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Role of the coordinating histidine in altering the mixed valency of CuA: An electron nuclear double resonance-electron paramagnetic resonance investigation

Lukoyanov D., Berry S., Lu Y., Antholine W., Scholes C.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

The binuclear CuA site engineered into Pseudomonas aeruginosa azurin has provided a CuA-azurin with a well-defined crystal structure and a CuSSCu core having two equatorial histidine ligands, His120 and His46. The mutations His120Asn and His120Gly were made at the equatorial His120 ligand to understand the histidine-related modulation to CuA, notably to the valence delocalization over the CuSSCu core. For these His120 mutants Q-band electron nuclear double resonance (ENDOR) and multifrequency electron paramagnetic resonance (EPR) (X, C, and S-band), all carried out under comparable cryogenic conditions, have provided markedly different electronic measures of the mutation-induced change. Q-band ENDOR of cysteine Cβ protons, of weakly dipolar-coupled protons, and of the remaining His46 nitrogen ligand provided hyperfine couplings that were like those of other binuclear mixed-valence CuA systems and were essentially unperturbed by the mutation at His120. The ENDOR findings imply that the CuA core electronic structure remains unchanged by the His120 mutation. On the other hand, multifrequency EPR indicated that the H120N and H120G mutations had changed the EPR hyperfine signature from a 7-line to a 4-line pattern, consistent with trapped-valence, Type 1 mononuclear copper. The multifrequency EPR data imply that the electron spin had become localized on one copper by the His120 mutation. To reconcile the EPR and ENDOR findings for the His120 mutants requires that either: if valence localization to one copper has occurred, the spin density on the cysteine sulfurs and the remaining histidine (His46) must remain as it was for a delocalized binuclear CuA center, or if valence delocalization persists, the hyperfine coupling for one copper must markedly diminish while the overall spin distribution on the CuSSCu core is preserved.