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Structure and Function of Dioxygenases. One Approach to Lignin Degradation

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The evolution of biosynthetic pathways leading to the formation of those structural natural products found in higher plants occurred in an oxygen-rich environment. Mixed function oxygenases evolved and functioned in catalyzing the insertion of molecular oxygen into basic structural polymers such as lignin, as well as natural products such as flavanoids and alkaloids. In addition to the critical role played by molecular oxygen in biosynthesis, oxidative degradative pathways evolved for the return of these natural products to the carbon cycle in the form of carbon dioxide. Oxidative degradation of natural products occurs by oxygen insertion reactions which are catalyzed by the mixed function oxygenases and by the dioxygenases. Reaction mechanisms for the mixed function oxygenases are now quite well understood, and a number of chemical model systems have been designed which catalyze such oxidation reactions. However, at the present time we do not have enough pertinent information on the mechanism of action of dioxygenases. This report provides some information to allow for the design of chemical model systems which mimic dioxygenase activity.

The appearance of molecular oxygen in the biosphere had an enormous influence on the evolution of electron-transfer enzymes. For example, the iron complex heme, which initially may have evolved to facilitate the reduction of soluble inorganic ions such as sulfate and nitrate, adapted to function in the reduction of oxygen to water. The latter reaction clearly evolved as a detoxification mechanism since "activated" molecular oxygen is exceedingly toxic to the anaerobic bacteria. With the onset of aerobic systems superoxide dismutase evolved as a defense mechanism to reduce superoxide anion (O_2^-) toxicity (Dagley, 1976; McCord et al., 1971). Anaerobic bacteria do not contain superoxide dismutase and therefore these organisms die when exposed to an environment which contains oxygen. Clearly the ability to cope with molecular oxygen became increasingly important in the evolution of higher forms of life.

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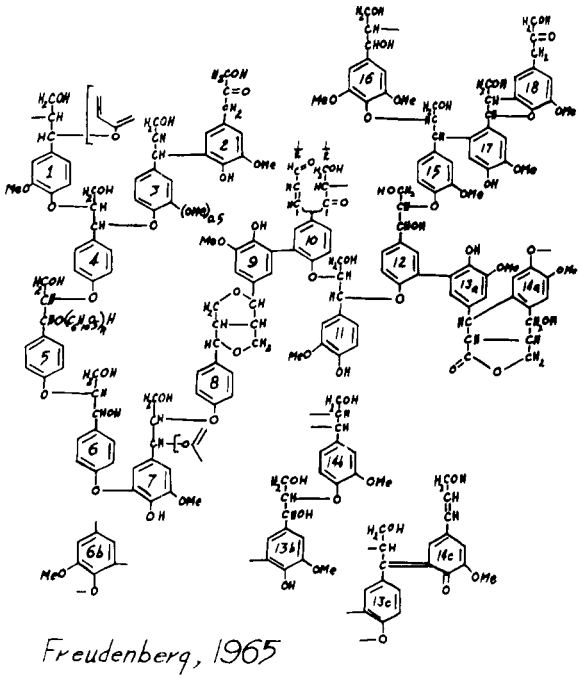

Figure 1. Schematic presentation of the structure of lignin showing extent of oxygen insertion into the polymer.

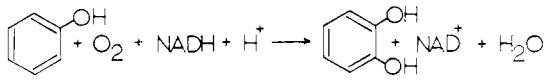

Figure 2. Flavoprotein catalyzed mixed function oxygenation of phenol to catechol.

The "fixation" of molecular oxygen into organic compounds represents a second important evolutionary step in the aerobic environment. In this particular case oxygen is not simply removed by reduction to water, but it is incorporated into natural products which now represent the majority of biomass on this planet (Dagley, 1975). Two groups of enzymes evolved to facilitate oxygen fixation: (i) the "mixed function oxygenases" and (ii) the "dioxygenases". Recently, it has been shown that a dioxygenase links the oxygen cycle to the carbon cycle in photosynthesis. Furthermore, the second most predominant biopolymer in nature (lignin) is synthesized as a result of a series of mixed-function oxygenase-catalyzed reactions. The inclusion of molecular oxygen into the basic polymeric structure of lignin is evident when one views the schematic presentation of this polymer (Figure 1). Since the biosynthesis of lignin, and related natural products in higher plants, had to occur in an oxygen-rich environment, then it is logical to assume that these natural products must be returned to the carbon cycle by oxidative degradation. At the present time there is no evidence of anaerobic biodegradation of lignin, flavanoids, or alkaloids. It appears that such natural products are only degraded by oxidation reactions. There was no reason for the evolution of reductive mechanisms for lignin degradation in the anaerobic environment.

