Nature and Mechanisms of Oxygenases

Enzymes involved in the biological fixation of molecular oxygen are discussed.

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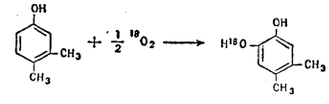
The role of molecular oxygen as a terminal electron acceptor in respiration has been recognized ever since Lavoisier initiated the study of the biological oxidation process some 200 years ago. In this process, electrons or hydrogen atoms are removed from the substrate and are transferred by dehydrogenases, through various carriers, to oxygen. The oxygen is reduced either to H_2O or H_2O_2. During this transfer reaction, energy may be conserved and used to synthesize adenosine triphosphate, a common carrier of chemical energy for mechanical, biosynthetic, and a host of other biological processes.

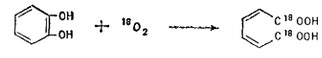
In 1867, Pasteur made the famous observation that anaerobically growing yeast cells degenerated in structure unless periods of aerobiosis were interspersed, although yeast had been known to be capable of maintaining life under strictly anaerobic conditions (*1*). This phenomenon suggested that molecular oxygen, besides supplying energy, is necessary for the biosynthesis of some compound or compounds essential to morphogenesis. In fact, subsequent experiments from a number of laboratories indicated that molecular oxygen is important for life, not only as the ultimate electron acceptor in the respiratory chain, but also as a biosynthetic device for essential metabolites (*2*). Nevertheless, the chemical basis of this oxygen-involved biosynthetic reaction had not been experimentally explored until enzymic oxygen-fixation reactions were discovered many years later.

In 1955, two groups of investigators independently and concurrently characterized a new type of enzymic reaction by which molecular oxygen is fixed into organic substrates. Mason and his collaborators (*3*), using ^{18}O-labeled oxygen and water, found that, during the oxidation of 3,4-dimethylphenol to dimethylcatechol catalyzed by a phenolase complex (E.C.1.10.3.1), the oxygen atom incorporated into the substrate molecule was derived exclusively from molecular oxygen, not from the oxygen of a water molecule (Eq. 1).



(1)

Hayaishi and associates (*4*) found that two atoms of oxygen, inserted into catechol by the action of pyrocatechase (E.C.1.13.1.1), were both derived exclusively from atmospheric oxygen (Eq. 2).



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In contrast to the previously known mechanism of biological oxidation, namely dehydrogenation, these types of oxidative reactions appeared to depend on the activation of molecular oxygen, which was then incorporated into organic substrates. It was therefore proposed that the enzymes which catalyze such oxygen-fixation reactions be named "oxygenases" (*5*). Since then, more and more new oxygenases have been discovered in animals, plants, and microorganisms. In addition to their ubiquitous distribution in nature, their versatile functions and unique properties have also been recognized not only in many biosynthetic reactions but also in the catabolic pathways of various substrates and in the metabolic disposal of foreign compounds.

In this article, we discuss the biological functions and interesting properties of oxygenases that have been discovered or characterized in animal and plant tissues, as well as in microorganisms. In addition, new developments in the study of reaction mechanisms and in current thoughts on the enzymic activation of molecular oxygen are presented.

Chemical Nature of Oxygenase-Catalyzed Reaction

Oxygenases participate in the biosynthesis and degradation of many metabolites, ranging from amino acids and lipids to porphyrins, vitamins, and hormones. They also take part in the metabolic disposal of a variety of drugs and foreign substances.

In general, oxygen-rich compounds, such as carbohydrates, are not favorable substrates for oxygenases, because these compounds usually have many reactive groups containing oxygen such as hydroxyl, carbonyl, or formyl, and they do not need to be oxygenated further. On the other hand, lipids and aromatic compounds are often metabolized by oxygenases, presumably because these compounds are generally oxygen-deficient and need to be oxygenated in order to become biologically active or more soluble in water. Yet because of the hydrophobic nature of lipids and aromatic compounds, molecular oxygen is the preferred hydroxylating agent, rather than water. In contrast, purines and pyrimidines with their hydrophilic ring systems, are usually hydroxylated by the addition of water, followed by dehydrogenation.

Two subclasses of oxygenases may be defined (*6*). Dioxygenases catalyze the incorporation of both atoms of molecular oxygen into a molecule of substrate, as in the case of pyrocatechase (Eq. 3). Monooxygenases, sometimes referred to as "mixed function oxygenases" (*7*) are responsible for the incorporation of a single atom of oxygen into the substrate, as in the case of phenolase (Eq. 4)

$$\text{Substrate} + O_2 \rightarrow \text{Substrate–}O_2 \quad (3)$$

$$\text{Substrate} + \tfrac{1}{2}O_2 \rightarrow \text{Substrate–O} \quad (4)$$

A major reaction catalyzed by dioxygenases is the cleavage of an aromatic double bond, which may be located (i) between two hydroxylated carbon atoms, (ii) adjacent to a hydroxylated carbon atom, or (iii) in an indole ring. Many monooxygenases, on the other hand, are concerned with hydroxylation reactions of aromatic as

