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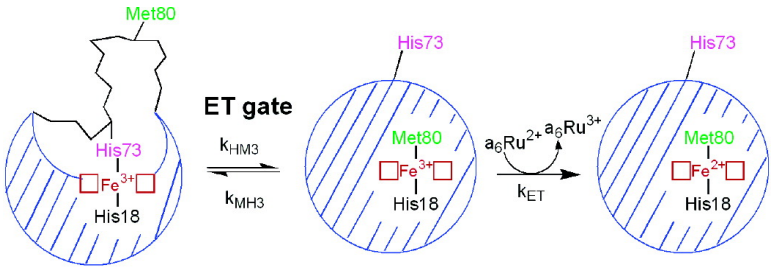

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Conformationally Gated Electron Transfer in Iso-1-cytochrome *c*: Engineering the Rate of a Conformational Switch

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Through the use of simple, rational protein engineering methods, we have increased the rate of an electron transfer (ET) conformational gate by nearly 500-fold. As a starting point, we used the well characterized, but slow (~30 s time scale), ET conformational gate due to the alkaline state (Lys−heme ligation) of oxidized cytochrome *c* (Cyt*c*).[1] We have developed a Lys 73 → His variant (H73) of yeast iso-1-Cyt*c* that significantly modulates the thermodynamics[2] and kinetics[3] of formation of the "alkaline" state of this protein and shifts the conformational change to physiological pH. Here, we study the intermolecular reduction ($Fe^{3+} \rightarrow Fe^{2+}$) of this protein with the small inorganic reagent, hexaammineruthenium(II) chloride (a_6Ru^{2+}). A 50−100 ms time scale conformational ET gate is observed, demonstrating that protein engineering can provide ET gates with tailored rates. The pH dependence of the ET rate constant for this kinetic phase matches well the independently measured rate constant for conversion of the His 73−heme conformation into the Met 80−heme conformation,[3] allowing unambiguous assignment of this conformational change to the ET gating step.

Conformational ET gates are likely important in regulating biochemical pathways.[4] For Cyt*c*, conformational changes involving substitution of the Met 80−heme ligand may play a role in modulating flow of electrons along the electron transport chain during oxidative phosphorylation.[5] Resonance Raman studies of complex formation between iso-1-Cyt*c* and cytochrome *c* oxidase provide support for this proposal.[6]

Theoretical treatments of conformationally gated ET are consistent with the kinetic scheme shown in Chart 1.[7,8] Evidence for conformationally gated ET has accrued in both protein and small molecule systems.[8−10] Decoupling of the rate constants in this scheme has been possible for Cyt*c*[1] and plastocyanin,[9a] where gating involves changes in metal ligation. Indirect methods, such as observation of a viscosity dependence for ET rates, are usually required to demonstrate conformationally gated ET.[4,9,10a,b] Only in the case of ET reactions of small inorganic complexes has it been possible to fully validate this kinetic mechanism.[8]

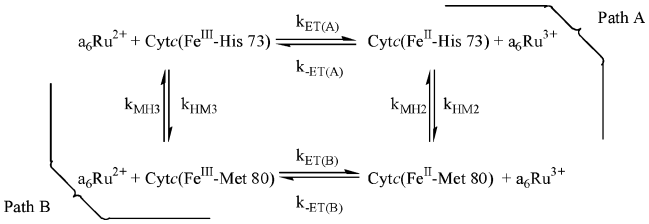
For H73 iso-1-Cyt*c*, path B in Chart 1 is expected to be dominant, except at high concentrations of a_6Ru^{2+}, because the reduction potential of the bis(His)−heme of Cyt*c*(Fe^{III}−His 73) should be near 0 mV versus NHE,[11] much smaller than the reduction potential of 290 mV versus NHE for the Met/His−heme of Cyt*c*(Fe^{III}−Met 80).[5a] With path B dominant, the rate law for the bimolecular reaction between a_6Ru^{2+} and the Fe^{III} state of H73 iso-1-Cyt*c* has the form given in eqs 1 and 2,[8] where the rate

$$-\mathrm{d}[\mathrm{Fe^{III}heme}]/\mathrm{d}t = k_{obs}[\mathrm{Cyt}c(\mathrm{Fe^{III}-His\ 73})][a_6Ru^{2+}] \quad (1)$$

$$k_{obs} = \{k_{ET(B)}k_{HM3}/(k_{ET(B)}[a_6Ru^{2+}] + k_{MH3})\} \quad (2)$$

constants are defined in Chart 1. If $k_{ET(B)}[a_6Ru^{2+}] \gg k_{MH3}$, then the rate law in eq 1 becomes independent of $[a_6Ru^{2+}]$ and k_{obs} becomes the rate of conversion from the Cyt*c*(Fe^{III}−His 73) to the Cyt*c*(Fe^{III}−Met 80) conformation.

