Mechanistic Information from the First Volume Profile Analysis for a Reversible Intermolecular Electron-Transfer Reaction Involving Pentaammine(isonicotinamide)ruthenium and Cytochrome *c*

Beate Bänsch,[1a] Martin Meier,[1a] Pablo Martinez,[1a,b] Rudi van Eldik,*,[1a] Chang Su,[1c] Ji Sun,[1d] Stephan S. Isied,[1d] and James F. Wishart,*,[1c]

Institute for Inorganic Chemistry, University of Witten/Herdecke, Stockumer Strasse 10, 5810 Witten, Germany, Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973, and Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

Received June 22, 1994[⊗]

The reversible intermolecular electron-transfer reaction between pentaammine(isonicotinamide)ruthenium(II/III) and horse-heart cytochrome *c* iron(III/II) was subjected to a detailed kinetic and thermodynamic study as a function of temperature and pressure. Theoretical calculations based on the Marcus–Hush theory were employed to predict all rate and equilibrium constants as well as activation parameters. There is an excellent agreement between the kinetically and thermodynamically determined equilibrium constants and associated pressure parameters. These data are used to construct a volume profile for the overall process, from which it follows that the transition state lies halfway between the reactant and product states on a volume basis. The reorganization in the transition state has reached a similar degree in both directions of the electron-transfer process and corresponds to a $\lambda^{\ddagger}$ value of 0.44 for this reversible reaction. This is the first complete volume profile analysis for a reversible intermolecular electron-transfer reaction.

Introduction

The application of high-pressure kinetic techniques in mechanistic studies of inorganic, organometallic, and bioinorganic reactions and the associated construction of volume profiles for the reactions under investigation have added a new dimension to the elucidation of intimate reaction pathways over the last decade.[2–6] In the case of symmetrical chemical reactions, such as solvent and ligand exchange processes, where no net chemical reaction occurs, the overall reaction volume is zero, and only the volume of activation, obtained from the pressure dependence of the observed rate constant, is needed to construct a volume profile for such a reaction. This is different in the case of nonsymmetrical chemical reactions, such as ligand substitution, isomerization, addition, elimination, etc., where the activation and reaction volumes are needed in order to construct a volume profile for the reaction under study. This has now been done for numerous examples[4,5] and has greatly assisted the interpretation of volumes of activation in terms of volume changes along the reaction coordinate for the overall chemical process. There are, however, numerous cases of nonsymmetrical reactions where it is experimentally not possible to measure the overall reaction volume and hence mechanistic information can only be obtained from an interpretation of the volumes of activation.[2,4–6]

A similar situation has been encountered in the case of symmetrical and nonsymmetrical intermolecular (outer-sphere) electron-transfer reactions.[7] In the case of self-exchange reactions, the overall reaction volume is zero and theoretical calculations based on the Marcus–Hush theory, as developed by Stranks and Swaddle, can be used to assist the interpretation of the observed volumes of activation for such processes (see refs 4,7, and 8–11 and the literature cited therein). Again the situation is totally different for nonsymmetrical electron-transfer reactions where it is essential to know the reaction volume in order to interpret the observed volumes of activation.[12,13] This is in many cases a difficult task since nonsymmetrical, intermolecular electron-transfer reactions are frequently nonreversible processes and undergo subsequent decomposition reactions that prevent the determination of the overall reaction volume.[7,13] In this respect we have started to study the effect of pressure on intramolecular and intermolecular electron transfer between cytochrome *c* and ruthenium ammine complexes,[14] but were up to now unable to construct the associated volume profiles due to the absence of reaction volume data. Nevertheless, the observed volumes of activation were rather encouraging and motivated further work in this area.

We have now been able to investigate the pressure dependence of intermolecular electron transfer between horse-heart cytochrome *c* and $[Ru(NH_3)_5(isonicotinamide)]^{2+/3+}$ in both directions, as well as the pressure dependence of the overall

---

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1994.

(1) (a) University of Witten/Herdecke. (b) On leave from the Departamento de Quimica Fisica Aplicada, Universidad Autonoma, Madrid 28049, Spain. (c) Brookhaven National Laboratory. (d) Rutgers, the State University of New Jersey.

(2) van Eldik, R.; Asano, T.; le Noble, W. J. *Chem. Rev.* **1989**, 89, 549.

(3) Akitt, J. W.; Merbach, A. E. *NMR Basic Princ. Prog.* **1990**, *24*, 189.

(4) van Eldik, R.; Merbach, A. E. *Comments Inorg. Chem.* **1992**, *12*, 341.

(5) van Eldik, R. In *Perspectives in Coordination Chemistry*; Williams, A. F., Floriani, C., Merbach, A. E. Eds.; VHCA, VCH: Basel, Weinheim, 1992; p 55.

(6) van Eldik, R. *Organometallics in Organic Synthesis* Vol. 4, Vieweg Verlag: Wiesbaden, Germany, 1993, p 27.

(7) van Eldik, R. *High Pressure Res.* **1991**, *6*, 251.

(8) Swaddle, T. W. *Inorg. Chem.* **1990**, *29*, 5017.

(9) Jolley, W. H.; Stranks, D. R.; Swaddle, T. W. *Inorg. Chem.* **1990**, *29*, 1948.

(10) Anderson, K. A.; Wherland, S. *Inorg. Chem.* **1991**, *30*, 624.

(11) Bänsch, B.; Martinez, P.; van Eldik, R. *J. Phys. Chem.* **1992**, *96*, 234.

