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Minireview

Crystal structure of human monoamine oxidase B, a drug target enzyme monotopically inserted into the mitochondrial outer membrane

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Abstract Monoamine oxidase B (MAO B) is an outer mitochondrial membrane protein that oxidizes arylalkylamine neurotransmitters and has been a valuable drug target for many neurological disorders. The 1.7 Å resolution structure of human MAO B shows the enzyme is dimeric with a C-terminal transmembrane helix protruding from each monomer and anchoring the protein to the membrane. This helix departs perpendicularly from the base of the structure in a different way with respect to other monotopic membrane proteins. Several apolar loops exposed on the protein surface are located in proximity of the C-terminal helix, providing additional membrane-binding interactions. One of these loops (residues 99–112) also functions in opening and closing the MAO B active site cavity, which suggests that the membrane may have a role in controlling substrate binding.
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Key words: Human monoamine oxidase B structure; Mitochondrial membrane; Monotopic; Flavin

1. Introduction

Eukaryotic monoamine oxidases (MAOs) catalyze the oxidative deamination of primary and secondary aromatic amines to imines, with concomitant reduction of oxygen to hydrogen peroxide. In mammals two isoforms of this enzyme have been identified as separate gene products, namely MAO A and MAO B [1,2]. The physiological roles of MAOs are related to the nature of their substrates. MAO A preferentially oxidizes the neurotransmitter serotonin and exhibits overlapping specificity with MAO B with adrenaline and dopamine. MAO B is most active with other arylalkylamines such as benzylamine, thus participating in the metabolism of exogenous amines to prevent their possible function as false neurotransmitters. MAOs are involved in depression and in a number of neurodegenerative disorders such as Parkinson's disease and, therefore, are well-known targets for drug treatment of these pathological conditions [3].

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Abbreviations: MAO, monoamine oxidase

MAO A and MAO B are integral outer mitochondrial membrane proteins [4]. Controlled proteolysis experiments suggest that these enzymes may be distributed in different orientations with respect to the membrane [5], although the exact orientation for each isozyme remains to be firmly established. Although the signals that mediate targeting and insertion of proteins into the outer mitochondrial membrane are poorly understood, MAOs have common features with other outer membrane mitochondrial proteins in that they have a C-terminal non-cleavable targeting signal sequence which is inserted into the membrane without proteolytic maturation [6,7]. Sequence analysis predicts that the C-terminal 32 amino acids (residues 489–520) form a transmembrane helix that anchors the protein to the mitochondrial membrane. Truncations at different levels of the C-terminus affect MAO B catalytic activity, which implies that this portion of the protein is important not only for membrane binding but also for enzyme functionality and stability [8].

2. The three-dimensional structure of human MAO B

We initially described the structure of human MAO B at 3.0 Å resolution (PDB code 1GOS) [9]. The structure was solved by X-ray crystallography using two crystal forms that were obtained under the same conditions with two different detergents (lauryldimethylamine oxide and Zwittergent 3–12). Crystal quality dramatically improved when the enzyme was crystallized in the presence of different competitive inhibitors. High resolution X-ray data (up to 1.7 Å) were collected and the coordinates of MAO B have been refined in a number of complexes with reversible and irreversible inhibitors (PDB codes 1OJA, 1OJB, 1OJC, 1OJD, and 1OJ9) [10].

The crystal structure of MAO B (520 amino acids) reveals a dimeric enzyme (Fig. 1A). Each monomer consists of a solvent-exposed globular structure (residues 1–488) anchored to the membrane by a C-terminal hydrophobic α-helix (residues 489–500). The solvent-exposed part of the protein resembles the structure of other flavin-dependent enzymes consisting of an FAD-binding domain (colored in blue in Fig. 1A) and a substrate-binding domain (in red in Fig. 1A). The latter domain accommodates a 700 $Å^3$ large cavity that originates from one side of the protein surface and extends deep into the protein, reaching the *re* face of the flavin cofactor. Analysis of the structure in complex with reversible and irreversible inhibitors revealed that this cavity forms the active site of this enzyme [10]. The MAO B dimer is identical in both crystal