Chart 1. Square Kinetic Scheme for Reaction of H73 Iso-1-cytochrome *c* with a_6Ru^{2+}



At pH 6.5, the equilibrium constant for formation of Cyt*c*(Fe^{III}−His 73) from Cyt*c*(Fe^{III}−Met80) is ~0.25,[2c] so at the appropriate $[a_6Ru^{2+}]$, both direct ET to Cyt*c*(Fe^{III}−Met 80) and conformationally gated ET involving Cyt*c*(Fe^{III}−His 73) should be observable. Figure 1 shows stopped-flow data for the reaction of

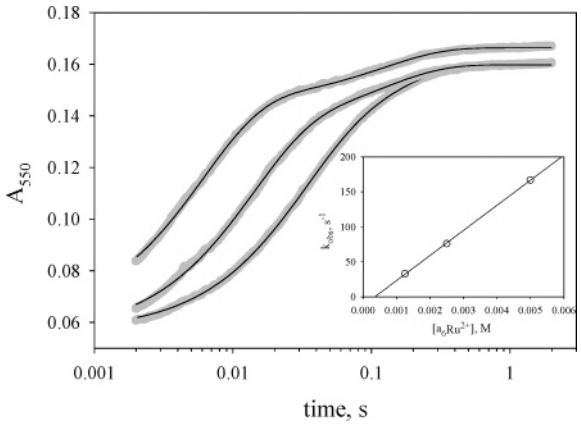


Figure 1. Stopped-flow data for the reduction of H73 iso-1-Cyt*c* by a_6Ru^{2+} in 10 mM MES, pH 6.5, 100 mM NaCl. The concentration of a_6Ru^{2+}, from left to right, is 5, 2.5, and 1.25 mM. The final concentration of H73 iso-1-Cyt*c* is 5 μM. The data points are shown in gray. The solid curves are a fit of the data to a double exponential rise to maximum equation. The inset shows the dependence of the fast phase rate constant on the concentration of a_6Ru^{2+}.

oxidized H73 iso-1-Cyt*c* with a_6Ru^{2+} at pH 6.5. There are clearly two kinetic phases. The fast phase is strongly dependent on $[a_6Ru^{2+}]$ (Figure 1, inset and Table 1). The slow (~100 ms) phase, within error, is independent of $[a_6Ru^{2+}]$. The bimolecular rate constant obtained for the fast phase is, within error, independent of pH, being $(3.9 \pm 0.5) \times 10^4\ M^{-1}\ s^{-1}$. In control experiments with wild-type (WT)[12] iso-1-Cyt*c* at the same set of pH values, no ~100 ms kinetic phase is observed, only a $[a_6Ru^{2+}]$-dependent fast phase. A bimolecular rate constant for reduction of the WT protein by a_6Ru^{2+} of $(4.8 \pm 0.4) \times 10^4\ M^{-1}\ s^{-1}$ was obtained. We assign this bimolecular reduction reaction to direct ET to the Met 80-

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Table 1. ET Data for a_6Ru^{2+} Reduction of H73 Iso-1-Cyt*c*[a]

		Slow phase		Fast phase	
pH	$[a_6Ru^{2+}]$ (mM)	k_{obs} (s^{-1})	amplitude (au)	k_{obs} (s^{-1})	amplitude (au)
6.0	1.25	11.6 ± 1.5	0.023 ± 0.004	41 ± 4	0.069 ± 0.004
	2.5	13.6 ± 1.5	0.014 ± 0.002	74 ± 2	0.075 ± 0.003
	5.0	14.8 ± 3.0	0.014 ± 0.003	174 ± 9	0.083 ± 0.001
6.5	1.25	7.6 ± 0.4	0.032 ± 0.002	33 ± 2	0.072 ± 0.003
	2.5	8.4 ± 0.2	0.024 ± 0.001	76 ± 2	0.079 ± 0.001
	5.0	8.7 ± 0.8	0.022 ± 0.001	167 ± 2	0.084 ± 0.001
7.5	1.25	6.1 ± 0.1	0.034 ± 0.001	33.6 ± 0.3	0.056 ± 0.001
	2.5	6.8 ± 0.4	0.028 ± 0.001	82.1 ± 1.1	0.060 ± 0.002
	5.0	7.9 ± 0.3	0.029 ± 0.001	196 ± 13	0.058 ± 0.003

[a] All data were acquired at 25 °C in 10 mM buffer at the indicated pH with 100 mM NaCl added (MES, pH 6.0 and 6.5; Tris, pH 7.5). Reported errors are the standard deviation from the average of at least four trials.