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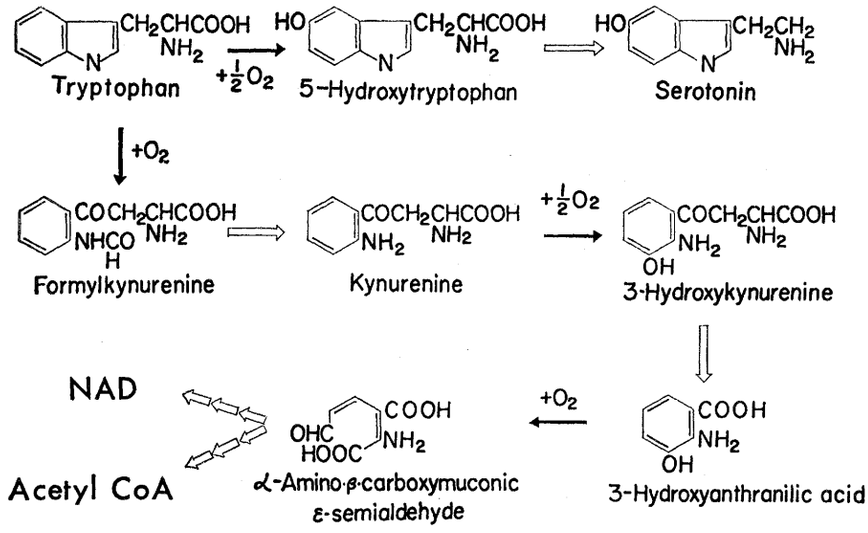


Fig. 1. Oxygenase reactions involved in the pathway of tryptophan metabolism. The black arrows denote oxygenase reactions and the white arrows represent reactions catalyzed by enzymes other than oxygenases.

well as aliphatic compounds. However, monooxygenases also catalyze a seemingly diverse group of reactions, including epoxide formation, dealkylation, decarboxylation, deamination, *N*- or *S*-oxide formation, and so forth. Furthermore, it has recently been discovered that, during enzymic hydroxylation of aromatic substrates, the substituent (deuterium, tritium, chlorine, bromine, and so forth) displaced by the entering hydroxyl group migrates to an adjacent position in the aromatic ring (*8*). Although the overall reactions catalyzed by various monooxygenases seem grossly different, the primary chemical events involved are basically identical, in the sense that these processes are all initiated by the incorporation of one atom of molecular oxygen into the substrate.

Oxygenases in Amino Acid Metabolism

The important role played by oxygenases in the catabolism of aromatic amino acids such as tryptophan, phenylalanine, tyrosine, and various phenolic compounds has been recognized for a long time (*9*). For example, tryptophan undergoes a variety of metabolic transformations. Among them, two of the most important are initiated by a monooxygenase and a dioxygenase, respectively (Fig. 1). Tryptophan serves as a precursor for the biosynthesis of serotonin, a potent vasoconstrictor substance and a neurohumoral agent. The enzyme tryptophan-5-monooxygenase catalyzes the initial step of this metabolic transformation and has been found and purified from the brain stem of various species of mammals (*10*). One atom of molecular oxygen is introduced into tryptophan to form 5-hydroxytryptophan, which is then decarboxylated to serotonin. Trytophan-2, 3-dioxygenase (E.C.1.13.1.12) catalyzes the insertion of two atoms of molecular oxygen into the pyrrole moiety of tryptophan, forming formylkynurenine as a product. Hence, it is sometimes referred to as tryptophan pyrrolase. This reaction is the initial step in a metabolic sequence which leads to the biosynthesis of a coenzyme, nicotinamide-adenine dinucleotide (NAD), from tryptophan in mammals as well as in some microorganisms and is probably a rate-limiting step and a likely site of regulation in this pathway. Formylkynurenine thus formed is converted to kynurenine which is then hydroxylated to 3-hydroxykynurenine by the action of a specific monooxygenase of mitochondria (Fig. 1). Kynurenine-3-monooxygenase (E.C.1.14.1.2) is localized in the outer membrane of rat liver mitochondria (*11*) and contains flavin-adenine dinucleotide (FAD) as its prothetic group (*12*). 3-Hydroxykynurenine is then transformed to 3-hydroxyanthranilic acid, which is then cleaved by a specific dioxygenase. This reaction is physiologically very important because the resulting compound can either be converted to acetyl-coenzyme A through various intermediates such as glutaryl-coenzyme A, or it can form a new pyridine ring yielding picolinic acid and quinolinic acid as the reaction products. Quinolinic acid was shown to yield nicotinic acid ribonucleotide which is a precursor of NAD (*13*). It is noteworthy that most, if not all, oxidative reactions of tryptophan metabolism involve oxygen fixation (*6*).

Similarly, the metabolism of phenylalanine and tyrosine is catalyzed by a number of oxygenases. For example, the enzymatic formation of tyrosine from phenylalanine and the formation of adrenaline, noradrenaline, melanin, and thyroxine are catalyzed by a number of consecutive reactions involving various mono- and dioxygenation reactions (*14, 15*). For many of the hereditary metabolic disorders that have been studied in human beings, the anomaly has been traced to the absence of activity of specific oxygenases in these pathways. In normal individuals, almost all of phenylalanine metabolism is channeled through tyrosine by the action of phenylalanine hydroxylase (E.C.1.14.3.1). Kaufman and his co-workers have shown that tetrahydropteridine derivatives serve as the direct hydrogen donor in this process (*14*). Hereditary deficiency of phenylalanine hydroxylase results in phenylketonuria, an inborn error of metabolism and a common cause for the mental retardation. Similarly, alkaptonuria is due to the hereditary deficiency of homogentisate dioxygenase (E.C.1.13.1.5), which catalyzes the conversion of homogentisic acid to 4-maleylacetoacetate.

