Biophysical Characterization of Allostery in the Heme-Dependent CO Sensor, CooA

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Allostery is a critical process for modulation of protein activity through non-covalent modification of structure, yet the thermodynamics that dictate these changes in living systems have not been characterized in detail. CooA is a heme-containing transcription factor found in the bacterium *Rhodospirillum rubrum* that allows the organism to control production of a provisional CO oxidation system. In the absence of light and oxygen, carbon monoxide binds to the heme cofactor in CooA, activating the protein for DNA binding. Three distinct conformations of CooA have been identified on native gels, each corresponding to a unique spectroscopically-characterized heme ligation state. To further explore the energetic relationship between the three states of CooA, unfolding studies with guanadinium hydrochloride were performed. Absorption spectroscopy has revealed significant differences in the unfolding behavior for each activation state; comparison of ΔG^{H_2O} values for the three states indicates that the active form has greater structural stability than the two inactive forms. The changes in protein structure are compensated by electronic and ligation changes at the heme cofactor. Cyclic voltammetry has been used to probe the heme environment, allowing for development of a thermodynamic cycle describing the early stages of CooA activation. These data for changes in both protein conformation and heme cofactor provide a preliminary outline for the complex process of allostery in CooA.