Relating Structure and Function in Two Homologous Cytochromes *c*

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Electron transfer is a fundamental reaction in biological energy transduction. Redox potential, $\mathcal{E}°$, is a major factor determining electron transfer energetics and rates. In this project, we aim to understand the structural basis for the different redox potentials of two homologous bacterial cytochromes *c, Hydrogenobacter thermophilus* cytochrome c_{552} (*Ht* cyt c_{552}) and *Pseudomonas aeruginosa* cytochrome c_{551} (*Pa* cyt c_{551}).

The redox potential of *Ht* cyt c_{552} (220 mV vs. NHE) is low relative to other proteins in the cyt c_8 subfamily, and is 65 mV lower than that of *Pa* cyt c_{551}. Primary sequences (69% similarity) and tertiary structures (0.7-Å RMSD) of *Pa* cyt c_{551} and *Ht* cyt c_{552} are highly homologous. Surprisingly, the major structural determinants of redox potential (axial ligands to the heme iron, percent solvent exposure of the heme group, interactions with heme propionates, and electrostatic potential at the heme iron active site) predict a higher redox potential for *Ht* cyt c_{552} relative to *Pa* cyt c_{551}. Hydrogen exchange (HX) studies, however, reveal that despite conservation of the fold of the Cys-X-X-Cys-His motif, there are substantial differences in the energetics of local unfolding events for residues within and near the motif between *Pa* cyt c_{551} and *Ht* cyt c_{552}. Analysis of the disparate HX behavior suggests that local unfolding events are more energetically accessible in *Ht* cyt c_{552} than in *Pa* cyt c_{551}. From this analysis, we hypothesize that the energetically accessible local unfolding events that occur in *Ht* cyt c_{552} allow for transient solvent access to the heme active site, which could explain the lower redox potential of *Ht* cyt c_{552} compared to *Pa* cyt c_{551}. We thus propose that energetics of protein structural fluctuations near redox cofactors may play a role in modulating metalloprotein redox potential.