Hydroxyprolines, primarily 4-hydroxyproline, together with a small amount of the 3-hydroxy isomer, are unique constituents of collagen, a major component of cartilage and other connective tissues, and the most abundant protein in the body. Early isotopic studies indicated that free hydroxyproline is not incorporated as such into collagen, but that collagen hydroxyproline is synthesized by the hydroxylation of some intermediate such as prolyl-transfer RNA or of proline-containing peptides. More recently, a proline-rich polypeptide precursor called protocollagen was shown to serve as substrate for the hydroxylation reaction by a partly purified monooxygenase which requires ferrous ion, ascorbate, and α-ketoglutarate (*16*). Although evidence suggests that the oxygenation reaction proceeds in polypeptides attached to ribosomes, it has not yet been established whether the true substrate is a nascent polypeptide chain attached to ribosomal complexes, or whether a complete polypeptide chain is released from ribosomes before proline is hydroxylated.

Oxygenases in Lipid Metabolism

The transformation of the open-chain polyolefin squalene into the tetracyclic substance lanosterol is initiated by a monooxygenase. Two independent groups of investigators have produced evidence that this monooxygenase incorporates one atom of oxygen into squalene, with the formation of an epoxide which then rearranges and cyclizes into the steroidal configuration (*17*) (Fig. 2). The prevalence of oxygenase reactions in the further metabolism of lanosterol to cholesterol, and also further transformation of the latter to bile acids and various steroid hormones, has already been reviewed (*18*). Almost all the oxidative steps in the catabolic pathway of cholesterol are catalyzed by various oxygenases. The degradation of the cholesterol side chain appears to be catalyzed by an oxygenase in mitochondria, while most monooxygenases in these pathways are localized in microsomes. The oxidation of glyceryl ether represents another unique example of a monooxygenase-catalyzed reaction in which pteridine derivatives have been suggested as a cofactor (*19*). Omega-oxidation of long-chain fatty acids, incorporation of oxygen atoms into various parts of hydrocarbons, as well as desaturation of fatty acids are also catalyzed by microsomal oxygenases.

Prostaglandins are a group of hormones active in stimulating contraction of intestinal smooth muscle and in reducing blood pressure. Furthermore, they antagonize the stimulatory effects of a number of other hormones, such as epinephrine, glucagon, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and so forth, on the release of free fatty acids and glycerol from epididymal fat pads. Experiments from Bergström's laboratory provided evidence that the biosynthesis of prostaglandins from unsaturated fatty acids is catalyzed by the action of two successive reactions involving a dioxygenase and a monooxygenase (*20*) (Fig. 3). Experiments with oxygen-18 indicated that the two oxygen atoms at the 9- and 11-positions of prostaglandins are derived from a single molecule of oxygen and the hydroxyl group at the 15-position seems to be incorporated after the pentacyclic ring is formed by the action of the dioxygenase.

The biosynthesis of vitamin A from β-carotene is mediated by a dioxygenase which requires ferrous ion (*21*). This reaction is unique in that the dioxygenase reaction cleaves a carbon-carbon double bond in an aliphatic compound.

Oxygenases in Drug Metabolism

The monooxygenase reactions catalyzed by liver microsomes have been the subject of intensive investigation by enzymologists, pharmacologists, and toxicologists, since this enzyme activity is inducible and is intimately related to the mechanism by which various drugs and other foreign substances are detoxified, and is also involved in the transformation of carcinogenic and carcinostatic substances. It has been shown by a number of investigators that the electrons from reduced nicotinamide-adenine dinucleotide phosphate (NADPH) are transferred to oxygen by way of a heme-like pigment, designated cytochrome P-450, and that the enzymes present in microsomes are responsible for a variety of reactions, including hydroxylation of various drugs and toxic compounds, *S*- and *N*-oxide formation, dealkylation, and other reactions. However, it is not yet known whether these various types of reactions are catalyzed by a single enzyme, or whether they are mediated by groups of enzymes which have different substrate specificity. Estabrook and his associates (*22*) have recently reviewed this topic.

Oxygenases in Plant Metabolism

Lipooxygenase (E.C.1.13.1.13) is prevalent in the plant kingdom and plays an important role in food processing and preservation. The enzyme catalyzes the conversion of lipids containing a *cis,cis*-1,4-pentadiene system to the corresponding hydroperoxides. Although lipooxygenase has been obtained in a crystalline form from soybeans (*23*), the nature of the prosthetic group and the reaction mechanism is not yet well understood.

By analogy with mammalian and bacterial systems, many oxygenase reactions are expected to be involved in plant metabolism since phenolic compounds are second only to carbohydrates in abundance in higher plants. They display a great variety of structure, ranging from derivatives of simple phenols, such as quinol, to complex polymeric materials, such as lignin. The overall pathways of biosynthesis of these compounds have been investigated in many laboratories, but very little is known about the detailed mechanisms of their enzymatic formation. Nevertheless, the accumulation of positive evidence from both tracer and enzymological investigations suggests that oxygenation reactions participate in the biosyntheses of these complex phenols and alkaloids.

