MOLECULAR AND FUNCTIONAL PROPERTIES OF THE MONOAMINE OXIDASES

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Abstract --- The role of monoamine oxidase A and B in the nervous system and in Parkinson's disease is reviewed. Recent advances made in the molecular and biochemical properties of MAO A are also discussed, and the putative functional regions within MAO A are identified based on sequence similarities in other proteins.

The monoamine oxidases (MAO A and B, collectively referred to as MAO) play a vital role in the oxidation of heterocyclic xenobiotics and the metabolism of biogenic amines in the central nervous system and peripheral tissues. MAO has long been thought to play a role in Parkinson's disease, depression, and alcoholism.[1-3] Current interest in these enzymes has been intensified by the discoveries of alterations in the genes that encode them. Defective MAO A, due to a mutation of CAG (a codon for glutamine) to TAG (a termination codon), has been identified in some members of a Dutch family who exhibited abnormal aggressive behavior.[4,5] Furthermore, an alternate allele of the MAO B gene has been identified in patients with Parkinson's disease,[6] a finding which suggests that an inherited variant form of MAO B may be associated with genetic predisposition for this neurological disorder.

MAO B Activates a Parkinsonism-Producing Neurotoxin

In addition to metabolizing biogenic amines, MAO B can oxidize xenobiotics. One striking example is the potent neurotoxin 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP; see Tables 1 and 2 for structures of MAO substrates and inhibitors). MPTP was originally identified from clinical studies of several individuals who used a synthetic heroin which contained MPTP as a contaminant.[7,8] When MPTP is self-administered in humans[7-9] or administered to nonhuman primates,[10,11] it selectively destroys neurons in the substantia nigra and produces clinical symptoms that are very similar to those of idiopathic Parkinson's disease (see reference 12 for a review of the MPTP model).

Several lines of evidence indicate that MPTP is metabolized by MAO B to yield an active toxin. Pretreatment of primates with MAO B inhibitors such as pargyline or deprenyl prevents the development of MPTP-induced parkinsonism,[13,14] and pretreatment with a substrate of MAO B reduces or eliminates MPTP's neurotoxicity.[15] Inhibitors of MAO A provide little or no protection in strains of mice that are susceptible to the effects of MPTP.[16] Thus, the activation of MPTP <u>in vivo</u> is dependent upon MAO B, not MAO A. Extracts of rat brain mitochondria[17] or highly purified human liver MAO B[18] oxidize MPTP to its corresponding pyridinium species,[19] 1-methyl-4-phenyl pyridium ion (MPP^+), through an unstable intermediate, 1-methyl-4-phenyl-dihydropyridinium ion ($MPDP^+$). MPP^+ has been identified as a major metabolite of MPTP in primate brain,[20] and MPP^+ is taken-up into regions containing substantia nigra and striatal cells *via* the major dopamine uptake system.[21] Pretreatment of mammals with uptake blockers of dopamine also provides protection against MPTP neurotoxicity.[21,22] Thus, the oxidation of MPTP by MAO B and the uptake of MPP^+ into neurons in the substantia nigra represent two critical steps in the generation of MPTP neurotoxicity.

Previous studies on the combined use of levodopa and deprenyl resulted in prolonged survival of parkinsonian patients.[23,24] Prompted by these results and the role played by MAO B in MPTP-induced parkinsonism, Tetrud and Langston[1] treated patients with deprenyl alone and found that this MAO B inhibitor significantly delayed the need for levodopa treatment in early

stage Parkinson's disease, apparently by slowing the rate of disease progression. These results were confirmed by a large study of over 800 patients by the Parkinson Study Group.[25,26]

Search for Compounds that Act Like MPTP

Several laboratories have attempted to identify environmental or endogenous compounds, resembling MPTP in biological effects, that can be activated by MAO B to produce a neurotoxic metabolite. 1,2,3,4-Tetrahydroisoquinoline is a naturally occurring substance that can cause behavioral deficits and reductions in dopamine, biopterin and tyrosine hydroxylase in the substantia nigra of primates, but the role played by MAO B has not yet been defined.[27] Several ß-carbolines[28] found in Japanese sake and soy sauce, and a series of 1,2,3,4-tetrahydro-, 3,4-dihydro- and fully aromatic isoquinolines[29] have been tested as potential substrates or inhibitors of MAO A and B. Substance YS (perlolyrine), but not flazin, was identified as a moderately potent inhibitor of MAO A, but MAO B was unaffected. Many of the isoquinoline derivatives were found to inactivate MAO A, including stereo selective inhibition by (+)-(R)-salsolinol, and a few were effective against MAO B. However, none of the compounds tested served as substrates for MAO A or B. Thus, naturally occurring compounds that resemble the action of MPTP remain to be identified.

