Ruthenium-Modified Cytochrome *c*: Temperature Dependence of the Rate of Intramolecular Electron Transfer

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Abstract: The ruthenium-modified horse heart cytochrome *c*, Ru(III)–cyt *c*(III), where the ruthenium is bound to the His-33 residue has been synthesized and characterized by ruthenium analysis, UV–vis and CD spectra, and differential pulse polarography and cyclic voltammetry. The intermediate Ru(II)–cyt *c*(III) has been generated by pulse radiolysis with use of four different radicals, $CO_2^{-}\cdot$, $(CH_3)_2COH\cdot$, $(CH_2OH)_2\dot{C}CHOH$, and $^{-}O_2CCH(OH)\dot{C}(OH)CO_2^{-}$. The rate of intramolecular electron transfer within the Ru(II)–cyt *c*(III) complex

$$\text{Ru(II)–cyt } c\text{(III)} \xrightarrow{k_u} \text{Ru(III)–cyt } c\text{(II)}$$

and its temperature dependence were determined over a 40 °C temperature range with the $CO_2^{-}\cdot$ radical. At 25 °C, these values are $k_u = 53 \pm 2\ s^{-1}$ (pH 7, 0.1 M phosphate buffer, 0.1 M $NaHCO_2$), $\Delta H^{\ddagger} = 3.5 \pm 0.2$ kcal mol^{-1}, and $\Delta S^{\ddagger} = -39 \pm 1$ eu.

There is a considerable body of evidence that electron transfer in biological molecules takes place rapidly over long distances (ca. >10 Å) without direct coupling of π-conjugated molecules between the electron donor and the electron acceptor.[1] One of the important themes in studying the mechanisms of electron transfer in biological systems is to understand the factors that control this long-range electron-transfer process.[2] We have attempted to understand the dependence of the rate of electron transfer on distance by measuring the intramolecular rate and temperature dependence of electron transfer between a donor and an acceptor separated by a number of peptide residues.[3] In a recent communication[3] we have extended this work to a modified protein donor–acceptor complex, the ruthenium-modified horse heart cytochrome *c* (Figure 1). In this protein the $[(NH_3)_5Ru^{II/III}-]$ ion is covalently bound to the imidazole moiety of His-33. The distance between the ruthenium site and the iron, estimated from the crystal structure of the oxidized tuna cytochrome *c*, is between 14 and 16 Å.[4] In this paper *we report on the temperature dependence of the rate of intramolecular electron transfer over a range of 40 °C and comment on its origin.* Different radicals, generated by pulse radiolysis, form the intermediate, Ru(II)–cyt *c*(III), for which intramolecular electron transfer was measured. Some differences are observed between our results and those of Winker et al.,[5] who studied the same electron-transfer reaction with use of flash photolysis and found the rate to be temperature independent between 0.5 and 60 °C.

Experimental

Horse heart cytochrome *c* (type VI) (Sigma) was purified by cation exchange chromatography[6] on CM-52 cellulose (Whatman). Ruthenium-modified cytochrome *c* was prepared by using our earlier procedure[7] (the Rutgers preparation). The ruthenium–cytochrome *c* derivative was further purified by using a second cation exchange resin (Bio Rex 70, column 15 × 1 cm) and eluted with 0.080 M phosphate buffer (pH 7.0) containing 0.25 M NaCl. Colorimetric ruthenium analysis was carried out on the ruthenium–cytochrome *c* derivative with use of about 3 mg of protein.[8] The heme iron was determined spectrophotometrically by using published extinction coefficient values.[9] Cyclic voltammetry and differential pulse polarography on cytochrome *c* and ruthenium–cytochrome *c* were performed by using a gold electrode modified with an adsorbed layer of 4,4′-bipyridyl.[10] The circular dichroism (CD) spectra of the ruthenium–cytochrome *c* and the cytochrome *c* were obtained on a Cary 60 spectrometer with a 10 mm path length cell (visible) and a 1 mm path length cell (ultraviolet). High-pressure liquid chromatography of the reduced and oxidized cytochrome *c* and the ruthenium derivative was done on a Waters Associates HPLC instrument with two M 6000 A pumps and a M 660 solvent programmer and a Perkin-Elmer LC 75 variable-wavelength detector. A weak cation exchange column (Synchrome CM 300, 250 × 5 mm) was used. For the HPLC a gradient of 60 → 80% Buffer B (5 mM NaH_2PO_4, 0.5 M NaAc, 1% acetonitrile, pH 6.1) was used in 4 min (1 mL/min) with Buffer A (5 mM NaH_2PO_4, 1% acetonitrile, pH 6.1) (Buffers A and B were adjusted with HAc and NaOH, respectively) (λ 410 nm).

