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Review

Structures and mechanism of the monoamine oxidase family

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Abstract

Members of the monoamine oxidase family of flavoproteins catalyze the oxidation of primary and secondary amines, polyamines, amino acids, and methylated lysine side chains in proteins. The enzymes have similar overall structures, with conserved flavin adenine dinucleotide (FAD)-binding domains and varied substrate-binding sites. Multiple mechanisms have been proposed for the catalytic reactions of these enzymes. The present review compares the structures of different members of the family and the various mechanistic proposals.

Keywords: enzyme mechanism; flavoproteins; L-amino acid oxidase; lysine-specific demethylase; monoamine oxidase; polyamine oxidase; protein structure; spermine oxidase.

List of abbreviations

MAO, monoamine oxidase; PHBH, *p*-hydroxybenzoate hydroxylase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; QSAR, quantitative structure-activity relation; PAO, polyamine oxidase; SMO, spermine oxidase; LAAO, L-amino acid oxidase; DAAO, D-amino acid oxidase; TMO, tryptophan monooxygenase; LSD, lysine-specific demethylase.

Introduction

Monoamine oxidase (MAO) family members oxidize a variety of amine substrates, including small-molecule monoamines and polyamines and modified amino acids within proteins. Enzymes belonging to the MAO family share similar overall structures (Figure 1), with nearly identical flavin adenine dinucleotide (FAD) binding domains resembling the folding pattern of p-hydroxybenzoate hydroxylase (PHBH) (1, 2), but contain varied substrate-binding sites (Figure 2). As flavoprotein oxidases, they catalyze substrate oxidation via two half-reactions; in the reductive half-reaction, the flavin cofactor is reduced when it accepts a hydride equivalent from the substrate, while in the oxidative step, the reduced flavin is reoxidized by molecular oxygen (Scheme 1).

Due to the ability of the flavin cofactor to accept one or two electrons, several mechanisms have been proposed for the transfer of electrons from the substrate to the cofactor (Scheme 2). The single electron transfer mechanism (Scheme 2A) involves formation of semiquinone flavin and aminium cation radical intermediates, with subsequent transfer of a hydrogen atom equivalent (3). Direct hydrogen atom transfer from the substrate α-carbon to the flavin either directly or a via non-flavin radical (Scheme 2B) is another possible mechanism for substrate oxidation (4). Variations of a nucleophilic mechanism (Scheme 2C), in which the amino group of the substrate attacks the C4a of the flavin, forming a covalent intermediate, followed by proton abstraction by an active site base, have also been proposed (5). Finally, the reaction could occur by direct hydride transfer from the substrate to the flavin (Scheme 2D) (6).

While the flavin increases the number of possible enzymatic mechanisms, its presence simplifies the study of flavin-dependent reactions due to its characteristic spectrum, which changes as the isoalloxazine ring system gains and loses electrons. The spectrum of oxidized flavin has maxima around 380 and 460 nm, which diminish when the cofactor is reduced. Monitoring the change in absorbance during a reaction allows for measurement of individual kinetic rate constants and facilitates detection of reaction intermediates. Furthermore, even though the monoamine oxidase family members interact with two substrates, an amine and oxygen, the oxidation of the amine substrate is effectively irreversible (7), and oxygen only reacts with the reduced enzyme, making the analysis of steady-state kinetic parameters less complicated.

Monoamine oxidases A and B

Monoamine oxidases A and B (MAO A and MAO B) are outer mitochondrial membrane proteins that catalyze the oxidation of primary, secondary, and tertiary amines, including several neurotransmitters, to the corresponding imines; the oxidized products are hydrolyzed nonenzymatically to the respective aldehydes or ketones (8) (Scheme 3). The two enzymes share 70% amino acid identity, and both contain a covalently-bound FAD cofactor attached to an enzyme cysteine via the 8α-methylene of the isoalloxazine ring (9). MAO A metabolizes serotonin (5-hydroxytryptamine), norepinephrine, and dopamine, while MAO B preferentially oxidizes benzylamine, dopamine and phenylethylamine and only metabolizes norepinephrine and serotonin slowly (10–13). MAO B also forms the neurotoxin 1-methyl-4-phenyl-pyridinium, a causative agent of Parkinson's disease,

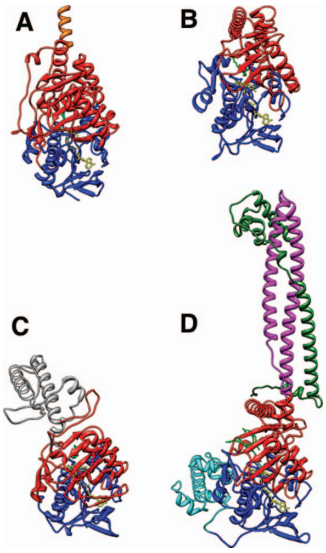


Figure 1 Ribbon representations of human MAO B (A), maize PAO (B), bacterial LAAO (C), and human LSD1 (D).
Images were generated using the program Chimera (115) and the following Protein Data Bank (PDB) files: 1OJA, 1H83, 2JB2, and 2UXN. The FAD-binding domains are colored blue and substrate-binding domains are red; substrate analogs or inhibitors are colored green and the flavin cofactors are yellow. The C-terminal membrane-binding α-helix of MAO B is colored orange; the helical domain of LAAO is gray. The tower domain of LSD1 (magenta) is shown interacting with a portion of CoREST, colored dark green, and the SANT domain of LSD1 is cyan.

from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (14). Inhibitors of the monoamine oxidases have been used clinically for the treatment of depression, as well as Parkinson's, Alzheimer's and other neurodegenerative diseases (15, 16).

