Volume 37
Number 24
November 30, 1998

Inorganic Chemistry

Articles

Mechanistic Information from the First Volume Profile Analysis for Intramolecular Electron-Transfer Reactions: Tetraammine−Ruthenium(Ligand) Complexes of Cytochrome *c*

Ji Sun,[†] Chang Su,[†] Martin Meier,[‡] Stephan S. Isied,[§] James F. Wishart,*[,†] and Rudi van Eldik*[,‡]

Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973-5000, Institute for Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany, and Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08854

Received August 19, 1998

The kinetics and thermodynamics of a series of reversible intramolecular electron-transfer reactions in systems of the type *trans*-$(NH_3)_4$(L)Ru(His33)−cyt *c*(hh) and *trans*-$(NH_3)_4$(L)Ru(His39)−cyt *c*(Ck), where L represents NH_3, isonicotinamide, 4-ethylpyridine, 3,5-lutidine, and pyridine, were studied as a function of pressure in order to construct the first complete volume profiles for such processes. The volume profiles demonstrate a significant partial molar volume increase associated with the reduction of the ruthenium center. In contrast to earlier results on a series of intermolecular reactions involving cytochrome *c* and the corresponding pentaammine complexes, for which the volume profiles are completely symmetrical (*Inorg. Chem.* **1996**, *35*, 1564), the studied intramolecular reactions exhibit asymmetric volume profiles. The overall volume changes can be accounted for in terms of electrostriction effects centered around the ammine ligands on the ruthenium center. Explanations in terms of electronic and nuclear factors are offered to account for the asymmetrical nature of the volume profile.

Introduction

The application of high-pressure kinetic techniques in mechanistic studies and the associated construction of volume profiles for the reactions under investigation have added a new dimension to the elucidation of reaction mechanisms.[1−5] In the case of symmetrical self-exchange reactions 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 rate constant is needed to construct a volume profile for such a reaction. The case is different for nonsymmetrical electron-transfer reactions, where the activation and reaction volumes are needed in order to construct the volume profile. Only then can the electron-transfer process be described and interpreted in terms of partial molar volume changes along the reaction coordinate.

With this end in view, we initiated a series of high-pressure studies of intermolecular and intramolecular electron-transfer reactions of cytochrome *c* (cyt *c*)with a series of ruthenium-ammine complexes.[6−9] During the course of our studies, we employed kinetic techniques such as pulse radiolysis and

* To whom correspondence should be addressed.
[†] Brookhaven National Laboratory.
[‡] University of Erlangen-Nürnberg.
[§] Rutgers, The State University of New Jersey.

(1) van Eldik, R.; Asano, T.; le Noble, W. J. *Chem. Rev.* **1989**, *89*, 549.
(2) van Eldik, R.; Merbach, A. E. *Comments Inorg. Chem.* **1992**, *12*, 341.
(3) van Eldik, R. In *Perspectives in Coordination Chemistry*; Williams, A. F., Floriani, C., Merbach, A. E., Eds.; VCH: Basel, Weinheim, 1992; p 55.
(4) van Eldik, R.; Hubbard, C. D. *Instrum. Sci. Technol.* **1995**, *23*, 1.
(5) Kowall, T.; Foglia, F.; Helm, L.; Merbach, A. E. *Chem. Eur. J.* **1996**, *2*, 285.
(6) Wishart, J. F.; van Eldik, R.; Sun, J.; Su, C.; Isied, S. S. *Inorg. Chem.* **1992**, *31*, 3986.
(7) Bänsch, B.; Meier, M.; Martinez, P.; van Eldik, R.; Su, C.; Sun, J.; Isied, S. S.; Wishart, J. F. *Inorg. Chem.* **1994**, *33*, 4744.
(8) Meier, M.; Sun, J.; Wishart, J. F.; van Eldik, R. *Inorg. Chem.* **1996**, *35*, 1564.
(9) Sun, J.; Wishart, J. F.; van Eldik, R.; Shalders, R. D.; Swaddle, T. W. *J. Am. Chem. Soc.* **1995**, *117*, 2600.

10.1021/ic980999f CCC: $15.00
Published on Web 11/04/1998

stopped-flow and thermodynamic techniques such as UV−vis spectrophotometry, cyclic voltammetry, and differential pulse voltammetry, all at pressures up to 200 MPa (2 kbar).

The first profiles were recently reported for a series of low-driving-force intermolecular electron-transfer reactions between $Ru(NH_3)_5L^{3+/2+}$ (L = pyridine (py), 4-ethyl-pyridine (4-etpy), 3,5-lutidine (3,5-lut), and isonicotinamide (isn)) and cytochrome *c*.[7,8] The volume profiles demonstrated that the transition state was located exactly halfway between the reactant and product states on a volume basis. The observed volume effects could mainly be ascribed to changes in electrostriction about the ruthenium complexes, whereas a very small volume contribution resulted from changes on cytochrome *c* itself.[9] This means that solvent reorganization occurs to a similar extent on the ruthenium center, independent of the direction in which the electron-transfer process occurs. By way of comparison, similar results were found for a series of intermolecular electron-transfer reactions between cytochrome *c* and $CoL_{2/3}^{3+/2+}$ (L = 2,2′:6′,2″-terpyridine, 1,10-phenanthroline, and 2,2′-bipyridine).[10,11]

