Conformationally-Gated Electron Transfer in Iso-1-Cytochrome *c*

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Conformational gating of protein electron transfer (ET) reactions has been of interest recently as a means of modulating metabolic pathways involving ET reactions.[1] In the case of cytochrome *c*, spectroscopic data suggest that conformational changes involving exchange of the heme ligand, methionine 80, for another ligand could act to gate the biologically important ET reactions of this protein.[2] We have recently developed a Lys 73 → His variant of iso-1-cytochrome *c* that stabilizes an alternate conformation of the protein where His 73 replaces Met 80 as a heme ligand near physiological pH.[3] This form of the protein is expected to have a redox potential ~0.25 V lower than the native form of the protein. Thus, reduction of the Fe^{3+}-heme should require a conformational gating step involving isomerization to the Met 80 conformation for ET to be efficient.

To study conformational gating in this protein, we carried out intermolecular ET reactions of His 73 iso-1-cytochrome *c* with the small inorganic reagent, pentaammineruthenium(II) chloride, a_6Ru^{2+}. Reactions were monitored by stopped flow methods at three concentrations of a_6Ru^{2+} and pH values from 6 to 7.5. At pH 7.5, where the Met 80/His 73 equilibrium constant is ~0.5, three kinetics phases are observed. The fastest phase is strongly concentration dependent and is consistent with direct ET with a bimolecular rate constant of ~40,000 $M^{-1}s^{-1}$. The intermediate phase is independent of the a_6Ru^{2+} concentration and correlated well with the pH dependence of the kinetics of formation of the Met 80-heme conformer from the His 73-heme conformer, measured independently by pH jump stopped flow methods.[4] The observed correlation provides direct linkage between a conformational change and a gated ET reaction. The slow phase is consistent with ET gated by the same conformational change, but slowed by a required cis/trans isomerization of a proline.

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