During the last decade a great deal of effort has been made to determine the structure and function of mixed function oxygenases. Elegant work has been done particularly by the Gunsalus and Coon groups and the Collman and Holm groups on the cytochrome P450 system. However, the majority of mixed function oxygenases which have been purified to homogeneity are flavoproteins (Strickland and Massey, 1973; Takemori et al., 1969; White-Stevens and Kamin, 1972; Ohta and Ribbons, 1970; Hesp et al., 1969; Howell et al., 1972). Those enzymes

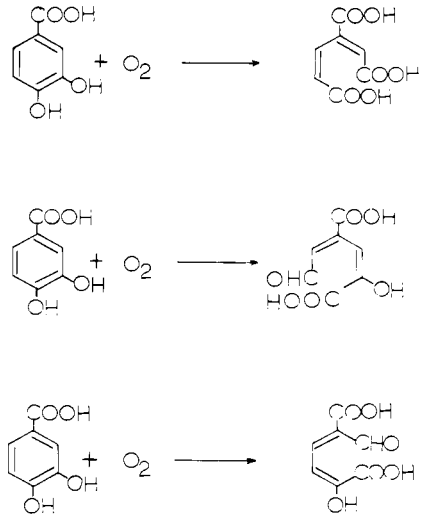

Figure 3. Dioxygenase reactions catalyzed by 3,4-PCase, 4,5-PCase, and 2,3-PCase, respectively.

responsible for hydroxylation of aromatic compounds have all been characterized as flavoproteins, for example (Figure 2).

Recent studies on both mixed function oxygenase and dioxygenase systems indicate that oxygen can be activated to a peroxy intermediate. Since peroxy compounds are very toxic to all living systems, higher plants have evolved a group of heme-containing enzymes called peroxidases. These enzymes maintain low steady-state concentrations of peroxy compounds in plants. Therefore, one function of peroxidases is to play a similar role in higher plants to that occupied by superoxide dismutase in the animal kingdom.

Hydroxylation of the benzene ring is a very important step in the biosynthesis of aromatic natural products as well as in their subsequent return to the carbon cycle by biodegradation. After the introduction of two hydroxyl groups, either ortho or para to each other, the aromatic nucleus is prepared for ring cleavage by dioxygenases. The majority of dioxygenases are non-heme iron proteins which convert aromatic compounds to aliphatic carboxylic acids capable of entering the tricarboxylic acid cycle.

Dioxygenases play a crucial role in the oxidative degradation of aromatic natural products including lignin. Therefore, if we understand the mechanism of action of this group of enzymes we are in a position to design catalysts capable of solubilizing aromatic polymers by the oxidation of polymeric catechols to aliphatic carboxylic acids.

RESULTS

In 1950 Hayaishi and Hashimoto discovered that enzyme-catalyzed oxidative ring cleavage of the aromatic nucleus of catechol occurred to give *cis,cis*-muconic acid as the reaction product. In 1955 it was shown by $^{18}O_2$ studies that both atoms of the same oxygen molecule were incorporated into the product, indicating the direct addition of molecular oxygen to the aromatic substrate to give an aliphatic dicarboxylic acid as the product. Since these earlier studies a large number of dioxygenases have been isolated and classified on the basis of their specificity. For example, three different enzymes have been isolated which cleave 3,4-dihydroxybenzoic acid [protocatechuic acid (PCA)] at different positions on the aromatic nucleus (Fujisawa and Hayaishi, 1968; Zabinski et al., 1972; Crawford, 1975) (Figure 3). The specificity of these three enzyme systems serves as an example of how natural systems have evolved which catalyze reactions hitherto

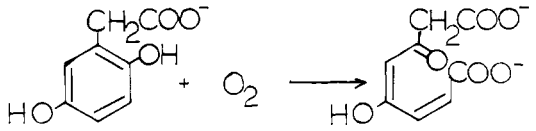

Figure 4. Cleavage of the para diphenol homogentisate by 1,2-HGase.

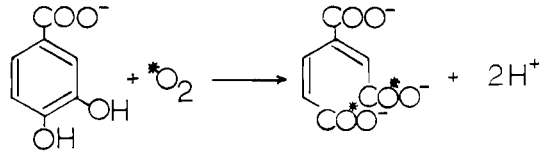

Figure 5. Incorporation of oxygen ($^{18}O_2$) by 3,4-PCase.

unattainable in chemical model systems.

Enzymes which cleave catechol by extradiol and intradiol mechanisms [metapyrocatechase (2,3-CTase)] and [pyrocatechase (1,2-CTase)], as well as those which cleave protocatechuic acid (3,4-PCase and 4,5-PCase), have been purified to homogeneity (Nozaki et al., 1970; Nozaki et al., 1963; Nozaki et al., 1968; Takemori et al., 1971; Dagley et al., 1968; Kojima et al., 1967; Nakazawa et al., 1969; Fujisawa and Hayaishi, 1968).