Up to now, no volume profile has been reported for an intramolecular electron-transfer reaction. The driving force for such reactions is usually so high that their kinetics can only be studied in one direction. In addition, subsequent reactions may lead to the decomposition of the redox products. It is therefore important to select reactions in which it is possible to determine the overall reaction volume from the pressure dependence of the equilibrium constant using spectrophotometric or electrochemical techniques. In our first study,[6] we reported volumes of activation of -17.7 ± 0.9 and -18.3 ± 0.7 cm^3 mol^{-1} for electron transfer in horse heart $(NH_3)_5Ru^{II}$−His33 and *Candida krusei* $(NH_3)_5Ru^{II}$−His39, respectively. From electrochemical measurements performed later,[9] we concluded that the reaction volume associated with the electron-transfer reaction in the horse heart system amounted to -31.7 ± 1.2 cm^3 mol^{-1}. This suggests that the transition state is again located approximately halfway between the reactant and product states on a volume basis, with the volume collapse being mainly due to electrostriction effects accompanying the oxidation of Ru(II) to Ru(III).

We have now studied a series of intramolecular electron-transfer reactions from cytochrome *c* to ruthenium in complexes of the type *trans*-$(NH_3)_4(L)Ru^{III}$−His33−cyt c^{II}, where L = isonicotinamide, 4-ethylpyridine, 3,5-lutidine, and pyridine. The activation volumes for the electron-transfer reactions were obtained from high-pressure pulse radiolysis experiments, whereas the overall reaction volumes were determined from differential pulse voltammograms and/or equilibrium determinations by UV−vis spectroscopy recorded as a function of pressure. The reported volume profiles permit a detailed analysis of the effects responsible for the observed pressure dependences and emphasize the important role of solvent electrostriction. Remarkably, the reduction of Ru(III) in these systems exhibits a significantly smaller pressure dependence than the reverse oxidation of Ru(II), i.e., the reaction volume profile is not symmetric. Explanations for this behavior are presented. One interpretation is that increased pressure reduces the nonadiabaticity of the reaction (i.e., increases the electronic coupling between the ruthenium and heme centers). Increased pressure may also reduce the reorganization energy. The implications of activation volume asymmetry on the earliness or lateness of the transition state are considered as well.

Experimental Section

Materials. Ruthenium-modified cytochromes *trans*-$(NH_3)_4(L)Ru$−cyt *c*, L = NH_3, isonicotinamide, 3,5-lutidine, 4-ethylpyridine, and pyridine, were prepared as previously reported.[12,13] Preparations of fully reduced ruthenium-modified cytochrome *c* solutions for pulse radiolysis experiments were performed in an argon-filled glovebox (Vacuum Atmospheres). The modified cytochrome *c* was reduced with an excess of sodium dithionite (50 mM), which was then removed by repeated ultrafiltration over a 3000 Da molecular weight cutoff membrane using argon-saturated 50 mM phosphate buffer. Meanwhile, 15−20 mL of phosphate buffer solution containing 1 mM sodium azide was saturated with N_2O in a bubbler flask in the glovebox. The reduced cytochrome *c* solution was diluted with N_2O-saturated buffer to the desired concentration and placed in a syringe for transfer into a quartz pillbox cell, which was first flushed several times with the solution. After the kinetic measurements, the final concentration of ruthenated cytochrome *c* in the solution (normally 2−20 μM) was verified using a Hewlett-Packard 8452A diode array spectrophotometer to measure the absorbance of a sample as fully reduced protein after addition of sodium ascorbate (ϵ_{416} = 129 100 M^{-1} cm^{-1}, ϵ_{550} = 27 000 M^{-1} cm^{-1}).[14]

High-Pressure Pulse Radiolysis. Electron pulse radiolysis transient-absorption experiments were carried out with the 2 MeV Van de Graaff accelerator at Brookhaven National Laboratory using a PC-controlled, CAMAC-based data acquisition and control system. For each pressure-dependence experiment, a sample was placed in a quartz pillbox cell, as described above, inside a thermostated, four-window, high-pressure vessel.[15] One window of the vessel was modified as described elsewhere[6,16] in order to enable a sufficient electron pulse to penetrate the sample solution. The solutions were stirred between kinetic measurements with the aid of a magnetic bar inside the pillbox cell in the high-pressure vessel. Azide radicals were generated in solution by the reaction of $^{\bullet}OH$ radicals with azide ion (1−10 mM NaN_3) in N_2O-saturated solution.[13]

The intramolecular electron-transfer rates were measured by an oxidative scheme using the pulse-radiolytically generated azide radical (E° = +1.33 V vs NHE). Direct oxidation of the ferrocytochrome center (reaction 1, $k \approx 1 \times 10^9$ M^{-1} s^{-1}) is slower than the oxidation of the ruthenium center (reaction 2, $k \approx 2 \times 10^9$ M^{-1} s^{-1}), permitting observation of a sufficient amount of the kinetic intermediate that decays according to reaction 3.

$$(NH_3)_4(L)Ru^{II}-\text{cyt } c^{II} + N_3^{\bullet} \rightarrow (NH_3)_4(L)Ru^{II}-\text{cyt } c^{III} + N_3^- \quad (1)$$

$$(NH_3)_4(L)Ru^{II}-\text{cyt } c^{II} + N_3^{\bullet} \rightarrow (NH_3)_4(L)Ru^{III}-\text{cyt } c^{II} + N_3^- \quad (2)$$

$$(NH_3)_4(L)Ru^{III}-\text{cyt } c^{II} \rightarrow (NH_3)_4(L)Ru^{II}-\text{cyt } c^{III} \quad (3)$$

