Molecular biological studies of monoamine oxidase: structure and function
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The two forms of monoamine oxidase (MAO) are defined by their substrate and inhibitor affinities [1]. This specificity must be reflected in the amino acid sequences and molecular structure of the active sites. A precise knowledge of those amino acid residues that form the active sites of MAO-A and MAO-B may enable new specific inhibitors of MAO to be rationally designed. Until recently the only approach to identifying those amino acid residues important for enzymic specificity was by protein chemical methods and from the large amounts of data describing the substrate and inhibitor specificities of the two forms. However, the recent isolation of cDNA clones for both forms of MAO from several different species initiates a molecular biological approach to structural and functional studies [2-4]. The translated protein sequences of MAO-A and MAO-B cDNAs showed that the two iso-enzymes differed in size; the MAO-A cDNA coded for a protein of 527 amino acid residues ($M_r$ 59700 Da) and MAO-B cDNA coded for a protein of 520 residues ($M_r$ 58 800 Da). This difference in size is in agreement with biochemical data obtained from the comparison of [3H]pargyline-labelled polypeptides on denaturing polyacrylamide gels [5]. The hydrophobicity profile of the two forms are very similar, suggesting that they share a similar structure.

Comparison of the nucleic acid sequences of the cDNAs for MAO-A and MAO-B shows that the different forms of MAO are the products of two related but different genes. In humans and probably in other mammals the two genes are closely linked on the X-chromosome [6] lending support to the idea that these genes may have arisen from duplication of a single ancestral gene. Lower vertebrates express both forms of MAO [7] suggesting that any gene duplication event that led to the creation of the two genes for the MAOs must have occurred in early prevertebrate evolution. The genes for the two forms of MAO have therefore been separated for a long evolutionary period. Comparison of the sequences of the different forms with those from other species showed that there is a greater degree of similarity between the same form from different species than between different forms from the same species. For example rat and human MAO-B have approximately 90% of amino acid residues identical while human MAO-A and human MAO-B share only approximately 70% of amino acid residues. Some of those amino acid residues that are identical in different species can be inferred to have been conserved during evolution because they are functionally important. One region which is highly conserved in all species and forms of MAO is near the C-terminus of the proteins (Fig. 1). It includes the site of covalent attachment of the flavin cofactor (residue 406 for MAO-A and 397 for MAO-B; this is known from protein chemical studies. Another region near the centre of the protein sequence (residues 187-230 for MAO-A) is highly conserved, there being only two substitutions between all the published sequences. Although there is no known function for this region, these conserved regions probably form part of the active site. More importantly, those residues that are conserved between species and are also different in the different forms could be those residues that are important in conferring the properties of the various types (marked with an asterisk in Fig. 1). These residues are scattered throughout the amino acid sequence of the MAOs. However, there are two clusters of such residues situated at the N-terminus and around residue 360 of the MAO-A sequence.

Abbreviation used: MAO, monoamine oxidase.
Another useful approach to understanding structure and function is the comparison of sequences with that of other proteins. There are several sequences homologous to the MAOs revealed by computer search of databases. A region near the N-terminus shows significant similarity to several flavin binding proteins (Fig. 2). This region fulfills the criteria for a predictive fingerprint for a flavin-binding domain [8]. This structure is found in many FAD-binding and ADP-binding proteins and the predicted structure consists of a stretch of $\beta$-sheet (residues 1–6), an $\alpha$-helical sequence (residues 8–21), a loop of variable length and a second $\beta$-sheet terminating in an acidic residue. This FAD-binding and ADP-binding $\beta\alpha\beta$ fold is found typically at the N-terminus (the MAOs are not an exception) and it has been suggested that this structure acts as a 'nucleation centre'. During biosynthesis this domain will form first and the rest of the protein structure forms around it. The bacterial enzymes fumarate dehydrogenase and succinate dehydrogenase, which are compared with the MAOs in Fig. 2, bind FAD covalently at a histidine residue at positions 41 and 45 respectively. The site of attachment of the flavin for MAO is, in contrast, at cysteine residues at position 406 for MAO-A and 397 for MAO-B. While MAO-A and MAO-B are identical for those predictive residues for the flavin-binding domain (boxed in Fig. 2), some residues show differences between the forms of MAO that are conserved between species (these residues are marked with an asterisk). Indeed, surrounding this region are several such residues. It is tempting to speculate that these residues, as well as forming part of the active site of MAO, are responsible for the different activities of the two forms. There is another short region (starting at residue 438 for MAO-A) which shows some homology to other flavoproteins such as NADPH-cytochrome P-450 oxidoreductase and some bacterial flavodoxins. This region is conserved in all forms of MAO and in the flavodoxins is known to bind the phosphate residues of FMN. This region may therefore interact with the phosphate groups of the flavin cofactor.