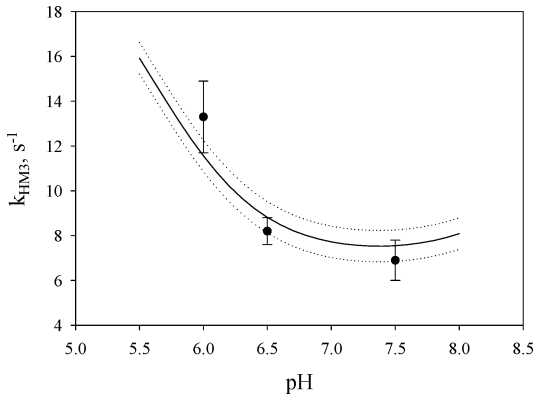

Figure 2. Comparison of the slow phase k_{obs} for reduction of H73 iso-1-Cyt*c* by a_6Ru^{2+} to the rate constant (k_{HM3}) of the His 73−Fe^{3+}−heme to Met 80−Fe^{3+}−heme conformational change as a function of pH. The solid circles with error bars are the average and standard deviation of k_{obs} at all $[a_6Ru^{2+}]$ for the slow phase reduction at each pH. The solid curve shows the pH dependence of the rate of formation of the Met 80−heme state from the His 73−heme state determined by pH jump methods (eq S11, ref 3). The dashed lines indicate the average standard deviation of the data points, which define this dependence.

ligated heme for both proteins ($k_{ET(B)}$ in Chart 1). The $k_{ET(B)}$ values compare well with $k_{ET} = (6.7 \pm 0.2) \times 10^4\ M^{-1}\ s^{-1}$, reported for the bimolecular reduction of horse Cyt*c* by a_6Ru^{2+}.[13]

The rate constant for the $[a_6Ru^{2+}]$-independent slow phase increases as pH drops (Table 1). The kinetics of the conformational change between the His 73- and Met 80-ligated states of the Fe^{3+}−heme of H73 iso-1-Cyt*c* monitored directly by pH jump stopped-flow methods show a similar pH dependence.[3] Figure 2 compares the pH dependence of k_{obs} for the slow reduction phase of H73 iso-1-Cyt*c* to the measured rate of the conformational change from the His 73-ligated to the Met 80-ligated Fe^{3+}−heme for this protein.[3] The correlation is very good, allowing direct assignment of the slow $[a_6Ru^{2+}]$-independent phase to conformationally gated ET mediated by the change from the low potential His 73−heme to the high potential Met 80−heme form of the protein (k_{HM3} in Chart 1). Interestingly, a very similar rate constant (17 s^{-1}) was observed for the His 82/Met 80 Fe^{3+}−heme ligand exchange in cyclic voltammetry studies on a Phe 82 → His variant of iso-1-Cyt*c*.[14] We also note that the amplitude of the slow phase in Table 1 decreases as pH decreases, consistent with the assignment of the slow reduction to the k_{HM3} process since thermodynamic studies show that the population of the His 73−heme form of this protein decreases as pH drops from 7.5 to 6.0.[2c]

On a 100 s time scale, an even slower reduction phase (k_{obs} of 0.062 ± 0.006 s^{-1}), independent of both pH and $[a_6Ru^{2+}]$, is observed. Its amplitude drops with decreasing pH, as observed for the slow phase observed on a 2 s time scale. Since the Lys 79 alkaline conformer of H73 iso-1-Cyt*c* is negligibly populated in this pH regime (see also Table S5 for WT iso-1-Cyt*c*),[2c] we assign this very slow phase to a cis to trans isomerization of Pro 76 required for Cyt*c*(Fe^{III}−His 73) to revert to Cyt*c*(Fe^{III}−Met 80).[3]

Broadly speaking, engineering metal ligation in ET proteins appears to be a fruitful target for modulating rates of ET gates, providing time scales from microseconds (plastocyanin, ∼50 μs)[9a] to milliseconds (His−heme, ∼100 ms, this work) to seconds (Lys−heme, ∼30 s).[1] Finer tuning could be achieved through local sequence manipulation (proline, etc.). Such flexibility should be useful in engineering both protein function and "designer" components for protein-based molecular electronics devices.

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Supporting Information Available: Stopped-flow experimental protocols, and tables of rate data for a_6Ru^{2+} reduction of the WT and H73 proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Greenwood, C.; Palmer, G. *J. Biol. Chem.* **1965**, *240*, 3660−3663. (b) Wilson, M. T.; Greenwood, C. *Eur. J. Biochem.* **1971**, *22*, 11−18. (c) Hodges, H. L.; Holwerda, R. A.; Gray, H. B. *J. Am. Chem. Soc.* **1974**, *96*, 3132−3137.