High-Pressure Spectrophotometry and Electrochemistry. For some systems, the overall reaction volume for reaction 3 could be determined from UV−vis spectra of equilibrium mixtures recorded as a function of pressure. For this purpose, the high-pressure vessel[15] was mounted in a Cary 210 spectrophotometer and reaction volumes were estimated as described elsewhere.[7,8] In addition, high-pressure differential pulse voltammetry was also employed to determine the reaction volume associated with the electron-transfer process in reaction 3. Details are given elsewhere.[9]

Results

The kinetics of the intramolecular electron-transfer reaction 3 were followed by measuring the decrease in the cyt c^{II} absorption at 550 nm and the increase in the absorbance of the

(10) Meier, M.; van Eldik, R. *Inorg. Chim. Acta* **1994**, *225*, 95.
(11) Meier, M.; van Eldik, R. *Chem. Eur. J.* **1997**, *3*, 39.
(12) Sun, J.; Wishart, J. F.; Gardineer, M. B.; Cho, M. P.; Isied, S. S. *Inorg. Chem.* **1995**, *34*, 3301.
(13) Wishart, J. F.; Sun, J.; Cho, M.; Su, C.; Isied, S. S. *J. Phys. Chem. B* **1997**, *101*, 687.
(14) Margoliash, E. *Biochem. J.* **1959**, *71*, 571.
(15) Spitzer, M.; Gärtig, F.; van Eldik, R. *Rev. Sci. Instrum.* **1988**, *59*, 2092.
(16) Wishart, J. F.; van Eldik, R. *Rev. Sci. Instrum.* **1992**, *63*, 3224.

Table 1. Summary of Activation Volume and Reaction Volume Data for a Series of Intramolecular Electron-Transfer Reactions on $(NH_3)_4(L)Ru-cyt\ c$ at 25 °C (Thermal Activation Parameters Were Taken from Ref 13)

L	electron transfer	$\Delta G°$ eV	k_{obs} s^{-1}	$\Delta H^{‡}$ kcal mol^{-1}	$\Delta S^{‡}$ eu	$\Delta V^{‡}$ cm^3 mol^{-1}	ΔV cm^3 mol^{-1}
				Horse Heart Cytochrome *c*			
NH_3	Ru → Fe	−0.125	53	3.5	−39.0	-14.5 ± 0.4^a $-17.7 \pm 0.9^{b,c}$	-31.7 ± 1.2^d
isn	Fe → Ru	−0.18	440	7.3	−22.0	3.2 ± 0.3^a 4.8 ± 1.0^a 3.2 ± 0.3^b 3.5 ± 0.5^b 3.9 ± 0.4^b	21.1 ± 1.0^d
Etpy	Fe → Ru	−0.08	76	9.0	−19.8	7.4 ± 0.6^a	24.9 ± 1.1^e 22.3 ± 0.8^e 22.8 ± 0.9^e
lut	Fe → Ru	−0.09	85	9.2	−18.9	5.8 ± 0.4^a	23.3 ± 0.9^e 26.4 ± 0.8^e 18.6 ± 0.4^d
py	Fe → Ru	−0.11	126	8.8	−19.3	7.2 ± 0.2^a	23.3 ± 0.6^d
				Candida krusei Cytochrome *c*			
NH_3	Ru → Fe	−0.18	154	2.3	−41	-18.3 ± 0.7^b	
isn	Fe → Ru	−0.13	220	6.4	−27	3.6 ± 0.7^a 4.1 ± 0.9^a 2.5 ± 0.8^b 4.2 ± 0.5^b	

[a] Measurements in Tris buffer. [b] Measurements in phosphate buffer. [c] Data taken from ref 6. [d] Determined electrochemically; see ref 9. [e] Measured spectrophotometrically in this study.

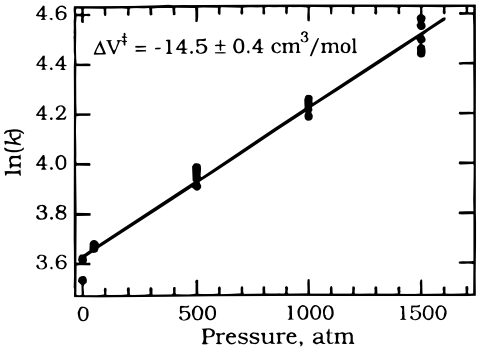

Figure 1. Plot of ln k_{obs} versus pressure for the reaction $(NH_3)_5Ru^{II}-cyt\ c^{III} \rightarrow (NH_3)_5Ru^{III}-cyt\ c^{II}$. Experimental conditions are as follows: [Ru−cyt *c*] = 16.5 μM; [HCOONa] = 90 mM; [Tris] = 10 mM; N_2O sat.; pH = 7.0.

