Special Seminar in Energy Research

at the

University of Pennsylvania

Biosynthesis of the Catalytic H-Cluster of [FeFe] Hydrogenase

Abstract

[FeFe] hydrogenase enzymes rapidly evolve H2 at a 6-Fe catalytic site termed the H-cluster, which consists of a traditional [4Fe-4S] cluster linked via a cysteine bridge to a dinuclear Fe subcluster [2Fe]H that possesses unusual biological ligands: two terminal CN- ligands, two terminal CO ligands, and azadithiolate and CO bridges, all of which are thought to be synthesized and installed by a set of Fe-S proteins denoted HydE, HydF, and HydG. With the James Swartz laboratory (Stanford University) we can generate [FeFe] hydrogenase in high yield using cell free synthesis methods, allowing for specific isotope labelling of its components as needed for definitive spectroscopic studies (1).

The radical S-adenosylmethionine (SAM) enzyme HydG lyses free L-tyrosine to produce CO and CN- for the assembly of the H-cluster. We use electron paramagnetic resonance (EPR) spectroscopy to detect and characterize HydG reaction intermediates generated with a set of 2H, 13C, and 15N nuclear spin labeled tyrosine substrates. 5′-deoxyadenosyl cleavage of tyrosine at the Calpha-Cbeta bond generates a transient 4-oxidobenzyl (4OB.) radical and a dehydroglycine bound to a C-terminal Fe-S cluster (2). Electron and proton transfer to this 4OB. radical forms p-cresol with the conversion of this dehydroglycine ligand to Fe-bound CO and CN-, a key intermediate in the assembly of the [2Fe] subunit of the H-cluster. We apply stopped-flow Fourier transform infrared (SF-FTIR) and electron-nuclear double resonance (ENDOR) spectroscopies to explore in detail the formation such species which are used to build the H-cluster (3). New X-ray crystallography and EPR studies reveal a unique site-differentiated structure for this C-terminal Fe-S moiety that clarifies its role in H-cluster synthesis. Many open issues remained to be explored in this unique facet of biological catalytic cluster synthesis, including the roles of the additional Fe-S proteins HydE and HydF (4).

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Thursday, October 30, 2014

3:00 PM