Since phenolase was found to catalyze the incorporation of one atom of oxygen into phenol compounds (*3*), participation of phenolase in the hydroxylation reactions of aromatic compounds during the biosynthesis of phenolic compounds has been suggested by a number of investigators. However, many questions, including the precise role of the enzyme in in vivo systems, remain to be answered. Likewise, peroxidase (E.C.1.11.1.7), rich in plant

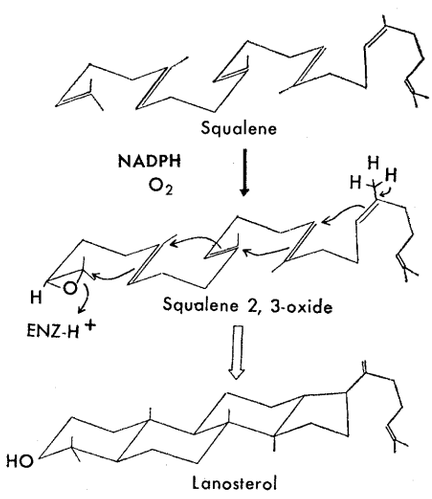


Fig. 2. Conversion of squalene to lanosterol.

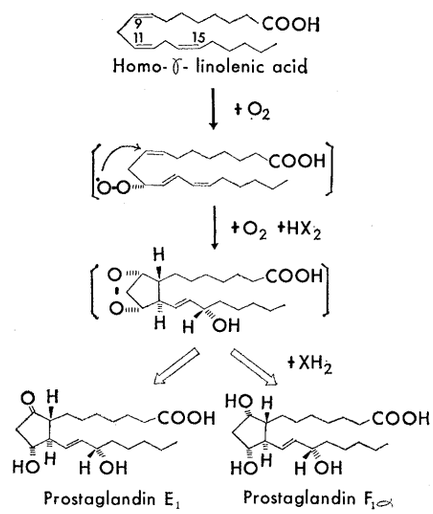


Fig. 3. Enzymic synthesis of prostaglandins E_1 and $F_{1\alpha}$ from homo-γ-linolenic acid. XH_2 denotes an electron donor.

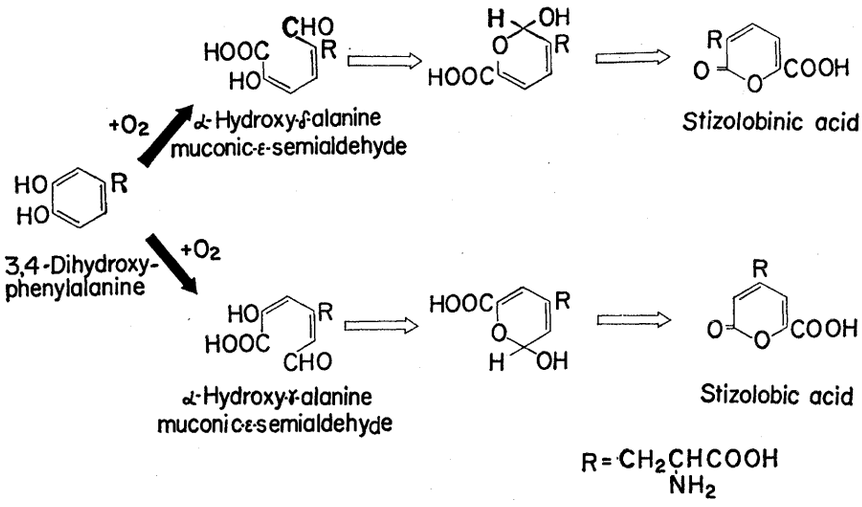


Fig. 4. Proposed pathways of biosynthesis of stizolobic and stizolobinic acids in plants.

tissues, was reported to catalyze various oxygenation reactions besides peroxidatic reactions. These are hydroxylation of aromatic compounds in the presence of dihydroxyfumarate and oxygen (*24*) and monooxygenation of amino acids, such as methionine (*25*) and tryptophan (*26*), to form the corresponding acid amide in the presence of pyridoxal phosphate and molecular oxygen. The physiological significance of these actions of peroxidase is obscure, but the overall reactions of the latter type are identical with those catalyzed by lysine monooxygenase (*27*) and other similar flavin-containing monooxygenases which will be described below.

Although many of the individual metabolic steps in plants have yet to be demonstrated conclusively, hydroxylation reactions appear to be involved in some cases. Nair and Vining (*28*) reported a cinnamic acid hydroxylase in the acetone precipitate of an extract of spinach leaves. The enzyme, like other monooxygenases, showed an absolute requirement for an external electron donor, which was satisfied by reduced pyridine nucleotide coenzyme plus tetrahydrofolic acid. Nair and Vining (*29*) have also characterized a phenylalanine hydroxylase from spinach, which is distinct from cinnamic hydroxylase. Such an enzyme would account for the conversion of phenylalanine to tyrosine that occurs in green plants. Anthranilic acid was converted to catechol by a chloroplast enzyme system from the leaves of *Tecoma stans*. The system was obtained in a soluble form and fractionated into three components. This enzyme system also required NADPH and tetrahydrofolic acid (*30*).

Hydroxylation and oxygenative cleavage of aromatic rings have been suggested for some steps in alkaloid biosyntheses, but many of these suggestions are admittedly speculative and tentative. Among them, Senoh *et al.* (*31*) suggested the possibility that the biosyntheses of pyridine, α-pyrone, and α-tetronic acid nuclei originate from metapyrocatechase-type oxidations, followed by some types of isomerization reactions. For example, stizolobic acid and stizolobinic acid, new amino acids isolated from the etiolated seedlings of *Stizolobium hassjoo*, are presumably derived from 3,4-dihydroxyphenylalanine in vivo by a metapyrocatechase-type cleavage reaction, and subsequent cyclization and dehydrogenation of the resultant α-hydroxymuconic semialdehyde derivatives (Fig. 4).