Ru(II) complex at 504 or 432 nm, which are isosbestic points for cyt c^{II} and cyt c^{III}. In the case of the pentaammine complex (L = NH_3), the energetically favorable direction of reaction 3 is reversed. For this purpose, the fully oxidized form of the complex, $(NH_3)_5Ru^{III}-cyt\ c^{III}$, was prepared and reduced to $(NH_3)_5Ru^{II}-cyt\ c^{III}$ with the carbon dioxide radical anion, CO_2^-, produced during the reaction of radiolytically generated hydroxyl radicals with formate ion (see ref 6 for more details on this procedure). Earlier experiments on the pentaammine system were performed in phosphate buffer[6] and repeated in Tris buffer in the present investigation. A typical set of experimental data is presented in Figure 1, from which it follows that the rate constant for the oxidation of Ru(II), i.e., the reverse of reaction 3, increases significantly with increasing pressure. The volume of activation of -14.5 ± 0.4 cm^3 mol^{-1} is slightly smaller than the value of -17.7 ± 0.9 cm^3 mol^{-1} reported earlier for the reaction in phosphate buffer.[6] We preferred to use Tris in the present study because its volume of protonation is close to zero[17] such that the pH of the Tris buffer does not change significantly with pressure. In addition, phosphate buffer has been shown to interact with the ruthenium−ammine complexes and lower their redox potentials.[13] For the experiments in Tris buffer, sufficient sodium azide or sodium formate concentrations were used to scavenge the $OH^•$ radicals and avoid the reaction of $OH^•$ with Tris.

The effect of pressure on reaction 3 for the series of systems $(NH_3)_4(L)Ru^{III}-cyt\ c^{II}$, L = isn, 4-etpy, 3,5-lut, and py, was significantly smaller than expected on the basis of our results for the pentaammine system[6] as well as for the series of intermolecular reactions studied before.[7,8] A typical set of data for the isonicotinamide system is reported in Supporting Information, Figure S1, from which it follows that the reduction of Ru(III) exhibits a slight deceleration with increasing pressure. The resulting volume of activation for this particular example is $+3.2 \pm 0.3$ cm^3 mol^{-1}. The results for the series of complexes are summarized in Table 1, where it is evident that the observed effects are highly reproducible and very similar for all complexes. Experiments were also performed on the isonicotinamide complex of *Candida krusei* cytochrome *c*. The results are included in Table 1 and indicate that a similar pressure dependence is observed for the intramolecular reduction of Ru(III), independent of whether it is attached to His33 in horse heart cytochrome *c* or to His39 in *Candida krusei* cytochrome *c*.

The overall reaction volume for reaction 3 was determined spectrophotometrically for L = 4-etpy and 3,5-lut. Typical examples of ln *K* versus pressure plots are reported in Figure 2 and Figures S2 and S3 in Supporting Information, respectively, from which it follows that no significant deviation from linearity is observed over the employed pressure range. In addition, the overall equilibrium constant decreases significantly with increasing pressure, which results in reaction volumes of ca. 22 cm^3 mol^{-1}. The results are included in Table 1. In some cases, the reaction volume was also determined from high-pressure electrochemical measurements,[9] and these data are also included in Table 1. It is immediately apparent from a comparison of the $\Delta V^{‡}$ and ΔV data that the volume of activation is not close to 50% of the overall reaction volume, as was found for a corresponding series of intermolecular reactions.[7,8] This dif-

(17) Kitamura, Y.; Itoh, T. *J. Solution Chem.* **1987**, *16*, 715.

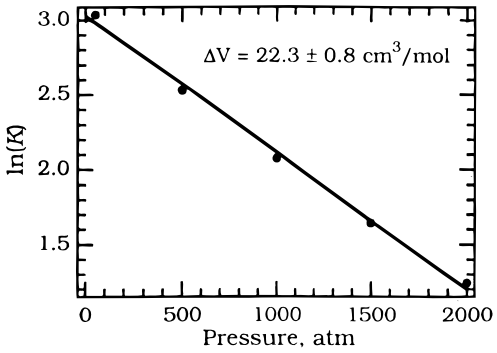

Figure 2. Plot of ln K versus pressure for the equilibrium $(NH_3)_4$(4-Etpy)Ru^{III}−cyt c^{II} ⇌ $(NH_3)_4$(4-Etpy)Ru^{II}−cyt c^{III}. Data were recorded during an increase in pressure. Experimental conditions are as follows: [Ru−cyt c] = 29 μM; [Tris] = 10 mM; [NaN_3] = 10 mM; [$NaClO_4$] = 80 mM; pH = 7.0.

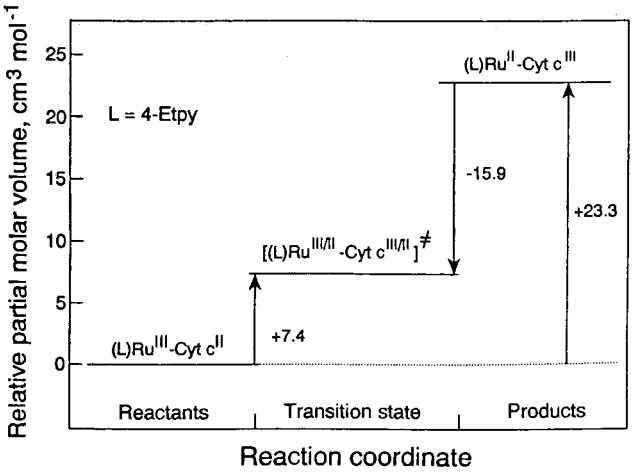

Figure 3. Volume profile for the overall reaction $(NH_3)_4$(L)Ru^{III}−cyt c^{II} ⇌ $(NH_3)_4$(L)Ru^{II}−cyt c^{III}. For experimental conditions see Figure 2.

ference points to some unique aspects of the intramolecular electron-transfer mechanism.