MAO A and B May Eliminate Extraneous Amines From Specific Subsets of Neurons

While the metabolic fate of MPTP implicates MAO B in Parkinson's disease, knowledge of the location of MAO A and B expression is necessary to further understand the role of these enzymes in vivo. Monoclonal antibodies specific for either MAO A or B have been particularly valuable for localizing these enzymes in regions of primate brain, including humans.[30,32] Using immunocytochemical staining techniques, MAO A was found in regions rich in catecholaminergic neurons while MAO B was localized in regions

abundant in serotonergic neurons. Non-neuronal glial cells (e.g., astrocytes) that are distributed throughout the brain contain mostly MAO B, suggesting that these cells can express or take up this protein. The regional expression of MAO A and B in neurons of primates is consistent with the location of these enzymes in rat brain using other histochemical methods.[33,34]

The pattern of MAO distribution in neurons was unexpected, based on previous studies of substrate preference. Although MAO B is preferentially localized in serotonergic neurons, MAO B has a very low affinity for serotonin. However, MAO B has a higher affinity for amines not used as neurotransmitters in serotonergic neurons such as phenylethylamine, dopamine, and norepinephrine.[35-38] Conversely, MAO A is preferentially localized in catecholaminergic neurons but has less affinity for the catecholamine norepinephrine than for serotonin, an amine not used as a neurotransmitter in catecholaminergic neurons.[34,35,38,39] These facts prompted us and others to conclude that the major role of MAO A and B in monoaminergic neurons may be to eliminate extraneous neurotransmitters (e.g., MAO A removes serotonin from catecholaminergic neurons, and MAO B removes catecholamines from serotonergic neurons).[30-34]

Monoamine Oxidase A and B are Encoded by Different Genes

In addition to substrate preference and tissue and cell distribution, MAO A and B can be distinguished by differences in inhibitor specificity and immunological properties (see reference 41 for a discussion of human liver and brain MAO A and B). The molecular basis of these differences was not definitively resolved, however, until the cDNAs that encoded the human enzymes were isolated and sequenced.[42,43] Examination of the deduced amino acid sequences showed that human MAO A and B contain 527 and 520 amino acid residues, respectively, with an overall sequence identity of 70%. However, different amino acids reside at the same position throughout these two

polypeptide chains, a finding which supports the interpretation that MAO A and B are derived from different genes and not from post-transcriptional, and/or post-translational modifications.[42] Interestingly, the sequences of MAO B in human platelet and frontal cortex are identical to human liver MAO B.[44]

Experiments with somatic cell hybrids show that the MAO A and B genes are located on the human X-chromosome.[45,46] *Furthermore,* in situ studies with cDNAs that encode these enzymes have confirmed that human MAO A and B are located in close proximity on Xp11.23-11.4.[47,48] Phage clones that span almost the entire human MAO A and B genes have been recently isolated and restriction mapped.[49-51] The human MAO A and B genes are larger than 70 kb in size and both have 14 introns and 15 exons organized identically, suggesting that they are derived from a common ancestral gene.[49]

Studies designed to uncover how the MAO genes are regulated have been initiated. The tissue distribution of human MAO A and B mRNA has been determined.[52] Studies of restriction site polymorphisms of the human MAO A alleles indicate that the MAO A gene is largely responsible for determining the level of MAO A activity.[53] Furthermore, the organization of the transcription elements in the promoter region of the MAO A and B genes appears to be different.[54] The MAO A promoter fragment contains three Sp1 elements but lacks a TATA box. In contrast, the MAO B promoter fragment contains an Sp1-CACCC-Sp1-TATA structure. These sequences presumably are responsible in part for the differences observed in the regulation of tissue- and neuronal-specific expression of these genes.

