Mechanistic Investigations on the Biosynthesis of the Lipoyl Cofactor

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Lipoyl synthase (LS) catalyzes the final step in the biosynthesis of the lipoyl cofactor, the sequential insertion of two sulfur atoms into the C-6 and C-8 positions of octanoic acid bound in an amide linkage to a conserved lysine residue on a designated lipoyl bearing protein. Genomic and biochemical studies indicate that LS belongs to a new class of metalloenzymes, in which an iron–sulfur cluster, coordinated by a conserved cysteine-containing motif, induces a reductive cleavage of S-adenosylmethionine (SAM), resulting in methionine and a 5'-deoxyadenosyl radical (5'-dA•). In LS, the 5'-dA• is proposed to abstract hydrogen atoms from C-6 and C-8 to allow subsequent insertion of sulfur atoms. We provide evidence that LS contains two [4Fe–4S] clusters per polypeptide, and suggest that one of the clusters donates the sulfur atoms during turnover. Consistent with this hypothesis, we show that LS that is isolated from minimal medium containing ^{34}S as the only sulfur source catalyzes the formation of ^{34}S-labeled lipoyl cofactor. Finally, we discuss the stoichiometry of the reaction with respect to SAM and LS itself, and possible intermediates on the catalytic pathway.