Spectroscopic Properties of CooA from *C. hydrogenoformans*

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CooA is a CO-sensing transcriptional activator that contains a protoheme as the active site for sensing its physiological effector. Recently, a CooA homologue from *C. hydrogenoformans* (Ch-CooA) has been found. The amino acid sequence of Ch-CooA displays 31% identity and 55% similarity with that of Rr-CooA. Cys and His, which are the fifth ligand of the Fe(III) and Fe(II) hemes of Rr-CooA (Cys75 and His77), are conserved in Ch-CooA at the corresponding positions (Cys80 and His82). However, the sixth ligand of the Fe(III) and Fe(II) hemes, Pro2 in Rr-CooA, is not conserved in Ch-CooA. This suggests that the coordination structure of the heme will be different between Rr-CooA and Ch-CooA. We therefore characterize the spectroscopic properties of Ch-CooA to find essential factors of CooA function.

The UV-Vis and Resonance Raman (RR) spectroscopies with mutagenesis studies reveal that His82 and N-terminus are coordinated to the Fe(III) and Fe(II) hemes as the axial ligands in Ch-CooA. When the CO-bound heme is formed, the N-terminus is replaced by CO upon CO binding. Two neutral ligands, His82 and the N-terminal α-amino group, are coordinated to the Fe(III) heme in Ch-CooA while two negatively charged ligands, a thiolate from Cys75 and the nitrogen atom of the N-terminal Pro, are the axial ligands of the Fe(III) heme in Rr-CooA. The difference in the coordination structure of the Fe(III) heme results in a large positive shift of the redox potentials of Ch-CooA compared with Rr-CooA. Comparing the properties of Ch-CooA and Rr-CooA demonstrates that the essential elements for CooA function will be: (i) the heme is six-coordinate in the Fe(III), Fe(II), and Fe(II)-CO forms; (ii) the N-terminal is coordinated to the heme as an axial ligand, and (iii) CO replaces the N-terminal bound to the heme upon CO binding.

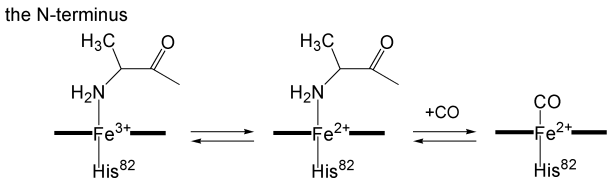

Figure. The coordination structure of Ch-CooA