Both MAOs are composed of an FAD-binding domain, conserved among a number of other flavoprotein oxidases, a substrate-binding domain, and a membrane-binding domain (Figure 1A) (17, 18). While pulsed electron paramagnetic resonance (EPR) studies have demonstrated that both forms of rat and human MAOs exist as dimers in solution (19), human MAO A crystallizes as a monomer (20). Both MAOs bind the outer mitochondrial membrane through a C-terminal α-helical region, with additional membrane interactions occurring with other hydrophobic residues (17, 18, 21). The FAD isoalloxazine ring exists in a strained, puckered state, in contrast to its planar conformation in solution; this difference has been suggested to make covalent addition at N5 or C4a more favorable by making these flavin atoms more 'sp^3-like' (18).

The substrate-binding sites of MAO A and MAO B (Figure 2A) are both mainly hydrophobic, encased by predominantly aromatic and aliphatic residues (17, 18). A notable exception is a conserved lysine (Lys296 in MAO B and Lys305 in MAO A) that interacts with a water molecule that also binds the N5 atom of the flavin cofactor (22). Tyrosines 398 and 435 in MAO B (Tyr407 and Tyr444 in MAO A) are located on opposite sides of covalently bound inhibitors and substrates in all of the MAOs, forming an aromatic 'sandwich', and mutation of these residues alters activity (23–25). These tyrosines have been proposed to orient the substrate for oxidation or to activate the amine by enhancing its nucleophilicity (26). The substrate-binding site of human MAO A consists of a cavity approximately 400 $Å^3$ in size (27, 28), while in MAO B a smaller hydrophobic cavity, termed the 'entrance cavity', is positioned between the main substrate-binding site and the protein surface; rotation of an isoleucine residue (Ile199) allows the two cavities to be fused into one larger 700 $Å^3$ site (22). The cavities remain separated or become joined depending on the nature of the substrate or inhibitor present (29). In MAO A, a phenylalanine (Phe208) replaces Ile199 of MAO B (Figure 3). Another difference in the substrate-binding sites of the two enzymes is Ile335 in MAO A vs. Tyr326 in MAO B; these residues contribute to the substrate and inhibitor selectivity of the two enzymes (25, 30). While structural studies initially suggested that substrate specificity differences were due to the conformation of a six-residue loop (residues 210–216 in human MAO A and 201–206 of human MAO B) (20, 28), subsequent studies showed that the loop is in the same conformation in both enzymes (Figure 3) (30).

MAOs preferentially bind the substrates with the amino group in the neutral from (31), consistent with deprotonation of the amine being required for oxidation (32). Cleavage of the amine substrate CH bond is rate-limiting with benzylamines (31) and phenethylamines (33) as substrates for MAO A, and with a series of benzylamines (7) and MPTP analogs (34) for MAO B.

Much of the initial mechanistic work with MAOs A and B was interpreted as favoring mechanisms in which a substrate radical was formed, either by single electron (Scheme 2A) or hydrogen atom transfer (Scheme 2B). The inactivation of the MAOs by cyclopropyl inhibitors is consistent with formation of a substrate radical (3, 35–37), leading Silverman and coworkers to propose a single electron transfer mechanism for oxidation of substrates, similar to the electrochemical oxidation of amines (3). Further support for the single electron transfer mechanism came from Yue et al. (38), who reported resonance Raman spectra consistent with an anionic flavin semiquinone in MAO B; however, they were not able to observe a flavin radical by EPR spectroscopy, a more definitive approach. Evidence of a radical was later found in the EPR spectrum of MAO B; however, this spectrum was not altered by either the inhibitor pargyline or by substrates and was not equivalent to that of the semiquinone formed by photoreduction, suggesting that the signal arose from another source (39). Miller and colleagues similarly failed to find any evidence of a radical in the EPR spectra of MAO A or MAO B (31), further suggesting that the previous results were the result of an impurity. Finally, stopped-flow absorbance spectroscopy of flavin reduction failed to detect an intermediate radical (7, 31). It is possible that the flavin semiquinone and aminium cation radical do not accumulate during the reaction, making them difficult to detect; thus, lack of evidence for an intermediate does not ensure that it does not form during the reaction. Nevertheless, lack of a consistently observable flavin semiquinone,

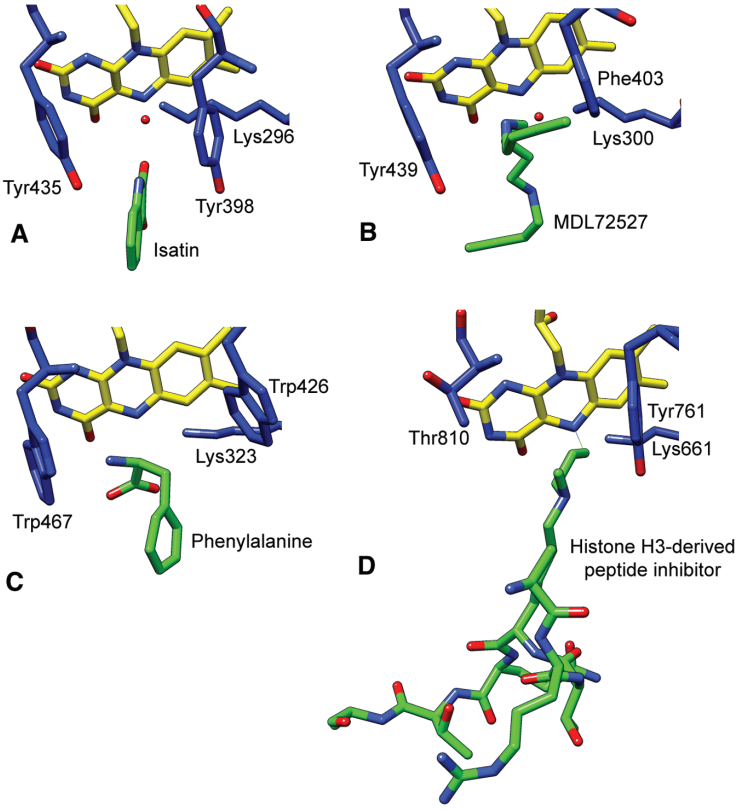
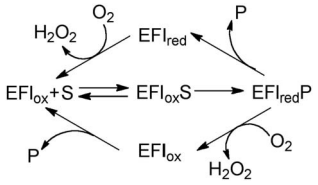