Pulse radiolysis experiments were carried out with a beam of 2-MeV electrons produced by a Van de Graaff accelerator at Brookhaven National Laboratories. Pulse lengths were usually in the range of 0.5–1 μs. Radical concentrations varying between 0.38 and 0.95 μM were used throughout these experiments. The analyzing light passed through a 2-cm (2 × 1 × 0.5) cell three times for a path length of 6.1 cm. The light was produced by a quartz–iodine lamp with a filter cutoff below 450 nm. Transients were detected spectrophotometrically (λ 550 nm) as described in ref 11. Data processing was done with computer programs developed by Dr. H. Schwartz.

In pulse radiolysis, irradiated water forms the following radicals[12,13]

$$H_2O \rightsquigarrow e^{-}_{(aq)}\ (2.8),\ \cdot OH\ (2.8),\ H\cdot\ (0.6) \qquad (1)$$

The numbers in parentheses denote *G* values, the number of radicals formed per 100 eV absorbed. When N_2O is present, the hydrated electron is converted to OH radicals,

$$H^{+} + e^{-}_{(aq)} + N_2O \rightarrow N_2 + \cdot OH \qquad k = 8 \times 10^9\ M^{-1}\ s^{-1} \qquad (2)$$

Reducing radicals $CO_2^{-}\cdot$, $(CH_3)_2\dot{C}OH$, and $(CH_3OH)_3C\dot{C}HOH$ were produced from the ·OH radicals by hydrogen atom abstraction from the

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(5) (a) J. Winkler, D. Nocera, K. Yocum, E. Bordignon, and H. B. Gray, *J. Am. Chem. Soc.*, **104**, 5798–5800 (1982). (b) K. Yocum, J. R. Winkler, D. G. Nocera, E. Bordignon, and H. B. Gray, *Chem. Scr.*, **21**, 29–33 (1983). (c) H. B. Gray, private communication, 1983.
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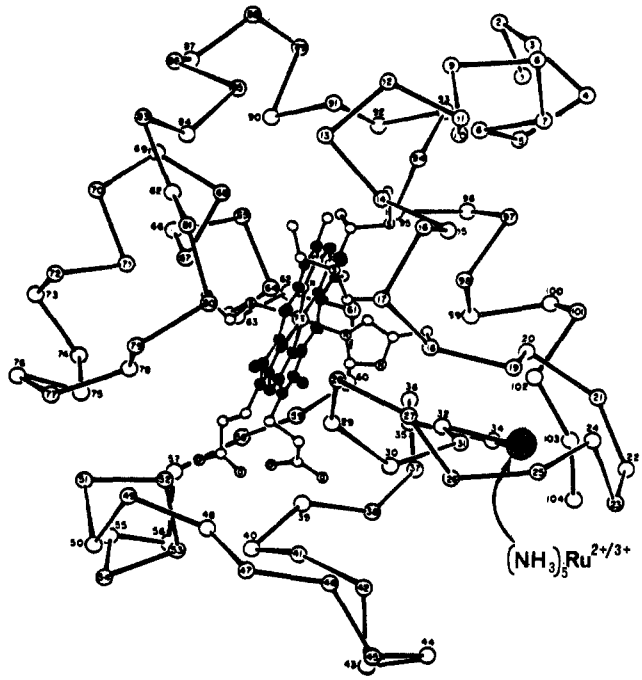

Figure 1. Histidine-33 ruthenium modified horse heart cytochrome *c*(III) (adapted from ref 23).

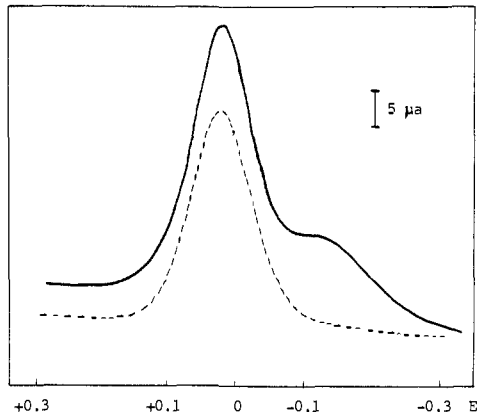

Figure 2. Reduction of [Ru(III)–cyt *c*(III)] (—) and [cyt *c*(III)] (---) by differential pulse polarography: volts vs. SSCE; 2 mm gold disk electrode; scan rate = 2 mV/s; pulse amplitude = 25 mV; 0.25 μmol [Ru(III)–cyt *c*(III)] or [cyt *c*(III)] in 0.1 M $NaClO_4$, 0.01 M bipyridine, 0.08 M phosphate buffer pH 7.

