35 GHz ENDOR Studies of Substrate and Inhibitor Radical Interactions with S-Adenosylmethionine Fragments in Lysine 2,3-Aminomutase.

Charles J. Walsby[1,3], Nicholas S. Lees[1], Dawei Chen[2], Elham Behshad[2], Perry A. Frey[2] and Brian M. Hoffman[1]

[1]Department of Chemistry, Northwestern University, [2]Department of Biochemistry, University of Wisconsin-Madison, [3]Department of Chemistry, Simon Fraser University

Lysine 2,3-aminomutase (LAM) utilizes a [4Fe-4S] cluster, *S*-adenosyl-L-methionine (SAM) and pyridoxal 5'-phosphate (PLP) to catalyze the isomerization of L-α-lysine to L-β-lysine via a radical mechanism. The reduced $[4Fe\text{-}4S]^{+}$ cluster has a unique non-cysteinyl coordinated Fe, which provides an electron to cleave SAM, initiating radical catalysis by formation of the 5'-deoxyadenosyl radical.

ENDOR spectroscopy has previously been used successfully to determine the relative positions of components in this enzymatic system at a point in the catalytic cycle prior to SAM cleavage [1]. In the current study, isotopic labeling of SAM and the use of both the true substrate lysine, and two substrate analogues, *trans*-4,5-dehydro-L-lysine (DHLys) and 4-thia-L-lysine (SLys), have allowed distances to be determined between the substrate and cleaved SAM components in both the substrate and product radical stages of the catalytic cycle. The results demonstrate the movement of the 5'-deoxyadenosine fragment away from the cluster following SAM cleavage, where it comes into close contact with the substrate.

[1] Chen, D.; Walsby, C. J.; Hoffman, B. M.; Frey, P. A. *J. Am. Chem. Soc.* **2003**, *125*, 11788.