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Inorganica Chimica Acta 225 (1994) 95–101

Inorganica Chimica Acta

Volume profile analysis for intermolecular electron transfer between cytochrome *c* and $Co(terpy)_2^{2+/3+}$

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Received 1 March 1994

Abstract

The kinetics and thermodynamics of the reversible outer-sphere electron-transfer reaction between $Co(terpy)_2^{2+/3+}$ (terpy = 2,2':6',2''-terpyridine) and horse-heart cytochrome $c^{III/II}$ was studied as a function of temperature and pressure. The activation volumes for the reduction of $Co(terpy)_2^{3+}$ and oxidation of $Co(terpy)_2^{2+}$ were found to be $+18.4 \pm 1.3$ and -18.0 ± 1.4 cm^3 mol^{-1}, respectively. The overall reaction volume for the reduction of $Co(terpy)_2^{3+}$ was determined to be $+33 \pm 3$ cm^3 mol^{-1}. These data are used to construct a volume profile, from which it follows that the transition state lies exactly halfway between the reactant and product states on a volume basis. The experimental results are in good agreement with the rate constant and activation volume calculated from the Marcus–Hush–Stranks–Swaddle relationships.

Keywords: Kinetics; Thermodynamics; Electron transfer; Cobalt complexes; Terpyridine complexes; Cytochrome *c*

1. Introduction

Electron transfer is one of the most important reactions in biological processes. In particular the electron-transfer reactions of the redox proteins such as cytochrome *c* are of general interest. A large number of mechanistic studies has been performed on this topic, including intramolecular [1–4] and intermolecular [5,6] electron-transfer reactions of cytochrome *c* or modified cytochrome *c*. We are especially interested in the effect of pressure on such reactions, since this will in principle enable us to construct a volume profile for the electron-transfer process, i.e. we can then locate the position of the transition state along the reaction coordinate on a volume basis. The construction of a volume profile can assist therefore the elucidation of the electron-transfer mechanism. Only a very few such studies have been performed on cytochrome *c* [7–9]. Furthermore such studies also allow a direct comparison with the theoretical position of the transition state based on the Marcus and related theories.

In the case of symmetrical electron-transfer reactions as for self-exchange reactions, we only need the activation volume in one direction since the overall reaction volume is zero. However, in the case of non-symmetrical reactions we need activation volumes for the reaction in both directions as well as the reaction volume [7], in order to be able to construct the volume profile. We are then restricted to reversible systems with a low driving force, i.e. systems with equilibrium constants close to unity.

In a recent study [9] we investigated the electron transfer between cytochrome *c* and $Ru(NH_3)_5isn^{2+/3+}$, where isn = isonicotinamide. In this system the driving force was much higher than in the $Co(terpy)_2^{3+/2+}$ system (terpy = 2,2':6',2''-terpyridine), which resulted in an unfavorable equilibrium situation for studying the reaction in both directions. Another problem with this system was the stability of the Ru(III) complex in aqueous solution at pH 7, since it tended to undergo disproportionation. In order to avoid these complications, we selected a complex with a redox potential very close to that of cytochrome *c*, with an equilibrium constant as close as possible to unity.

2. Experimental

2.1. Materials

Horse-heart cytochrome *c* from Sigma (Type VI) was oxidized with an excess of $K_3Fe(CN)_6$ in 0.1 M pH 7 phosphate buffer. This solution was passed over a Sephadex G25 column to remove the oxidizing reagent.

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SSDI 0020-1693(94)04035-T

Reduced cytochrome *c* was prepared by treating the oxidized protein with an excess of ascorbic acid. The final purification was done by ultrafiltration with an Amicon ultrafiltration cell. The concentrations were determined from $\epsilon = 27\,600\ M^{-1}\ cm^{-1}$ at 550 nm for cytochrome c^{II} and $\epsilon = 9100\ M^{-1}\ cm^{-1}$ at 550 nm for the oxidized protein.

Both cobalt complexes were prepared and recrystallized several times as described in the literature [10–12]. 2,2′:6′,2″-Terpyridine (= terpy) from Sigma was used without further purification in this synthesis. UV–Vis spectra and chemical analyses were in good agreement with those reported previously. All other chemicals were of analytical reagent grade. The investigated redox reactions were found to be independent of pH in the range 6.7–7.7. All solutions were prepared with deionized, argon-saturated Millipore water. 0.05 M Tris buffer, pH 7.3 and 0.05 M $LiNO_3$ were used as the reaction medium. All solutions were kept under Ar to avoid oxidation by dissolved oxygen.

