The Biosynthesis of Lipoic Acid: A Saga of Death, Destruction, and Rebirth

Abstract: Lipoyl synthase (LipA), a member of the radical S-adenosylmethionine (SAM) superfamily of enzymes, catalyzes the sequential insertion of sulfur atoms at C6 and C8 of an amide-linked octanoyl chain on a lipoyl carrier protein (LCP) to afford the lipoyl cofactor. This cofactor plays key roles in several multienzyme complexes that are involved in energy or amino acid metabolism. LipA contains two essential [4Fe–4S] clusters. One of the clusters interacts with SAM to generate two 5’-deoxyadenosyl 5’-radical intermediates that abstract hydrogen atoms from C6 and C8 of the octanoyl chain, while the second cluster is believed to provide the inserted sulfur atom. Therefore, LipA becomes inactivated upon one full turnover due to destruction of its iron-sulfur (Fe/S) cluster cofactor.

Herein, we provide strong evidence for this provocative role for an Fe/S cluster in enzymatic catalysis. When we conduct reactions under conditions wherein only one sulfur atom is inserted into the organic substrate, we find that the LCP substrate becomes cross-linked to LipA through the Fe/S cluster. Extensive characterization of the cross-linked species by Mössbauer spectroscopy and X-ray crystallography shows that it contains 3 iron ions and 3 sulfide ions in a cubane-like structure that is connected to the LCP by a bridging monothiolated octanoyl linker. Upon isolation of the species and its reintroduction into a new reaction mixture, formation of the lipoyl product is observed with kinetics that are consistent with the overall rate of catalysis.

Lastly, we show that accessory proteins can rebuild the cluster at a rate that is equal to or faster than the overall rate of turnover, conferring catalytic properties to the system.

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