

Characterization of Active Site Residues in Class I Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1

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Nitronate monooxygenases (NMOs) are flavin-dependent enzymes that utilize oxygen as a final electron acceptor in order to detoxify alkyl nitronates. Propionate 3-nitronate (P3N) was recently discovered as a physiological substrate of *Pseudomonas aeruginosa* NMO (PaNMO). P3N irreversibly inhibits succinate dehydrogenase – an important enzyme involved in energy production. Previous kinetic and spectroscopic studies have been conducted on both fungal and bacterial NMO, which demonstrate that P3N is oxidized with k_{cat} and k_{cat}/K_m values of 1000 s^{-1} and $\geq 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively.¹ These high kinetic parameters illustrate the importance of detoxifying the metabolic poison P3N.

Over 4,000 genes in a multitude of organisms are annotated as NMOs.¹ The crystal structures of both *Cyberlindnera saturnus* NMO and PaNMO have been resolved and demonstrate that both enzymes have conserved active sites. Multiple sequence alignments demonstrated that Tyr109, His133, His183, Tyr254, Tyr299, and Tyr303 are highly conserved residues in various prokaryotic and eukaryotic NMO's.¹ These residues are arranged in a constellation of aromatic residues juxtaposing the flavin. The role of these residues in substrate binding and enzymatic catalysis are being explored utilizing site-directed mutagenesis. Utilizing polymerase chain reaction, these residues have been substituted with various residues as confirmed by DNA sequencing. Utilizing steady state kinetics and spectroscopic characterization, the role of select residues will be investigated.

Keywords: *Pseudomonas aeruginosa*, nitronate monooxygenase, site-directed mutagenesis, kinetics, flavin.

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