Figure 2 The active sites of human MAO B (A, PDB file 1OJA), maize PAO (B, PDB file 1B5Q), bacterial LAAO (C, PDB file 2JB2), and human LSD1 (D, PDB file 2UXN) with bound substrates or inhibitors (green).

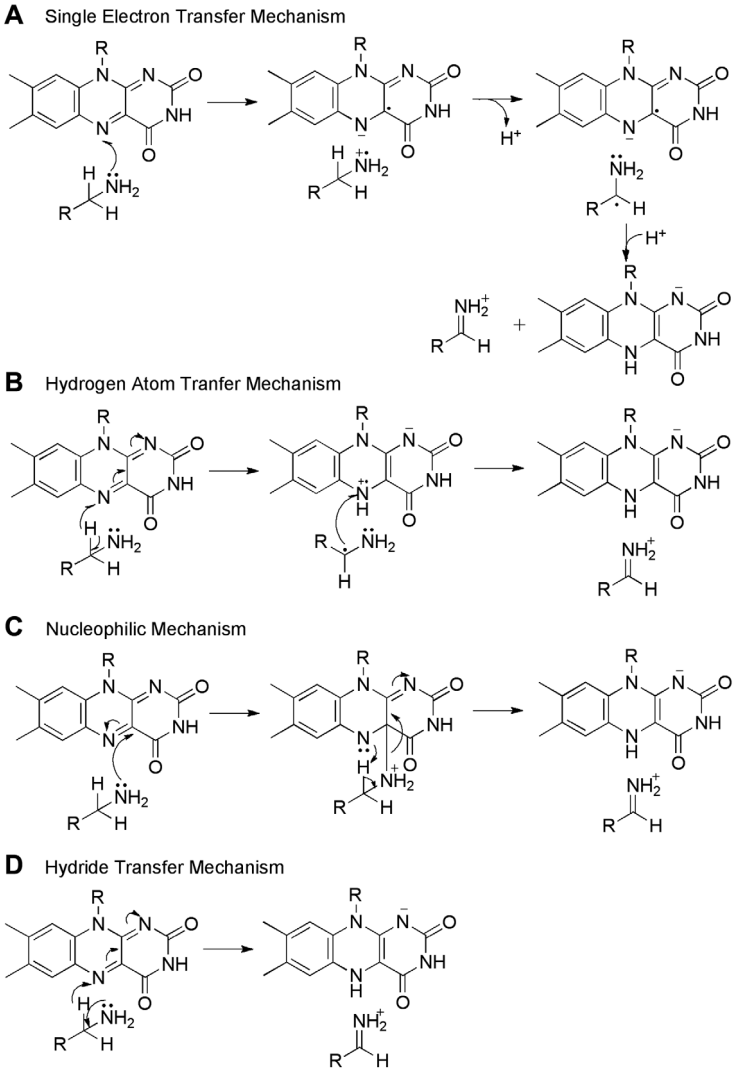
either by EPR spectroscopy or during stopped-flow analyses, provides a strong argument against any single electron mechanism. Furthermore, the thermodynamic barrier to single electron transfer is quite high, with the high redox potential of amines (E_m=+1.5 V) making them unlikely to be oxidized by the flavin cofactor (E_m=-0.2–0 V), and there is no alternate oxidant strong enough to abstract an electron from the amine substrate (31). Although substrate binding might increase the redox potential of the flavin cofactor in MAO A and B (40), the difference in potentials still remains unfavorable.

An alternative version of the hydrogen atom transfer mechanism (Scheme 2B) utilizes an active site radical rather than the flavin for abstraction of the hydrogen atom; spectroscopic evidence for tyrosyl radical formation was described by Rigby et al. for MAO A (41). While three tyrosines are located in the vicinity of the active site (Tyr69, Tyr407, and Tyr444 in MAO A and Tyr60, Tyr398, and Tyr435 in MAO B), the data were most consistent with radical formation at Tyr407 (Tyr398 in MAO B). Mutagenesis studies, however, showed that the Y444F mutation affects activity more significantly than the Y407F mutation (25), and mechanistic studies with the Y444F mutant established that mutating that residue did not alter the mechanism of C-H cleavage (23). Subsequent studies with mouse polyamine oxidase (PAO), another MAO family member, showed that mutation of PAO Tyr430, homologous to MAO A Tyr407, to phenylalanine only resulted in a 6-fold decrease in activity, again suggesting that tyrosyl radical formation was unlikely during catalysis for that enzyme (42). Thus, the functional evidence does not support the involvement of a tyrosine radical in amine oxidation by the MAO family.