Table 1. List of crystalline dioxygenases and monooxygenases

Names	Cofactors	Substrate	Product	Reference
		Dioxygenases		
Metapyrocatechase (E.C.1.13.1.2)	Ferrous ion	Catechol	α-Hydroxymuconic semialdehyde	(*51*)
3,4-Dihydroxy-phenylacetate 2,3-oxygenase (E.C.1.13.1.7)	Ferrous ion	3,4-Dihydroxy-phenylacetate	α-Hydroxy δ-carboxymethyl-muconic semi-aldehyde	(*52*)
Homogentisate dioxygenase	Ferrous ion	Homogentisate	Maleylacetoace-tate	(*53*)
Protocatechuate 3,4-dioxygenase (E.C.1.13.1.3)	Ferric ion	Protocatechuate	β-Carboxymuconic acid	(*54*)
		Monooxygenases		
Lactate mono-oxygenase (E.C.1.1.3.2)	FMN	Lactate	Acetate	(*37*)
Lysine mono-oxygenase	FAD	Lysine	δ-Amino-*n*-valer-amide	(*27*)
Imidazoleacetate monooxygenase (E.C.1.14.1.5)	FAD (NADH)	Imidazole-acetate	Imidazoloneacetate	(*41*)
p-Hydroxybenzoate hydroxylase	FAD (NADPH)	*p*-Hydroxy-benzoate	Protocatechuate	(*44*)

Microbial Oxygenases

The microbial oxidation of various aromatic compounds has been investigated by microbiologists; such studies have contributed to our knowledge of general pathways of metabolism and their regulation, and to our knowledge of genetics (see *32*).

The presence of physiologically important oxygenases in animal tissues is generally confined to specialized organs and tissues, and the amount is rather limited. Therefore, it is usually difficult to obtain mammalian oxygenases in large quantities. On the other hand, a number of oxygenases in bacteria are inducible and therefore serve as a much better starting material for enzyme studies. Almost all the oxygenases which have so far been crystallized were obtained from microbial origin. The names of these crystalline oxygenases are listed in Table 1, together with their cofactors, substrates, and reaction products.

Reaction Mechanism of Dioxygenases

Typical dioxygenases which have been obtained in crystalline form usually contain either ferric or ferrous ion as a sole cofactor (Table 1). In that sense, they belong to the so-called "nonheme iron proteins," although

they are devoid of labile sulfide and the molecular weights are much larger than ferredoxin and related proteins.

Some properties of typical nonheme iron-containing dioxygenases which have been crystallized in our laboratory (Fig. 5) are shown in Table 2. Although the molecular weight, valence state of iron, and the iron content per mole of enzyme protein are different, the number of iron atoms present roughly coincides with the number of subunits in the enzyme protein and the number of substrate molecules bound to the enzyme, indicating that each subunit may contain one atom of iron and that all the iron of the total molecule may be involved in catalysis as an interacting site for the substrate (*33*). Since inorganic iron is the only constituent of these typical dioxygenases besides enzyme protein, and since iron has been known to combine with and/or activate molecular oxygen, it has been tacitly assumed that iron is in the active center of these dioxygenases and plays a major role in the activation of oxygen as well as substrate. On the basis of analytical, kinetic, and inhibition experiments, which were reported elsewhere (*34*), we proposed that iron in the active center forms a ternary complex with oxygen and organic substrate. Within this ternary complex, both organic substrate and oxygen are activated in some way and react together to form an oxygenated end product (*6*).

Experiments with highly purified tryptophan-2,3-dioxygenase (pyrrolase) (E.C.1.13.1.12), a dioxygenase containing heme as a cofactor, provided further evidence which supports the following scheme as a plausible mechanism of the reaction (*35*). The organic substrate, tryptophan, combines with the enzyme, which may evoke a conformational change of the enzyme protein and an increase of the reactivity of the heme moiety (Fig. 6). The oxygen molecule is then bound to the heme to form a ternary complex of tryptophan, heme, and oxygen. Within this ternary complex, oxygen is presumably activated and reacts with the substrate to form an oxygenated product. The only enzymatically active species of oxygenated heme so far known is the compound III of peroxidase (*36*), in which case ferrous ion in the heme is oxygenated in the absence of its organic substrate.

In contrast, in the case of tryptophan pyrrolase, the organic substrate is necessary in order to form an oxygenated intermediate. The exact physicochemical characterization of the electronic structure of heme, oxygen, and tryptophan in this ternary complex and, in particular, the nature of active oxygen remain to be answered. But the spectral properties of the oxygenated heme in tryptophan pyrrolase appear to be similar to those of oxygenated forms of hemoglobin, myoglobin, and peroxidase. These experimental observations with tryptophan-2,3-dioxygenase provide further evidence to support the hypothetical reaction mechanism proposed on the basis of experimental findings with the nonheme iron-containing dioxygenases (*6*); in addition, they indicate that a ternary complex of iron, oxygen, and organic substrate is formed as an intermediate of a dioxygenase reaction. Although it may still be premature to generalize, this apparently ordered mechanism may be characteristic of other dioxygenases as well.

