The Iron-sulfur Cluster of Pyruvate Formate-lyase Activating Enzyme in Whole Cells:Cluster Interconversions and a Valence-localized $[4Fe\text{-}4S]^{2+}$ State

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Pyruvate formate-lyase activating enzyme (PFL-AE) catalyzes the generation of a catalytically essential glycyl radical on pyruvate formate-lyase (PFL). Purified PFL-AE contains an oxygen-sensitive, labile [4Fe-4S] cluster that has been shown to undergo cluster interconversions *in vitro*, with only the $[4Fe\text{-}4S]^{+}$ cluster state being catalytically active. Whole cells studies on overexpressing PFL-AE showed that approximately 50% of the total iron is present in $[4Fe\text{-}4S]^{2+}$ clusters, 5.5% in $[2Fe\text{-}2S]^{2+}$ clusters, and the remainder as mononuclear Fe(III) (24.5%) and Fe(II) (7%) species after a 2 hr aerobic induction of PFL-AE expression. Subsequent anaerobic incubation of the culture results in approximately 77% of the total iron being present as $[4Fe\text{-}4S]^{2+}$ clusters, with no detectable $[2Fe\text{-}2S]^{2+}$. Subsequent aerobic incubation of the culture converts the iron species nearly back to the original quantities. Furthermore, the Mössbauer spectra clearly reveal that the $[4Fe\text{-}4S]^{2+}$ cluster of PFL-AE in whole cells contains a valence localized Fe^{3+}-Fe^{2+} pair, which has not been previously observed in the purified enzyme. To explore the factors associated with valence-localization in the $[4Fe\text{-}4S]^{2+}$ cluster, Mössbauer spectra were obtained for purified PFL-AE in the presence of a series of small molecules, including potential AdoMet degradation products, substrates and products of PFL, and abundant cellular metabolites. The results indicate that addition of certain small molecules containing adenosyl moieties, including 5'-deoxyadenosine, AMP, ADP, and methylthioadenosine reproduce the valence-localized state of the $[4Fe\text{-}4S]^{2+}$ cluster of PFL-AE. Subsequent addition of excess amounts of AdoMet displaces these metabolites from the cluster to allow the preferential binding of AdoMet. It is important that the bound metabolites are easily displaced, as AdoMet is required in the radical catalysis of PFL-AE and the subsequent generation of the glycyl radical in PFL.