

Ultra High Resolution Crystallographic Studies of Cholesterol Oxidase: Correlation Between Structure and Catalytic Function

A. Vrielink¹, A. Y. Lyubimov²

¹*School of Biomedical Biomolecular and Chemical Sciences, University of Western Australia, Crawley, WA, Australia*

²*Molecular Cellular and Developmental Biology, University of California Santa Cruz, Santa Cruz, California, United States*

Cholesterol oxidase is a bifunctional bacterial flavoenzyme that catalyzes the oxidation and isomerization of Δ^5 -6-ene-3 β -ketosteroids to produce Δ^3 -4-ene-3 β -ketosteroids. The enzyme constitutes an important virulent factor in patients suffering from tuberculosis and coccobacillus infections. Sub-Ångstrom resolution structures of the enzyme from *Streptomyces* sp. SA-COO at different pH and in the presence of a substrate analogue provide an unprecedented view of the FAD cofactor and residues that are important for catalysis. The quality of the electron density maps reveals hydrogen positions for key active site residues and has enabled modeling of alternate conformations for a large number of residues in the enzyme. These structures reveal a narrow tunnel leading from the external surface of the molecule directly to the isoalloxazine portion of the FAD cofactor. The hydrophobic nature of this tunnel suggests it is the pathway for molecular oxygen to access the isoalloxazine group for the oxidative half reaction. Amino acid side chains are implicated as important for stabilization of the reduced cofactor and gating of the tunnel. In the presence of the substrate analog a group of three aromatic residues forces the oxidized isoalloxazine moiety to bend along the N5-N10 axis as a response to ligand binding in the active site. This suggests that some tuning of the FAD redox potential is caused by Michaelis complex formation during regular catalysis. These structural studies, revealing active site plasticity, have expanded our understanding of the interplay between the enzyme, cofactor and substrate.

This presentation will address how the high levels of detail visible in the electron density maps of cholesterol oxidase at sub-Ångstrom resolution contribute to a more detailed understanding of flavoenzyme redox chemistry.