Comparative Studies of MAO A and B among Different Species

While the bovine and rat MAO A and B genomic clones have not yet been isolated and characterized, nucleotide sequencing studies of cDNAs have yielded the deduced amino acid sequences for bovine adrenal MAO A,[55] rat liver MAO A[56,57] and rat liver MAO B.[58] Partial cDNA sequencing of rat adrenal

MAO A suggests that the adrenal and liver forms of MAO A may be identical.[57] Part of the sequence of bovine liver MAO B has been determined by direct amino acid sequencing.[55] Three regions in MAO A (residues 15-32, 187-228, and 389-458) and in MAO B (residues 6-23, 178-219, and 380-449) have sequence identities of 78%, 88%, and 86% respectively across rat, bovine, and human species. The high level of sequence identity between species implies the importance of these regions in enzyme function. These regions correspond to a dinucleotide-binding site (also referred to as the AMP- or ADP-binding site) positioned near the *N*-terminus, a region of unknown function, and the covalent FAD-attachment site at cysteine residue 406 in MAO A and 397 in MAO B. The *C*-terminus end contains a short stretch of mostly hydrophobic amino acids (residues 501-518 in MAO A and 492-511 in MAO B), followed by variable charged amino acids, a finding which suggests that this region may be responsible for anchoring MAO to the outer mitochondrial membrane. Using truncated cDNAs, Mitoma and Ito[59] confirmed this hypothesis by demonstrating that the targeting signal for the membrane is located within the carboxy-terminal 29 amino acid residues.

The Dinucleotide-binding Site Is Found in a Beta-Alpha-Beta Motif

The MAO A inhibitor moclobemide and the MAO B inhibitor deprenyl promise to be useful in the treatment of unipolar depression[60] and Parkinson's disease,[1] respectively. However, knowledge of the secondary and tertiary structure of MAO would be of great value for the design of drugs that have greater efficacy for these diseases. Since MAO is an integral protein of the outer mitochondrial membrane,[61] it has been difficult to obtain crystals suitable for X-ray diffraction studies. Alternatively, a search for sequence similarities between MAO And other proteins of known structure may be useful for predicting the secondary or tertiary structure of some of the motifs or domains within the MAO polypeptide.

A search for amino acid sequence similarities between MAO And other proteins that bind FAD either non-covalently or covalently revealed the presence of a dinucleotide-binding site,[62,63] which was found in 80% of the proteins examined that bind FAD (n=36, see Table 1). The dinucleotide-binding site is involved in non-covalent interactions with FAD or NAD.[62] Some FAD-binding proteins have both a dinucleotide-binding site and a covalent binding site, while others have one or the other or neither (see Table 1). MAO binds FAD covalently, but it also has a dinucleotide-binding site.

In MAO A, this dinucleotide-binding region spans residues 15-43, and this site in MAO B extends from residues 6-34. In this region, three glycine residues are highly conserved in proteins that bind FAD, and these residues are flanked on both sides by a stretch of hydrophobic residues (see Table 1). The dinucleotide-binding site is thought to consist of a ß sheet-α helix-ß sheet motif that interacts with the AMP moiety of FAD,[62] and may play a role in aligning the flavin for reduction and then oxidation during the catalysis of amines to their corresponding aldehydes.

FAD is Covalently Bound to Many Proteins in Procaryotes and Eucaryotes

Sequencing of FAD-linked peptide fragments revealed that FAD is bound covalently to cysteine in the pentapeptide SGGCY in MAO A and B.[64,65] Comparison of this sequence with the full-length deduced amino acid sequences indicates that FAD is bound to cysteine 406 in MAO A and cysteine 397 in MAO B.[42] MAO A and B share 20 identical amino acids around the FAD-bound cysteine. FAD is not bound to a nearby cysteine residue located eight amino acids upstream of the target cysteine in either of these enzymes, suggesting that the amino acid sequence flanking the recipient cysteine is critical for FAD coupling to the protein.

FAD is bound through an 8-methyl-S-cysteinyl bond in MAO And in flavocytochromes C552 and C553.[66,67] All other flavin-linked proteins,

however, contain FAD bound covalently through an 8-methyl-*N*-3 histidine or an 8-methyl-*N*-1 histidine bond,[68,69] or to a tyrosine residue.[70]

The mechanism by which FAD is coupled to any of these proteins remains unknown. In studies that attempt to address this question, Brandsch and Bichler[69] found that the apoenzyme of 6-hydroxy-D-nicotine oxidase can be converted into an enzymatically active holoenzyme by the covalent coupling of FAD. This step required the presence of an energy generating system and a crude extract from Escherichia coli.[71] These results suggest that covalent binding of FAD to protein is catalyzed by an uncharacterized enzyme. However, these results may not be applicable to MAO since 6-hydroxy D-nicotine oxidase does not contain the dinucleotide-binding site. It is possible that a coupling enzyme is necessary to covalently link FAD when the recipient protein does not have a dinucleotide-binding site.