(12) Heremans, K.; Bormans, M.; Snauwaert, J.; Vandersypen, H. *Faraday Discuss. Chem. Soc.* **1982**, *74*, 343.

(13) Krack, I.; van Eldik, R. *Inorg. Chem.* **1990**, *29*, 1700.

(14) Wishart, J. F.; van Eldik, R.; Sun, J.; Su, C.; Isied, S. S. *Inorg. Chem.* **1992**, *31*, 3986.

0020-1669/94/1333-4744$04.50/0

equilibrium constant for this reversible electron-transfer process. These data enable us to construct the first complete volume profile for such a system and also allow a direct comparison of the kinetically and thermodynamically determined reaction volumes. The resulting volume profile reveals a complete description of the reaction under study and assists in the interpretation of related activation volumes measured before.[14]

Experimental Section

Materials. Horse-heart cytochrome *c* (Sigma Type VI) was oxidized with $K_3Fe(CN)_6$ and purified on a CM-52 column by elution with 85 mM pH 7 sodium phosphate buffer. Reduced cytochrome *c* was prepared by reducing the purified oxidized cytochrome *c* with an excess of ascorbic acid followed by exhaustive ultrafiltration with degassed 50 mM phosphate buffer. Concentrations and redox states of the cytochrome solutions were confirmed by UV–vis absorption spectroscopy.

Pentaammine(isonicotinamide)ruthenium(II) perchlorate, $[Ru(NH_3)_5isn](ClO_4)_2$, was prepared by the method of Ford et al.[15] Pentaammine(isonicotinamide)ruthenium(III) perchlorate was prepared by addition of 206 mg of $[Ru(NH_3)_5isn](ClO_4)_2$ to a solution prepared by dissolving 67 mg of Ag_2O using a minimum of trifluoroacetic acid in ca. 3 mL of water. The silver metal precipitate was removed by filtration through a 0.2 μm syringe filter. Addition of an equal volume of saturated sodium perchlorate resulted in the precipitation of 101 mg of $[(NH_3)_5Ru(isn)](ClO_4)_3$, which was filtered, washed once with ethanol and 1:4 ethanol/ether, and washed twice with ether. Yield: 41%.

All solutions were prepared with deionized Millipore water, using Tris buffer and $LiClO_4$ to adjust the ionic strength. All solutions were saturated with Ar to avoid possible complications with dissolved oxygen and were protected from light to avoid photochemical decomposition.

Measurements. UV-vis spectra were recorded on a Shimadzu UV250 spectrophotometer. The most useful wavelengths for following the electron-transfer reaction at the selected concentrations for stopped-flow kinetics were 312 and 470 nm for the reduction and oxidation of cytochrome *c* by $[Ru(NH_3)_5isn]^{2+/3+}$, respectively. The kinetic measurements at ambient pressure were performed on a Durrum D110 stopped-flow system, whereas a homemade high-pressure stopped flow was used for measurements at elevated pressure.[16] Both instruments were thermostated to ±0.1 °C. A Biologic (Claix, France) personal computer-based data collection system was used. Kinetic data, consisting of 1000 points per run, were processed using the KINFIT set of programs (On-Line Instrument Systems, Jefferson, GA). All reactions were followed to completion, and the kinetic traces resulted in excellent first-order fits over 3–4 half-lives. The reported rate constants are the mean of at least six runs, and the quoted uncertainties are one standard deviation. Test solutions were transferred to the stopped-flow instrument in polyethylene syringes. The medium used for the stopped-flow experiments was 50 mM Tris buffer, pH 7.1, and 50 mM $LiClO_4$.

Equilibrium measurements were performed in the thermostated cell compartment of the Shimadzu spectrophotometer at ambient pressure and on a Zeiss DMR 10 spectrophotometer equipped with a high-pressure cell[17] for pressures up to 150 MPa.

The reduction potentials of horse heart ferricytochrome *c* and $[Ru(NH_3)_5isn]^{3+}$ were measured in nearly the same medium used for the stopped-flow experiments (50 mM Tris buffer, pH 7.0, and 50 mM $NaClO_4$) by differential pulse polarography at a polished gold disk electrode which had been treated with 4,4′-dipyridyl disulfide, $(NC_5H_4-S)_2$. Data were collected on a Bioanalytical Systems 100 electrochemical analyzer.

Results and Discussion

The oxidation of Cyt c^{II} by $[Ru^{III}(NH_3)_5isn]^{3+}$ proceeds according to the reversible reaction 1

$$Ru(NH_3)_5isn^{3+} + \text{Cyt } c^{II} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Ru(NH_3)_5isn^{2+} + \text{Cyt } c^{III} \qquad (1)$$

and is accompanied by characteristic spectral changes between 300 and 600 nm. The driving force for this reaction is 0.112 eV (see below), such that appropriate experimental conditions can be selected to follow the reaction in both directions. The kinetic data as a function of concentration, temperature, and pressure for both reactions are summarized in Table 1 along with the calculated activation parameters. On the basis of the measured driving force for reaction 1, the overall equilibrium constant $K = 79$ at 25 °C and 0.1 M ionic strength. All kinetic measurements were performed under pseudo-first-order conditions with an excess of Ru(II/III). Plots of k_{obs} versus Ru(III) concentration for the forward reaction in (1) are linear and exhibit no contribution from the reverse reaction, under the selected experimental conditions, with $k_f = 1.1 \times 10^5\ M^{-1}\ s^{-1}$ at 25 °C and 0.1 M ionic strength. This reaction rate is significantly decelerated with increasing pressure, with an average volume of activation of $+16.0 \pm 1.5\ cm^3\ mol^{-1}$.