2.2. Measurements

UV–Vis spectra at ambient pressure were recorded on a Hewlett Packard HP 8452 spectrophotometer, whereas a Zeiss DMR 10 spectrophotometer equipped with a high pressure cell [13] ($P \leq 150$ MPa) was used to record spectra as a function of pressure. The electron-transfer process was followed by monitoring the absorbance as a function of time at 550 nm. The kinetic measurements at ambient pressure were performed on a Durrum D110 stopped-flow system. The measurements at elevated pressure were performed on a homemade high pressure stopped-flow instrument [14,15]. Both instruments were thermostated to ±0.1 °C. Data collection and all subsequent calculations were performed on an online computer system using Olis Kinfit Programs. All kinetic traces showed excellent first-order behavior over the first 3 to 4 half-lives of the reaction. The rate constants reported in this paper are the mean of at least 6 kinetic runs. The corresponding uncertainties are the standard deviations of the mean value.

3. Results and discussion

The redox reactions of cytochrome *c* with small, inorganic reagents are accompanied by significant spectral changes between 400 and 600 nm. In this work we studied the reversible intermolecular electron-transfer process between cytochrome $c^{II/III}$ and $Co(terpy)_2^{3+/2+}$, Eq. (1). Due to the low driving force of this system ($\Delta G^\circ = 0.003$ eV) we were able to follow the reaction in both directions; viz. k_f and k_b.

$$\text{Cyt } c^{II} + Co(terpy)_2^{3+} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \text{Cyt } c^{III} + Co(terpy)_2^{2+} \quad (1)$$

The kinetic data as a function of concentration, temperature and pressure for the forward and back electron-transfer processes are given in Tables 1 and 2. Figs. 1 and 2 show the observed pseudo-first-order rate constant k_{obs} as a function of the $Co(terpy)_2^{3+/2+}$ concentration, respectively. Both plots exhibit no intercept, i.e. no contribution from the corresponding reverse reaction. Spectral (UV–Vis) measurements clearly indicated that the reaction goes to completion in both directions under the selected experimental conditions. The second-order electron-transfer rate constant k_f has a value of $1427 \pm 56\ M^{-1}\ s^{-1}$, and for the back reaction $k_b = 1704 \pm 46\ M^{-1}\ s^{-1}$ at 25 °C and 0.1 M ionic strength. The forward reaction is significantly decelerated by increasing pressure, with an activation volume of $+18.4 \pm 1.2\ cm^3\ mol^{-1}$. The back reaction is significantly accelerated by pressure with an activation volume of $-18.0 \pm 1.4\ cm^3\ mol^{-1}$. The corresponding plots of ln k_f and ln k_b versus pressure are linear within the

Table 1
Kinetic data for the electron-transfer reaction of $Co^{III}(terpy)_2(ClO_4)_3$ with cytochrome c^{II} (horse heart)[a]

T (°C)	p (MPa)	$[Co^{III}]$ (M)	k_{obs} (s^{-1})	k_f ($M^{-1}\ s^{-1}$)
25.0	0.1	8.10×10^{-5}	0.134 ± 0.005	1427 ± 36
		1.52×10^{-4}	0.242 ± 0.004	
		1.66×10^{-4}	0.244 ± 0.004	
		3.10×10^{-4}	0.47 ± 0.01	
		4.18×10^{-4}	0.61 ± 0.01	
20.0	0.1	1.52×10^{-4}	0.185 ± 0.005	1217
25.0			0.242 ± 0.004	1592
30.0			0.333 ± 0.004	2191
35.0			0.443 ± 0.008	2914
40.0			0.577 ± 0.006	3796
20.0	0.1	1.66×10^{-4}	0.190 ± 0.009	1145
25.0			0.244 ± 0.004	1470
30.0			0.335 ± 0.005	2018
35.0			0.435 ± 0.005	2620
40.0			0.57 ± 0.01	3434
25.0	10	3.20×10^{-4}	0.58 ± 0.02	
	50		0.43 ± 0.02	
	75		0.37 ± 0.02	
	100		0.30 ± 0.01	
25.0	10	4.8×10^{-4}	0.82 ± 0.05	
	50		0.62 ± 0.02	
	75		0.52 ± 0.01	
	100		0.41 ± 0.01	

[a]Experimental conditions: $[Cyt\ c^{II}] = 0.75 \times 10^{-5}$ M, pH = 7.2, ionic strength = 0.1 M, [Tris] = 0.05 M, $[LiNO_3] = 0.05$ M, $\lambda = 550$ nm. Calculated activation parameters for k_f: $\Delta H^{\neq} = 40.5 \pm 1.2\ kJ\ mol^{-1}$; $\Delta S^{\neq} = -47 \pm 4\ J\ K^{-1}\ mol^{-1}$; $\Delta G^{\neq}(298\ K) = 54.8\ kJ\ mol^{-1}$; $\Delta V^{\neq} = 18.4 \pm 1.2\ cm^3\ mol^{-1}$.