When human liver MAO A is expressed in yeast, FAD is covalently attached.[72] Weyler and colleagues suggest that FAD may be bound without the aid of an enzyme since no known proteins in yeast contain covalently attached FAD. It is possible that the dinucleotide-binding site in MAO facilitates the covalent attachment of FAD without the aid of a flavin-coupling enzyme.

Other Regions Putatively Involved in Binding FAD and Substrate

In addition to the dinucleotide-binding site, a search for amino acid sequence similarities between MAO and other proteins revealed regions putatively involved in either FAD or substrate binding. The three-dimensional structures and deduced amino acid sequences of Escherichia coli fumarate reductase and succinate dehydrogenase indicate that the sequence Y--GI-T interacts with FAD.[73-78] This sequence also is found in other FAD-binding proteins such as Proteus vulgaris fumarate reductase and the flavoprotein subunit of Ascaris summ mitochondrial complex II.[79] MAO contains this sequence just four amino acids downstream of the covalent

attachment site for FAD. This finding raises the possibility that this region in MAO is involved in interaction with FAD.

Another sequence in MAO, FAGTET,(residues 432-437 in MAO A and 423-428 in MAO B) was found to be identical to a region in some cytochrome P-450 proteins. This region in cytochrome P-450s is thought to be involved in substrate interactions as indicated by crystal structural and site-directed mutagenesis studies.[80,81] The sequence FAGTET may be involved in substrate binding in MAO. In support of this notion, there exists some evidence that MAO and cytochrome P-450s have similar substrate binding sites. First, cytochrome P-450s have some substrates that are structurally similar to MAO substrates (for example, serotonin is a substrate for MAO A and zoxazolamine is a substrate for cytochrome P-450). Furthermore, both cytochrome P-450 and MAO B metabolize MPTP.[82] Finally, MAO's catalytic function (deamination) is one of many activities demonstrated by cytochrome P-450s.

Substitution of Selected Amino Acids by Site-Directed Mutagenesis

The role of the nine cysteine residues in the catalytic activity of MAO A and B has been examined by site-directed mutagenesis.[83] Each cysteine residue was converted to a serine residue, and the mutant cDNAs were expressed in COS cells and compared to the control MAO A and B cDNAs. Substitution of two cysteine residues in MAO A (C-374 and C-406) and three in MAO B (C-156, C-365 and C-397) resulted in complete loss of catalytic activity, suggesting that these residues are constituents of the active site or contribute to the proper folding and/or conformation of the molecules. Since cysteine residues 406 in MAO A and 397 in MAO B are the sites where FAD is covalently linked to these enzymes, substitution at these positions would be expected to inactivate the enzymes. Cysteine residues 374 in MAO A and 156 or 365 in MAO B, however, could be the targets of members of certain classes of irreversible inhibitors (e.g., cyclopropylamines.[84] In fact, cysteine was identified as the amino acid residue to which a labile adduct

was bound during inactivation of MAO B by 1-phenylcyclopropylamine.[85] Recent work in our laboratory[86] has examined the role played by glutamic acid residue 34 in MAO B activity. Three mutant cDNAs were prepared, where glutamic acid (encoded by GAA) was changed to glutamine (CAA), aspartic acid (GAT) on alanine (GCA) and then expressed in COS cells. All three mutants were devoid of catalytic activity, confirming the putative crucial role of glutamic acid in binding FAD in the dinucleotide binding site. In other studies[87] which examined a chimera of MAO A with the *N*-terminus 36 amino acids of MAO B and a chimera of MAO B with the *N*-terminus 45 amino acids of MAO A, no significant difference in catalytic properties or sensitivity to inhibitors was found compared to the wild type MAO A and B enzymes. Since these chimeras contained the dinucleotide binding site, it appears that this region of the molecule is not responsible for determining the different substrate specificities of these isoenzymes.