Other dioxygenases which cleave ortho diphenols have been purified to homogeneity (3,4-dihydroxyphenylacetate 2,3-dioxygenase (Fujisawa and Hayaishi, 1968), 2,3-dihydroxybenzoate dioxygenase (Kita, 1965), and 7,8-dihydroxyflavone 8,9-dioxygenase (Schultz et al., 1974; Schultz and Wood, 1977). The majority of dioxygenases purified to date cleave the benzene nucleus of ortho diphenols. Relatively few dioxygenases that cleave para diphenols have been purified. This is an unfortunate oversight, since this class of oxygenases appears to be ubiquitous among natural microbiol populations (Crawford, 1975a,b; Hopper and Chapman, 1970; Adachi et al., 1966) and in mammalian tissues (Adachi et al., 1966). For example, homogentisate 1,2-dioxygenase (1,2-HGase) has been found in both mammalian tissues, plants, and in bacteria; this enzyme plays an important role in the metabolism of tyrosine (Hopper and Chapman, 1970; Flamm and Crandall, 1963; Durand and Zenk, 1974) (Figure 4).

Protocatechuate 3,4-dioxygenase (3,4-PCase) from *Pseudomonas aeruginosa* (EC 1.13.11.3) catalyzes the cleavage of protocatechuate to β-carboxy-*cis*,*cis*-muconate with the incorporation of two atoms of molecular oxygen (Figure 5). In the past decade, this enzyme has been studied extensively by Hayaishi and his co-workers (Bloch and Hayaishi, 1966). Molecular weights of 700 000 and 660 000 have been estimated for the crystalline enzyme by sedimentation equilibrium and total amino acid composition, respectively. Reportedly eight identical subunits, each containing one iron atom, comprise the holoenzyme (Nozaki, 1974). Each subunit was shown to contain one atom of high-spin Fe^{3+} (Fujisawa et al., 1972). The native enzyme is easily isolated and crystallized from cell extracts of *Pseudomonas aeruginosa* grown with *p*-hydroxybenzoic acid as carbon source. We have been able to obtain 0.5 g of crystalline enzyme from a 20-L culture of this organism routinely. The native enzyme is red in color having a λ_{max} at 450 nm with a broad absorption band between 400–650 nm. When protocatechuic acid is added to the enzyme in the absence of O_2 there is an increase in extinction of the chromophore accompanied by a shift to the red (λ_{max} 480 nm). By using stopped flow techniques Fujisawa et al. (1972) have shown that the introduction of oxygen gives a new spectral intermediate with a λ_{max} near 520 nm. This complex breaks down rapidly to give β-carboxy-*cis*,*cis*-muconic acid and the native enzyme (450 nm). Based on this kinetic study, the following reaction pathway has been proposed by the Japanese group (Fujisawa et al., 1972):

$$E + S = ES$$

$$ES + O_2 \rightarrow ES\text{-}O_2$$

$$ESO_2 \rightarrow E + P$$

where E = 3,4-PCase, S = protocatechuic acid, and P = β-carboxy-*cis*,*cis*-muconic acid.

During the last six months we have reinvestigated the subunit structure of 3,4-PCase and we have made a number of new observations on subunit size and interactions.

Our studies on the native enzyme showed that it dissociates into identical subunits (mol wt 66 000) in base. When these subunits are treated with 1.0% sodium dodecyl sulfate (SDS) at pH 10.5, further dissociation occurs to give equal fractions of two smaller units of molecular weights 15 000–16 500 and 18 000–20 500 respectively. Sequence analysis of these nonidentical subunits has yielded the following residues from the amino terminal: peptide 1, Pro-Ile-Glu-Leu- and peptide 2, Pro-Ala-Gln-Asp-. At the present time we are isolating large quantities of peptides 1 and 2 for complete sequence analyses.

Resolution of 28 tryptic peptides together with the amino acid composition and UV absorbance of the apoenzyme suggest that the molecular weight of the minimum repeating unit is about 37 000. The molecular weight of the base dissociable subunit determined by SDS gel electrophoresis and Bio-Gel P-200 column fractionation is approximately 66 000. Iron reconstitution studies show that up to 10 active iron atoms and 10–14 inactive iron atoms can be retained by the enzyme in similar sites. These results suggest each base dissociable subunit is an $\alpha_2\beta_2$ structure with one active iron site and perhaps one inactive iron site (Lipscomb et al., 1976).

EPR spectroscopy has firmly established that the irons in the native enzyme are in a high-spin ferric state ($S = 5/2$). 3,4-PCase exhibits a prominent feature near $g = 4.3$, typical of high-spin ferric iron in a "rhombic" environment. A low-temperature EPR spectrum is presented in Figure 6.