The activation entropy $\Delta S^{\ddagger}$ for the forward reaction is $-75 \pm 3\ J\ K^{-1}\ mol^{-1}$, and that for the reverse reaction is $-87 \pm 12\ J\ K^{-1}\ mol^{-1}$. In order to compare the observed activation entropy with other reactions, it is necessary to correct it[18] by $-\Delta S°/2$ for the contribution of the net reaction entropy ($\Delta S° = +12 \pm 12\ J\ K^{-1}\ mol^{-1}$). The corrected activation entropy of $-81 \pm 7\ J\ K^{-1}\ mol^{-1}$ is typical for redox reactions of this type between a metal complex and a metalloprotein.[18] Outer-sphere exchange reactions between like-charged octahedral metal complexes usually have significantly more negative corrected activation entropies (-100 to $-150\ J\ K^{-1}\ mol^{-1}$) due to increased electrostriction in the precursor complex, while reactions between metalloproteins show small negative or even positive corrected activation entropies (-5 to $+10\ J\ K^{-1}\ mol^{-1}$), primarily due to protein desolvation in the precursor complex.[18]

It is a standard feature of contemporary electron transfer studies to compare the observed rate constant with that predicted by the Marcus cross-relation:[19-21]

$$k_{12} = (k_{11}k_{22}K_{12}f_{12})^{1/2}W_{12} \qquad (2)$$

$$\ln f_{12} = (\ln K_{12})^2/(4\ln(k_{11}k_{22}/Z^2)) \qquad (3)$$

$$W_{12} = \exp[-(w_{12} + w_{21} - w_{11} - w_{22})/2RT] \qquad (4)$$

The collision frequency Z in eq 3 is assumed to be $\sim 1 \times 10^{11}\ M^{-1}\ s^{-1}$. The electrostatic work correction term W_{12} is given in (4),[19] where the individual terms w_{12} and w_{21} represent the electrostatic work required to bring the reactants or products to the separation distance in the transition state and w_{11} and w_{22} represent the electrostatic work involved in the self-exchange reactions.

$$w_{ij} = z_iz_je_0^2N_A/4\pi\epsilon_0\epsilon\sigma(1 + \chi\sigma) \qquad (5)$$

In eq 5, z_i and z_j are the charges on the ions, e_0 is the electronic charge, ϵ_0 is the permittivity of vacuum, ϵ is the bulk dielectric constant, σ is the contact distance of the ions ($\sigma = r_i + r_j$), and χ is the reciprocal Debye–Hückel length. For aqueous solution

---

(15) Ford, P. C.; Rudd, De F. P.; Gaunder, R.; Taube, H. *J. Am. Chem. Soc.* **1968**, *90*, 1187.

(16) (a) van Eldik, R.; Palmer, D. A.; Schmidt, R.; Kelm, H. *Inorg. Chim. Acta* **1981**, *50*, 131. (b) van Eldik, R.; Gaede, W.; Wieland, S.; Kraft, J.; Spitzer, M.; Palmer, D. A. *Rev. Sci. Instrum.* **1993**, *64*, 1355.

(17) Fleischmann, F. K; Conze, E. G.; Stranks, D. R.; Kelm, H. *Rev. Sci. Instrum.* **1974**, *45*, 1427.

(18) Sutin, N. *Adv. Chem. Ser.* **1977**, *No. 162*, 156–172.

(19) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–322.

(20) Sutin, N. *Prog. Inorg. Chem.* **1983**, *30*, 441.

(21) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891.

Table 1. Summary of Rates and Activation Parameters for the Reversible Reaction[a]

$$\mathrm{Ru(NH_3)_5isn^{3+} + Cyt}\ c^{\mathrm{II}} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{Ru(NH_3)_5isn^{2+} + Cyt}\ c^{\mathrm{III}}$$

temp, °C	P, MPa	[Ru(III)], M	k_{obs},[b] s^{-1}	k_f,[c] $M^{-1}\ s^{-1}$	activation params
25.0	0.1	1.98×10^{-4}	23.3 ± 1.6	$(1.08 \pm 0.03) \times 10^5$	
		4.00×10^{-4}	46.2 ± 3.1		
		5.93×10^{-4}	65.8 ± 6.0		
10.0	0.1	4.00×10^{-4}	27.4 ± 2.0	6.85×10^4	$\Delta G^{\ddagger}_{298} = 44.1$ kJ mol^{-1}
15.0			33.9 ± 3.6	8.47×10^4	
20.0			38.5 ± 2.6	9.62×10^4	$\Delta H^{\ddagger} = 21.8 \pm 0.9$ kJ mol^{-1}
25.0			46.2 ± 3.1	11.5×10^4	
30.0			54.9 ± 2.5	13.7×10^4	$\Delta S^{\ddagger} = -75 \pm 3$ J $K^{-1}\ mol^{-1}$
15.0	5	3.96×10^{-4}	35.5 ± 2.6		$\Delta V^{\ddagger} = +14.2 \pm 1.5$ $cm^3\ mol^{-1}$
	50		25.8 ± 1.8		
	100		20.2 ± 1.3		
15.0	10	3.96×10^{-4}	38.3 ± 1.0		$\Delta V^{\ddagger} = +18.0 \pm 0.5$ $cm^3\ mol^{-1}$
	40		30.6 ± 1.9		
	70		23.9 ± 1.9		
	100		19.6 ± 1.4		
15.0	10	3.96×10^{-4}	33.7 ± 2.6		$\Delta V^{\ddagger} = +15.9 \pm 0.7$ $cm^3\ mol^{-1}$
	40		27.1 ± 1.4		
	70		20.9 ± 2.5		
	100		18.6 ± 1.5		