There have been a number of reports that claim that MAO is a dimer of two similar but non-identical subunits. These arose out of analysis of the flavin composition of purified preparation and suggested that the enzyme consisted of a complex of a flavin-binding subunit and an unmodified subunit. Some experiments by Lan et al. [9] address this question; they transfected expression vectors containing the sequences of MAO-A or MAO-B into mammalian cells that express only low levels of MAO. MAO is a dimer of two similar but non-identical subunits. These arose out of analysis of the flavin composition of purified preparation and suggested that the enzyme consisted of a complex of a flavin-binding subunit and an unmodified subunit. Some experiments by Lan et al. [9] address this question; they transfected expression vectors containing the sequences of MAO-A or MAO-B into mammalian cells that express only low levels of MAO. MAO is a dimer of two similar but non-identical subunits. These arose out of analysis of the flavin composition of purified preparation and suggested that the enzyme consisted of a complex of a flavin-binding subunit and an unmodified subunit. Some experiments by Lan et al. [9] address this question; they transfected expression vectors containing the sequences of MAO-A or MAO-B into mammalian cells that express only low levels of MAO.
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MAO, and analysed the resulting enzyme activity obtained. The transfected cells expressed monoamine oxidase activity of the correct form showing that a single subunit is sufficient to generate activity and that the identified cDNAs code for enzymically active type A and type B monoamine oxidases.

The successful expression of the monoamine oxidase cDNAs in a mammalian cell enables the direct study of hypotheses of structure and function. The production of chimaeric forms of monoamine oxidase consisting of the N-terminal sequences of MAO-A and the C-terminal sequences of MAO-B, for example, could be illuminating. When these constructs are transfected into mammalian cells enzymic assays may reveal which domains of the MAOs determine the enzyme specificity. More specific site-directed mutation of individual amino acid residues will identify those amino acids forming the active site.


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The use of mechanism-based inactivators to probe the mechanism of monoamine oxidase

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Monoamine oxidase (MAO; EC 1.4.3.4) is one of the enzymes responsible for the catabolism of various biogenic amine neurotransmitters as well as for the metabolism of certain exogenous amines [1]. The enzyme exists in two different isozymic forms known as MAO-A and MAO-B [2], which differ in substrate specificity, distribution among tissues and in their structures [3–5]. Non-specific or MAO-A selective inhibitors have been shown to be clinically useful as antidepressants; the clinical usefulness of MAO-B-selective inhibitors in the treatment of depression remains to be demonstrated [6]. However, MAO-B-selective inhibitors are being used as adjuncts to the l-dopa treatment of Parkinson's disease [7, 8]. Furthermore, the administration of MAO-B inhibitors alone early in the course of this disease can alleviate the need for other drugs, presumably by slowing the progression of the disease [7].

Abbreviation used: MAO, monoamine oxidase.

We have been interested in the use of mechanism-based inactivators [9] of MAO as a probe for the mechanism of this enzyme and in the design of new classes of inactivators. Mechanism-based inactivators have been shown to be quite useful in the study of enzyme mechanisms because they are really nothing more than substrates for an enzyme which happened to be converted into products that react with the enzyme and inactivate it. But since the mechanism by which these compounds are converted into the reactive products proceeds, at least initially, by the normal catalytic mechanism, any information that is obtained regarding the inactivation mechanism is directly related to the catalytic mechanism. That is the approach that we have taken to elucidate the mechanism for mitochondrial MAO.

MAO is a dimeric flavoenzyme in which one FAD is covalently attached to each subunit of the apoprotein [10]. It catalyses the oxidation of amines to the corresponding imines with concomitant