corresponding molecules. The ·OH radical also decays by dimerization and reaction with H atoms. The rate of reaction of ·OH with cytochrome *c* is k(OH + cyt *c*(III)) = 4×10^{10} M^{-1} s^{-1}.[13]

Experiments were carried out at concentrations of 0.1 M sodium formate, 0.1 M isopropanol, and 0.1 M pentaerythritol, and 0.05 M potassium tartrate at pH 7.0 (0.1 M phosphate buffer) and at concentrations of ruthenium–cytochrome *c*(III) of 0.8×10^{-6} to 3.2×10^{-6} M.

Results and Discussion

(a) Characterization of the Ru(III)–Cytochrome *c* (III) Species. The ruthenium-modified cytochrome *c* was characterized as reported earlier by using UV–vis spectra, NMR, cyclic voltammetry, and peptide mapping experiments.[7] The species used in this study was characterized by using Fe and Ru analysis (Ru/Fe 1.0 ± 0.05). Differential pulse polarography gave two overlapping peaks

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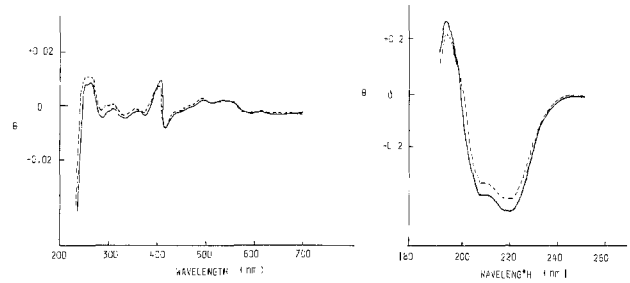

Figure 3. Circular dichroism (CD) spectra of ruthenium-modified horse heart cytochrome *c*(III) (—) and native horse heart cytochrome *c*(III) (---) both at 1.0×10^{-5} M: (A) UV–vis region, 1-cm cell; (B) expanded UV region, 0.1-cm cell. θ refers to measured ellipticity.

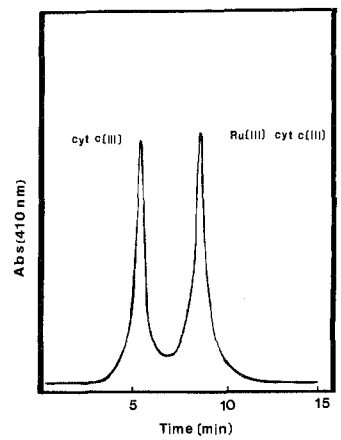

Figure 4. HPLC separation of cytochrome *c*(III) and ruthenium-modified cytochrome *c*(III) (see Experimental for conditions).

Scheme I

$$A\cdot + \text{Ru(III)-cyt } c\text{(III)} \xrightarrow{k_1} \text{Ru(II)-cyt } c\text{(III)} + A \quad (3)$$

I

$$\downarrow k_u$$

$$A\cdot + \text{Ru(III)-cyt } c\text{(III)} \xrightarrow{k_2} \text{Ru(III)-cyt } c\text{(II)} + A \quad (4)$$

II

at +0.26 and +0.13 V (vs. NHE) corresponding to the reduction of the heme and the ruthenium sites, respectively (Figure 2). CD spectra of the ruthenium(III)–cytochrome *c*(III) were identical with those of the native cytochrome *c*(III) in the UV and visible region. Figure 3 shows both CD spectra for a solution of 1.0×10^{-5} M. High-pressure liquid chromatography of the native cytochrome *c*(III) and the ruthenium(III)–cytochrome *c*(III) derivative on a weak cation exchange column is shown in Figure 4. Figure 4 shows the resolution obtained thus far on HPLC. Further work on the NMR and the interaction of the ruthenium–cytochrome *c* derivative with cytochrome *c* oxidase is in progress.