Future Studies

Previous investigations with xenobiotics such as MPTP and with MAO inhibitors such as deprenyl emphasize the importance of elucidating the structure of MAO. Searches for sequence similarities between MAO and other proteins have revealed a dinucleotide-binding domain and other regions possibly involved in interacting with FAD and substrate. Site-directed mutagenesis studies and biochemical characterization of the mutants will help clarify the roles of specific amino acid residues in substrate and FAD binding, and aid in designing drugs with greater efficacy for disorders like Parkinson's disease and depression. Ultimately, the three-dimensional structure of MAO A and B is needed to understand fully the structure-function relationships of these important amine-degrading enzymes.

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FOOTNOTES

[1]The abbreviations used are: monoamine oxidase A (MAO A), monoamine oxidase B (MAO B), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenyl-pyridium ion (MPP^+), 1-methyl-4-phenyldihydropyridium ion ($MPDP^+$). When MAO is used, it refers to both MAO A and B.

[2]For conciseness, only recent or crucial publications to the discussion have been cited here. References to older work can be found in more comprehensive reviews.[12,88-91]

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Table 1

Some MAO Substrates

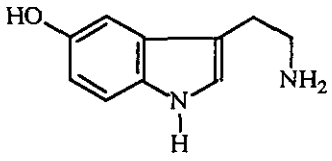
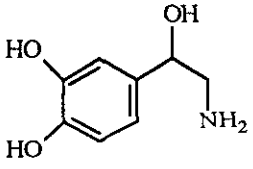
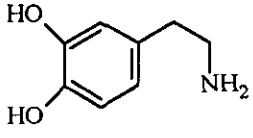
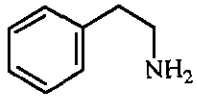
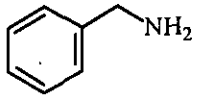
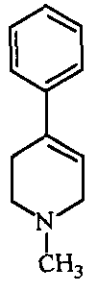
Compound	Structure	Selectivity
Serotonin		MAO A
Norepinephrine		MAO A
Dopamine		MAO A and B
Phenylethylamine		MAO B
Benzylamine		MAO B
MPTP		MAO B

Table 2

Some MAO Substrates

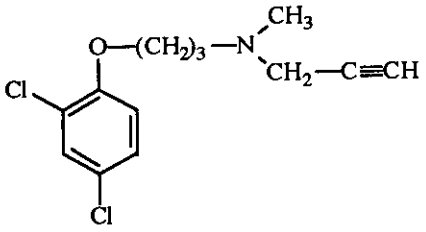
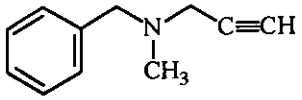
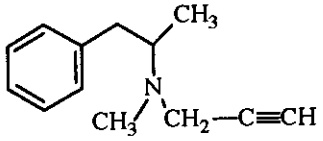
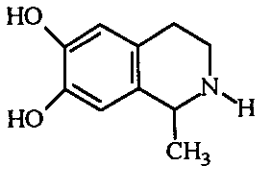
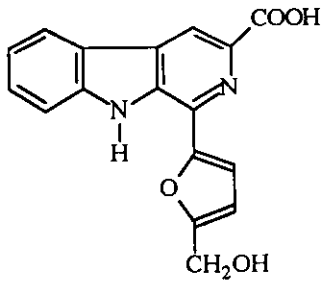
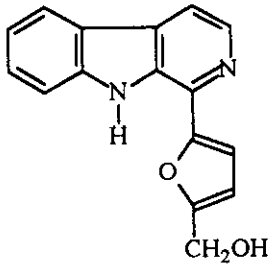
Compound	Structure	Selectivity
Clorgyline		MAO A
Pargyline		MAO B
Deprenyl		MAO B
Salsolinol		MAO A
Flazin		No Inhibition
Perlolyrine		MAO A

Table 3

Dinucleotide-binding Sites in some Flavoproteins

(Flavoproteins in which FAD is Covalently Bound)

