In vivo States of the Iron Sulfur Cluster of Pyruvate Formate-Lyase-Activating Enzyme

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Pyruvate formate-lyase-activating enzyme (PFL-AE) is a member of a novel superfamily of enzymes that reductively cleave S-adenosyl-L-methionine (SAM or AdoMet) to generate the extremely reactive 5'-deoxyadenosyl radical, which further generates the catalytically essential glycyl radical on pyruvate formate-lyase (PFL) for subsequent radical catalysis. A site-differentiated [4Fe-4S] cluster has been identified in PFL-AE with $[4Fe\text{-}4S]^{1+}$ cluster as the catalytically active form while the $[4Fe\text{-}4S]^{2+}$ state is the oxidized counterpart during turnover. The ENDOR studies of PFL-AE with various isotopically labeled AdoMets have demonstrated direct coordination of AdoMet to the unique non-cysteine coordinated Fe.

All studies of PFL-AE to date have focused on the purified enzyme, in this study we shift our attention on *in vivo* form. Two expression vectors one with and one without *pflA* gene inserted, were transformed into the same BL21(DE3)plysS cells. The cells were grown and induced in 57Fe-enriched medium, and then either harvested or subjected to anaerobic and aerobic cycling by bubbling with N_2 or air. Samples were removed at different times at each stage and the cells were harvested by centrifugation. EPR and Mössbauer studies of these whole cell samples demonstrated oxygen-dependent cycling of cluster states.