(b) Kinetics and Temperature Dependence of the Intramolecular Electron-Transfer Process. In an earlier communication[3] we reported our preliminary results on the rate of reduction of Ru(III)–cyt *c*(III) with $CO_2^{-}\cdot$ to generate the Ru(II)–cyt *c*(III) intermediate with use of pulse radiolysis. The rate of intramolecular electron transfer from Ru(II) to the heme was measured to be $k_u = 82 \pm 20$ s^{-1} (pH 6.7, 2 mmol phosphate buffer, 0.1 M sodium formate buffer). In Scheme I the sequence of reactions leading to Ru(II)–cyt *c*(III) intermediate is shown where A· is the reducing radical produced at a concentration lower than that of the Ru(III)–cyt *c*(III) by more than one order of magnitude. At that radical concentration, A· selects between the [$(NH_3)_5$-Ru^{III}-] site and the heme site.

The Ru(II)–cyt *c*(III) intermediate was generated with use of four different reducing radicals, A = $CO_2^{-}\cdot$, $(CH_3)_2C(\cdot)OH$, $(CH_2OH)_3CCHOH\cdot$, and $(^{-}O_2CCH(OH)\dot{C}(OH)CO_2^{-})$. Figure

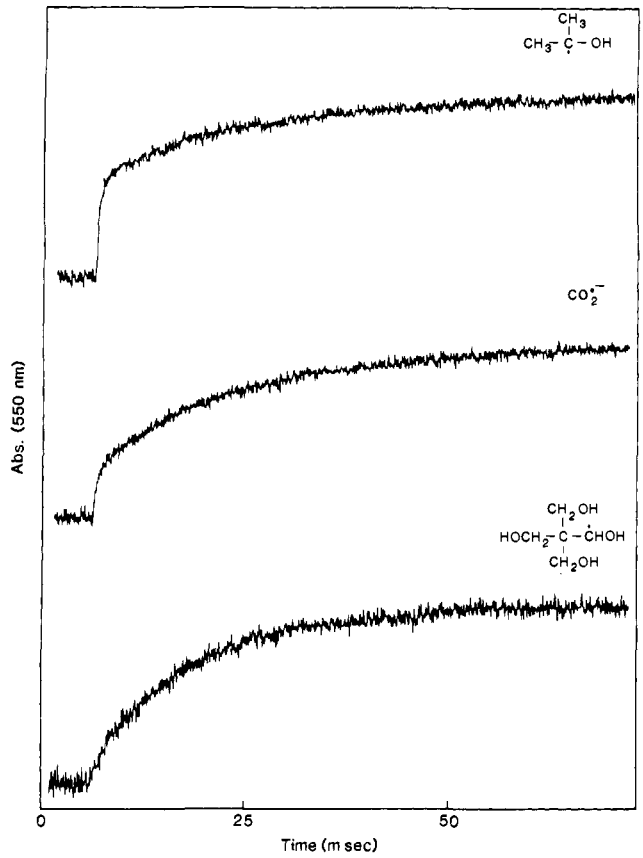

Figure 5. Reduction of ruthenium(III)–cytochrome *c*(III) by $(CH_3)_2\dot{C}OH$, $CO_2^{-}\cdot$, and $(CH_2OH)_3C\dot{C}HOH$ radicals (see Experimental for conditions).

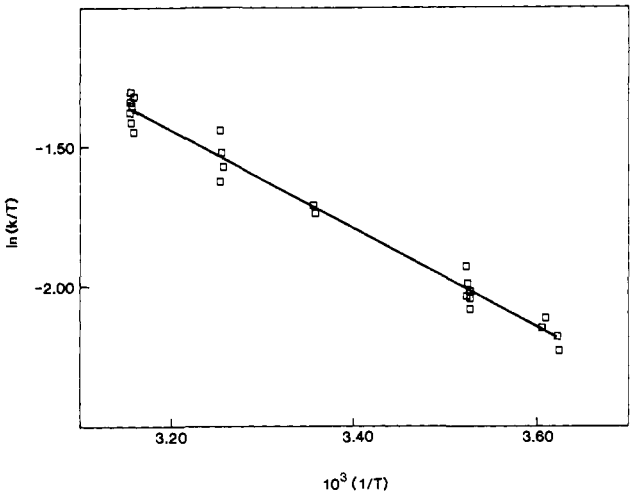

Figure 6. Temperature dependence of the intramolecular electron-transfer rate constant in histidine-33 ruthenium modified cytochrome *c*.

5 shows the absorbance vs. time profile for three of these radicals.