	+	*	*	*	*	#	*	#		*	#	*		@		*										*		*	*	&							
1	D	V	V	V	I	G	G	G	I	S	G	L	S	A	A	K	L	L	A	E	H	E	_	_	_	_	_	V	N	V	L	V	L	E	A	R	E	R
2	D	V	V	V	I	G	G	G	I	S	G	L	A	A	A	K	L	L	S	E	Y	K	_	_	_	_	_	I	N	V	L	V	L	E	A	R	D	R
3	D	V	V	V	I	G	G	G	I	S	G	L	S	A	A	K	L	L	T	E	Y	G	_	_	_	_	_	V	S	V	L	V	L	E	A	R	D	R
4	D	V	V	V	V	G	G	G	I	S	G	M	A	A	A	K	L	L	H	D	S	G	_	_	_	_	_	L	N	V	V	V	L	E	A	R	D	R
5	K	V	V	V	V	G	G	G	T	G	G	A	T	A	A	K	Y	I	K	L	A	D	P	_	_	_	S	I	E	V	T	L	I	E	P	N	T	K
6	P	A	V	V	I	G	T	G	Y	G	A	A	V	S	A	L	R	L	G	E	A	G	_	_	_	_	_	V	Q	T	L	M	L	E	M	G	Q	L
7	E	T	V	I	I	G	G	G	C	V	G	V	S	L	A	Y	H	L	A	K	A	G	M	_	_	_	_	R	D	V	V	L	L	E	K	S	F	L
8	D	A	V	V	I	G	A	G	G	A	G	I	A	R	L	A	Q	I	S	Q	S	G	_	_	_	_	_	Q	T	C	A	L	L	S	K	V	F	P
9	S	I	I	V	V	G	G	G	L	A	G	L	M	A	T	I	K	A	A	E	S	G	_	_	_	_	_	M	A	V	K	L	F	S	I	V	P	V
10	D	L	A	I	V	G	A	G	G	A	G	L	R	A	A	I	A	A	A	Q	A	N	P	_	_	_	N	A	K	I	A	L	I	S	K	V	Y	P

(Flavoproteins in which FAD is not Covalently Bound)

	+	*	*	*	*	#	*	#		*	#	*		@		*										*		*	*	&							
11	D	V	V	V	I	G	A	G	P	G	G	Y	V	A	A	I	R	A	A	Q	L	G	_	_	_	_	_	L	K	T	A	C	I	E	K	Y	I	G
12	D	Y	L	V	I	G	G	G	S	G	G	L	A	S	A	R	R	A	A	E	L	G	_	_	_	_	_	A	R	A	A	V	V	E	S	H	K	L
13	R	V	V	V	I	G	A	G	V	I	G	L	S	T	A	L	C	I	H	E	R	Y	H	_	_	_	_	S	V	L	Q	P	L	D	V	K	V	Y
14	Q	V	A	I	I	G	A	G	P	S	G	L	L	L	G	Q	L	L	H	K	A	G	_	_	_	_	_	I	D	N	V	I	L	E	R	Q	T	P
15	Q	V	A	V	I	G	S	G	G	A	A	M	A	A	A	L	K	A	V	E	Q	G	_	_	_	_	_	A	Q	V	T	L	I	E	R	G	T	I
16	K	L	L	I	L	G	S	G	P	A	G	Y	T	A	A	V	Y	A	A	R	A	N	L	Q	_	_	P	V	L	I	T	G	M	E	K	G	G	Q
17	D	A	I	V	V	G	G	G	F	S	G	L	K	A	A	R	D	L	T	N	A	G	_	_	_	C	E	V	L	P	E	G	G	E	R	L		
18	D	V	L	I	V	G	A	G	P	A	G	L	M	A	A	R	V	L	S	E	Y	V	R	Q	K	P	D	L	K	V	R	I	I	D	K	R	S	T
19	R	K	L	N	Q	G	T	E	R	H	L	M	H	L	E	L	D	I	S	D	S	K	_	_	_	_	_	I	R	Y	E	S	G	D	H	V	A	V
20	K	I	V	I	V	G	G	G	A	G	G	L	E	M	A	T	Q	L	G	H	K	L	G	R	K	K	K	A	K	I	T	L	V	D	R	N	H	S
21	R	V	A	I	V	G	A	G	V	S	G	L	A	S	I	K	C	C	L	E	E	G	_	_	_	_	_	L	E	P	T	C	F	E	R	S	D	D

(Flavoproteins in which the nature of FAD-binding is uncertain)

