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STRUCTURE OF HUMAN MONOAMINE OXIDASE B, A DRUG TARGET FOR NEUROLOGICAL DISORDERS

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The mitochondrial outer membrane Monoamine Oxidases A and B (MAO A and MAO B) have been targets for neuro-protective drug research over the past 40 years since they are part of the catabolism of neurotransmitters such as serotonin and dopamine. Structure determination of either enzyme has been precluded mainly due to their membrane-association. In the past year, we have solved the structure of human MAO B by multicrystal averaging based on two crystal forms grown from different detergents. This is one of the first structures of a monotopically inserted membrane protein to be completed. MAO-B structure shows the membrane interacting regions as a C-terminal transmembrane helix, plus apolar loops located at various positions in the sequence. The overall structure is a dimer, but does not involve helix-helix interactions within the membrane inserted segments. The structure was solved in complex with pargyline, which occupies the substrate binding site. A smaller 'entrance' cavity forms an entry way between the active site cavity and the protein surface. The amino acid residues separating these two cavities in MAO B differ in MAO A and mutagensis experiments targeting these residues on MAO B result in a enzyme with properties closer to MAO A than to MAO B. The opening of the entrance cavity is toward the membrane surface suggesting substrate access is from the membrane bilayer. MAO B structure rationalizes previous QSAR data and provides basic information for the rational design of drugs that would exhibit a high degree of specificity.

Keywords: NEUROTRANSMITTERS, MEMBRANE PROTEIN, ENZYME INHIBITORS

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THREE DIMENSIONAL STRUCTURAL VIEW OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION

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The metabotropic glutamate receptors (mGluRs) are key receptors in the modulation of excitatory synaptic transmission in the central nervous system. To gain insight into the ligand binding mechanism of the mGluR family, we determined the three crystal structures of the ligand binding region (LBR) of mGluR1 (m1-LBR) in a complex with glutamate and in two unliganded forms. They all showed disulfide-linked homo-dimers, whose 'active' and 'resting' conformations are modulated through the novel dimeric interface by a packed α-helical structure. The bilobed protomer architectures flexibly change their domain arrangements between an 'open' or 'closed' conformation. The structures imply that glutamate binding stabilizes both of the 'active' dimer and 'closed' protomer in dynamic equilibrium. The movements of the four domains within the dimer are likely to affect the separation of the transmembrane and intracellular regions, and thereby activate the receptor [1]. More recently, we have solved the crystal structures of m1-LBR complexed with an antagonist, (S)-(a)-methyl-4-carboxyphenylglycine, and with both glutamate and Gd^{3+} ion. This crystallographic study has revealed that the surface of the C-terminal domain contains the acidic patch, which inhibits the dimer formation by the two open protomers in the active state. Taken together, these findings provide a clearer structural basis to describe the link between ligand binding and the dimer interface.

References
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TUNNELS AND CAVITIES SUSTAIN ACTIVITY IN 'TRUNCATED HEMOGLOBINS'

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Truncated hemoglobins (trHbs) are recently discovered small (115-130 amino acids) oxygen-binding hemeproteins, widespread in (often pathogenic) microorganisms, but also present in higher plants. trHb tertiary structure is based on a 2-on-2 a-helical sandwich structurally related to the 3-on-3 a-helical sandwich of the classical globin fold. Specifically, trHbs lack most of the N-terminal A helix, and substitute the heme-proximal F-helix with an elongated polypeptide segment. The crystallographic analysis of members of the trHb homology family shows that folding of a short polypeptide chain around the heme group gives rise to protein packing defects, which may have been adopted by evolution to code for new functionalities. In particular, we have found that in *Mycobacterium tuberculosis* and in *Chlamydomonas eugametos* trHbs a continuous hydrophobic tunnel (about 20 Å long) through the protein matrix connects the solvent space to the heme distal cavity. Sequence analyses indicate that this may be a conserved structural feature, suggesting a possible functional role. Indeed, diffusion experiments show that Xe atoms and ligands such as butyl-isocyanide can travel through the protein matrix along the identified tunnel path. Remarkably, independent microbiological analyses have shown that M. tuberculosis trHb-N supports, *in vivo* and *in vitro*, fast conversion of NO into NO_3^-, hinting at a pseudo-enzymatic role for this protein in the mycobacterium defense mechanisms.

Keywords: HEME PROTEINS, NITRIC OXIDE, OXYGEN

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CRYSTALLOGRAPHIC STUDIES OF HEME ENZYMES ENGINEERED FOR LIGAND BINDING

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Our laboratory is exploring the use of artificial protein cavities as specific ligand-binding templates. We have introduced several such cavities, each of which binds small molecules with specificities that mimic the removed structure. These engineered proteins may be useful for creating new catalysts and biosensors.

Recent results in the Gray laboratory have demonstrated the design of “molecular wires” or sensitizer-linked substrates (SLS) which bind to the substrate channel of P450cam with high affinity. These wires allow photochemically induced electron transfer (ET) to be directed through the wire into the buried active site. In collaboration with the Gray group, we will report new high resolution structures of several SLS probes bound to P450cam. These studies have suggested that it may be possible to excise the natural ET pathway from a metalloprotein and replace it with synthetic “molecular wires”. Toward this goal, we will report the designed removal of the proposed ET pathway from yeast cytochrome c peroxidase to leave an open, ligand-binding channel in its place. Small molecules bind to the channel with affinities that can be rationalized based on the structures. Peptide based SLS probes are being designed to replace the native ET pathway. Initial studies show evidence for both reversible and kinetic trapping of a peptide based SLS. Replacement of the natural ET pathway with SLS probes will allow rapid and direct placement of oxidizing or reducing equivalents into the active site and enable the trapping of reactive intermediates and study of ET pathway specificity and determinants.

Keywords: PROTEIN ELECTRON TRANSFER LIGAND BINDING PROTEIN ENGINEERING