A typical example of a volume profile, the first reported for an intramolecular reaction, is presented in Figure 3. The asymmetrical nature of the volume profile results from the fact that the volume of activation associated with the reduction of Ru(III) is significantly smaller in magnitude than the volume of activation associated with the oxidation of Ru(II) for the series of complexes (not including L = NH_3) in Table 1. The volume of activation for the forward reaction in (3) merely represents ca. 25% of the overall volume increase associated with the reduction of Ru(III). The position of the transition state is such that the forward reaction in (3) could be interpreted to have an "early", and the reverse reaction a "late", transition state. This unexpected and rather remarkable finding calls for a more detailed analysis of the volume profiles in terms of the underlying electron-transfer mechanism.

Discussion

Reaction Volume Data. We start our more detailed interpretation with an analysis of the overall reaction volumes summarized in Table 1. As mentioned in the Introduction, the reported volume effects are mainly thought to arise from changes in electrostriction on the ruthenium center through hydrogen bonding of the coordinated ammonia ligands with the surrounding solvent molecules.[7−9] Electrostriction effects have been demonstrated to be proportional to the squares of the charges on coordination complexes.[18,19] In the case of similarly charged species, the number of NH_3 ligands on the ruthenium center interacting with the surrounding solvent molecules must be responsible for the observed electrostriction. The observed volume effects suggest a ratio of approximately 3:2 in terms of the number of ammonia ligands effectively interacting with the surrounding solvent molecules in the intramolecular reactions of $(NH_3)_5Ru$−cyt c and *trans*-$(NH_3)_4$(L)Ru−cyt c, respectively. This could be related to specific site effects experienced by the ammine ligands when the different complexes are bound to cyt c. In the case of the *trans*-$(NH_3)_4$(L)Ru−cyt c complexes, the less hydrophilic, substituted pyridine ligands L (trans to the binding site and oriented generally toward the solvent water) will exclude some water molecules from the region of the ruthenium center, resulting in significantly less electrostriction than the fifth ammonia ligand on the $(NH_3)_5Ru$−cyt c complex. It is safe to assume that the remaining four ammonia molecules in both systems will partly interact with groups on the surface of the protein such as the C-terminal glutamate residue, which could decrease their influence on the surrounding solvent by as much as half. Then the pentaammine complex could have effectively three ammonia ligand equivalents in terms of electrostriction interactions with surrounding water, as compared to only two in the case of the tetraammine complexes. In this way, we can account for the ratio of approximately 3:2 observed in the associated reaction volumes.

Activation Volume Data. a. Interpretation within the Marcus−Hush Electron-Transfer Formalism. The volumes of activation for the reverse reaction in (3), measured directly for L = NH_3 but calculated from the difference between the activation volumes for the forward reaction and the overall reaction volumes for all other L, i.e., $\Delta V_b^{\ddagger} = \Delta V_f^{\ddagger} - \Delta V$, are all rather similar and vary between −14 and −18 cm^3 mol^{-1}. Thus, intramolecular electron transfer from Ru^{II} to cyt c^{III} is accompanied by a significantly negative activation volume, similar to those observed for the intermolecular reactions.[7,8] In combination with the small, positive activation volume for electron transfer in the opposite direction, an asymmetric volume profile results, *unlike* that observed in the intermolecular case.

For an *adiabatic*, intermolecular electron-transfer reaction, the activation volume can be considered to be the sum of terms as outlined by Stranks[20] and Swaddle[21] and indicated in eq 4.

$$\Delta V^{\ddagger}_{inter} = \Delta V^{*}_{COUL} + \Delta V^{*}_{DH} + \beta RT + \Delta V^{*}_{SR} + \Delta V^{*}_{IR} + \lambda^{*}\Delta V \quad (4)$$

ΔV^{*}_{COUL} represents the Coulombic work to bring the reactants together at infinite dilution, ΔV^{*}_{DH} the Debye−Hückel correction for finite ionic strength, βRT the contribution of the preexponential factor[21] (β is the isothermal compressibility of the solvent), 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}),[20] and $\lambda^{*}\Delta V$ the contribution due to net volume changes for an asymmetrical electron-transfer reaction. The transition-state electron probability density parameter λ^{*} (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

(18) Kitamura, Y.; van Eldik, R. *Ber. Bunsen-Ges. Phys. Chem.* **1984**, *88*, 418.
(19) Sachinidis, J. I.; Shalders, R. D.; Tregloan, P. A. *Inorg. Chem.* **1994**, *33*, 6180.
(20) Stranks, D. R. *Pure Appl. Chem.* **1974**, *38*, 303.
(21) Jolley, W. H.; Stranks, D. R.; Swaddle, T. W. *Inorg. Chem.* **1990**, *29*, 1948.

coordinate.

$$\lambda^* = 0.5\{1 + (\Delta G^\circ_{12} + w_{21} - w_{12})/\lambda_{12}\} \qquad (5)$$

For *nonadiabatic*, intramolecular electron-transfer reactions, the first three terms of eq 4 and the work terms in eq 5 drop out but an additional term ΔV^*_{NA} is added[21] to reflect the pressure dependence of the electronic coupling between the two redox centers.

$$\Delta V^{\ddagger}_{intra} = \Delta V^*_{NA} + \Delta V^*_{SR} + \Delta V^*_{IR} + \lambda^* \Delta V \qquad (6)$$