temp, °C	P, MPa	[Ru(II)], M	k_{obs},[b] s^{-1}	k_b,[d] $M^{-1}\ s^{-1}$	activation params
25.0	0.1	2.46×10^{-4}	1.03 ± 0.04	1520 ± 130	
		4.88×10^{-4}	1.53 ± 0.04		
		7.39×10^{-4}	1.83 ± 0.04		
		9.86×10^{-4}	2.18 ± 0.07		
10.0	0.1	2.56×10^{-4}	0.799 ± 0.028	1.04×10^3	$\Delta H^{\ddagger} = 28.0 \pm 3.7$ kJ mol^{-1}
15.0			0.929 ± 0.015	1.39×10^3	$\Delta S^{\ddagger} = -87 \pm 12$ J $K^{-1}\ mol^{-1}$
20.0			0.995 ± 0.020	1.91×10^3	
25.0			1.20 ± 0.06	2.03×10^3	
30.0			1.39 ± 0.04	2.47×10^3	
10.0	0.1	10.0×10^{-4}	1.57 ± 0.20		
15.0			1.96 ± 0.19		
20.0			2.42 ± 0.11		
25.0			2.71 ± 0.17		
30.0			3.23 ± 0.12		
25.0	10	9.96×10^{-4}	2.48 ± 0.11	1.82×10^3	$\Delta V^{\ddagger} = -17.2 \pm 1.5$ $cm^3\ mol^{-1}$
	40		2.95 ± 0.23	2.28×10^3	
	70		3.55 ± 0.35	2.95×10^3	
	100		4.02 ± 0.34	3.35×10^3	
25.0	10	9.96×10^{-4}	2.56 ± 0.19		
	40		3.00 ± 0.26		
	70		3.67 ± 0.19		
25.0	10	2.56×10^{-4}	1.13 ± 0.03		
	40		1.26 ± 0.05		
	70		1.37 ± 0.03		
	100		1.54 ± 0.03		

[a] Experimental conditions: [Cyt c] = 2×10^{-5} M; pH = 7.1; [Tris buffer] = 0.05 M; [$LiClO_4$] = 0.05 M; ionic strength = 0.1 M; wavelength = 470 nm for Ru^{III} + Cyt c^{II}, 312 nm for Ru^{II} + Cyt c^{III}. [b] Mean value of at least 6 kinetic runs. [c] k_f calculated from the slope of k_{obs} versus [Ru(III)]. [d] k_b calculated from the slope of k_{obs} versus [Ru(II)]—see Discussion.

at 25 °C, $\epsilon = 78.5$ and $\chi = 3.29\mu^{1/2}$ nm^{-1} with the ionic strength μ in mol dm^{-3}.[22]

To estimate $k_{f,calc}$, the following values were employed (μ = 0.1 M, pH = 7). For the Ru(III)/Ru(II) couple: $E°_{11} = 0.385$ V,[23] $k_{11} = 1.1 \times 10^5$ $M^{-1}\ s^{-1}$,[24] radii 0.40/0.42 nm.[25] For the Cyt c^{III}/Cyt c^{II} couple: $E°_{22} = 0.273$ V,[23] $k_{22} = 350$ $M^{-1}\ s^{-1}$,[26] radius 1.66 nm,[27] charges +7.5/+6.5.[27] From the difference in reduction potentials, the free energy change for the overall reaction $\Delta G°_{12} = -10.8$ kJ mol^{-1} and $K_{12} = 78.6$. The various work terms from eq 5 are as follows: $w_{12} = 5.3$, $w_{21} = 4.0$, $w_{11} = 7.0$, and $w_{22} = 5.8$ kJ mol^{-1} at 25 °C and 0.1 M ionic strength. Taking the resulting work correction factor of W_{12} = 2.0 and $f_{12} = 0.866$, we obtain $k_{f,calc} = 1.0 \times 10^5$ $M^{-1}\ s^{-1}$, in excellent agreement with the observed value of 1.1×10^5 M^{-1} s^{-1}.

Due to the unfavorable equilibrium constant for measurement of the rate of electron transfer in the reverse direction (k_b, Ru(II)-to-Fe(III)), it is important to consider the effect of the forward step (k_f, Fe(II)-to-Ru(III)) when interpreting the kinetic

---

(22) Martinez, P.; Mohr, R.; van Eldik, R. *Ber. Bunsen. Phys. Chem.* **1986**, *90*, 609.

(23) Data measured in this study. The cytochrome potential agrees with that reported in: Drake, P. L.; Hartshorn, R. T.; McGinnis, J. M.; Sykes, A. G. *Inorg. Chem.* **1989**, *28*, 1361.

(24) Brown, G. M.; Krentzien, H. Z.; Abe, M.; Taube, H. *Inorg. Chem.* **1979**, *18*, 3374.

(25) Mean values estimated from a comparison with other pentaammineruthenium complexes.

(26) Gupta, R. K. *Biochim. Biophys. Acta* **1973**, *292*, 291–295. Calculated for 25 °C.

