Biochemical Characterization of HydF, a Scaffolding Enzyme, in the Synthesis of the Hydrogenase Active Site Metal Center: Implications Towards the Evolution of Biocatalysts from Mineral-Based Components on Early Earth

Benjamin R. Duffus, Eric M. Shepard, Shawn E. McGlynn, Alexandra L. Bueling, Mark A. Winslow, John W. Peters and Joan B. Broderick

Contributions from the Astrobiology Biogeocatalysis Research Center and the Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana 59717, USA

Iron-sulfur (Fe-S) catalytic centers observed in modern enzymes represent highly tuned remnants from ancient, modified Fe-S mineral complexes that were capable of catalyzing electron transfer reactions and small molecule interconversions on early earth. By examining the synthesis of complex Fe-S clusters such as the H-cluster of [FeFe]-hydrogenase, the connection between Fe-S minerals, Fe-S enzymes and biocatalysts can be further understood. [FeFe]-hydrogenase contains a unique active site composed of a [4Fe-4S] cubane linked to a 2Fe unit ligated by CN^-, CO, and a bridging dithiolate. H-cluster synthesis invokes radical chemistry initiated by the radical S-adenosylmethionine (SAM) enzymes HydE and HydG, as well as a GTPase and scaffold protein, HydF. The GTPase functionality of HydF indicates that the energy coupled to GTP hydrolysis is likely linked to precursor cluster synthesis on the HydF scaffold by HydE and HydG. Spectroscopic characterization of HydF shows remarkable affects upon GTP binding, suggesting that the N-terminal GTPase domain, containing a typical P-loop structure proposed to be the most ancient peptide nest for nucleotide binding (Milner-White, E. J.; Russell, M. J. *Origin. Life. Evol. Biosph.* **2005,** *35,* 19-27), is in communication with the Fe-S cluster domain. These possibilities open up exciting new avenues of research and bring to light the remarkable possibility that the ancestor of HydF may have been one of the earliest examples of a protein that coupled the chemistry of an Fe-S peptide nest with a nucleotide binding nest.