For small ion−ion intermolecular electron-transfer reactions in water, the Coulombic, Debye−Hückel, and βRT terms in eq 4 tend to cancel each other, leaving ΔV^*_{SR} and $\lambda^* \Delta V$ as the dominant contributions to $\Delta V^{\ddagger}_{inter}$. Although we have made such assumptions in the past,[7,8] it is not demonstrated that they hold true when one of the reactants is a large, low-dielectric species such as cytochrome *c*. In both the inter- and intramolecular cases, the driving forces are relatively small, so the transition-state parameter λ^* does not depart far from the symmetric value of 0.5 to induce a large asymmetric contribution from the $\lambda^* \Delta V$ term. For example, in the intramolecular cases, the offsets from $\Delta V/2$ due to this term are as follows (in cm^3 mol^{-1}): NH_3, +1.5; etpy, −0.9; lut, −0.9; py, −1.3; isn, −1.8.

The asymmetric volume profiles observed in the intramolecular cases may be due to a significant ΔV^*_{NA} term from the effect of pressure on the degree of electron-transfer coupling between the two centers. An increase in coupling with pressure would make a negative contribution to the activation volume in both directions. Increased pressure is unlikely to impact electronic coupling through a covalently bonded network unless it were to induce a conformational transition substantial enough to rehybridize orbitals along the pathway. Instead, coupling through van der Waals interactions, hydrogen bonds, and through-space jumps is more likely to be affected by pressure. Pressure-induced electron-transfer rate enhancement through an apparent decrease in the distance of a through-space pathway has been reported.[22] In the present case, activation volumes for both electron-transfer directions are approximately 6 cm^3 mol^{-1} more negative than they would be if the volume profile was symmetrical. There is no through-space jump along the putative pathway between the heme iron and the ruthenium complex bound at His33; however, a hydrogen bond link is part of the pathway. Another pathway containing a hydrogen bond link connects the two centers in *Candida kreusei* cyt *c*, where similar activation volume effects are observed.

In an effort to quantify these effects, we compared the data at 150 MPa with those at ambient pressure obtained in this report and others previously reported[13] in the form of a Marcus plot ($\ln k$ vs driving force, Figure 4). If one assumes that the reorganization energy λ remains constant, the 150 MPa data are indeed shifted upward, which corresponds to a higher k_{max} and greater coupling. The average upward shift corresponds to a rate ratio ($k_{150}/k_{0.1}$) of 1.24 or $\Delta\Delta V^{\ddagger} = -3.5$ cm^3 mol^{-1} (=ΔV^*_{NA}?). Alternatively, these data can be interpreted such that the Marcus curve is shifted to the left; i.e., λ decreases as the pressure is increased. A decrease in λ of only 0.025 eV would account for the differences in observed rates between 150 and 0.1 MPa. Thus, the negative offset in activation volumes may be the result of an increase in the coupling matrix element H_{AB}, a decrease in λ, or both effects.

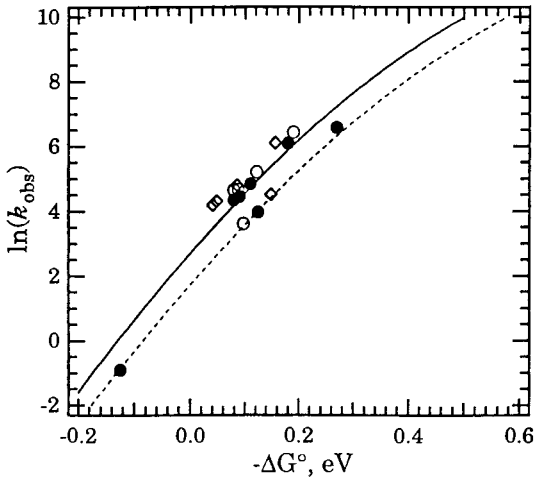

Figure 4. Marcus plot for the intramolecular electron transfer in $(NH_3)_4$-Ru(L)-modified horse heart cytochrome *c*. Solid circles: data from ref 13 obtained at 1 atm in phosphate buffer. Open circles: data obtained in Tris buffer as described in this paper. Open diamonds: data obtained at 1500 atm in Tris buffer as described in this paper. Driving forces for the Tris buffer measurements at 1 and 1500 atm were obtained from the high-pressure electrochemical experiments reported in ref 9 or spectroscopic equilibrium determinations reported here. Solid and dotted lines: Marcus curves for $k_{max} = 3.9 \times 10^5$ s^{-1} and $\lambda = 1.0$ and 1.1 eV, respectively (ref 13).

b. Consideration of a Discrete Intermediate. Another explanation for this behavior is that the transition state is "late" in the Ru^{II} to cyt c^{III} direction such that most of the electrostrictive solvent reorganization has occurred before the transition state is reached. Conversely, little release of electrostriction would occur before the transition state in the cyt c^{II} to Ru^{III} direction. One way this may occur is if the intramolecular electron-transfer reaction proceeds via an intermediate in which the electron is located on the protein and both metal centers are in the oxidized state. In this case, electron transfer from the ruthenium center to the intermediate state, i.e., the reverse of reaction 3, will cause significant changes in electrostriction on the ruthenium center. However, electron transfer from the iron center to the intermediate, i.e., the forward reaction 3, will not cause a significant volume change on the ruthenium center, in agreement with the observed volumes of activation. Reaction 3 is therefore analyzed in terms of a two-step mechanism shown in a simplified manner in (7)

