Investigation of the unique iron site of the [4Fe-4S] cluster in Pyruvate Formate-Lyase-Activating Enzyme

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Pyruvate formate-lyase-activating enzyme (PFL-AE) belongs to the "Radical-SAM" superfamily of enzymes which share a common CXXXCXXC sequence motif. It activates the enzyme pyruvate formate-lyase (PFL) via a radical mechanism utilizing S-adenosylmethionine (SAM) as a required co-substrate of PFL-AE. Initial investigations have shown that the $[4Fe\text{-}4S]^{1+}$ cluster of PFL-AE is involved in the generation of the glycyl radical of PFL.[1] One of the iron atoms of the [4Fe-4S] cluster is quite labile as shown by the quick conversion between $[4Fe\text{-}4S]^{2+}$ to $[3Fe\text{-}4S]^{+}$ and this unique labile site has been shown to interact with SAM.[2-4] The conservation of a 3-cysteine motif in the radical SAM enzyme suggests a special role for a site-differentiated cluster in the radical generation process.

^{57}Fe-enriched PFL-AE purified under strictly anaerobic conditions contains only $[4Fe\text{-}4S]^{2+}$ clusters, as determined by Mössbauer spectrosocopy. This $[4Fe\text{-}4S]^{2+}$ cluster could be easily oxidized to $[3Fe\text{-}4S]^{+}$ by the addition of potassium ferricyanide within 10 minutes at room temperature. ^{57}Fe was introduced into the natural abundance $[3Fe\text{-}4S]^{+}$ cluster resulting in reconstituted $[3^{56}Fe^{57}Fe\text{-}4S]^{2+}$. Analysis of the reconstituted $[3^{56}Fe^{57}Fe\text{-}4S]^{2+}$ cluster with and without SAM by Mössbauer spectroscopy clearly suggests that the unique Fe interacts with SAM.

References

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