Proteins containing Rieske-type [2Fe-2S] clusters with two histidyl and two cysteinylic ligands play crucial roles in many biological electron transfer reactions such as aerobic respiration, photosynthesis, and biodegradation of various alkene and aromatic compounds. The distinct biological function of this protein family is in part associated with the cluster redox potential, for which its approximate correlation with number of hydrogen bonds to the cluster has been proposed. Despite this fundamental importance, spectroscopic techniques for the specific characterization of the $N_e$ and peptide nitrogens are very limited, and evaluation of the contribution of structure to function for hydrogen bond network around the cluster is often difficult to address experimentally in many iron-sulfur proteins, because several are contributed by peptide backbone.

The $^{14}$N electron spin echo envelope modulation (ESEEM) spectra from Rieske-type [2Fe-2S] proteins show dominant contributions of two coordinated histidyl $^{14}$N$_δ$ ligands. Other weakly coupled nitrogens around cluster, i.e. $N_e$ and peptide nitrogens $N_p$, did not exhibit readily recognizable lines in the spectra, due to the influence of nuclear quadrupole interaction requiring special relations between nuclear Zeeman frequency and hyperfine coupling. To overcome this problem, we have applied the orientation-selected two-dimensional ESEEM, called hyperfine sublevel correlation (HYSCORE) spectroscopy, to the uniformly $^{15}$N-labeled, high- and low-potential Rieske proteins from hyperthermophilic archaea, with the specific aim to detect, characterize, and compare weakly coupled $^{15}$N$_ε$ and peptide $^{15}$N$_p$ in their immediate cluster environment, because $^{15}$N does not possess the quadrupole moment.

In this presentation, we demonstrate that the cross-peaks from coordinated $^{15}$N$_δ$ (couplings $\sim$6-8 MHz), remote $^{15}$N$_ε$ ($\sim$0.2-0.4 MHz) and peptide $^{15}$N$_p$ ($\sim$1 MHz) nuclei around the Rieske clusters are well resolved in the $^{15}$N HYSCORE spectra. These results can be used to discuss the protein environment affecting the electronic structure of reduced clusters via variations of unpaired spin density distribution over the histidyl ligands and hydrogen bonds involving peptide nitrogens. We suggest that the HYSCORE experiment with $^{15}$N-labeled proteins can offer a new practical tool, applicable for the detailed structure-mechanism studies of a wide range of the biological redox protein system involving weakly coupled nitrogens.

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