$$Ru^{III}-Fe^{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Ru^{III}-Fe^{III}, e^-] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Ru^{II}-Fe^{III} \qquad (7)$$

where the intermediate has the electron located somewhere on the protein and both metals are in the oxidized form. For the forward and reverse reaction steps, applying steady-state conditions to the intermediate, $k_f = k_1k_2/(k_{-1} + k_2)$ and $k_b = k_{-2}k_{-1}/(k_{-1} + k_2)$, respectively. If $k_2 \gg k_{-1}$, then $k_f = k_1$ and $k_b = k_{-1}/K_2$. This will mean that the forward reaction in (3) will involve hardly any change in electrostriction on the ruthenium center, whereas the reverse reaction in (3) will involve almost complete oxidation, accompanied by a large increase in electrostriction, of the ruthenium center. A crucial aspect of our suggestion is the assumption that $k_2 \gg k_{-1}$. This is true for the tetraammine complexes, where the driving force for the overall reaction 7 is such that it favors the reduction of the Ru(III) center, since $k_2/k_{-1} \approx 4-30$ for L = etpy to L = isn. This means that once the electron is located on the protein it will favor the reduction of Ru(III) instead of Fe(III) in these systems. Furthermore, in this treatment, it is assumed that $k_f = k_1$, from

(22) Meier, M.; van Eldik, R.; Chang, I.-J.; Mines, G. A.; Wuttke, D. S.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **1994**, *116*, 1577.

which it follows that k_f should be rather insensitive to the nature of the Ru complex. However, it has been shown[13] that the electron-transfer rates in this system vary with driving force according to Marcus theory, implying direct involvement of the ruthenium center in the rate-determinig step. Therefore, the two-step electron-transfer mechanism can be discounted.

A two-step hole-transfer mechanism (8) provides another explanation for our observations.

$$Ru^{III}-Fe^{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Ru^{II}-Fe^{II}, h^+] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Ru^{II}-Fe^{III} \qquad (8)$$

According to this scheme, the Ru(III) center is reduced to Ru(II) and a positive hole, h^+, is formed, which is then transferred to the Fe(II) site. To account for the observed volume profile, this model will only be valid if the separation of $Ru^{II}-h^+$ is small enough in the rate-determining step to have little effect on electrostriction. Hole formation and separation will then be accompanied by a minor volume increase, the actual change will be associated with the effective separation of $Ru^{II}-h^+$. The rest of the formal treatment is very similar to that outlined above for the two-step mechanism involving electron instead of hole transfer. Since the ruthenium center is involved in the rate-determining redox step (k_1), this mechanism predicts a rate dependence on the driving force.

The question arises of the identity of the oxidized species "h^+". An upper limit for the reduction potential of this species can be obtained from the observed activation free energies for the electron-transfer reactions and the reduction potentials of the pendant ruthenium complexes in the following way:

$$E(h^+) \leq \Delta H^{\ddagger} - T\Delta S^{\ddagger} + E(Ru) \qquad (9)$$

Maximum $E(h^+)$ values thus obtained are +0.91 V vs NHE for L = NH_3 and +0.99−+1.04 V vs NHE for all the other derivatives. Assuming that nearly all the activation free energy is used to produce "h^+", only tyrosine residues ($E_{1/2}$ = 0.8 V vs NHE[23]) have sufficiently low redox potentials, i.e., $E_{1/2}$ < 0.9 V vs NHE, to qualify as the oxidized intermediate. There are four tyrosine residues in horse heart cytochrome *c*, at positions 48, 67, 74, and 97. Of these, Tyr48 and Tyr97 are located about 10 Å from the ruthenium-modified His33 residue.[24] In comparison, the shortest distance between the imidazole rings of His18 and His33 (coordinated to the iron and ruthenium centers, respectively) is about 11 Å. To more carefully evaluate the possibility of an oxidized Tyr48 or Tyr97 intermediate, pathway calculations[25] were performed to estimate the relative couplings between all of the sites. The results indicate that the couplings for His33−Tyr48 and Tyr48−Heme iron are slightly better than those for His33−Heme iron (factors of 2.0 and 2.8, respectively, relative to His33−Fe).[26] Coupling between Tyr97 and His33 or heme iron is comparable to the Tyr48 values. Although the electronic couplings slightly favor the two-step mechanism involving oxidation of Tyr48 or Tyr97, the small improvement in the coupling prefactor is not large enough to compensate for the large activation barrier imposed by the highly unfavorable driving force for the oxidation of tyrosine. For this reason, a hole-transfer mechanism with a discrete intermediate should be considered a highly unlikely candidate to explain the observed volume profile.

A remaining possible explanation for the asymmetrical volume profiles for these intramolecular electron-transfer reactions may be related to kinetic and spectroscopic evidence reported in the literature for conformational changes that occur during the redox reactions of cytochrome *c*. Rush et al.[27] have reported kinetic evidence for the participation of a highly reactive form, which is thought to be a conformation of the reduced protein with an open crevice, during the oxidation of cyt *c* by $Co(phen)_3^{3+}$. Tabushi et al.[28] have reported evidence using stopped-flow circular dichroism for conformational changes in the protein during reduction of cyt c^{III}. A distinct conformational intermediate was observed during the reduction of cyt c^{III}, whereas no appreciable intermediate was observed during the oxidation of cyt c^{II}. In more recent work,[29−31] the combination of infrared and electrochemical techniques has revealed further spectroscopic evidence for redox-linked conformational changes on cyt c, as evidenced by the IR spectra of the tyrosine residues coupled to changes in hydrogen bonding.