(27) Heremans, K. In *Inorganic High Pressure Chemistry: Kinetics and Mechanisms*; van Eldik, R., Ed.; Elsevier: Amsterdam, 1986; p 376.

data for the reverse reaction as a function of Ru(II) concentration. In fact, it would have been desirable to study the reverse reaction in the presence of excess Ru(III) to ensure pseudo-first-order conditions for both reaction steps. However, under these conditions the absorbance changes become too small to obtain accurate kinetic data. We therefore worked at the highest possible Ru(II) concentration to ensure pseudo-first-order conditions and to minimize the contribution of the forward step. The data for the reverse reaction in Table 1 were therefore used to obtain k_b from the slope of k_{obs} versus Ru(II) concentration, which results in a value of 1.5×10^3 $\mathrm{M^{-1}\ s^{-1}}$ at 25 °C. It follows that the overall equilibrium constant $K = k_f/k_b = 71 \pm 7$, which is very close to the thermodynamic value reported above. The reverse reaction is significantly accelerated by pressure, and the observed effect strongly depends on the Ru(II) concentration due to the interference of the forward reaction. The pressure dependence of k_b results in a $\Delta V^{\ne}$ value of -17.2 ± 1.5 $\mathrm{cm^3\ mol^{-1}}$, which indicates a significant volume collapse on going to the transition state.

Further analysis of reaction 1 requires consideration of the elementary steps involved in the outer-sphere electron-transfer process. The standard Marcus–Hush model[19–21,28] describes a three-step process: outer-sphere precursor formation (K_{OS}), rate-determining electron-transfer (k_{ET}), and successor dissociation, as outlined in (6)–(8).

$$\mathrm{Ru(NH_3)_5isn^{3+}} + \mathrm{Cyt}\ c^{\mathrm{II}} \overset{K_{\mathrm{OS}}}{\rightleftharpoons} \{\mathrm{Ru(NH_3)_5isn^{3+}}\text{·}\mathrm{Cyt}\ c^{\mathrm{II}}\} \quad (6)$$

$$\{\mathrm{Ru(NH_3)_5isn^{3+}}\text{·}\mathrm{Cyt}\ c^{\mathrm{II}}\} \overset{k_{\mathrm{ET}}}{\rightleftharpoons} \{\mathrm{Ru(NH_3)_5isn^{2+}}\text{·}\mathrm{Cyt}\ c^{\mathrm{III}}\} \quad (7)$$

$$\{\mathrm{Ru(NH_3)_5isn^{2+}}\text{·}\mathrm{Cyt}\ c^{\mathrm{III}}\} \rightleftharpoons \mathrm{Ru(NH_3)_5isn^{2+}} + \mathrm{Cyt}\ c^{\mathrm{III}} \quad (8)$$

This mechanism is related to the overall reaction 1 by $k_f = K_{OS}k_{ET}$. For the system investigated here it is not possible to separate K_{OS} and k_{ET} kinetically. Theoretical approaches to prediction of these quantities for protein-small molecule redox reactions are detailed in ref 19. The expression for precursor complex formation (eq 9, in SI units) includes a steric factor S due to the fact that the active site covers only a small part of the protein surface.

$$K_{\mathrm{OS}} = SK_{\mathrm{A}} = S(4000\pi N_{\mathrm{A}}\sigma^3/3)\mathrm{e}^{-w_{12}/RT} \quad (9)$$

$$k_{\mathrm{ET}} = \kappa_{\mathrm{el}}\nu_{\mathrm{n}}\mathrm{e}^{-\Delta G^*_r/RT} \quad (10)$$

The electron transfer rate k_{ET} within the precursor complex depends on the electronic transmission coefficient κ_{el}, the nuclear frequency factor ν_n, and the free energy barrier of reorganization ΔG^*_r.

$$\Delta G^*_{\mathrm{r}} = (\lambda_{12}/4)\left(1 + \frac{\Delta G^{\circ}_{12} + w_{21} - w_{12}}{\lambda_{12}}\right)^2 \quad (11)$$

$$\lambda_{12} = (\lambda_{11} + \lambda_{22})/2 \quad (12)$$

Several methods for estimating σ, S, κ_{el}, and ν_n are discussed in refs 19 and 20. Marcus and Sutin[19] have inferred from the maximum value of the rate constant, observed for ruthenium and osmium polypyridine complexes in the barrierless regime ($-\Delta G^{\circ} \approx \lambda$), that the product $SK_A\kappa_{el}\nu_n \approx (1-3) \times 10^8$ $\mathrm{M^{-1}\ s^{-1}}$ for reactions of the type discussed here. Taking the higher value and their estimates of the reorganization energies for a ruthenium complex similar to the one used here ($[\mathrm{Ru^{II/III}(NH_3)_5(histidine)}]$, $\lambda_{11} = 108$ $\mathrm{kJ\ mol^{-1}}$) and for cytochrome *c* ($\lambda_{22} = 100$ $\mathrm{kJ\ mol^{-1}}$), we calculate $k_{f,est} = 8.3 \times 10^4$ $\mathrm{M^{-1}\ s^{-1}}$ $k_{b,est} = 6.3 \times 10^2$ $\mathrm{M^{-1}\ s^{-1}}$. These estimated rate constants are within a factor of 2–3 of the observed values, supporting the validity of the general approach.