Variable-temperature EPR spectroscopy on native 3,4-PCase has determined that the high-spin ferric iron exhibits a zero-field splitting parameter, D, of 1.6 cm^{-1} (Blumberg and Peisach, 1973). This is similar to that found for ferric rubredoxin. 3,4-PCase and rubredoxin are unique among iron proteins which exhibit a $g = 4.3$ resonance in that they exhibit values of D much larger than the other proteins. This similarity had led to the suggestion that 3,4-PCase may have a rubredoxin-like environment, i.e., a tetrahedral coordination of cysteines (Jensen, 1973). The saturation field (H_{sat}) obtained from the Mössbauer spectra on native 3,4-PCase suggests otherwise (Figure 7). Rubredoxin exhibits an H_{sat} of −370 kG (Rao et al., 1972; Debrunner et al., 1977), while 3,4-PCase exhibits an H_{sat} of −525 kG (Que et al., 1976). This H_{sat} further suggests that cysteine may not be coordinated to the metal at all. Mössbauer data on iron complexes having one mercaptide coordination show that these complexes all reflect fields of ca. −450 kG (Koch et al., 1975). Our Mössbauer data further eliminate an octahedral oxygen coordination, since these complexes all exhibit much smaller D values (ca. 0.5 cm^{-1}) (Oosterhuis, 1974).

Mössbauer spectra taken on dithionite reduced 3,4-PCase show that the irons are in a high-spin ferrous state. The data obtained for the reduced protein further sub-

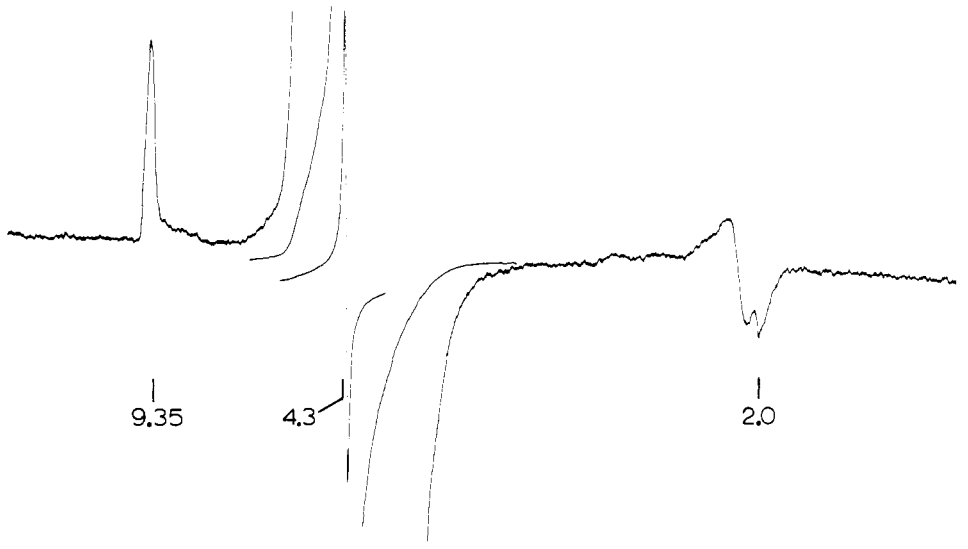

Figure 6. EPR spectrum of polycrystalline 3,4-PCase, packed in its mother liquor. Conditions: T = 12 K; microwave frequency, 9.196 GHz; microwave power, 3 mW; modulation amplitude, 10 G; sweep rate, 1000 G/min; time constant, 0.3 s; receiver gain, 3200 in the low-field region and 320 and 32 in the g = 4.3 region, respectively. The feature in the g = 2 region is due to some adventitiously bound copper (≤0.1 spin/mol).