Reaction Mechanism of Monooxygenases

The simplest type of monooxygenase catalyzes the incorporation of a single atom of molecular oxygen, concomitant with the reduction of the other oxygen atom to water by the electrons derived from the substrate. Thus the overall stoichiometry may be expressed by the following equation

$$SH_2 + O_2 \rightarrow SO + H_2O \qquad (5)$$

Here SH_2 denotes the substrate molecule. Since the reducing agent is internally supplied, these monooxygenases may be referred to as internal monooxygenases. The first of these to be crystallized was the lactate oxidative decarboxylase (E.C.1.1.3.2) from *Mycobacterium phlei* (*37*). This enzyme catalyzes the conversion of lactate to acetate with the incorporation of one atom of oxygen and the evolution of one mole of CO_2.

L-Lysine monooxygenase, which has

Table 2. Properties of some nonheme iron dioxygenases.

Enzyme	Mol. wt.	Iron		Subunits (No.)	Substrate binding to enzyme (mole/mole)
		Valence	Content (atom/mole)		
Metapyrocatechase	140,000	Fe^{+2}	3	$2 \sim 3$	$2 \sim 3$
Protocatechuate-3,4-dioxygenase	700,000	Fe^{+3}	~ 8	~ 8	~ 8

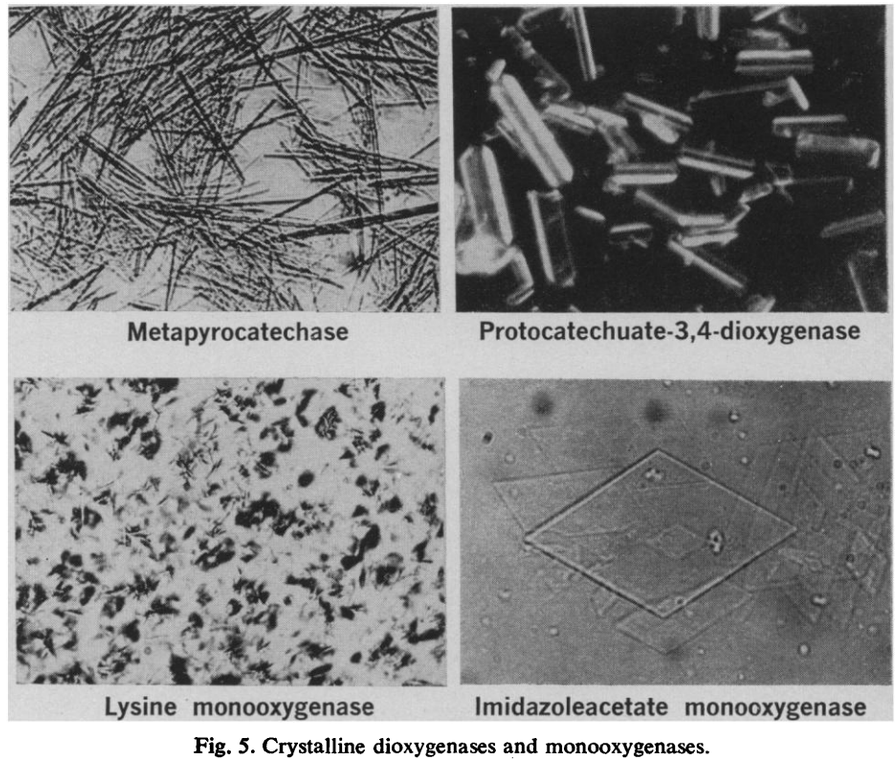


Fig. 5. Crystalline dioxygenases and monooxygenases.

been crystallized from *Pseudomonas fluorescens* in our laboratory (*27*), is also an internal monooxygenase (Fig. 5). L-Lysine is metabolized by this enzyme to δ-amino-*n*-valeramide, with the insertion of one atom of oxygen and the evolution of 1 mole of CO_2. When L-lysine was incubated with a stoichiometric amount of this enzyme under anaerobic conditions, reduction of the enzyme-bound FAD was observed, indicating that lysine serves as an internal electron donor, being dehydrogenated itself. The dehydrogenated product of lysine is presumably the α-imino acid analog of lysine, which is identical to a proposed intermediate for the L-amino acid oxidase reaction, since the overall reaction catalyzed by lysine monooxygenase under anaerobic conditions seems identical to that catalyzed by amino acid oxidase (*38*). Therefore, the first step of the reaction of lysine monooxygenase is, very probably, the transfer of two hydrogen atoms from the α-position of L-lysine forming an enzyme-bound α-imino acid intermediate and reduced FAD ($FADH_2$). In the presence of oxygen, both the imino acid and $FADH_2$ will react with molecular oxygen, probably in a ternary complex resulting in the formation of δ-amino-*n*-valeramide, CO_2, and water (Fig. 7).

The enzyme catalyzing the formation of acid amide and CO_2 from the corresponding amino acid may be widely distributed in nature, because arginine (*39*) and tryptophan (*40*) are metabolized in a similar manner. Although H_2O_2 could not be found in the reaction, an enzyme-bound H_2O_2 or its equivalent may be involved, because similar reactions are catalyzed by crystalline horseradish peroxidase as described before.

Table 3. Classification of monooxygenases.

