

Mechanistic Investigation of D-Arginine Dehydrogenase from *Pseudomonas aeruginosa*

Giovanni Gadda^{‡§¹}, Hongling Yuan[‡], Swathi Gannavaram[‡], Guoxing Fu[§], Irene Weber[§]

Departments of [‡]Chemistry and [§]Biology and ¹The Center for Biotechnology and Drug Design, Georgia State University, Atlanta, Georgia 30302-4098

D-arginine dehydrogenase (DADH) catalyzes the oxidation of D-amino acids to the corresponding iminoacids, which are non-enzymatically hydrolyzed in solution to α -ketoacids and ammonia. The enzyme prefers D-arginine and D-lysine, but is active with all D-amino acids except for glycine, aspartate and glutamate. D-leucine is the slowest amongst the substrates for which the steady state kinetic parameters are available.^{1,2}

Here, the chemical mechanism of leucine oxidation catalyzed by DADH was investigated with pH, substrate, solvent and β -secondary kinetic isotope effects (KIE) and proton inventories by using rapid kinetics in which the enzyme was reduced with D-leucine in a stopped-flow spectrophotometer. Moreover, the role of the active site Tyr249 was investigated by replacing this residue with phenylalanine and characterizing the resulting mutant enzyme with steady state and rapid reaction approaches. The data presented will be discussed in the context of the crystallographic structures at high resolutions (<1.3 Å) of the enzyme in complex with iminoarginine or iminohistidine,¹ allowing us to propose a chemical mechanism of substrate oxidation where a hydride ion is transferred from the unprotonated amino substrate to the flavin N(5) atom.

This study was supported in part by NSF-CAREER MCB-0545712(G.G.).

(1) Fu, G.; Yuan, H.; Li, C.; Lu, C. D.; Gadda, G.; Weber, I. T. *Biochemistry*, 49, 8535-45.

(2) Yuan, H.; Fu, G.; Brooks, P. T.; Weber, I. T.; Gadda, G. *Biochemistry*, 49, 9542-50