The Role of a $[2Fe\text{-}2S]^{2+}$ Cluster in Carbon-Sulfur Bond Formation as Catalyzed by Biotin Synthase

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Biotin synthase is an AdoMet-dependent radical enzyme that catalyzes the insertion of sulfur into unactivated CH bonds in dethiobiotin to form the thiophane ring of biotin. The recent crystal structure of biotin synthase from *E. coli* shows two FeS clusters bound within the core of an $(\alpha\beta)_8$ barrel. A $[4Fe\text{-}4S]^{2+}$ cluster binds AdoMet and is proposed to catalyze reductive cleavage of the AdoMet sulfonium and generation of a 5′-deoxyadenosyl radical. A $[2Fe\text{-}2S]^{2+}$ cluster is bound in the core of the barrel ~4.7 Å from the substrate dethiobiotin, and is proposed to play a role in addition of sulfur to generate the thiophane ring. This cluster is bound within a unique coordination environment that involves 3 cysteine and 1 arginine residues. Mutation of these residues suggests that while the presence of the $[2Fe\text{-}2S]^{2+}$ cluster is required for activity, only the arginine is essential for activity. One sulfide within this cluster is readily labeled with $^{34}S^{2-}$ from the buffer, and we show that it is this bridging sulfide that is transferred to generate the biotin thiophane ring. Biotin synthesis is accompanied by destruction of the $[2Fe\text{-}2S]^{2+}$ cluster and partial unfolding of the protein barrel. We demonstrate that the native cluster content can be regenerated through the action of proteins from the iron-sulfur cluster (ISC) assembly system, with the chaperone HscA playing an indispensable role in mediating cluster transfer. We propose a mechanism for in vivo biotin biosynthesis that requires ISC-mediated repair of the biotin synthase $[2Fe\text{-}2S]^{2+}$ cluster following every turnover.