complex of the monodentate 4-methylphenolate ligand. The middle spectrum is of the ferric-salen complex of the monoprotonated 4-methylcatecholate ligand. Note that two isomers are present, because the two oxygen atoms on the ligand are inequivalent, and either may be used to make the monodentate complex. The bottom spectrum is of the ferric-salen complex of the fully deprotonated 4-methylcatecholate ligand, which binds to the iron in a bidentate fashion. Note the smaller span of isotropic shifts, which has been demonstrated to be diagnostic of bidentate coordination. (B) Paramagnetically shifted ^{1}H-NMR spectra of enzyme-substrate complexes of the dioxygenase enzymes catechol 1,2-dioxygenase (CTD) and protocatecholate 3,4-dioxygenase (PCD). Note the resemblance between the position of the methyl resonance in the complex of 4-methylcatechol with CTD and that of one of the methyl resonances in the middle spectrum in (A), indicating that 4-methylcatechol binds to the ferric center in CTD in a monodentate fashion through the O-1 oxygen only. By contrast, the spectrum of 4-methylcatechol with PCD resembles the bottom spectrum in (A), indicating that here the 4-methylcatecholate ligand is bidentate.[66]

These results contradict an early hypothesis that the mode of substrate binding, i.e., monodentate versus bidentate, might be a crucial factor in activating the substrate for reaction with dioxygen.[67]

Spectroscopic observations of the enzymes during reactions with substrates and substrate analogues have enabled investigators to observe several intermediates along the catalytic pathway. Such studies have led to the conclusion that the iron center remains high-spin Fe^{III} throughout the entire course of the reaction. This conclusion immediately presents a problem in understanding the nature of the interaction of dioxygen with the enzyme, since dioxygen does not in general interact with highly oxidized metal ions such as Fe^{III}. The solution seems to be that this reaction represents an example of *substrate* rather than *dioxygen* activation.

Studies of the oxidation of ferric catecholate coordination complexes have been useful in exploring mechanistic possibilities for these enzymes.[71] A series of ferric complexes of 3,5-di-t-butyl-catechol with different ligands L have been found to react with O_2 to give oxidation of the catechol ligand (Reaction 5.58)

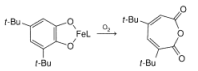

Figure 5.9) and that complexes of ligands that are poor donors tend to favor electron donation from catechol to Fe III, thus increasing the relative amount of minor form B. It should be noted that the spectroscopic characteristics of these complexes are nevertheless dominated by the major resonance form A, regardless of the nature of L.

All these studies of the enzymes and their model complexes have led to the mechanism summarized in Figure 5.9.[66] In this proposed mechanism, the catechol substrate coordinates to the ferric center in either a monodentate or a bidentate fashion, presumably displacing the water or hydroxide ligand. The resulting catechol complex then reacts with dioxygen to give a peroxy derivative of the substrate, which remains coordinated to Fe^{III}. The subsequent rearrangement of this peroxy species to give an anhydride intermediate is analogous to well-characterized reactions that occur when catechols are reacted with alkaline hydrogen peroxide.[72] The observation that both atoms of oxygen derived from O_2 are incorporated into the product requires that the ferric oxide or hydroxide complex formed in the step that produces the anhydride does not exchange with external water prior to reacting with the anhydride to open it up to the product diacid.

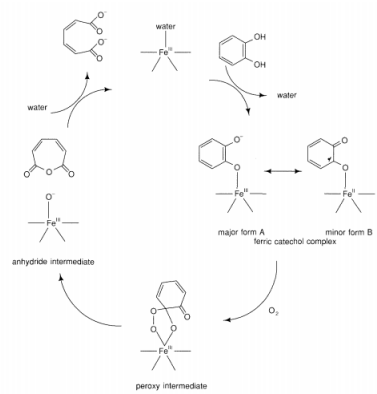

Figure 5.9 - Proposed mechanism for catechol dioxygenases (modified from Reference 45). The major form A is shown here with the catechol bound in a monodentate fashion. It may sometimes be bound in a bidentate fashion as well (see text).

It is interesting to consider how the intradiol dioxygenase enzymes overcome the kinetic barriers to oxidations by dioxygen, and why this particular mechanism is unlikely to be applicable to the monooxygenase enzymes. The first point is that the ferric catechol intermediate is paramagnetic, with resonance forms that put unpaired electron density onto the carbon that reacts with dioxygen. The spin restriction is therefore not a problem. In addition, the catechol ligand is a very good reducing agent, much more so than the typical substrates of the monooxygenase enzymes (see next section). It is possible, therefore, that the reaction of dioxygen with the ferric catechol complex results in a concerted two-electron transfer to give a peroxy intermediate, thus bypassing the relatively unfavorable one-electron reduction of O_2.