Electron donor	Cofactor	Example
	Internal monooxygenase	
Substrate	Flavin	Lysine monooxygenase
	External monooxygenase	
Ascorbic acid	Cu	Dopamine β-hydroxylase
Pyridine nucleotide	Flavin	Imidazoleacetate monooxygenase
Pyridine nucleotide	Pteridine	Phenylalanine hydroxylase
Pyridine nucleotide	Flavin nonheme iron (and/or heme)	ω-Hydroxylation system

While the internal monooxygenases do not require external reducing agents, more common types of monooxygenases require various kinds of electron donors. The overall reaction may be schematically represented by the following equation (Eq. 6)

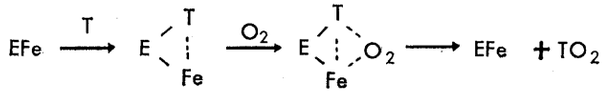
$$S + O_2 + H_2X \rightarrow SO + X + H_2O \quad (6)$$

where S denotes the substrate molecule, and H_2X represents an electron donating system. The nature of the reductant may serve as a basis for the subclassification of external monooxygenases (Table 3).

An external monooxygenase which catalyzes the NADH-dependent conversion of imidazoleacetate to yield imidazoloneacetate has been purified from a pseudomonad and crystallized (Fig. 5) (*41*). The enzyme, which has a typical flavoprotein spectrum, can be dissociated into its protein and coenzyme moieties by acid ammonium sulfate treatment and then reactivated by FAD, but not by flavin mononucleotide (FMN). Reduction of enzyme-bound FAD is observed by the addition of NADH under anaerobic conditions, either in the presence or absence of the substrate imidazoleacetate. The reduced flavin thus obtained is shown to serve as an ultimate hydrogen donor for the hydroxylation reaction of the substrate (*42*).

These findings are consistent with the reaction scheme shown in Fig. 8. First, electrons are transferred from NADH to FAD, forming $FADH_2$, which interacts with oxygen and the substrate, imidazoleacetate, to form imidazoloneacetate and water. All these steps are catalyzed by a presumably single enzyme.

A similar reaction mechanism is also

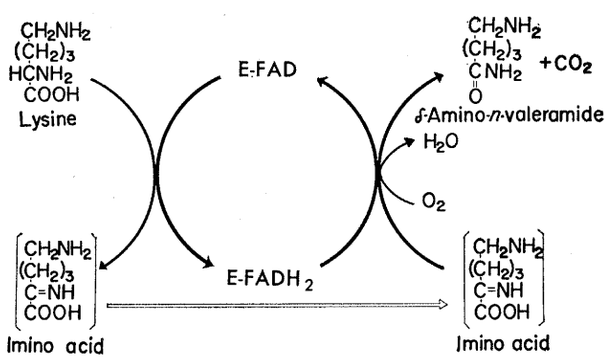


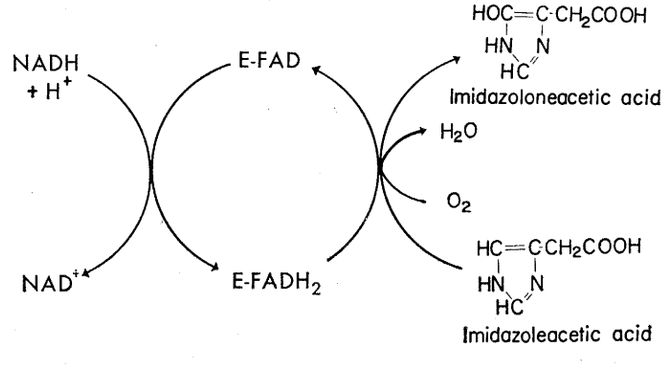
EFe : Tryptophan 2, 3 dioxygenase
T : Tryptophan
TO_2: Formylkynurenine

Fig. 6 (left). Proposed reaction mechanism of tryptophan-2,3-dioxygenase.

Fig. 7 (below left). Proposed reaction mechanism of lysine monooxygenase.

Fig. 8 (below right). Proposed reaction mechanism of imidazoleacetate monooxygenase.





proposed for salicylate hydroxylase (*43*) and *p*-hydroxybenzoate hydroxylase (*44*), other flavin-dependent external monooxygenases. In the former reaction, reduced enzyme-bound FAD produced either by chemical or by photochemical reduction is also shown to serve as an ultimate electron donor for the hydroxylation of salicylate (*45*).

All monooxygenases which have been obtained in crystalline form from bacteria are characterized as flavoproteins (Table 1). Since they are devoid of metal components, flavin seems to be a sole cofactor of these enzymes (*46*). Some of the external monooxygenases are, however, characterized by the involvement of copper, pteridine derivatives, or a more complicated electron transfer system (Table 3). For example, bacterial oxygenation of hydrocarbon and long-chain fatty acids at the terminal methyl carbon atom has been shown to involve at least three protein components (*47*). Electrons are transferred by a rubredoxin-NADH reductase, probably a flavoprotein, from NADH to rubredoxin. Rubredoxin, isolated from a pseudomonad, is a red nonheme iron protein, whose molecular weight is 22,000; it contains 2 gram-atoms of ferric ion per mole of enzyme. The reduced rubredoxin provides the reducing power for the action of the hydroxylase on the substrate. Ferredoxins, which differ from rubredoxin because they contain labile sulfide, participate in steroid hydroxylation in adrenal mitochondrial fractions (*48*), in fatty acid desaturation in Euglena and spinach (*49*), and in the *d*-camphor methylene hydroxylase system (*50*).

