Investigating the Interaction Between the [4Fe-4S] Cluster of Pyruvate Formate-Lyase-Activating Enzyme (PFL-AE), a Radical SAM Enzyme, with S-Adenosylmethionine Via EPR and ENDOR Spectroscopic Studies

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Pyruvate formate-lyase-activating enzyme (PFL-AE) belongs to the "radical SAM" protein superfamily. Enzymes in this superfamily catalyze diverse reactions, which include unusual methylation, isomerization, sulfur insertion, ring formation, anaerobic oxidation and protein radical formation reactions. PFL-AE, which activates pyruvate formate-lyase (PFL), utilizes an Fe-S cluster and *S*-adenosylmethionine (AdoMet) to generate catalytically essential radicals. During generation of a glycyl radical on PFL, AdoMet is converted stoichiometrically to methionine and 5'-deoxyadenosine. An AdoMet-derived adenosyl radical has been implicated as the intermediate responsible for abstraction of the pro-S hydrogen atom of PFL Gly734.

In order to probe the mechanism by which the Fe-S cluster interacts with AdoMet to generate an adenosyl radical intermediate, we have undertaken an investigation of the interaction of PFL-AE with isotopically labeled AdoMets and AdoMet analogs. AdoMets labeled with ^{15}N, ^{13}C, ^{2}H, and ^{17}O at specific sites were employed to probe its interaction with the Fe-S cluster of PFL-AE. AdoMet analogs will give us additional insight into the formation of the adenosyl radical. Syntheses and characterization of the labeled AdoMets and AdoMet analogs will be presented, along with the results of the EPR and ENDOR spectroscopic studies aimed at probing the interaction of AdoMet with the [4Fe-4S] cluster of PFL-AE. Binding studies of AdoMet with PFL-AE from equilibrium dialysis experiments will also be presented.