

P011 Mechanistic intermediates of nitric oxide synthase

Gazur, B., Popale, D., and Daff, S.

University of Edinburgh

Nitric oxide synthase (NOS) catalyzes nitric oxide (NO) production in a two step reaction. The first step involves L-arginine being oxidised to N^G-hydroxy-L-arginine (NOHA) before it is oxidised to NO and L-citrulline in the second step. The three mammalian isozymes are homodimeric enzymes and each subunit is composed of a reductase and an oxygenase domain. The oxygenase domain contains the arginine binding site, one cysteine ligated heme thiolate, and one H₄B molecule ((6R)-5,6,7,8-Tetrahydrobiopterin). The enzyme's substrates are O₂, L-arginine (or NOHA) and NADPH. H₄B is an essential cofactor for NO production by NOS and is important in dimerization and as a redox cofactor. The mechanism of the reaction between L-arg and oxygen in the active site is currently uncertain. Site-directed mutagenesis was conducted: Glycine 586 of nNOS was replaced by a serine residue (G586S nNOS_{oxy}) and expressed and purified from *E.coli*. Stopped flow kinetic experiments showed the formation of a novel reaction intermediate during G586S nNOS_{oxy} catalysis in the presence of H₄B and substrate, subsequent to the formation of the oxy-ferrous compound.