The radicals derived from sodium formate ($CO_2^{-}\cdot$), isopropanol ($(CH_3)_2\dot{C}OH$), potassium tartrate ($^{-}O_2CCH(OH)\dot{C}(OH)CO_2^{-}$) and pentaerythritol ($C(CH_2OH)_3\dot{C}HOH$) reduce cyt *c*(III) with second-order rate constants of 1.3×10^9, 3.8×10^8, 1.7×10^8, and $<10^6$ M^{-1} s^{-1}, respectively.[14] The ratio of the fast rise in absorbance (λ 550 nm) to the slow rise (Figure 5) is an indication of the initial distribution of the reducing electron between the heme site and the ruthenium site. For the four radicals studied, the intermediate Ru(II)–cyt *c*(III) concentration ranges from ~35% (2-propanol) to >95% (pentaerythritol), depending on the choice of the radical. The selectivity of the different radicals for the heme and the ruthenium sites is a function of the charge and solvation properties. The hydrophobic 2-propanol radical has the highest preference for the hydrophobic heme site, while the negatively charged formate and tartrate radicals prefer the hydrophilic ruthenium site. The polyhydroxylic pentaerythritol radical selects the ruthenium site almost exclusively. Earlier work by Simic et al.[14] reported an upper limit on the rate of reduction of cyt *c*(III) with $(CH_2OH)_3CCHOH$ of $<10^6$ M^{-1} s^{-1}. They postulated that steric and hydration properties of pentaerythritol prevent close approach of this radical to the heme site in cyt *c*(III).

The radical derived from formate ($CO_2^{-}\cdot$) was selected for the bulk of our studies because it is known to react with cyt *c*(III) quantitatively.[14] For $CO_2^{-}\cdot$, k_2 was determined to be 1.8×10^9 M^{-1} s^{-1}. With use of this value and the ratio of the slow and fast absorbance rises for $CO_2^{-}\cdot$ (Figure 5), k_1 (eq 3) is calculated to be 5.4×10^9 M^{-1} s^{-1}.

The rate of intramolecular electron transfer between the ruthenium site and the heme site was studied at different Ru(III)–cyt *c*(III) concentrations, different radical concentrations, and over a temperature range of 3–44 °C, all at constant ionic strength

Table I. Temperature Dependence of the Intramolecular Electron-Transfer Reaction from the Ru(II) → heme(III) Site in Ru(II)-cyt *c*(III)[a]

T, °C	k_u, s^{-1}	*T*, °C	k_u, s^{-1}
43.8	77.0	24.8	52.0
43.5	74.5	24.9	53.8
43.5	84.6	24.8	52.3
43.8	85.6		
43.7	81.8	10.8	41.1
43.9	82.9	10.6	38.7
43.9	79.8	10.4	35.3
		10.4	36.7
33.9	63.5	10.7	37.0
34.1	67.0	10.4	37.7
34.2	60.2		
34.2	72.3	4.2	32.3
		3.9	33.4
		2.9	31.2
		2.8	29.8

[a] $CO_2^{-}\cdot$ radical as reductant, [Ru(III)-cyt *c*(III)] = 1.6×10^{-6} M? in 0.1 M $NaHCO_2$, 0.1 M phosphate buffer, pH 7.0.

Table II. Effect of [Ru(III)-cyt *c*(III)] Concentration (M) on the Rate of Intramolecular Electron Transfer[a]

[Ru(III)-cyt *c*(III)]	k, s^{-1}
0.80×10^{-6}	54.8
	54.4
	55.3
	54.9
	54.8
1.6×10^{-6}	53.8
	52.0
	52.3
3.2×10^{-6}	52.0
	52.3
	51.7
	52.0

[a] *T* = 25 °C, 0.1 M $NaHCO_2$, 0.1 M phosphate buffer, pH 7.0.

Table III. Effect of Radical Type on the Rate of Intramolecular Electron Transfer[a]

radical	k, s^{-1}
$CO_2^{-}\cdot$	53 ± 2
$(CH_3)_2\dot{C}OH$	57 ± 2
$^{-}O_2CCH(OH)\dot{C}(OH)CO_2^{-}$	61 ± 2
$(CH_2OH)_3C\dot{C}HOH$	65 ± 7

[a] *T* = 25 °C, [Ru(III)-cyt *c*(III)] = 1.6×10^{-6}, 0.1 M [radical] in 0.1 M phosphate buffer, pH 7.0.

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(Tables II and III). Figure 5 is a plot of ln (k/T) vs. $1/T$ between 3 and 44 °C.