	+	*	*	*	*	#	*	#		*	#	*		@		•										*		*	*	&							
22	D	I	I	V	V	G	G	G	S	T	G	C	C	I	A	G	R	L	A	N	L	D	D	_	_	Q	N	L	T	V	A	L	I	E	G	G	E	N
23	D	I	L	V	L	G	G	G	S	S	G	S	C	I	A	G	R	L	A	N	L	D	H	_	_	_	S	L	K	V	G	L	I	E				
24	Q	I	C	V	V	G	S	G	P	A	G	F	Y	T	A	Q	H	L	L	K	H	H	S	_	_	_	R	A	H	V	D	I	Y	E	K	Q	L	V
25	D	N	V	I	V	G	T	G	L	A	G	V	E	V	A	F	G	L	R	A	S	G	W	_	_	E	G	N	I	R	L	V	G	D	A	T	V	I
26	A	I	V	V	V	G	A	G	T	A	G	V	N	A	A	F	W	L	R	Q	Y	G	Y	K	_	G	E	I	R	I	F	S	P	E	S	V	A	P
27	E	L	V	R	E	G	G	T	G	T	V	R	H	L	I	F	D	I	S	G	G	D	_	_	_	_	_	L	R	Y	L	E	G	Q	S	I	G	I
28	D	Y	I	I	A	G	G	G	L	T	G	L	T	T	A	A	R	L	T	E	N	P	_	_	_	_	N	I	S	V	L	V	I	E	S	G	S	Y

FAD-binding consensus sequence

\# Highly conserved glycines
@ Highly conserved alanine residue
& Highly conserved glutamate or aspartate involved in hydrogen bonding to FAD
\+ Conserved hydrophilic residue (usually acidic or basic)
* Usually a hydrophobic residue

		Reference
1	Bovine liver MAO A (15)	43
2	Rat liver and adrenal MAO A (15)[a]	56,57
3	Human liver MAO A (15)	42
4	Human liver MAO B (6)	42

5	C. Vinosum Flavocytochrome C (4)	92
6	Streptomyces Cholesterol oxidase (7)	93
7	Rat liver dimethylglycine dehydrogenase (44)	94
8	E. coli Succinate dehydrogenase (8)	73
9	B. subtillus Succinate dehydrogenase (4)	78
10	E. coli Fumarate reductase (7)	74
11	P. fluorescens lipoamide dehydrogenase (5)	95
12	Human erythrocyte glutathione reductase (22)	96
13	Pig kidney D-amino acid oxidase (2)	97
14	P. fluorescens p-hydroxybenzoate hydroxylase (4)	98
15	P. aeruginosa Mercuric reductase (100)	99
16	E. coli Thioredoxin reductase (7)	100
17	A. oxidans 6-hydroxy-L-nicotine oxidase (3)[b]	101
18	T. cutaneum Phenol hydroxylase (9)	102
19	Rat liver NADPH-Cytochrome P-450 oxidoreductase (290)	103
20	E. coli NADH dehydrogenase (7)	104
21	Pig liver flavin-containing monooxygenase (4)	105
22	H. polymorpha alcohol oxidase (8)	106
23	P. pastoris alcohol oxidase (8)	107
24	Bovine Adrenodoxin reductase (8)	108
25	P.putida Putidaredoxin reductase (6)	109
26	P. oleovorans rubredoxin reductase (2)	110
27	Spirulina Ferredoxin-NADP$^+$ reductase (25)	111
28	A. Niger Glucose oxidase (21)	112

Numbers in parentheses indicate the position of the starting residues

Flavoproteins which lack the dinucleotide-binding site but have FAD covalently bound

A. oxidans 6-hydroxy-D-nicotine oxidase[b]	101
P. putida p-cresol methylhydroxylase	113
Rat Liver L-gulono-γ-lactone oxidase	114

Flavoproteins which lack the dinucleotide-binding site but have FAD non-covalently bound

Human erythrocyte NADH-cytochrome B5 reductase	115

Flavoproteins which lack the dinucleotide-binding site but have FAD bound

S. typhimurium NADPH-sulfite reductase	116
P. flourescens phenol hydroxylase	117
A. thaliana Nitrate reductase	118

[a]Note that the rat liver and adrenal forms of MAO A have identical dinucleotide-binding sites.

[b]Note the difference in sequence between the enzymes that metabolize the L and D forms of 6-hydroxy-nicotine.

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