CO-dependent Activity Controlling Mechanism of NPAS2 Revealed by Resonacen Raman Spectroscopy

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Neuronal PAS domain protein 2, which was recently established to be a heme protein, acts as a CO-dependent transcription factor. The protein consists of the basic helix-loop-helix domain, and two heme-containing PAS domains (PAS-A and PAS-B). In this study, we prepared wild-type and mutant versions of the isolated PAS-A domain, and measured resonance Raman spectra of these proteins. When the ferric wild-type protein was excited at 363.8 nm, a band assignable to the Fe^{3+}-S stretching was observed at 334 cm^{-1}, which was upshifted by 2.0 cm^{-1} for the ^{54}Fe-labeled protein. Furthermore, this band was drastically weaker in the spectrum of the C170A mutant, suggesting that Cys170 is an axial ligand of the ferric heme. The Raman spectrum of the reduced form of wild-type was mainly of 6-coordinate low-spin, and the ν_{11} band, which is sensitive to the donor strength of the axial ligand, was lower than that of reduced cytochrome c_3, suggesting coordination of a strong ligand, and thus a deprotonated histidine. In the reduced forms of H119A and H171A, the 5-coordinate species became more prevalent, whereas no such changes were observed for C170A, indicating that His119 and His171, but not Cys170, are axial ligands in the ferrous heme. This means that ligand replacement from Cys to His occurs upon heme reduction as observed in another CO-sensor protein, CooA. The CO-bound form displayed $\nu_{Fe\text{-}CO}$ and $\nu_{C\text{-}O}$ bands at 496 and 1967 cm^{-1}, respectively. The $\nu_{Fe\text{-}CO}$ *vs* $\nu_{C\text{-}O}$ correlation plot indicates that a neutral His is a *trans* ligand of CO. Our results support a mechanism in which CO binding disrupts the hydrogen bonding of His171 with surrounding amino acids, which induces conformational changes in the His171-Cys170 moiety, leading to physiological signaling as shown in Figure 1.

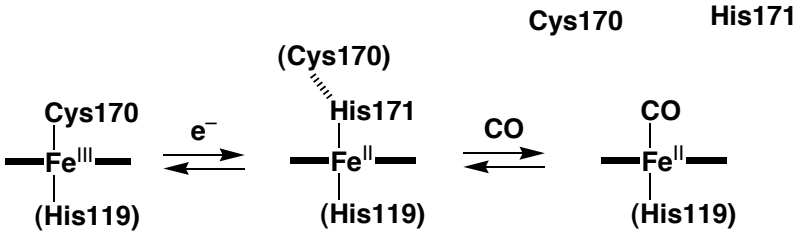

Figure 1 Expected heme environmental structure of the PAS-A domain of NPAS2