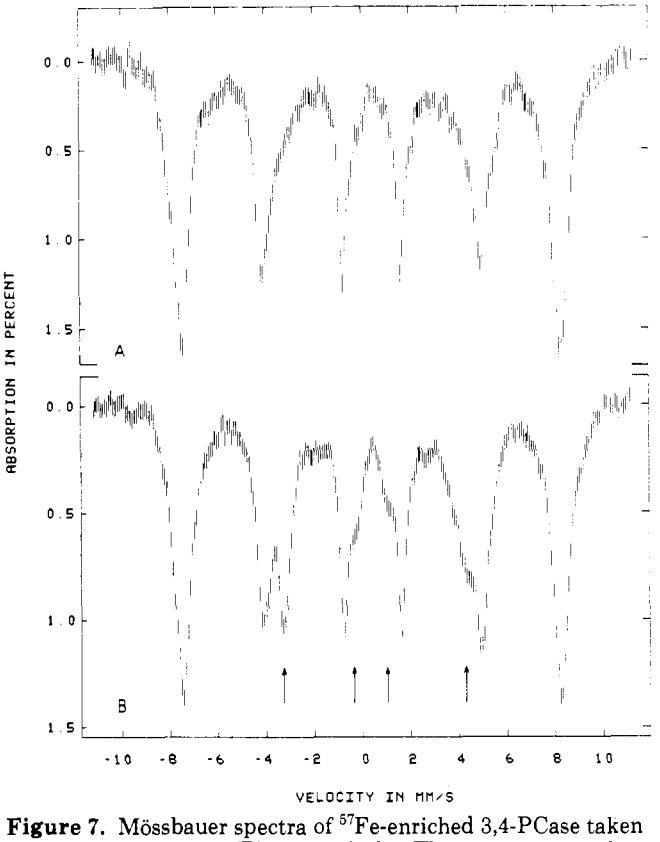

Figure 7. Mössbauer spectra of ^{57}Fe-enriched 3,4-PCase taken at 1.5 K (A) and 4.2 K (B), respectively. The spectra were taken in a field of 600 G applied parallel to the Mössbauer radiation. The absorption lines of the "g = 4.3" Kramers doublet are indicated by arrows (for details, see Que et al., 1976).

stantiate the dissimilarity of 3,4-PCase with rubredoxin. The characteristic isomer shift for reduced rubredoxin (δ = 0.68 mm/s) reflects its more covalent, tetrasulfur environment while that of 3,4-PCase (δ = 1.21 mm/s) suggests more ionic, oxygen–nitrogen type of environment.

The Mössbauer parameters for reduced 3,4-PCase are quite similar to those of deoxyhemerythrin (Okamura and Klotz, 1973). In fact, there is good agreement in their isomer shifts (δ = 1.21, 1.15 mm/s, respectively), suggesting that the irons may reside in similar environments. X-ray diffraction studies on myohemerythrin and methemerythrin implicate histidine and tyrosine residues for iron binding (Hendrickson et al., 1975; Stenkamp et al., 1976). The involvement of tyrosine in iron binding is also suggested by the optical spectrum of 3,4-PCase (Fujisawa and Hayaishi, 1968). The native enzyme exhibits an intense absorption centered near 450 nm ($\epsilon \sim 3$ mM^{-1} cm^{-1}/Fe). The large extinction coefficient indicates a charge transfer transition rather than a d–d transition which would have a much smaller extinction coefficient (ca. 50 μM^{-1} cm^{-1}). With cysteine as an unlikely ligand, tryosine is most probably responsible for this absorption. Similar optical properties have been observed for transferrin (λ_{max}, 480 nm, ϵ 2.6 mM^{-1} cm^{-1}) (Aisen, 1973). Resonance Raman studies on transferrin have implicated tyrosine in iron coordination (Gaber et al., 1974).

Optical absorption spectra for oxygenated intermediates of 3,4-PCase have been reported by Fujisawa et al. (1972). While the nature of such complexes has been extensively investigated for heme proteins, very little work has been published for such intermediates in dioxygenase reactions. We have characterized the ternary complex of 3,4-PCase with 3,4-dihydroxyphenylpropionate and molecular oxygen by EPR and Mössbauer spectroscopy. Our investigation reveals a high-spin ferric complex whose electronic structure gives rise to a large and negative zero-field splitting. To our knowledge, such parameters have not been observed for any other iron protein. Our data show clearly that the electronic state of the iron in the enzyme is changed upon oxygen binding.

EPR spectra of the oxygenated complex are displayed in Figure 8. The upper trace shows a spectrum of the oxygenated complex frozen after steady-state conditions had been achieved.

The most prominent features in Figure 8A are strong resonances at g = 6.7 and g = 5.3. Such resonances can result from a high-spin ferric ion in an environment of almost axial symmetry; they result commonly from the ground Kramers doublet of high-spin ferric heme proteins, for which $D > 0$. Figure 9 shows a Mössbauer spectrum of an oxygenated sample prepared under the same conditions as the EPR sample for which the upper trace in Figure 8 was obtained. The spectrum in Figure 9 was taken at 4.2 K in a magnetic field of 600 G applied parallel