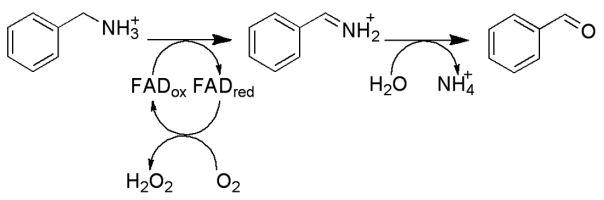
Scheme 1 The reductive and oxidative half-reactions of flavoprotein oxidases.

Quantitative structure-activity relation studies (QSARs) of the MAOs have yielded conflicting results. Substituent σ values are a quantitative measure of the electron-donating or withdrawing properties of the substituent. Comparing rates with the σ values for a series of substrates can indicate whether development of charge occurs in the transition state for the reaction. A positive correlation, or ρ value, corresponds to a development of negative charge, whereas a negative ρ value is associated with a buildup of positive charge; lack of a correlation, or a ρ value around zero, indicates little charge development. Studies with a series of para- and meta-substituted benzylamine analogs with MAO B (7), as well



Scheme 2 Mechanism proposed for amine oxidation by the MAO family of enzymes.

as with a series of phenethylamine analogs and MAO A (33), showed no correlation of activity with the substrate σ values, suggesting that no charge builds up during the reaction. Development of negative charge would be expected with mechanisms involving proton abstraction, namely single electron transfer and nucleophilic mechanisms; thus, these studies instead support direct hydrogen atom abstraction from the substrate or a hydride transfer mechanism. In contrast, in QSAR studies with MAO A and a series of 16 para-substituted benzylamine analogs, the activity correlated positively with the substituent σ values, with a ρ value of 2.0 for 12 of the analogs studied, but a ρ value of 0.5 for the remaining four analogs; the reason for the discrepancies was not clear. These results suggest a buildup of negative charge on the substrate, consistent with one version of the nucleophilic mechanism (Scheme 2C) (31); neither a flavin-substrate adduct nor a radical intermediate was observed during the reaction. Miller and Edmondson (31) attributed the differences between the results of QSAR studies with benzylamines for MAO A and B to different timing of proton abstraction in the transition state. The transition state for MAO B would be early, so that the small amount of negative charge on the benzyl carbon could be offset by positive charge created on the amine nitrogen. In MAO A, abstraction would occur later, so the charge development would be more sensitive to the substituent. Furthermore, these authors argued that substrate binding differences in the two enzymes could change the orientation of the π-orbitals of the benzene ring with respect to the π orbital of the breaking C-H bond, decreasing or preventing transmission of electronic effects



Scheme 3 Reaction catalyzed by MAO.

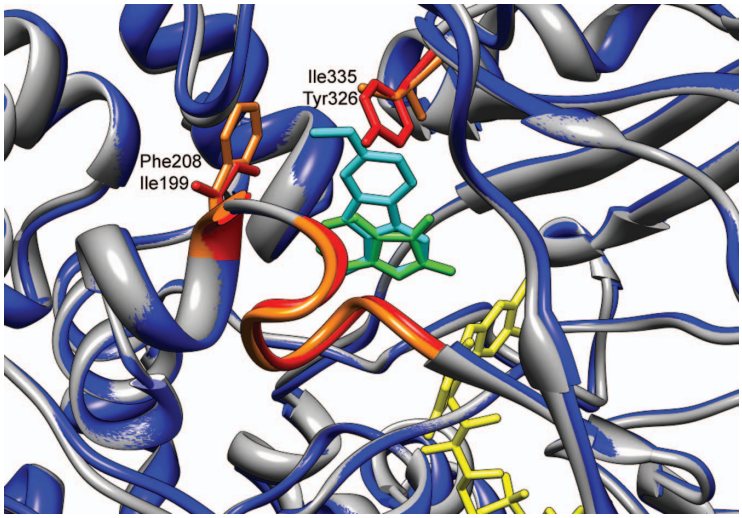


Figure 3 An overlay of the substrate-binding sites of human MAO B (PDB file 1OJA) and human MAO A (PDB file 2Z5X). The figure highlights residue differences in the active sites of these two enzymes that affect substrate specificity, and identifies the substrate-cavity forming loop that appears to be conserved in the two enzymes. MAO B is colored blue, and Ile199, residues 201–206, and Tyr326, are colored red; the inhibitor isatin is green. MAO A is pictured in dark gray, with Phe208, residues 210–216, and Ile335 colored orange; the inhibitor harmine is cyan. The flavin cofactor is shown in yellow.

from the substituents to the amino group, leading to small ρ values in MAO B. The differences in the results for MAO A with benzylamines and phenethylamines were attributed to reduction of the substituent electronic effects in the phenylethylamine analogs from the methylene group between the aromatic ring and the reaction site causing electronic effects to be undetectable (33); However, this effect should only be 2–3-fold (43). An alternative explanation to the different ρ values in the reactions of MAO A and B with benzylamines is that different steps in the overall reaction are determining the rate of the reaction. As noted above, the neutral amine is the substrate for MAO, and the pK_a values of benzylamines are affected by substituents in the aromatic ring (44). The reactions of benzylamines with MAO A and B were studied at a pH below the pK_a of benzylamine even when bound in the enzyme active site (32), so that the ρ value would reflect the fraction of the substrate that is deprotonated in addition to the rate constant for amine oxidation. Different contributions from these two effects could account for the different results with MAO A and B.