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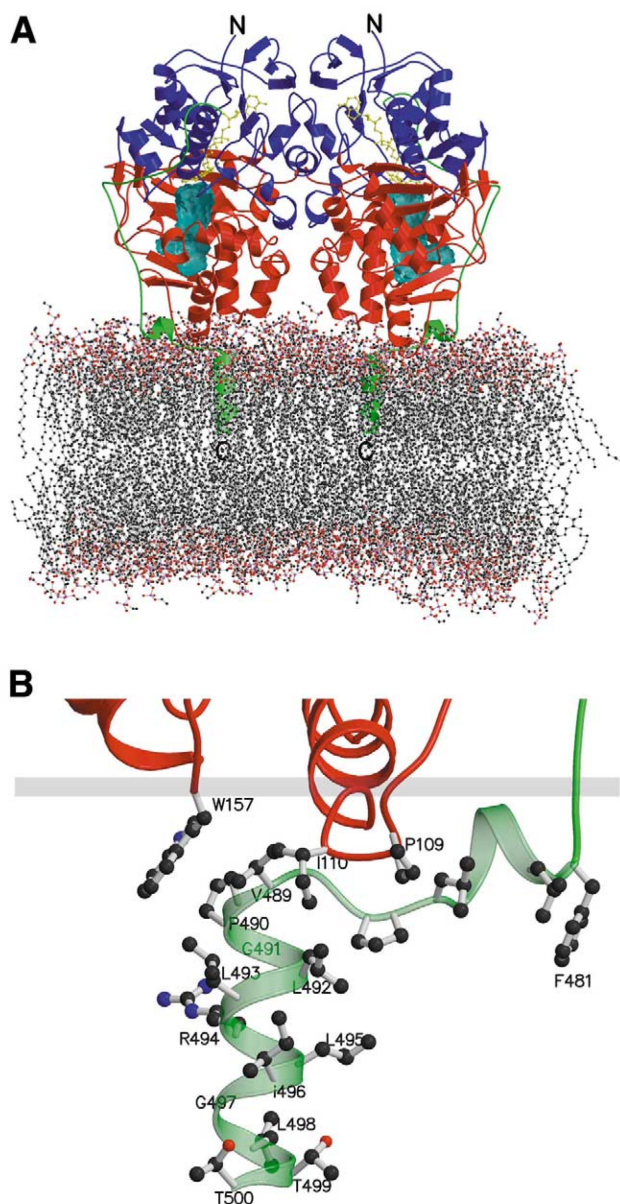

Fig. 1. Three-dimensional structure of human MAO B. A: Structure of MAO B dimer bound to the phospholipid bilayer. Each monomer has a two-domain overall topology, consisting of the FAD-binding domain in blue and the substrate-binding domain in red. The amino- and carboxy-termini of each monomer are highlighted as 'N' and 'C', respectively. The FAD cofactor is represented in yellow ball-and-stick. The C-terminal membrane-binding region is depicted in green. In each monomer the active site cavity is colored in light blue. B: The C-terminal transmembrane helix and the neighboring apolar sites involved in membrane binding. The secondary structures are colored as in A. Residue side chains are in ball-and-stick representation, with carbon atoms represented in black, nitrogens in blue, oxygens in red. The gray line is indicative of the upper boundary of the membrane but it is not intended to represent the exact depth of protein insertion into the membrane.

forms and involves a significant fraction of the protein surface (15% of the monomer accessible surface). These structural data predict that MAO B exists as a dimer in its membrane-bound form. Current kinetic data show the respective active sites are independent which is consistent with the entrances to the two active site cavities being located opposite with respect to the dimer interface (Fig. 1A).

