Function of the Tunnel Network in Acetyl-Coenzyme A Synthase/Carbon Monoxide Dehydrogenase

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Acetyl-coenzyme A synthase/carbon monoxide dehydrogenases (ACS/CODH) are found in evolutionarily ancient acetate-producing bacteria and methane-producing archaea. The X-ray crystal structure of ACS/CODH from *Moorella thermoacetica* revealed a 310 Kda $\alpha_2\beta_2$ tetramer with an extensive hydrophobic tunnel network. This tunnel allows CO to migrate from the active-site C-cluster, where CO_2 is reduced to CO, to the active site A-cluster, where CO reacts with Coenzyme A and a methyl group to produce acetyl-CoA. The C-cluster is located in the β subunit while the A-cluster located in the α subunit *ca*. 70 Å away. The tunnel also connects the two C-clusters within the tetramer, which are separated by *ca*. 40 Å. The A-cluster consists of a [Ni_p Ni_d] dimer bridged to an [Fe_4S_4] cluster while the C-cluster is a novel {[Ni Fe][Fe_3S_4]} cluster. The structure of the α subunits changes with each catalytic cycle. When the subunit is in the open conformation, the tunnel is blocked; when the subunit is in the closed conformation, the tunnel is functional. Thus, protein conformational changes regulate the delivery of CO through the tunnel. Site-directed mutagenesis was used to prepare recombinant mutants designed to block the tunnel at different points along the region between the two C-clusters or between A and C clusters. EPR spectroscopy, Stopped-flow kinetics, and activity assays were used to explore the function of the tunnel as well as the site at which CO_2/CO enter/exit the enzyme. Results suggest that the tunnel regulates delivery of CO to the A-cluster, and that these gases enter/exit the enzyme at the ββ interface. Details of experiments supporting these conclusions will be presented.