Support for the nucleophilic mechanism initially came from model studies with methyllumiflavins, which oxidize amines via a mechanism that involves a covalent intermediate between the flavin and amine, with subsequent proton transfer from the substrate (5). Proton transfer requires the presence of an active site base; a base strong enough to accept a proton from the benzyl substrate, however, is missing in either MAO. To circumvent the lack of the required base, Miller and Edmondson (31) proposed an altered nucleophilic mechanism in which amine attack of the flavin C4a leads to formation of a strong base at N5 of the flavin, which then accepts a proton from the substrate (Scheme 2C).

At present, the nucleophilic and radical mechanisms both have strong advocates, but there is a lack of definitive evidence to support either. In addition, neither fully accounts for all the mechanistic data on MAOs. A more complete mechanistic picture for this enzyme family comes from consideration of the other family members described below.

The mechanism of oxidation of reduced MAO by O_2 has not been studied in detail. When monitored by stopped-flow absorbance, the reactions of reduced flavoprotein oxidases with O_2 are typically found to be single exponential processes with rate constants directly proportional to the O_2 concentration and no discernible intermediates. Based on model studies (45), the oxidation reaction is generally assumed to involve two single-electron transfers, forming a short-lived superoxide-flavin semiquinone radical pair (46). More detailed studies of the oxidation mechanism have been carried out with flavoprotein oxidases from other structural families. Protonation of an active site histidine in glucose oxidase is required for rapid reaction of the reduced enzyme with O_2 (47), presumably by stabilizing the negative charge on oxygen as it is converted to superoxide anion. A lysine in monomeric sarcosine oxidase similarly must be protonated for rapid oxidation of the reduced form of that enzyme (48, 49). This lysine is also part of a water channel that extends to the surface and may provide the proton needed in the oxidation reaction. Whether the active site lysine in MAO (Figure 2A) has a similar role has not been established.

Polyamine oxidases

Polyamine oxidases (PAOs) participate in the catabolism of spermine and spermidine and their acetyl derivatives. Increased levels of the polyamines spermine and spermidine are found in rapidly proliferating cells, and polyamines are essential for cell growth (50), yet excess accumulation of

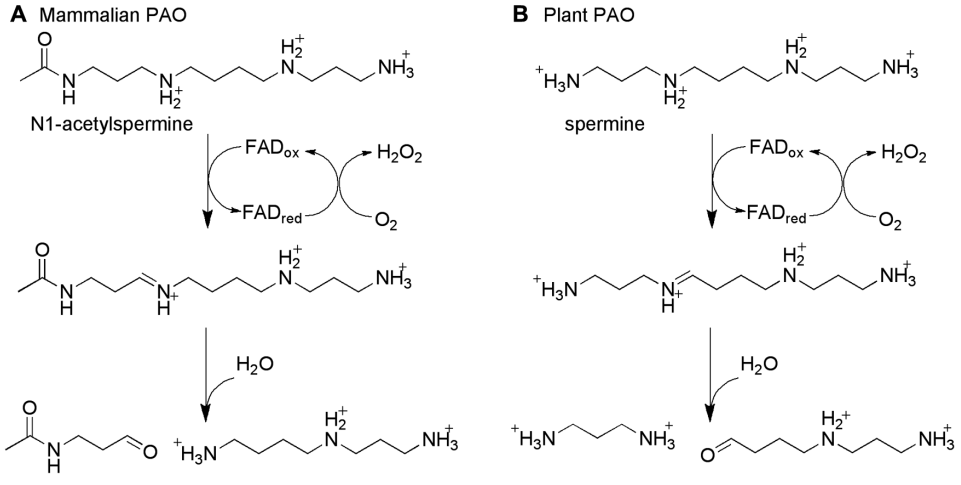
polyamines in cells causes cytotoxicity (51). The polyamine metabolic pathways are deregulated in several cancers, and polyamine analogs can act as antineoplastic agents (52–54) by altering the regulation of polyamine metabolic and catabolic pathways, ultimately reducing the availability of the natural polyamines. The term PAO is often reserved for enzymes preferring acetylated spermine or spermidine, while enzymes that preferentially oxidize spermine itself are referred to as spermine oxidases (SMOs). This terminology is not consistently used, resulting in some confusion as to the preferred reaction of individual enzymes. Mammalian PAOs convert N1-acetylspermine and N1-acetylspermidine to spermidine and putrescine, respectively, plus N-acetyl-3-aminopropanaldehyde and H_2O_2 (Scheme 4) (55, 56). In contrast, plant (57) and protozoan (58) PAOs oxidize their substrates on the *endo* side of the secondary N(4)-amino group, oxidizing spermine and spermidine to 3-aminopropyl-4-aminobutyraldehyde and 4-aminobutyraldehyde, respectively, plus 1,3-diaminopropane and H_2O_2 (Scheme 4). While mammalian polyamine oxidase is constitutively expressed, the expression of mammalian SMO is induced by polyamines, including antitumor polyamine analogs (59–61). Induction of SMO also has cytotoxic effects, possibly due to the increase in production of H_2O_2 (62, 63). Conversely, treatment of SMO with the competitive inhibitor MDL72527 inhibits production of H_2O_2, leading to decreased oxidative DNA damage and thereby decreasing the mutagenic changes associated with cancer progression (64).

Structures are only available for PAOs from maize and yeast. Unlike the MAOs, maize PAO is monomeric and contains a non-covalently bound FAD (Figure 1B) (65–67). A structure of maize PAO in complex with MDL72527 (Figure 2B) reveals several similarities with the structure of the MAOs, including homologous FAD-binding sites, a bent orientation for the flavin cofactor, the presence of the water molecule that interacts with a conserved lysine residue (Lys 300 in maize PAO) and the N5 of the flavin cofactor, and the 'aromatic sandwich' for the reactive nitrogen of the substrate (66). Unlike the MAOs, the substrate-binding site consists of a U-shaped cavity that is approximately 30 Å long (66). The cavity has several acidic residues at one entrance that likely guide the positively charged substrate to the active site, while the other entrance is slightly more narrow and is lined by backbone carbonyl groups (66). The inhibitor forms a series of hydrogen bonds and van der Waals interactions with the protein.