In each MAO B monomer an extended loop (residues 461–488) originates from the FAD-binding domain and connects the main body of the protein to the C-terminal α-helix (in green in Fig. 1A). The conformation of this loop is stabilized by a number of interactions with the amino acid side chains of both substrate- and cofactor-binding domains. This loop leads to the C-terminal hydrophobic helix that departs perpendicularly from the base of each monomer. Most remarkably, the helical axis is parallel to the dimer two-fold axis (Fig. 1A). This suggests that the dimer is oriented with its axis perpendicular to the plane of the membrane, with the C-terminal helices rooted into the bilayer. This model is in agreement with hydropathy plots (MPEx, http://blanco.biomol.uci.edu/mpex) that identify residues 489–515 with significant free energy values for transmembrane segments. The side chain of Arg494 is properly oriented to establish electrostatic interactions with the polar head groups of the membrane phospholipids (Fig. 1B). In the dimeric structure, the transmembrane helices are too far from each other to establish any direct interactions.

The MAO B substrate-binding domain bears some solvent-exposed hydrophobic sites (Pro109–Ile110, Trp157) that are in close proximity to the C-terminal helix and therefore are likely to be involved in membrane binding (Fig. 1B). Indeed, it has been shown that truncations of the C-terminal tail (residues 461–520) at different levels do not prevent a partial binding of MAO B to the membrane [8]. These data agree with hydropathy plots that identify additional segments with free energy values compatible with these polypeptide stretches being at the bilayer interface. These hydrophobic patches may help to create a more rigid orientation of the protein to the membrane surface.

The full-length MAO B (520 amino acids) was used for crystallization in the presence of different inhibitors. In all structures the electron density is interrupted at Thr500, implying that residues 501–520 are disordered in the crystals. In the outer mitochondrial membrane, the disordered residues are predicted to extend the C-terminal helix such that it can span the membrane, with the last five residues (516–520) being exposed on the opposite side with respect to the main body of the protein (Fig. 2, right panel). However, another possibility is that the last 20 residues, rather than extending the transmembrane helix, may turn back to position the C-terminus on the same side of the outer membrane surface where the main body of the MAO B dimer is located (Fig. 2, left panel). So far, no experimental evidence is available to distinguish between these two models. Interestingly, a small two-residue deletion with respect to MAO B (deletion of residues 501–502 in MAO B) is observed in the sequence of the highly homologous MAO A isozyme. A chimera of MAO B in which the C-terminus (residues 393–520) was replaced with the MAO A C-terminus (residues 402–527) shows no catalytic activity [11] which suggests the MAO A C-terminus cannot function in protein folding and/or membrane insertion in the place of the MAO B sequence. These differences suggest that the regular path of the C-terminal helix may be interrupted at residue 500.

The relationship between the mitochondrial outer membrane localization and physiological roles of MAOs remains to be elucidated. Other amine oxidases such as polyamine