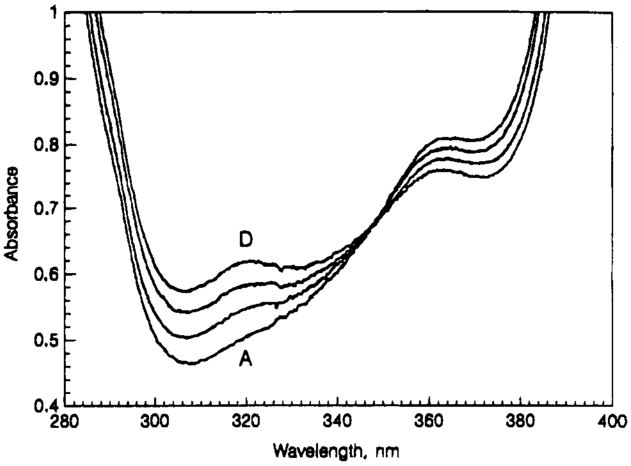

Figure 1. UV–vis spectra recorded as a function of pressure for the overall equilibrium $\mathrm{Ru(NH_3)_5isn^{3+}} + \mathrm{Cyt}\ c^{\mathrm{II}} \rightleftharpoons \mathrm{Ru(NH_3)_5isn^{2+}} + \mathrm{Cyt}\ c^{\mathrm{III}}$. Experimental conditions: [Cyt *c*] = 1.0×10^{-5} M; [Ru] = 2.5×10^{-4} M; pH = 7.0; [Tris buffer] = 0.05 M; ionic strength = 0.10 M; T = 26.5 °C; pressure = 5 (A), 50 (B), 100 (C), 150 (D) MPa.

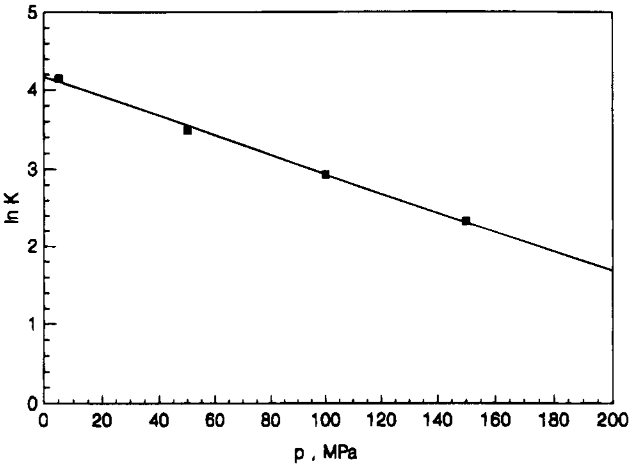

Figure 2. Plot of ln K versus pressure for the overall equilibrium $\mathrm{Ru(NH_3)_5isn^{3+}} + \mathrm{Cyt}\ c^{\mathrm{II}} \rightleftharpoons \mathrm{Ru(NH_3)_5isn^{2+}} + \mathrm{Cyt}\ c^{\mathrm{III}}$. Experimental conditions are as in Figure 1.

The overall equilibrium constant for reaction 1 was measured spectrophotometrically by the systematic addition of $\mathrm{Ru(NH_3)_5isn^{2+}}$ to Cyt c^{III}. An increase in the absorbance of the mixture at 312 nm as a function of [Ru(II)] was observed. The difference in absorbance before and after mixing the two reactants was used to estimate the equilibrium constant. This resulted in $K = 67 \pm 11$ at 25 °C, which is in close agreement with that obtained from the kinetic and potential data. The pressure dependence of K was determined by recording the spectrum of an equilibrium mixture of reaction 1 as a function of pressure as shown in Figure 1. The observed spectral changes are reversible when the pressure is systematically decreased again. It can clearly be seen that the spectra exhibit a clean isosbestic point at 350 nm and an increase in pressure causes a decrease in [Cyt c^{III}] and an increase in [Cyt c^{II}]. These spectral changes can be used to estimate the value of K at various pressures, which results in the data in Figure 2, from which it follows that $\Delta\bar{V}(K) = +31 \pm 1$ $\mathrm{cm^3\ mol^{-1}}$. This value is in excellent agreement with that estimated from the kinetic data, viz. $\Delta\bar{V}(K) = \Delta V^{\ne}(k_f) - \Delta V^{\ne}(k_b) = (16.0 \pm 1.5) - (-17.2 \pm$

---

[28] Hush, N. S. *Trans. Faraday Soc.* **1961**, *57*, 557.

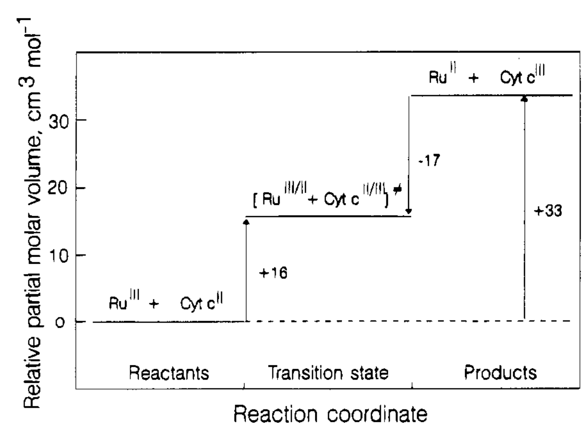

Figure 3. Volume profile for the equilibrium process $Ru(NH_3)_5isn^{3+}$ + Cyt $c^{II} \rightleftharpoons Ru(NH_3)_5isn^{2+}$ + Cyt c^{III}.

1.5) = +33 ± 2 $cm^3\ mol^{-1}$, and demonstrates the good correlation between thermodynamic and kinetic data obtained from pressure dependence studies.

