

P005 Choreographing radical propagation in a B₆ and B₁₂ enzyme

Kirsten Wolthers, Steve Rigby, Nigel Scrutton

*Faculty of Life Sciences, University of Manchester, MIB,
131 Princess Street, Manchester, M1 7DN*

Ornithine 4, 5 aminomutase (OAM) is an adenosylcobalamin (AdoCbl)- and pyridoxal-L-phosphate (PLP)-dependent enzyme that converts D-ornithine to 2,4-diaminopentanoic acid by a 1, 2 amino shift. UV-vis stopped-flow (sf) spectroscopy revealed that substrate binding induces transimination and homolysis of the AdoCbl Co-C bond with similar observed rate constants. Rupture of the Co-C bond does not lead to a detectable steady-state level of cob(II)alamin; however, sf traces show its transient formation. Binding of the inhibitor, D-2, 4-diaminobuteryic acid (DAB) leads to stable formation of cob(II)alamin. The EPR spectra of the OAM-DAB complex shows strong electronic coupling between cob(II)alamin and an organic radical, indicating a interspin distance of $< 6 \text{ \AA}$. For OAM reconstituted with PLP (PLP-OAM), transimination occurs in three resolvable steps with four spectral intermediates (A \leftrightarrow B \leftrightarrow C \leftrightarrow D). The individual components represent the internal aldimine (λ_{max} 416 nm; A), two unliganded PLP-states of the enzyme (λ_{max} at 409 nm; B and C) and the external aldimine (λ_{max} 426 nm; D). D-ornithine and DAB generate both tautomeric forms of external aldimine; however, with D-ornithine, the equilibrium is shifted towards the ketoimine state. The equilibrium distribution of the PLP prototrophic isomers may influence the drive towards homolytic rupture and stabilization of PLP-bound radical intermediates.