Mechanism of Acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase: a Bifunctional Enzyme with Ni-Fe-S Active Sites Connected by a Tunnel Network

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Acetyl-Coenzyme A synthase/Carbon Monoxide Dehydrogenase (ACS/CODH) is found in evolutionarily primitive living systems, including chemoautotrophic anaerobes such as methanogenic archaea and acetogenic bacteria. The enzyme is bifunctional; it catalyzes the reversible reduction of CO_2 to CO and the synthesis of acetyl-CoA from CO, CoA, and a methyl group transferred from a corrinoid-iron-sulfur protein. The former reaction occurs at a Ni-Fe-S cluster called the *C-cluster* while the latter reaction occurs at a distinct Ni-Fe-S cluster called the *A-cluster*. The enzyme from *Moorella thermoacetica* is a 310 Kda $\alpha_2\beta_2$ tetramer containing with an extensive tunnel network that serves to channel CO from the C-cluster to the A-cluster. Curiously, the tunnel also connects the two C-clusters. In this presentation, a combination of spectroscopic, structural, kinetic, genetic and computational methods will be used to address the mechanism of catalysis and properties of the tunnel. In one group of experiments, stopped-flow kinetics and Mössbauer spectroscopy have been used to evaluate the magnetic and redox properties of the A-cluster in states capable (and incapable) of accepting a methyl group. In other experiments, the catalytic abilities of various site-directed mutagenesis, which were structurally designed to block the tunnel in different positions, were used to explore the properties of the tunnel. In a third group of experiments, mathematical modeling of stopped-flow kinetic data has been used to evaluate details of the catalytic mechanism.