The volumes of activation determined for the forward and back reactions in (1) can be used to construct the volume profile in Figure 3. To our knowledge, this is the first complete volume profile for a nonsymmetrical, reversible, intermolecular electron-transfer reaction. The overall reaction is accompanied by a large volume increase, and the volume of the transition state is halfway between those of the reactant and product species. This means that the extents of reorganization of the reactant and product species for this reversible process are very similar for the forward and back reactions, respectively. A comparison with data for related systems involving the Cyt $c^{II/III}$ couple in Table 2 reveals some interesting trends. Reduction of Ru(III) to Ru(II) is always accompanied by a positive $\Delta V^{\neq}$ value, compared to a negative value for the oxidation of Ru(II) to Ru(III) independent of whether we are dealing with an intermolecular or intramolecular process. Very similar effects were observed[12] for the reduction of Co(III) and oxidation of Co(II), which involves the same metal ion charge types as the Ru(II/III) system. However, in the case of the $Fe(CN)_6^{3-/4-}$ system[12] the reduction of Fe(III) is accompanied by a volume decrease and the oxidation of Fe(II) by a volume increase, i.e. exactly opposite to that observed in the case of Ru and Co complexes. These trends clearly demonstrate that the observed $\Delta V^{\neq}$ is mainly controlled by electrostriction effects on the redox partner of the Cyt *c* system. Reduction of Ru(III) and Co(III) complexes will result in a large volume increase due to charge neutralization, and vice versa for the oxidation reactions. On the other hand, reduction of $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ will be accompanied by a large volume decrease due to the increase in electostriction, and the opposite is true for the oxidation reaction. A direct comparison of the $\Delta V^{\neq}$ values in Table 2 can only be made for related systems involving the same metal center. The intramolecular ruthenium systems quoted in Table 2 show similar behavior to that reported here, whereas the cobalt and iron systems provide additional evidence that electrostriction dominates the volume profiles of redox reactions of metal complexes with cytochrome *c*.

A direct comparison of the $\Delta\bar{V}$ value measured for reaction 1, viz. between +31 and +33 $cm^3\ mol^{-1}$, with that measured electrochemically for the reduction of $Ru(NH_3)_6^{3+}$, viz. +29 ± 1 $cm^3\ mol^{-1}$,[29] indicates that the observed volume changes are governed by changes on the Ru center. This further supports the conclusion that the redox process causes no major volume change on the Cyt *c* molecule. A similar conclusion was arrived at in earlier work.[12,14] However, in a very recent paper[30] electrochemical measurements on the cytochrome *c* system at elevated pressure revealed a volume decrease of 24 $cm^3\ mol^{-1}$ on the reduction of Cyt c^{III} to Cyt c^{II}. This finding is at variance with that reported in this paper and elsewhere.[12,14] Possible reasons for this discrepancy are presently being explored through further electrochemical experiments.[31]

It is possible to estimate the volumes of activation theoretically according to the treatments outlined by Stranks,[32] Swaddle et al.,[33,34] and Wherland et al.[35,36] as indicated in (13). In this

$$\Delta V^{\neq} = \Delta V^{*}_{COUL} + \Delta V^{*}_{DH} + \Delta V^{*}_{SR} + \Delta V^{*}_{IR} + \beta RT + \lambda^{*}\Delta\bar{V} \quad (13)$$

equation ΔV^{*}_{COUL} represents the Coulombic work to bring the reactants together at infinite dilution, ΔV^{*}_{DH} the Debye–Hückel correction for finite ionic strength (0.1 M), ΔV^{*}_{SR} the rearrangement of the surrounding solvent molecules, ΔV^{*}_{IR} the inner-sphere rearrangement which is small enough to be neglected (≤0.5 $cm^3\ mol^{-1}$),[32] βRT the contribution of the preexponential factor[34] (β is the isothermal compressibility of the solvent), and $\lambda^{*}\Delta\bar{V}$ the contribution due to net volume changes for a nonsymmetrical electron-transfer reaction. A detailed account of the equations employed to calculate the various contributions is given elsewhere.[11,33] The transition-state electron probability density parameter λ^{*} (or $|m|$,[37] not to be confused with the reorganization energy λ) has values between 0 and 1 depending on the location of the transition state along the reaction coordinate.[28] It can be calculated from eq 9, which results in $\lambda^{*} = 0.44$.

$$\lambda^{*} = {}^{1}/_{2}\left(1 + \frac{\Delta G^{\circ}_{12} + w_{21} - w_{12}}{\lambda_{12}}\right) \quad (14)$$

If the same parameter values used for the rate calculations above are applied, the results obtained for the various volume contributions are (in $cm^3\ mol^{-1}$) are as follows: $\Delta V^{*}_{COUL} = -5.4$, $\Delta V^{*}_{DH} = +6.5$, $\Delta V^{*}_{SR} = -5.7$, $\lambda^{*}\Delta\bar{V} = 0.44(33) = 14.6$, and $\beta RT = +1.3$. These values result in $\Delta V^{\neq}(k_f) = +11.3$ $cm^3\ mol^{-1}$, which is considerably smaller than the experimental value of +16.0 ± 1.5 $cm^3\ mol^{-1}$. For the back reaction we find $\lambda^{*} = 0.56$ and the volume contributions (in $cm^3\ mol^{-1}$) to be as follows: $\Delta V^{*}_{COUL} = -4.1$, $\Delta V^{*}_{DH} = +5.0$, $\Delta V^{*}_{SR} = -5.5$ and $\lambda^{*}\Delta\bar{V} = 0.54(-33) = -18.4$, and $\beta RT = +1.3$. These values result in $\Delta V^{\neq}(k_b) = -21.5$ $cm^3\ mol^{-1}$, which is considerably more negative than the experimental value of −17.2 ± 1.5 $cm^3\ mol^{-1}$.