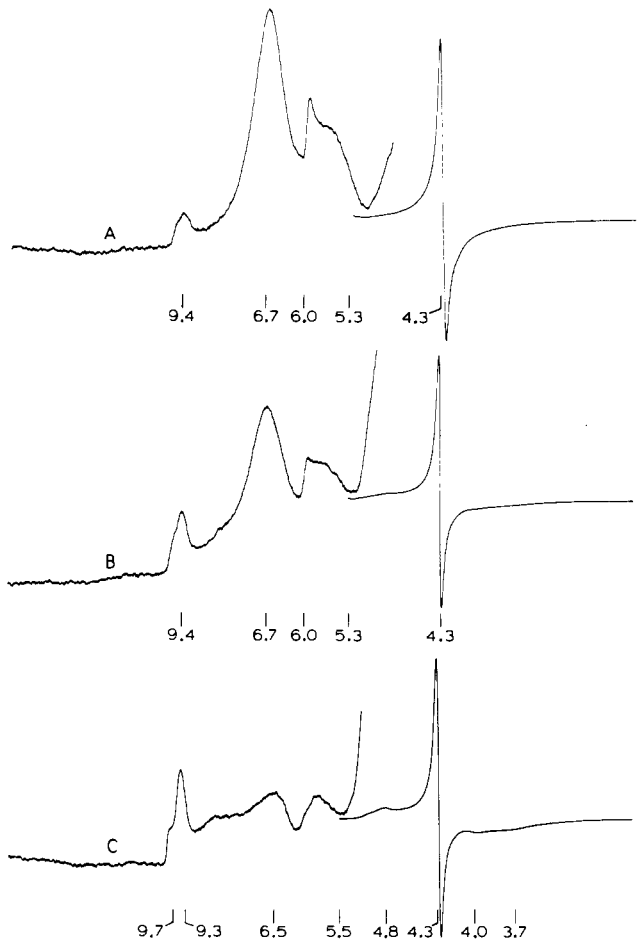

Figure 8. EPR spectra of the ternary complex of 3,4-PCase with 3,4-dihydroxyphenylpropionate and O_2. Conditions: T = 12.3 K; 9.196 GHz, 1 mW; modulation amplitude, 10 G; sweep rate, 1000 G per min; receiver gain, 3200 in the low-field region, 160 in the g = 4.3 region in A and 63 in B and C. The magnetic field increases linearly to the right and selected values of the frequency-to-field ratio (g value) are given on the abscissa. (A) The sample was frozen after steady-state conditions had been achieved. (B) The sample was frozen after the ternary complex had decayed for 1 half-life (4 min) as monitored by optical spectroscopy. (C) The sample was frozen after the ternary complex had decayed for 4 half-lives (for details, see Que et al., 1976). The decay of the g = 6.7 resonance parallels product formation and the decay of the oxygenated complex as observed with optical spectroscopy.

to the Mössbauer radiation. The intensities of the six prominent lines did not change when the field was applied perpendicular to the Mössbauer radiation. This observation implies that this spectrum results from a Kramers doublet for which $g_x \simeq g_y << g_z$; such doublets yield either no EPR signal (if $g_x = g_y = 0$) or only extremely weak signals. On the other hand, a Kramers doublet with g values at 6.7 and 5.3 has to yield a Mössbauer spectrum with intensities depending quite sensitively on the direction of the applied field. Thus the EPR and Mössbauer spectra result from two different Kramers doublets. We have shown that these observations can be reconciled by assuming a high-spin ferric system with almost axial symmetry and with a negative zero-field splitting (Que et al., 1976). By measuring the temperature dependence of the g = 6.7 resonance (which results from the upper Kramers doublet of the $S = 5/2$ system) we have determined the zero-field splitting parameter $D \simeq -2$ cm^{-1}.

As to date we do not fully understand the nature of enzyme–substrate complexes. These are certainly in a high-spin ferric state characterized by *positive* zero-field

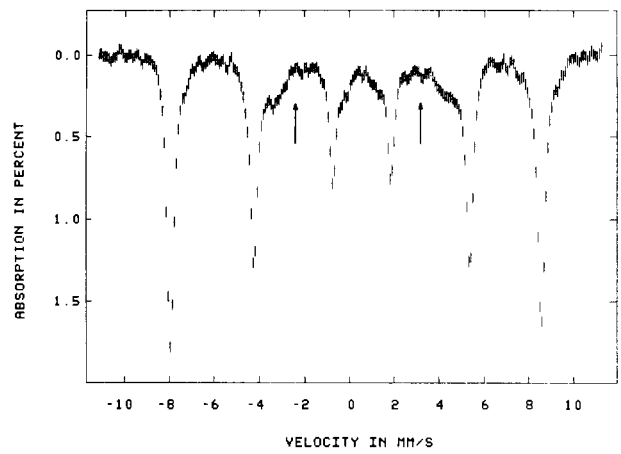

Figure 9. Mössbauer spectrum of the ternary complex of 3,4-PCase with 3,4-dihydroxyphenylpropionate and O_2 taken at 4.2 K in a 600-G parallel field. The prominent six-line pattern results from the ground Kramers doublet of the ternary complex.

splittings. What is puzzling is that we observe at least two species no matter what substrate is used. These species appear in the same ratio at low and high substrate concentrations. However rudimentary our understanding of the enzyme–substrate complexes, addition of oxygen causes dramatic changes of the electronic configuration of the iron. This, however, does not necessarily imply that the oxygen molecule is directly coordinated to the iron. The details of oxygen binding clearly need further elucidation.