The asymmetric volume profiles reported in the present study exhibit a much larger absolute volume of activation associated with the reduction of cyt c^{III} than that associated with the oxidation of cyt c^{II}. In general, conformational changes on proteins are known to result in volume changes,[32] which could in principle lead to an offset in the position of the transition state and result in an asymmetrical volume profile. Such a complication can be analyzed by considering a two-step mechanism similar to that used in (7) and (8), in which the first step involves slow electron transfer and the second a rapid conformational change. To account for a reaction volume of ca. 25 $cm^3\ mol^{-1}$ associated with the overall redox process, the electron-transfer step is expected on the basis of earlier measurements[7−9] to contribute ca. 20 $cm^3\ mol^{-1}$, whereas the conformational change will contribute ca. 5 $cm^3\ mol^{-1}$.[9] These numbers will lead to a volume of activation for the forward reaction of +10 $cm^3\ mol^{-1}$ and for the back reaction −15 $cm^3\ mol^{-1}$; on the basis that, the absolute volume changes associated with the forward and reverse electron transfer reactions are the same. These volumes of activation differ significantly from those reported in Table 1 and suggest that the volume change associated with the electron-transfer reaction must be significantly smaller, which is unreasonable on the basis of the available data.[7−9] A similar treatment of a two-step mechanism, in which the first reaction involves a slow conformational change (associated with a small volume change) and the second reaction involves rapid electron transfer (associated with a large volume change), can in principle account for the observed data in Table

(23) Jovanovic, S. V.; Steenken, S.; Simic, M. G. *J. Phys. Chem.* **1991**, *95*, 684. DeFelippis, M. R.; Murthy, C. P.; Broitman, F.; Weinraub, D.; Faraggi, M.; Klapper, M. H. *J. Phys. Chem.* **1991**, *95*, 3416.
(24) Brookhaven Protein Data Bank file 1HRC.
(25) Pathway v0.973, courtesy of Dr. Jeffrey Regan. Regan, J. J.; Risser S. M.; Beratan, D. N.; Onuchic, J. N. *J. Phys. Chem.* **1993**, *97*, 13083. Coupling factors were calculated between "states" A/33/HIS/CD2-CG, A/48/TYR/CZ-OH, A/97/TYR/CE2-HE2, A/97/TYR/CD1-HD1, and BH/105/HEM/FE.
(26) Notably, the best path between His33 and the hydroxyl group of Tyr48 includes a water molecule of crystallization (HOH 125), which improves the coupling by a factor of 1.67 over the best pathway with the waters of crystallization removed.
(27) Rush, J. D.; Koppenol, W. H.; Garber, E. A. E.; Margoliash, E. *J. Biol. Chem.* **1988**, *263*, 7514.
(28) Tabushi, I.; Yamamura, K.; Nishiya, T. *Tetrahedron Lett.* **1978**, *49*, 4921. Tabushi, I.; Yamamura, K.; Nishiya, T. *J. Am. Chem. Soc.* **1979**, *101*, 2785.
(29) Moss, D.; Nabedryk, E.; Breton, J.; Mäntele, W. *Eur. J. Biochem.* **1990**, *187*, 565.
(30) Hellwig, P.; Behr, J.; Ostermeier, C.; Richter, O.-M. H.; Pfitzner, U.; Odenwald, A.; Ludwig, B.; Michel H.; Mäntele, W. *Biochemistry* **1998**, *37*, 7390.
(31) Behr, J.; Hellwig, P.; Mäntele, W.; Michel, H. *Biochemistry* **1998**, *37*, 7400.
(32) Heremans, K. in *Chemistry under Extreme or Nonclassical Conditions*; van Eldik, R., Hubbard, C. D., Eds.; Wiley/Spektrum: New York/Heidelberg, 1997; Chapter 12.

1 and the asymmetric nature of the volume profile. However, this model is not in agreement with the driving-force dependence of the overall reaction. We therefore conclude that conformational gating cannot account for the asymmetric nature of the reported volume profile.

Summary

We conclude that the most plausible explanation for the observed volume profiles involves a pressure-induced increase in electronic coupling, which underscores the importance of nonbonding interactions in long-distance electron-transfer reactions. Alternatively, these data may indicate that λ is smaller at higher pressure in the intramolecular case, as compared to that for the intermolecular reactions. Such information was not revealed by the thermal activation and thermodynamic parameters reported for these systems.[13] The volume profile analysis therefore has enabled unique insight to be gained into the nature of the intramolecular electron-transfer mechanism in these ruthenated cytochromes *c*.

Acknowledgment. Work at the 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. R.v.E. gratefully acknowledges financial support from the Volkswagen Foundation and the Deutsche Forschungsgemeinschaft that enabled him to participate in this work. S.S.I. acknowledges support from the Center for Advanced Food Technology (CAFT) at Rutgers University.

Supporting Information Available: Plots of ln k_{obs} and ln K versus pressure for electron transfer in $(NH_3)_4$(isn)Ru^{III}−Cyt c^{II}, $(NH_3)_4$(4-Etpy)Ru^{III}−Cyt c^{II}, and $(NH_3)_4$(3,5-lut)Ru^{III}−Cyt c^{II} (4 pages). Ordering information is given on any current masthead page.

IC980999F