Modeling Proline Ligation in the Heme-Dependent CO Sensor, CooA, Using Small Molecule Analogs

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CooA, the only protein known to employ proline as a heme ligand, is a CO-activated transcription factor in the bacterium *Rhodospirillum rubrum*. The protein utilizes the heme cofactor to bind CO, and the ligation state of the heme iron changes as CooA is activated. Reduction of the heme causes a ligand switch, adjusting the heme pocket to allow for CO binding by displacing an endogenous proline ligand. The proposed role of proline is to stabilize the heme pocket during the reduction and ligand switch, while maintaining a bond that is weak enough to allow CO to displace it. In order to probe ligand properties, binding affinity studies were conducted using pyrrolidine and imidazole as analogs of proline and histidine, respectively. The equilibrium binding affinities were measured in titration experiments where changes in heme ligation were monitored by electronic absorption spectroscopy. To more accurately define the possible steric effects of proline, 2-methypyrrolidine was used to determine how additional steric constraint affects binding affinity. Three different protein models: CooA variant (ΔP3R4), myoglobin, and $P450_{cam}$, were used to explore the binding properties of proline in different protein environments. These binding studies provide a direct thermodynamic comparison between proline and histidine as heme ligands, giving a more complete understanding of proline ligation in the protein context.