^{57}Fe ENDOR Spectroscopy on the Iron-Sulfur Cluster Involved in Substrate Reduction of Heterodisulfide Reductase

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Heterodisulfide reductase (Hdr) from methanogenic archea is an iron-sulfur protein that catalyses the reversible two-electron reduction of the mixed disulfide CoM-S-S-CoB to the thiol coenzymes coenzyme M (CoM-SH) and coenzyme B (CoB-SH). Unusually, this enzyme uses an iron-sulfur cluster to mediate disulfide reduction in two one-electron steps via site-specific cluster chemistry. Upon half-reaction of the oxidized enzyme with CoM-SH in the absence of CoB-SH, a paramagnetic intermediate is formed, designated CoM-Hdr. The S = 1/2 species can be reduced in a one-electron step but not oxidized. The g-values, i.e. 2.013, 1.991, 1.938 (for Hdr from *M. Marburgensis*), and signal broadening in the ^{57}Fe-enriched enzyme as well as with ^{33}S labelled CoM-SH had previously suggested that the intermediate is a novel substrate-bound [4Fe-4S]$^{3+}$ cluster. In the present work we report ^{57}Fe pulsed ENDOR at two very different frequencies, 9 and 94 GHz, that identify the iron sites of CoM-Hdr. We find direct evidence for a [4Fe-4S]$^{3+}$ cluster and we determine the sign of the ^{57}Fe hyperfine couplings from the high field data. We compare the ^{57}Fe hfc values with the ones reported for [4Fe-4S]$^{3+}$ model systems and those found in the substrate bound [4Fe-4S]$^{3+}$ cluster of a NEM-alkylated form of ferredoxin:thioredoxin reductase (FTR), an enzyme which catalyses a disulfide cleavage in two one-electron steps involving [4Fe-4S] chemistry. We find compelling differences between the hfc values, which indicate unique structural properties of the cluster in HDR.