The results presented here are in substantial agreement with those of our preliminary report carried out under slightly different conditions ($k_u = 83 \pm 20$ s^{-1}, pH 6.7, 2 mM phosphate buffer, 0.1 M sodium formate).[3] The new results with $CO_2^{-\cdot}$ (Tables II and III) have all been obtained in 0.1 M phosphate buffer and 0.1 M $NaHCO_2$, pH 7. Under these conditions the rate of the intramolecular electron transfer ($k_u = 53 \pm 2$ s^{-1}) clearly exhibits a temperature dependence ($\Delta H^\ddagger = 3.5 \pm 0.2$ kcal mol^{-1}) and a large negative entropy of activation $\Delta S^\ddagger = -39 \pm 1$ eu). The temperature dependence of the rate of electron transfer was also studied with the 2-propanol radical over the temperature range of 7–41 °C. The temperature dependence obtained for the electron transfer rate when 2-propanol was used was identical (within experimental error; $\Delta H^\ddagger = 3.3 \pm 0.2$ kcal mol^{-1}, $\Delta S^\ddagger = -39$ eu) with that obtained when CO_2^- was used (i.e., the same temperature dependence was obtained when two different radicals, two different chemistries, were used to generate the Ru(II)–cyt *c*(III) intermediate).

Our results differ from those of Winkler et al.[5] where flash photolysis was used to study the same electron-transfer process. Their reported rate constant ($k = 22$ s^{-1}) did not show any temperature dependence between 0.5 and 60 °C.[5b] However, results of the flash photolysis have an error limit in $\Delta H^\ddagger$ of 0 ± 1 kcal mol^{-1}.[5c] The conditions used for the flash photolysis work ([Ru(III)–cyt *c*(III)] = 5×10^{-6} M, [EDTA] = 5×10^{-3} M) are different from the conditions described here, which possibly may account for the small differences. In our experiments, when 1.25×10^{-3} M EDTA is added to the 0.1 M formate solution of Ru(III)–cyt *c*(III) (in 0.1 M phosphate buffer), no detectable change in the kinetics of electron transfer is observed at 25 °C. This led us to conclude that the EDTA does not have an effect on the rate of electron transfer of our species at room temperature.

It is of interest to comment on the rate and temperature dependence of this intramolecular electron-transfer reaction for this unique modified protein. The activation barrier for intramolecular electron transfer in this molecule can be attributed to a number of nuclear and electronic factors.[24] The first to be considered is the *work term* for the reactant Ru^{II}–Fe^{III} pair and the product Ru^{III}–Fe^{II} pair. At the distance of separation between these two sites, the work term is expected to be negligible unless one considers the low local dielectric medium of the polypeptide between the two sites. The second factor of importance is the *inner-sphere and outer-sphere* reorganization energies, $\Delta G^\ddagger_{in}$ and $\Delta G^\ddagger_{out}$. The inner-sphere reorganization energy for the ruthenium site in a closely related complex is ≤ 1 kcal.[1] For the heme site, it is also expected to be small on the basis of the crystal structure of the reduced and oxidized cytochrome *c*.[4] The outer-sphere reorganization energy for the $Ru^{II/III}$ site at the distance of closest approach is estimated to be ~ 6 kcal mol^{-1}.[1] Although no value for the outer-sphere reorganization energy for cytochrome *c* is available, it is expected to be small. The third factor of importance in determining the rate of electron transfer is the *thermodynamic driving force* for the reaction; this can be approximated as the difference in the reduction potential between the $Ru^{II/III}$ site and the $heme^{II/III}$ site. This value can be obtained from the differential pulse polarography data (Figure 2). If one assumes that the ruthenium and heme sites are far enough apart not to have any significant effect on one another, one can approximate the thermodynamic quantities ($\Delta H°$ and $\Delta S°$) for the heme site by using the value for native cytochrome *c*[15,16] in reaction 5

$$\text{cyt } c^{III} + {}^1/_2H_2 \rightarrow \text{cyt } c^{II} + H^+ \qquad (5)$$

$\Delta H° = -14.4$ kcal mol^{-1}; $\Delta S° = -28$ eu ($I =$ 0.1 M, pH 7, 25 °C)

and those for ruthenium site by using the value for $[Ru(NH_3)_6]^{2/3+}$ in reaction 6[17,18]

$$[Ru^{III}(NH_3)_6] + {}^1/_2H_2 \rightarrow [Ru^{II}(NH_3)_6] + H^+ \qquad (6)$$