(2) (a) Godbole, S.; Dong, A.; Garbin, K.; Bowler, B. E. *Biochemistry* **1997**, *36*, 119−126. (b) Godbole, S.; Bowler, B. E. *Biochemistry* **1999**, *38*, 487−495. (c) Nelson, C. J.; Bowler, B. E. *Biochemistry* **2000**, *39*, 13584−13594. (d) Nelson, C. J.; LaConte, M. J.; Bowler, B. E. *J. Am. Chem. Soc.* **2001**, *123*, 7453−7454.

(3) Martinez, R. E.; Bowler, B. E. *J. Am. Chem. Soc.* **2004**, *126*, 6751−6758.

(4) (a) Davidson, V. L. *Biochemistry* **2002**, *41*, 14633−14636. (b) Davidson, V. L. *Acc. Chem. Res.* **2000**, *33*, 87−93.

(5) (a) Rosell, F. I.; Ferrer, J. C.; Mauk, A. G. *J. Am. Chem. Soc.* **1998**, *120*, 11234−11245. (b) Döpner, S.; Hildebrant, P.; Rosell, F. I.; Mauk, A. G. *J. Am. Chem. Soc.* **1998**, *120*, 11246−11255.

(6) Döpner, S.; Hildebrant, P.; Rosell, F. I.; Mauk, A. G.; von Walter, M.; Buse, G.; Soulimane, T. *Eur. J. Biochem.* **1999**, *261*, 379−391.

(7) (a) Hoffman, B. M.; Ratner M. A.; Wallin, S. A. In *Advanced Chemical Series*; Johnson, M. K., King, R. B., Kurtz, D. M., Jr., Kutal, C., Norton, M. L., Scott, R. A., Eds.; American Chemical Society: Washington, DC, 1990; Vol. 226, pp 125−146. (b) Brunschwig, B. S.; Sutin, N. *J. Am. Chem. Soc.* **1989**, *111*, 7454−7465.

(8) (a) Meagher, N. E.; Juntunen, K. L.; Salhi, C. A.; Ochrymowycz, L. A.; Rorabacher, D. B. *J. Am. Chem. Soc.* **1992**, *114*, 10411−10420. (b) Wijetunge, P.; Kulatilleke, C. P.; Dressel, L. T.; Heeg, M. J.; Ochrymowycz, L. A.; Rorabacher, D. B. *Inorg. Chem.* **2000**, *39*, 2897−2905.

(9) (a) Di Bilio, A. J.; Dennison, C.; Gray, H. B.; Ramirez, B. E.; Sykes, A. G.; Winkler, J. R. *J. Am. Chem. Soc.* **1998**, *120*, 7551−7556. (b) Kostić, N. M.; Ivković-Jensen, M. M. *Biochemistry* **1997**, *36*, 8135−8144. (c) Mei, H.; Wang, K.; Peffer, N.; Weatherly, G.; Cohen, D. S.; Miller, M.; Pielak, G.; Durham, B.; Millet, F. *Biochemistry* **1999**, *38*, 6846−6854.

(10) (a) Lui, L.; Hong, J.; Ogawa, M. Y. *J. Am. Chem. Soc.* **2004**, *126*, 50−51. (b) Lasey, R. C.; Lui, L.; Zang, L.; Ogawa, M. Y. *Biochemistry* **2003**, *42*, 3904−3910. (c) Davis, W. B.; Ratner, M. A.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2001**, *123*, 7877−7886.

(11) (a) Raphael, A. L.; Gray, H. B. *J. Am. Chem. Soc.* **1991**, *113*, 1038−1040. (b) Reid, L. S.; Taniguchi, V. T.; Gray, H. B.; Mauk, A. G. *J. Am. Chem. Soc.* **1982**, *104*, 7516−7519.

(12) The WT and the H73 variant have a Cys 102 → Ser mutation to prevent intermolecular disulfide bond formation during physical studies.

(13) Nocera, D. G.; Winkler, J. R.; Yocum, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145−5150. In our hands, we obtain an identical k_{ET} of (6.4 ± 0.1) × $10^4\ M^{-1}\ s^{-1}$ for the reduction of horse cytochrome *c* by a_6Ru^{2+} under the same conditions (0.1 M sodium phosphate, pH 7). The slightly faster rate for the horse protein appears to be a buffer effect. In 0.1 M sodium phosphate, pH 7, we obtain k_{ET} = (9.4 ± 1.4) × $10^4\ M^{-1}\ s^{-1}$ for the a_6Ru^{2+} reduction of WT iso-1-Cyt*c*.

(14) (a) Feinberg, B. A.; Liu, X.; Ryan, M. D.; Schejter, A.; Zhang, C.; Margoliash, E. *Biochemistry* **1998**, *37*, 13091−13101. (b) Hawkins, B. K.; Hilgen-Willis, S.; Pielak, G. J.; Dawson, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 3111−3112.

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