Chemistry Seminar Program - Fall 2005

Friday October 28th (3:15)

6th Fall Seminar

Rob Cicchillo

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(PhD Candidate)

"Biochemical, Biophysical, and Mechanistic Investigations of Lipoyl Synthase from Escherichia coli: A Member of the "Radical SAM" Family of Enzymes"

Abstract

Lipoic acid (6,8-thioctic acid) is an essential cofactor in several multienzyme complexes that are involved in energy metabolism, such as the pyruvate dehydrogenase complex, the α-ketoglutarate dehydrogenase complex, the branched-chain keto-acid dehydrogenase complex, and the glycine cleavage system. The biosynthesis of this cofactor involves the insertion of two sulfur atoms into two completely unactivated carbon atoms of protein bound octanoyl groups. Lipoyl synthase, the protein thought to catalyze sulfur insertion, has been cloned and purified by immobilized metal affinity chromatography. Indeed, both lipoyl synthase and biotin synthase belong to a superfamily of enzymes that use S-adenosyl-L-methionine (AdoMet) and iron sulfur clusters to generate high-energy carbon-centered radicals that are intermediates in catalysis. Proteins that are members of this superfamily have a conserved iron sulfur cluster-binding motif consisting of cysteine residues in a CX_3CX_2C pattern. Lipoyl synthase deviates from other members in this class in that it contains an additional conserved set of cysteines lying in the motif CX_4CX_5C. We have shown, through site-directed mutagenesis, UV-visible spectroscopy, EPR Spectroscopy, and Mössbauer spectroscopy, that the active enzyme contains two [4Fe-4S] clusters that lie within the conserved cysteine motifs. We have also demonstrated that the synthesis of one molecule of lipoic acid requires the consumption of two equivalents of AdoMet. Recently we have obtained experimental evidence through isotope labeling studies that lipoyl synthase itself donates the sulfur atoms that are inserted into positions C6 and C8 of the octanoyl group.