$\Delta H° = -5.8$ kcal mol^{-1}; $\Delta S° = -14.2$ eu ($I =$ 0.5–1.0 M, 25 °C)

The driving force ($\Delta G°$) for reaction 7 is -4.5 kcal mol^{-1}, while the enthalpy, $\Delta H°$, for the same reaction is -8.6 kcal mol^{-1} (almost twice the value of the $\Delta G°$) and $\Delta S°$ is -13.8 eu. The difference

$$\text{cyt } c(III) + [Ru^{II}(NH_3)_6] \rightarrow \text{cyt } c(II) + [Ru^{III}(NH_3)_6] \qquad (7)$$

in the temperature dependence of the two equilibrium constants (eq 5 and 6) is a reflection of the differences in solvation energy between the hydrophilic ruthenium site and the hydrophobic heme site. This thermodynamic difference appears in the temperature dependence of the rate as in eq 8, where $\Delta H^\ddagger_{12}$ is the enthalpy

$$\Delta H^\ddagger_{12} = \frac{\Delta H^\ddagger_{11} + \Delta H^\ddagger_{22}}{2} + \frac{\Delta H°_{12}}{2} \qquad (8)$$

of activation of reaction 7, $\Delta H^\ddagger_{11}$ and $\Delta H^\ddagger_{22}$ are the self-exchange enthalpies for the cyt *c* and Ru complex, respectively, and $\Delta H°_{12}$ is -8.6 kcal mol^{-1}, the enthalpy of reaction 7. The large negative value of $\Delta H°_{12}$ (-8.6 kcal mol^{-1}) will decrease the apparent activation barrier, resulting in a smaller temperature dependence of the rate of electron transfer than is normally expected.

The fourth parameter that contributes to the magnitude of the rate constant of the intramolecular electron transfer is the *electronic interaction* between the ruthenium and the heme sites. According to Hopfield,[19] the electronic interaction will decrease exponentially with distance, as the reaction becomes nonadiabatic. Experimentally this parameter can be determined from the entropy of activation after correction for the nuclear and thermodynamic effects.[24] By using the values above (eq 5–7), one can calculate the temperature-independent electronic contribution to the reaction barrier as $\Delta S^\ddagger \sim -32$ eu (assuming negligible contribution from the work and reorganizational energy terms at room temperature).

The above parameters can also be used to rationalize the rate of *inter*molecular electron-transfer reaction between $[Ru(NH_3)_6]^{2+}$ and native cytochrome *c*(III). This can be done because the work term for the reaction is very small (ca. ≤ 1 kcal mol^{-1}). For this reaction a bimolecular rate constant of 3.8×10^4 M^{-1} s^{-1} (at 25 °C, pH 7, phosphate buffer 0.1 M), $\Delta H^\ddagger = 2.9$ kcal mol^{-1}, and $\Delta S^\ddagger = -28$ eu have been measured.[20] Here again, the low-temperature coefficient can be attributed in part to the difference in the thermodynamic entropies between the heme site and the ruthenium site. These two examples of inter- and intramolecular electron transfer, and possibly many others, show that caution should be exercised in interpreting low-temperature dependencies of electron-transfer reactions as due to electronic effects alone. This can be done only after careful examination of the temperature dependence of the redox potential of both the donor and acceptor sites.

Studies by Cruetz and Sutin[21] on the kinetics of the reduction and the kinetics of ligand binding to ferricytochrome *c* revealed the presence of a unimolecular process with a rate constant of ~ 30–60 s^{-1}. This has been attributed to a crevice opening resulting from a dissociation of the Fe(III)–thioether bond. Recent work using stopped flow circular dichroism on ferricytochrome *c*[22] indicated the presence of an unstable transient upon reduction

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of cytochrome c(III) by hemin Fe(II). A conformational change (k = 17 s^{-1}, 28 °C) was reported.[22] It was also observed that the oxidation of ferrocytochrome c did not result in the same reversible conformational change. As a result of such observations, one should question whether the unimolecular rate observed in this Ru(II)-cyt c(III) intermediate refers directly to the intramolecular electron-transfer process or to different rate-limiting processes as in eq 9a and 9b. In this case the rate-determining step is a

$$\text{Ru(II)-cyt } c\text{(III)} \xrightarrow{k_u} \text{Ru(II)-cyt } c\text{(III)}^* \quad (9a)$$

$$\text{Ru(II)-cyt } c\text{(III)}^* \rightarrow \text{Ru(III)-cyt } c\text{(II)} \quad (9b)$$

conformational change and not the electron-transfer process. The interesting point in the observations reported here is the similarity between the intramolecular electron-transfer rate constant (53 s^{-1}) and the unimolecular rate constants reported for these other processes (k = 15-60 s^{-1}). We are currently devising experiments to answer this question. The sensitivity of the rate of electron transfer to driving force will show if the intramolecular electron-transfer step is rate limiting. This can be studied by changing the ligand environment around either the heme site or the ruthenium site. These experiments will be the subject of a future report.

