

Infrared Evidence that the Only Adduct Formed when Acetyl-Coenzyme A Synthase is Reacted with CO is the NiFeC Complex

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Carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) is a bifunctional enzyme which enables archaea and bacteria to grow autotrophically on CO and H₂/CO₂ using the Wood-Ljungdahl pathway. CO produced from reduction of CO₂ by CODH is transferred to the active site of ACS through an intramolecular tunnel, where it combines with Coenzyme A and a methyl cation to produce acetyl-CoA. The active site of ACS contains a single [Fe₄S₄] cubane bridged by a cysteine sulfur atom to a binuclear center. The binuclear center is composed of two Ni atoms bridged by two separate cysteine sulfurs. The Ni site attached to the [Fe₄S₄] is referred to as proximal Ni (Ni_p), while the other Ni atom, which assumes a square-planar geometry, is referred to as the distal site (Ni_d). We report the characterization of the carbonylated form of highly active (0.67 spins/mol) heterologously expressed monomeric ACS from *C. hydrogenoformans* in *E. coli* by rapid-freeze quench EPR (RFQ-EPR) and stopped-flow infrared spectroscopies (SF-IR). The reaction of ACS with CO produces a single metal-carbonyl species whose formation rate, measured by SF-IR, correlates with the rate of formation, measured by RFQ-EPR, of the paramagnetic state of the enzyme (*NiFeC species*). These results indicate that the *NiFeC* species is the predominant form observed in solution when ACS reacts with CO. The *NiFeC* species contains Ni_p in the +1 redox state and [Fe₄S₄] in the +2 state, thus there is no evidence for either a Ni⁰ or a Ni^{II} state in the carbonylated form of the enzyme.