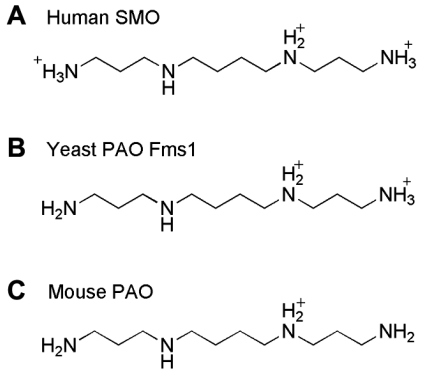
The yeast PAO Fms1 oxidizes spermine and N1-acetylspermine, but not spermidine, forming spermidine and 3-aminopropanal or N-acetyl-3-aminopropanaldehyde, respectively, and functions in pantothenic acid production (68). Fms1 and maize PAO share only 20% amino acid sequence identity, but have very similar overall structures (69). Unlike maize PAO, Fms1 crystallizes as a dimer and also forms a dimer in solution, but like maize PAO the enzyme contains a tunnel with two entrances that forms the substrate-binding site (69). The substrate-binding site is hydrophilic on each of the two ends and hydrophobic in the middle, with the substrate bound through hydrogen bonds and hydrophobic interactions. Lys296 of Fms1 is conserved with Lys300 of maize PAO, and the FAD-binding domain is similar to that of the MAOs. The FAD cofactor, however, is planar.

Structural modeling of mouse SMO based on the structure of maize PAO suggests that the overall structural features, including the FAD and substrate-binding sites, are generally the same, although the substrate and inhibitor specificities differ (60, 70). Like the MAOs, the reaction kinetic mechanism is ping-pong for both Fms1 and human SMO, but the rate-limiting step is product release for these two enzymes (71, 72).

Mouse PAO, human SMO, and the yeast PAO Fms1 differ from one another in the protonation states of their substrate nitrogens required for optimal activity (Scheme 5). For all three enzymes, results of pH studies are consistent with a requirement that the substrate nitrogen at the site of C-H bond cleavage be uncharged for oxidation (71–73), consistent with observations with the MAOs. Whether the remaining nitrogens must be neutral or charged differs among the three enzymes, as shown in Scheme 5 for spermine. For



Scheme 4 Reactions catalyzed by mammalian and plant PAOs.



Scheme 5 Required protonation states of spermine as substrate for different PAOs.

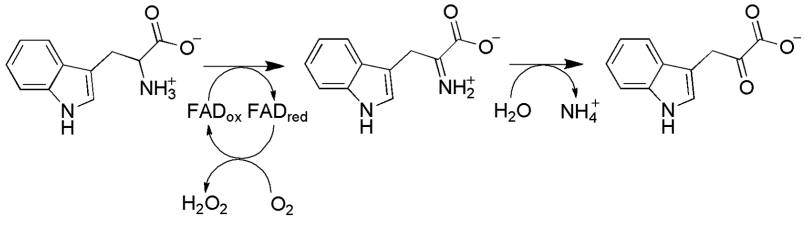
human SMO, the effects of pH on steady-state and rapid-reaction kinetic parameters are consistent with the reactive form of spermine having all three non-reacting nitrogen atoms protonated (72). Similar analyses of Fms1 support a preference for the substrate form with only two protonated nitrogens (71). Finally, mouse PAO preferentially binds the singly charged forms of substrates (73). These differences likely play a role in determining the substrate specificity of the enzymes. For example N1-acetylspermine is a 50-fold worse substrate for SMO than spermine; this makes sense because the N1 atom of N1-acetylspermine cannot be protonated (72). Fms1 prefers N1-acetylspermine over spermine by less than 10-fold (71); this is consistent with the requirement that N1 be neutral. Finally, mouse PAO prefers N1-acetylspermine over spermine by over 100-fold (73); this can be attributed to the extra protonatable nitrogen in the latter.

QSAR studies with mouse PAO have been used to differentiate among the various proposed mechanisms for amine oxidation. With *N,N′*-dibenzyl-1,4-diaminobutanes as the substrate, substrate oxidation is the rate-limiting step for enzyme. The effect of substituents of seven para-substituted N,N′-dibenzyl-1,4-diaminobutanes on activity yielded small negative ρ values (-0.59 at pH 8.6 and -0.09 at pH 6.6), consistent with little or no accumulation of charge in the transition state and with either a hydride transfer mechanism or a hydrogen atom abstraction mechanism (74). This is similar to the results with MAO B with benzylamines (7) and with MAO A with phenethylamines (33). The more positive ρ value at pH 6.6 in the case of PAO can be attributed to the effect of the substrate protonation state when the reactions are carried out below the pK_a of 8 for the substrate bound to the enzyme (74). As is the case with MAO A and B, rapid reaction studies of amine oxidation by PAO, SMO, and Fms1 failed to detect a semiquinone or flavin adduct intermediate (71–74), contrary to the expectations of radical and nucleophilic mechanisms. Thus, the data for the PAOs are fully consistent with a hydride transfer mechanism (Scheme 2D).