The goal of this study is the elucidation of the catalytic mechanism. Clearly any mechanism proposed has to be consistent with the magnetic resonance data on the iron center. In addition, we have to determine the mode of substrate binding. Enzyme inhibition studies have been useful in determining the relative importance of various functional groups on substrate binding. Protocatechuic acid could coordinate to the iron through the carboxylate group, or one hydroxy group, or chelate to the iron with both hydroxy groups, or it may not coordinate at all. K_I determinations have been made for a large number of competitive inhibitors. These data are presented in Figure 10.

It is clear that the carboxylate group is important for binding (see XVII and XVIII). Using protocatechuic aldehyde (X) we have preliminary evidence that the carboxylate binding site is an ϵ-amino group of lysine, because the aldehyde forms a Schiff's base which is reduced to an enamine derivative with sodium borohydride. The carboxylate group does not coordinate to the iron because similar spectral changes to those seen with the substrate occur when catechol (XVIII) binds to the active site.

The inhibition data indicate that only the para hydroxy group is involved in iron binding, since there are large differences in inhibition between the compounds having para OH and meta OH groups (compare XI and XII, XIII and XIV, XV and XVI). Significant spectral changes occur when the para isomer is added to the enzyme while only small perturbations of the original optical and EPR spectra are observed when the meta isomer is added.

On the basis of this kinetic study, together with our studies on the iron center, we can now propose a mechanism for 3,4-PCase (Figure 11).

In the proposed mechanism, the iron serves to bind substrate and promote the polarization of electrons toward C-4. The iron also catalyzes what, in the absence of the metal ion, would be a spin-forbidden reaction, the reaction of oxygen (a spin-triplet) to the substrate (a spin-singlet)

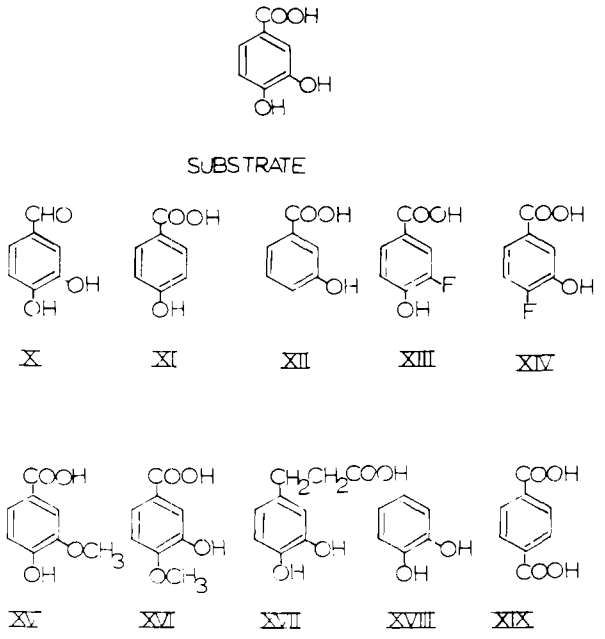

Figure 10. Kinetic studies with 3,4-PCase. K_m for substrate = 3.0×10^{-5} M. The following K_I values were determined for competitive substrate analogues: X = 1.4×10^{-5} M, XI = 2.5×10^{-4} M, XII = 4.5×10^{-3} M, XIII = 1×10^{-6} M, XIV = 8.0×10^{-4} M, XV = 3×10^{-3}M, XVI = 2×10^{-2} M, XVII = 1.8×10^{-5} M, XVIII = 7.0×10^{-4} M, XIX = not determined precisely.

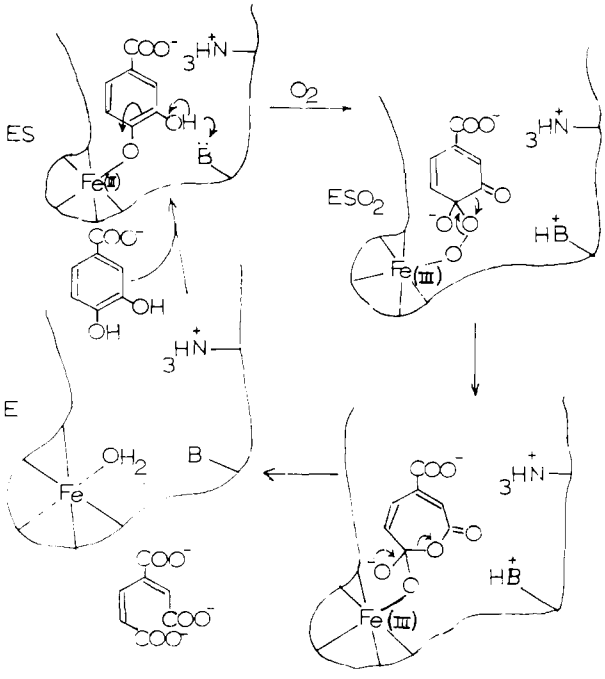

Figure 11. Proposed reaction mechanism for 3,4-PCase.

through an ionic mechanism. The iron possibly binds the peroxide which is formed, though this remains to be demonstrated. This oxygenated intermediate then rearranges to give product.