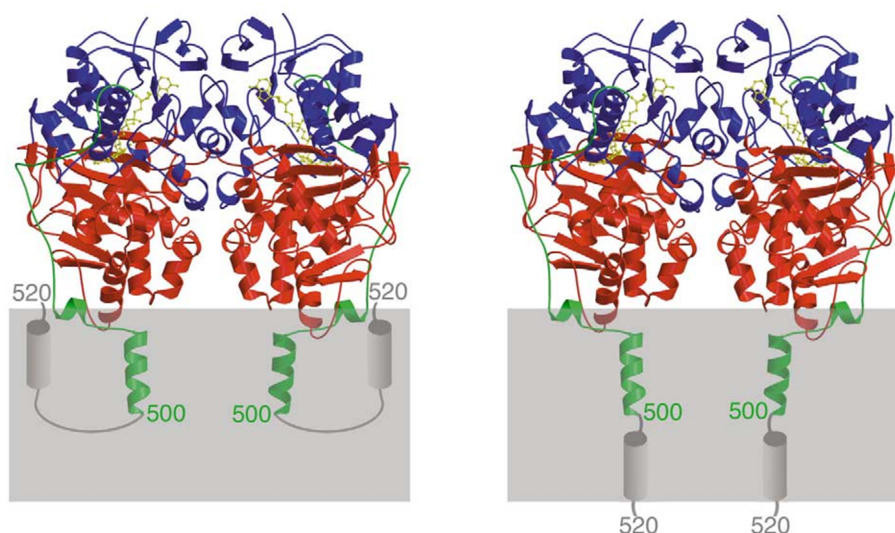

Fig. 2. Proposed hypotheses for the structure of the C-terminal tail (residues 500–520). MAO B dimer is represented as in Fig. 1A. The gray square represents the membrane, while the two possible conformations of the C-terminal part are depicted as gray cylinders. In the right panel the helix is suggested to span the entire bilayer; in the left panel another hypothesis is proposed in which the helix is interrupted, with the polypeptide chain turning back with the C-terminus positioned on the same side of the membrane with respect to the main body of the protein. All pictures have been produced with the programs Molscript [19] and Raster3D [20].

oxidase are not membrane proteins [12]. The crystal structure shows that the active site of MAO B is located in the globular part of the protein. This raises the question of why MAOs are membrane-bound. In the MAO B dimer, the substrate-binding site of each monomer faces the membrane surface and partly interacts with it (Fig. 1A). In particular, loop 99–112, which functions as a gate to the active site cavity, is expected to be partly embedded in the membrane (Fig. 1B). The membrane may have a role in controlling the function of this loop in gating the active site. In this regard it is important to point out that the protonated amino group of MAO B substrates may be attracted to the membrane surface and to the catalytic site by electrostatic interactions with the negatively charged phosphate head groups. These considerations would imply that the membrane may have a role in increasing the local substrate concentration at the active site of MAO by electrostatic interactions.

3. Comparison with other monotopic membrane proteins

MAOs are classified as integral membrane proteins because digestion of membrane phospholipids is needed for detergent extraction of the protein from its membrane environment [13]. The high resolution crystal structure of human MAO B provides the basis for a model consisting of a soluble globular core anchored to the membrane through a hydrophobic C-terminal helix that protrudes perpendicularly from the base of the structure. To date no other structures with similar membrane-binding domains have been described. Among monotopic membrane proteins the structures of prostaglandin H_2 synthase and of squalene cyclase have been solved. Prostaglandin H_2 synthase (also known as cyclooxygenase) is characterized by three amphipathic helices that lie approximately in a plane at the base of the structure, creating an extensive hydrophobic surface that interacts with the membrane hydrophobic core [14,15]. The squalene cyclase structure shows a very similar architecture with an α-helix together with three loops forming a large non-polar plateau encircled by a ring of positively charged amino acids [16,17]. In MAO B the C-terminal helix is perpendicular to the plane of the membrane and hydrophobic amino acids are distributed along the helix, with only one positively charged residue, Arg494, whose side chain points towards the membrane polar heads.

Another monotopic membrane protein whose structure has been recently described is fatty acid amide hydrolase [18], which is predicted to have an N-terminal transmembrane domain. The structure of a truncated form of this protein (lacking the N-terminal portion) reveals a dimeric enzyme. Two α-helices at the base of each monomer could interact with the membrane in a manner similar to what is found in prostaglandin H_2 synthase and squalene cyclase. At opposite sides of the dimer two long N-terminal helices run parallel to the molecular two-fold axis, with each N-terminus situated in a position appropriate to be preceded by the predicted transmembrane helix in the intact enzyme. This putative N-terminal transmembrane helix could reinforce the membrane interactions of the two helices at the base of the protein.

4. Conclusions

Few structures of monotopic membrane proteins are known. The MAO B structure reveals the presence of a C-terminal hydrophobic α-helix that is oriented properly for transmembrane insertion, while the other monotopic structures are characterized by amphipathic helices that face the base of the protein on one side and the membrane on the other. In this respect, MAO B is presently unique among the structurally characterized monotopic membrane proteins.

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