The role of the conserved lysine-water-flavin N5 atom interaction has been studied by site-directed mutagenesis in mouse and maize PAO with conflicting results. In maize polyamine oxidase, mutagenesis of this lysine to methionine results in a 1400-fold decrease in the rate constant for flavin reduction (75), suggesting that this residue plays an essential role in substrate oxidation in members of this family of enzymes. In mouse PAO, however, the similar mutagenesis of the conserved lysine to methionine has no effect on the kinetics of reduction (73). Instead, the mutation decreases the rate of flavin reoxidation; the effects of pH and D_2O as solvent have been interpreted in favor of a mechanism in which the neutral form of the lysine coordinates a water molecule that accepts a proton during flavin oxidation (76). The need for a neutral lysine is in contrast to the need for a positively charged residue for rapid reaction of sarcosine and glucose oxidase with O_2, so that this conserved lysine is not playing such a role in mouse PAO. The reason for the divergent effects of the identical mutation in the maize and mouse enzymes is not clear, but may reflect differences in the specificities of the plant and animal enzymes.

L-Amino acid oxidases

L-Amino acid oxidases (LAAOs) catalyze the oxidative deamination of an L-amino acid substrate to an α-keto acid, ammonia, and hydrogen peroxide (Scheme 6). The structures of the enzymes from the snake *Calloselasma rhodostoma* and the bacterium *Rhodococcus opacus* (Figure 1D) have been solved in the presence of ligands and substrates and establish that the LAAOs are in the MAO structural family (77–79). The structures show that the enzymes are both dimers, but dimerize differently. LAAO from *R. opacus* contains a helical domain responsible for dimerization, while the enzyme from *C. rhodostoma* dimerizes via interactions between residues in several different domains (Figure 4). Both enzymes contain a FAD-binding site similar to those in other members of the MAO family, as well as the conserved lysine residue near the N5 atom of the FAD (Figure 2C). The deep substrate-binding site in LAAO resembles the substrate-



Scheme 6 Reaction catalyzed by LAAO.

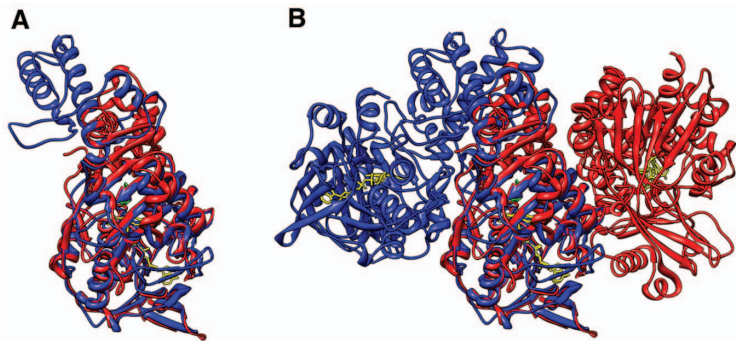
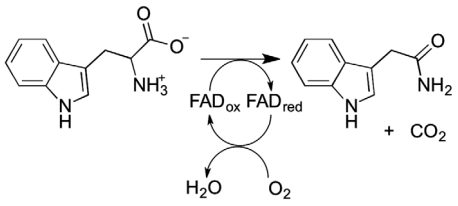


Figure 4 An overlay of the structures of monomers (A) and dimers (B) of L-amino acid oxidases (LAAOs) from the snake *C. rhodostoma* (red, PDB file 2IID) and the bacterium *R. opacus* (blue, PDB file 2JB2).

binding tunnel in PAO, but in LAAO the tunnel is only open on one end. LAAOs prefer hydrophobic amino acids, including phenylalanine, tryptophan, and tyrosine, as substrates (80); this is reminiscent of the preference of MAOs for amines with aromatic groups such as serotonin and catecholamines. Consistent with this substrate preference, the active site contains a hydrophobic pocket for the substrate side chain. LAAOs differ from MAOs and PAOs in that the substrates contain a carboxylate in addition to an amino moiety. The active sites of LAAOs contain a conserved arginine that forms a salt bridge with the carboxylate and a conserved tyrosine that forms a hydrogen bond with it (77, 78). The substrate-binding site is a mirror image of that in D-amino acid oxidase (DAAO), a member of a different structural family of flavin amine oxidases that catalyzes the same chemistry; a significant number of mechanistic studies have led to the conclusion that hydride transfer is the mechanism for substrate oxidation by DAAO (81, 82).

Tryptophan monooxygenase (TMO) from *Pseudomonas savastonai* is representative of a subfamily of LAAOs that catalyzes the oxidative decarboxylation of amino acids; the physiological reaction catalyzed by the enzyme is the oxidation of tryptophan to indoleacetamide, carbon dioxide, and water (Scheme 7) in the two-step pathway for production of indoleacetic acid (83). Homologous genes have been identified in other bacteria (84).While the structure of (TMO) has not been solved, modeling studies demonstrated that LAAO and TMO are homologous proteins, and results of mutagenesis support this conclusion (85–87). Like other LAAOs, the enzyme most efficiently oxidizes amino acids with large hydrophobic side chains, such as tryptophan and phenylalanine, although methionine and alanine are also substrates (88).



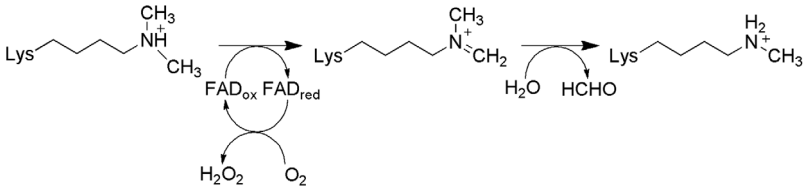
Scheme 7 Reaction catalyzed by TMO.