In the case of internal monooxygenases, an ultimate electron donor, flavin, is first reduced by the substrate itself. The resulting reduced flavin and dehydrogenated substrate react with oxygen, forming a ternary complex and thus producing the oxygenated product and water. On the other hand, in the case of external monooxygenases, an ultimate hydrogen carrier may be first reduced by an external hydrogen- or electron-donating system. Whatever the ultimate hydrogen or electron carrier may be, the reduced carrier will react with substrate and oxygen to form a ternary complex, and the reaction may proceed in a way quite similar to that of the internal monooxygenases. Whether the complex formation proceeds in a concerted or a successive manner is still unknown.

Summary

The discovery some 10 years ago of oxygenases, a class of enzymes which bring about the direct interaction of gaseous oxygen with organic molecules, added another dimension to our knowledge of oxygen metabolism in general. Oxygenases participate in numerous metabolic transformations by catalyzing degradative and biosynthetic processes in animals, plants, and many microorganisms. The enzymes are also involved in the metabolic disposal of a variety of drugs and foreign substances. Selected samples of metabolic pathways in which oxygenase reactions are involved are presented.

Several di- and mono-oxygenases have now been obtained in highly purified or crystalline form from microorganisms, thus permitting a critical analysis of the mechanisms of their actions. Studies on the mechanism of oxygenase action are focused at present on two related aspects of the general problem—the nature of "active oxygen" and the mechanism by which it is formed. Recent evidence is consistent with the view that in most, if not all, dioxygenase reactions, iron is involved and that iron-bound oxygen appears to be the activated form. In a classical formulation, this complex may be expressed as $Fe^{+2}O_2 \rightleftharpoons Fe^{+3}O_2^-$. The presence of substrate appears to play a crucial role in this activation process. On the other hand, in monooxygenase reactions, molecular oxygen, organic substrate, and reducing agent, either internal or external, form a ternary complex. The oxygenation of organic substrate and the reduction of one oxygen atom to water seem to occur simultaneously. However, there is some evidence that, in the monooxygenase reactions too, molecular oxygen is activated by the reducing agent to O_2^- or even to O_2^{--} or their equivalent. Iron or copper may be involved in at least some monooxygenase reactions, whereas these metals may not be involved in others.

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Violence and Man's Struggle To Adapt

Violence, one form of aggressive behavior, produces maladaptive results in this technological age.

Marshall F. Gilula and David N. Daniels

The need is not really for more brains, the need is now for a gentler, a more tolerant people than those who won for us against the ice, the tiger, and the bear (*1*).

Violence waits in the dusty sunlight of a tenement yard and in the shadows of a distraught mind. Violence draws nearer in the shouts of a protest march and in ghetto rumblings. Violence erupts from Mace-sprinkled billy clubs and a homemade Molotov cocktail. Violence of war explodes the peace it promises to bring. Hourly reports of violence bring numbness, shock, confusion, sorrow. We live in a violent world (*2*).

Violence surrounds us, and we must try to understand it in the hopes of finding alternatives that will meet today's demand for change. Do we benefit from violence? Or is violence losing whatever adaptive value it may once have had? We present two theses. (i) Violence can best be understood in the context of adaptation. Violence is part of a struggle to resolve stressful and threatening events—a struggle to adapt. (ii) Adaptive alternatives to violence are needed in this technological era because the survival value of violent aggression is diminishing rapidly.

The shock of Robert F. Kennedy's death prompted the formation of a committee on violence (*3*) in the Department of Psychiatry, Stanford University School of Medicine. We committee members reviewed the literature on violence and then interpreted this literature from the point of view of psychiatrists and psychologists. We discussed our readings in seminars and sought answers to our questions about violence. This article presents a synthesis of our group's findings and observations and reflects our view of adaptation theory as a unifying principle in human behavior.

We define pertinent terms and describe the adaptation process before we examine violence as it relates to individual coping behavior and collective survival. We then describe three theories of aggression and relate them to adaptation. Next, we discuss relevant examples of violence as attempted coping behavior and factors that foster violence and illustrate the urgent need for other ways of expressing aggression. Finally, we consider the changing nature of adaptation and suggest ways of coping with violence.

Definition of Terms

Two groups of terms require definition: (i) aggression and violence; and (ii) adaptation, adjustment, and coping. We found that these terms have quite different meanings for different disciplines.

We here define aggression (*4, 5*) as the entire spectrum of assertive, intrusive, and attacking behaviors. Aggression thus includes both overt and covert attacks, such defamatory acts as sarcasm, self-directed attacks, and dominance behavior. We extend aggression to include such assertive behaviors as forceful and determined attempts to master a task or accomplish an act. We choose a broad definition of aggression rather than a restrictive one because relations between the underlying physiological mechanisms and the social correlates of dominant, assertive, and violent behavior are still poorly understood. Hence, our definition encompasses but is broader than the definition of aggression in animals that is used in experimental biology (*6, 7*), which says that an animal acts aggressively when he inflicts, attempts to inflict, or threatens to inflict damage upon another animal. Violence (*4*) is destructive aggression and involves inflicting physical damage on persons or property (since property is so often symbolically equated with the self). Violent inflicting of damage is often intense, uncontrolled, excessive, furious, sudden, or seemingly purposeless. Furthermore, violence may be collective or individual, intentional or unintentional, apparently just or unjust.

By adaptation we mean the behavioral and biological fit between the species and the environment resulting

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