A Role for Iron-Sulfur Cluster Assembly in Biotin Biosynthesis: Transfer of Sulfur from a $[2Fe\text{-}2S]^{2+}$ Cluster to Biotin and ISC-Mediated Cluster Repair

Joseph T. Jarrett,* Michael R. Reyda, Devrim Eren, Robyn Broach, Michael E. Dotson

Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104, USA.

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 ISC assembly system, with 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.