Model studies on the oxidation of catechols tend to support this mechanism. Organic chemists have been able to isolate products from O_2 oxidation of catechols in alkaline media, to form the expected *cis,cis*-muconic acid (Grinstead, 1964; Baldwin, 1976). In particular, Jack Baldwin's work in this area may shed light on the enzymatic mechanism. The catechol (VIII) was synthesized (Figure 12). At −50 °C VIII was shown to isomerize to IX in the absence of O_2. Upon exposure to O_2, the corresponding *cis,cis*-muconic acid was formed. This experiment emphasizes that O_2 can add to an organic substrate and that a peroxy intermediate may indeed be formed. If this mechanism is correct, then the role of the iron in 3,4-PCase is to catalyze the isomerization presented in Figure 12. Therefore, the catechol is activated so that it can react with molecular oxygen to give a peroxy intermediate. We believe that this enzyme provides the first evidence for substrate "activation", as a prerequisite to oxygen "activation".

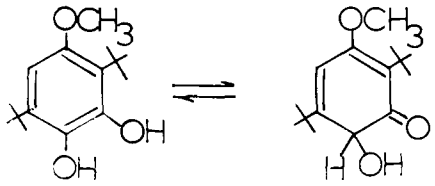

Figure 12. Isomerization of substituted catechol (VIII) to a substituted keto alcohol (IX).

GENERAL CONCLUSIONS

Our present technology for the separation of lignin from cellulose requires the use of unnatural oxidizing agents which yield lignin-based waste products which are harmful to the environment and in some cases dangerous to the public health. Biological systems are capable of the oxidation of lignin by using molecular oxygen as the chemical reagent. Although biological processes for the delignification of cellulose appear to be slow, this important process does occur in natural aerobic ecosystems. Clearly if we understand the catalytic mechanisms involved in solubilizing lignin by oxidation, then we can design low molecular weight catalysts capable of delignification with greater efficiency. The sixty-five thousand dollar question is "Can we improve on nature?". The compact structure of the lignin polymer makes it difficult to wet and difficult to penetrate even by microorganisms which may be only 2 μm in size. Therefore, it seems logical that if we build small molecular catalysts which mimic oxygenase active sites in both their structure and functionality; then we could improve on nature, because we will have overcome slow diffusion-rate controlled processes. Further studies of the type reported in this manuscript should provide enough basic information for the design of catalysts which are capable of oxidative fission of the benzene nucleus. Catalysts which mimic mixed function oxygenases have already been prepared, and these catalysts do prepare the benzene ring for cleavage by the dioxygenases. Therefore, we conclude that catalytic delignification of cellulose is likely to be accomplished in the not too distant future.

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Biodegradation of Natural and Man-Made Recalcitrant Compounds with Particular Reference to Lignin

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Novel procedures are described for study of the biological decomposition of natural and man-made recalcitrant molecules. Six different lignocelluloses were prepared containing ^{14}C in primarily their lignin components. Degradation of these lignin-labeled lignocelluloses by the microfloras of soil and lake water was observed by monitoring evolution of $^{14}CO_2$ from incubation mixtures. The standard most-probable-number technique for enumeration of specific microbial groups within natural habitats was adapted for the enumeration of degraders of recalcitrant, insoluble, or other organic compounds. Evolution of $^{14}CO_2$ from ^{14}C-labeled substrates was shown to be an acceptable, readily recognizable transformation for use in scoring dilution replicates positive or negative for degradation of a specified compound. Use of this radioisotopic modification of the most-probable-number technique allowed enumeration of lignin degraders, 2,4-D (2,4-dichlorophenoxyacetate) degraders, and hexachlorobiphenyl degraders in several soil and water environments.

There are many chemical structures in the biosphere that are to various degrees resistant to microbiological degradation in soil and water. Such compounds are often identified as being "recalcitrant" (Alexander, 1965a,b, 1967, 1975). These biologically resistant molecules may enter the biosphere either through the activities of industrial societies or via natural, biological processes (Alexander, 1975).

Probably the most common slowly decomposed, natural compound on the earth is the plant polymer lignin. Lignin is a structural polymer found in all vascular plants where it performs numerous, essential physiological functions

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