Transient Absorbance Spectroscopy Studies of XO Ligand Rebinding for the Heme Based Oxygen Sensor SmFixL

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Heme-sensor proteins are critical in control of a number of physiological processes. The coordination of diatomic ligands such as O₂, CO and NO to the heme provoke a response that allows the organism to adapt to the availability of the ligand. FixL, from Sinorhizobium meliloti is a bacterial 2-component oxygen sensor that responds to changes in aerobicity by turning on the transcription of a number of genes responsible for nitrogen fixation as well as microaerobic metabolism. The protein comprises a heme binding domain and a linked kinase domain. Previous ligand rebinding rate measurements on the SmFixL-CO system revealed a conformational transition that was hypothesized to be involved in signal transduction. (Rodgers, K. R. et al. Biochemistry, 2001, 40, 12932)

To further assess the role(s) of ligation-coupled conformational dynamics in the signal transduction mechanism in SmFixL, new transient absorbance spectroscopic studies have been performed on a number of SmFixL-XO systems. (where XO = CO, NO and O₂). The diatomic ligands were removed by photolysis and ligand rebinding studied on timescales ranging from nanoseconds to milliseconds. Kinetic parameters have been obtained both from fitting of single wavelength measurements and global fitting of time resolved ΔA spectra. The mechanistic implications of these results will be discussed.