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Registry No. $CO_2^{-\cdot}$, 14485-07-5; $(CH_3)_2COH\cdot$, 5131-95-3; $(CH_2OH)_3\dot{C}CHOH$, 54954-29-9; $^-O_2CCH(OH)\dot{C}(OH)CO_2^-$, 64672-61-3.

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Electron Transfer across Polypeptides. 2. Amino Acids and Flexible Dipeptide Bridging Ligands[1]

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Abstract: A series of cobalt(III)-L-ruthenium(III) complexes (I-VIII) with bridging amino acid and dipeptides derivatized with an isonicotinoyl (iso) group at the N-terminal has been synthesized, $[SO_4(NH_3)_4Ru\text{-}(iso\text{-}(AA)_n)\text{-}Co(NH_3)_5]^{3+}$ (I-VIII; n = 0, 1, 2), where for n = 1, AA = Gly, Phe, and Pro and for n = 2, $(AA)_2$ =GlyGly, GlyPhe, GlyLeu, and PhePhe. The effect of these flexible bridging groups on the rate of intramolecular electron transfer and its temperature dependence have been studied. The intramolecular electron-transfer rates for the Gly, Pro, and Phe amino acid complexes were compared with that of the parent isonicotinic acid complex. The unimolecular rate constant (25 °C, 1 M HTFA), $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ for the intramolecular electron transfer from the Ru(II) site to the Co(III) site for the Gly, Pro, and Phe amino acid complexes are 3.8×10^{-5} s^{-1}, 19.9 kcal/mol, -12 eu; 9.9×10^{-5} s^{-1}, 18.0 kcal/mol, -16 eu; and 3.9×10^{-5} s^{-1}, 19.4 kcal/mol, -14 eu, respectively. For the GlyPhe, GlyLeu, GlyGly, and PhePhe dipeptide complexes, the corresponding unimolecular rate constants, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, are 8.6×10^{-6} s^{-1}, 20.3 kcal/mol, -13.5 eu; 15×10^{-6} s^{-1}, 14.6 kcal/mol, -31.5 eu; 9.9×10^{-6} s^{-1}, 13.3 kcal/mol, -37 eu; and 11.6×10^{-6} s^{-1}, 11.2 kcal/mol, -44 eu, respectively. For the amino acid cases the rates were insensitive to the amino acid side chain. In the dipeptide cases the rate constants are very similar, but the differences between the four flexible dipeptides studied were reflected in the temperature dependence of the rate constant. These differences in the activation parameters are related to the differences in the peptide conformation and hydration properties. The slowness of electron transfer in this series of complexes is attributed to the high reorganizational energy around the cobalt site and to the unfavorable driving force. The reactions, however, go to completion because of the rapid release (ca. $t_{1/2}$ < microseconds) of the ligands from the Co(II) site.

Electron transfer in biological systems takes place through the mediation of a number of proteins, which contain a variety of active sites. The active sites (heme, Fe-S, Cu, and flavin) are generally protected from the solvent, to varying degrees, by a hydrophobic environment created by the polypeptide chain. Recent crystal structures of these electron-transfer proteins[2] have stimulated many speculations concerning the role that the polypeptide chain plays in the electron mediation process. Considerable evidence indicates that rapid electron transfer occurs over long distances (ca. ≥10 Å) between these proteins and their biological partners.[3] However, little is known about the variety of pathways by which peptides participate in the electron-transfer process.

The peptide structure can play a number of roles in the electron-transfer process.[3] One role can be simply *structural*, where the polypeptide chain and the rest of the secondary structure can adjust distances between the sites undergoing electron transfer. Another role that the peptide chain can play is as a *recognition factor*, where a segment of a polypeptide chain, e.g., with positively charged amino acids, helps orient the protein toward a segment of another protein, e.g., with negatively charged amino acids.[4] The *electronic structure of the polypeptide backbone*[5] can be important

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