The observed differences between the experimental and calculated $\Delta V^{\neq}$ values may arise from overestimation of the contribution of the solvent rearrangement term, ΔV^{*}_{SR}, which may be much smaller for a bulky molecule as cytochrome *c* than in the case of model complexes. On the basis of the

---

(29) Sachinidis, J.; Shalders, R. D.; Tregloan, P. A. Presented at the 9th National Convention of the Royal Australian Chemical Institute, Melbourne, 1992; WI-32.

(30) Cruanes, M. T.; Rodgers, K. K.; Sligar, S. G. *J. Am. Chem. Soc.* **1992**, *114*, 9660.

(31) Sun, J.; Wishart, J. F.; van Eldik, R.; Shalders, R. D.; Swaddle, T. W. Submitted for publication.

(32) Stranks, D. R. *Pure Appl. Chem.* **1974**, *38*, 303.

(33) Spiccia, L.; Swaddle, T. W. *Inorg. Chem.* **1987**, *26*, 2265.

(34) Jolley, W. H.; Stranks, D. R.; Swaddle, T. W. *Inorg. Chem.* **1990**, *29*, 385.

(35) Wherland, S. *Inorg. Chem.* **1983**, *22*, 2349.

(36) Murguia, M. A.; Wherland, S. *Inorg. Chem.* **1991**, *30*, 139.

(37) For the sake of consistency with the literature, we follow the notation of Hush[28] and Stranks[32] in this instance. In the Marcus notation, the absolute value of the Lagrangian parameter *m* would be used.

Table 2. Summary of Rate and Activation Parameters for Related Intramolecular and Intermolecular Electron-Transfer Reactions

reacn[a]	$-\Delta G°$, eV	k_{298}	$\Delta H^{\ddagger}$, kJ mol^{-1}	$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$, cm^3 mol^{-1}	$\Delta \bar{V}$, cm^3 mol^{-1}	ref
Intramolecular Reactions							
A_5Ru^{II}–His(33)hh^{III}	0.13	39 s^{-1}	14.6	−163	−17.7		14
A_5Ru^{II}–His(39)ck^{III}	0.18	87 s^{-1}	9.6	−171	−18.3		14
Intermolecular Reactions							
$A_6Ru^{II} + hh^{III}$	0.27	6.3×10^4 M^{-1} s^{-1}	3.8	−142	−15.6		14
$hh^{II} + A_5Ru^{III}isn$	0.11	1.1×10^5 M^{-1} s^{-1}	22	−75	+16.0	+33,[b] +31[c]	this work
$A_5Ru^{II}isn + hh^{III}$	−0.11	1.5×10^3 M^{-1} s^{-1}	28	−87	−17.2		
$hh^{II} + Co^{III}(phen)_3^{3+}$	0.12	1.9×10^3 m^{-1} s^{-1}			+8.5	+20	12
$Co^{II}(phen)_3^{2+} + hh^{III}$	−0.12				−11.5[b]		
$hh^{II} + Fe^{III}(CN)_6^{3-}$	0.15	3.0×10^6 M^{-1} s^{-1}			−24[b]	−37	12
$Fe^{II}(CN)_6^{4-} + hh^{III}$	−0.15	1.2×10^4 M^{-1} s^{-1}			+13		

[a] Abbreviations: A = NH_3, hh = horse-heart Cyt *c*, ck = *Candida krusei* Cyt *c*. [b] Estimated from $\Delta \bar{V} = \Delta V^{\ddagger}$(forward) − $\Delta V^{\ddagger}$(back). [c] Measured directly—see Discussion.

arguments presented earlier, the solvent rearrangement contribution will mainly come from the ruthenium complex and not from cytochrome *c*. On the basis of the ratio of the radii, this would reduce the ΔV^{*}_{SR} contribution to about 20% of the calculated value, about −1.1 cm^3 mol^{-1}. This in turn will increase the theoretical estimates of $\Delta V^{\ddagger}(k_f)$ and $\Delta V^{\ddagger}(k_b)$ to +15.9 and −17.3, respectively. This correction brings the theoretically estimated $\Delta V^{\ddagger}$ values very close to the experimentally observed values.

We have shown that the major volume effects in this system result from changes at the ruthenium center and not cytochrome *c*. The significant observation that the transition state for the process investigated in this study lies almost exactly halfway between the reactant and product states on a volume basis (see Figure 3) is in excellent agreement with that expected on the basis of the λ^{*} parameter. In the present and previous[14] studies we dealt with redox processes that have a relatively low driving force. For systems with higher driving force than the present study, the reorganization effects on the Ru centers may become less important, such that the $\Delta V^{\ddagger}$ data may reflect other factors which control the electron-transfer process such as electronic coupling.

Acknowledgment. R.v.E. gratefully acknowledges financial support from the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Volkswagen Foundation. The work at Brookhaven National Laboratory was carried out under Contract DE-AC02-76CH-00016 with the U. S. Department of Energy and supported by its Division of Chemical Sciences, Office of Basic Energy Sciences. S.S.I. acknowledges support from the Charles and Joanna Busch Foundation and the National Science Foundation (Grant CHE-840552). The authors thank Dr. Norman Sutin and Dr. Bruce Brunschwig for helpful comments.