The mechanism of amine oxidation by TMO has been studied in much more detail than that of the other LAAOs, making this a model enzyme for LAAOs in general. Similar to the PAOs, product release is rate-limiting for turnover of the physiological substrate tryptophan (89). The pH dependence of kinetic parameters established that the neutral form of the amine is required for catalysis, similar to the situation with MAO and PAOs (86, 90). No intermediates have been detected by stopped-flow absorbance spectroscopy during the oxidation of amino acids by TMO (88, 90). A series of deuterium and nitrogen kinetic isotope effects established that rehybridization of the substrate nitrogen atom occurs during C–H bond cleavage, in contrast to the expectation for the nucleophilic mechanism of Scheme 2C (90). *Ab initio* calculations of the transition states and energetics of the different mechanisms in Scheme 2 showed that the magnitudes of the isotope effects measured for TMO are only consistent with a hydride transfer mechanism (91).

Lysine-specific demethylase

Lysine-specific demethylase 1 (LSD1) removes methyl groups from lysyl residues in the N-terminal tail of histone H3 (92, 93) and the tumor suppressor protein p53 (94) by catalyzing oxidation of the carbon-nitrogen bond between the methyl group and the epsilon amine of the lysine, forming an imine intermediate that is nonenzymatically hydrolyzed to produce formaldehyde and the demethylated lysine (Scheme 8). The enzyme plays a role in epigenetic regulation of gene expression in cells, modulating cellular activities including growth and differentiation (95–98). Altered expression of LSD1 has been correlated with proliferation of neuroblastoma (99) and prostate cancer (100). Nonselective MAO inhibitors, such as tranylcypromine and propargylamine, as well as more specific mechanism-based inhibitors, inactivate LSD1 (101–106).

The structure of LSD1 (Figure 1) has been solved with and without peptide substrates and in the presence of inhibitors (102, 103, 107, 108). The amine oxidase domain of LSD1 which is homologous to the other MAO family members is divided by an insert, called the tower domain, that protrudes out of the core of LSD1 and interacts with



Scheme 8 Reaction catalyzed by LSD1.

CoREST (RE1-silencing transcription factor corepressor-1), a regulatory protein (Figure 1D) (107, 108). LSD1 also contains an N-terminal SWIRM (Swi3p, Rsc8p and Moira) domain with affinity for DNA (109). LSD1 has a large substrate-binding pocket that accommodates the N-terminal tail of the histone, which appears to fold inside the substrate-binding cavity, forming several interactions with active-site residues (103, 107, 110). LSD1 is very specific in its histone demethylase activity, exclusively demethylating lysine 4 of histone H3 *in vitro*, with activity towards lysine 9 of histone H3 reported only in the presence of the androgen receptor (111). The interactions between substrate and enzyme position the fourth lysine of the peptide substrate directly in front of the flavin cofactor (107). LSD1 also contains the conserved active site lysine (Lys661) (Figure 2D) (112), and mutation of this residue to alanine eliminates LSD1 activity (110). LSD1 lacks the 'aromatic cage' of MAO. In LSD1, Tyr761 corresponds to one of the aromatic residues in MAO; however, a threonine replaces the other (113).

Mechanistic studies with LSD1 are more difficult, due to the complexity of the substrate; thus, kinetic studies have been carried out with peptide substrates corresponding to the N-terminal tail of histone H3. The turnover rate for LSD1 is about 200–1000 times slower than that for other amine oxidases (113). Due to its regulatory activities, LSD1 may have evolved for increased specificity, not optimum activity. C-H bond cleavage is the rate-limiting step in the oxidation of a 21-mer peptide by LSD1 (114). Just as with the other members of the family, stopped-flow studies failed to show evidence of intermediates between oxidized and reduced enzyme during oxidation of the peptide substrate by LSD1. Based on these results and analyses of kinetic isotope effects on LSD1 kinetics, it was concluded that the mechanism of amine oxidation by LSD1 is hydride transfer (114).

Expert opinion

Because of its physiological importance, the mechanism and structure of monoamine oxidase has been subject to intense study for decades. In recent years it has become apparent that enzymes with similar structures catalyze the oxidation of other amine substrates, presumably using a common mechanism. The monoamine oxidase family members share structural features, including a conserved FAD-binding domain and a lysine-water-flavin triad. The substrate-binding sites, however, reflect the different substrates. In each case, there is evidence that the deprotonated amine is the functional substrate. While, nucleophilic and radical mechanisms have been proposed for oxidation of amines by MAO, the accumulation of structural and mechanistic evidence supports a common hydride transfer mechanism for all members of the MAO family.

Outlook

The last decade has seen major advances in our understanding of the MAO family of flavoproteins. Future studies are likely to focus on obtaining additional structural information and development of specific inhibitors of the different family members. These efforts will complement continuing studies of the mechanism of these enzymes and provide insight into the structural bases for the differences in specificity of the family members. The growing interest in the mechanism by which flavoprotein oxidases catalyze the reaction of the reduced flavin with oxygen will likely lead to studies of the oxidative half-reaction of the MAO family.

Highlights

- The monoamine oxidase family of flavoenzymes catalyze the oxidation of primary and secondary amines, polyamines and amino acids.
- Despite low levels of sequence identity, structures of the MAO family are homologous, with a conserved FAD-binding site and a novel lysine-water-flavin interaction.
- The substrate binding sites of family members are quite diverse, corresponding to the varied structures of the substrates.
- Mechanistic studies support a common hydride transfer mechanism for all members of the MAO family.

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