Table 2
Kinetic data for the electron-transfer reaction of $Co^{II}(terpy)_2(ClO_4)_2$ with cytochrome c^{III} (horse heart)[a]

T (°C)	p (MPa)	$[Co^{II}]$ (M)	k_{obs} (s^{-1})	k_b ($M^{-1}\ s^{-1}$)
25.0	0.1	1.15×10^{-4}	0.205 ± 0.002	1704 ± 46
		2.10×10^{-4}	0.382 ± 0.008	
		2.60×10^{-4}	0.452 ± 0.004	
		3.80×10^{-4}	0.660 ± 0.008	
20.0	0.1	1.15×10^{-4}	0.187 ± 0.002	1626
25.0			0.205 ± 0.002	1783
30.0			0.242 ± 0.003	2104
35.0			0.267 ± 0.003	2322
40.0			0.292 ± 0.002	2539
20.0	0.1	2.60×10^{-4}	0.431 ± 0.008	1658
25.0			0.452 ± 0.004	1738
30.0			0.521 ± 0.006	2004
35.0			0.580 ± 0.008	2231
40.0			0.624 ± 0.006	2400
25.0	10	3.60×10^{-4}	$0.62\ \pm0.02$	
	50		$0.78\ \pm0.03$	
	100		$1.26\ \pm0.02$	
	150		$1.67\ \pm0.02$	
25.0	10	3.60×10^{-4}	$0.58\ \pm0.02$	
	50		$0.86\ \pm0.03$	
	100		$1.26\ \pm0.03$	
	150		$1.59\ \pm0.04$	

[a]Experimental conditions: $[Cyt\ c^{III}]=(1.0–2.0)\times10^{-5}$ M, pH = 7.2, ionic strength = 0.1 M, [Tris] = 0.05 M, $[LiNO_3]$ = 0.05 M, λ = 550 nm. Calculated activation parameters for k_b: $\Delta H^{\neq}=14\pm1$ kJ mol^{-1}; $\Delta S^{\neq}=-136\pm4$ J K^{-1} mol^{-1}; $\Delta G^{\neq}(298\ K)=54.4$ kJ mol^{-1}; $\Delta V^{\neq}=-18.0\pm1.4$ cm^3 mol^{-1}.

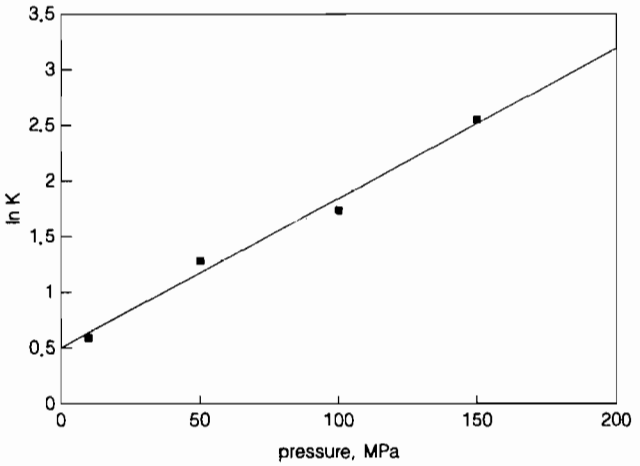

Fig. 1. Concentration dependence of k_{obs} for the reduction of Cyt c^{III}. Experimental conditions: $[Cyt\ c^{III}]=1.0\times10^{-5}$ M, pH = 7.2, ionic strength = 0.1 M, [Tris] = 0.05 M, $[LiNO_3]$ = 0.05 M, λ = 550 nm, temp. = 25.0 °C.

experimental error limits (Fig. 3). Both reactions exhibit significantly negative $\Delta S^{\neq}$ values of -47 ± 4 and -136 ± 4 J K^{-1} mol^{-1}, respectively. These are typical values for an outer-sphere electron-transfer process [16]. The $\Delta S^{\neq}$ value for the back reaction is significantly

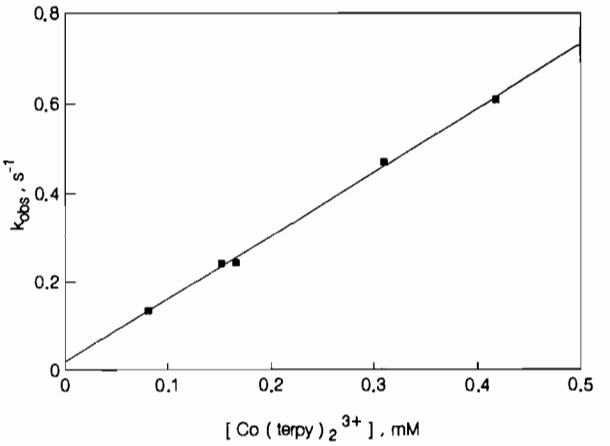

Fig. 2. Concentration dependence of k_{obs} for the oxidation of Cyt c^{II}. Experimental conditions: $[Cyt\ c^{II}]=0.75\times10^{-5}$ M, pH = 7.2, ionic strength = 0.1 M, [Tris] = 0.05 M, $[LiNO_3]$ = 0.05 M, λ = 550 nm, temp. = 25.0 °C.

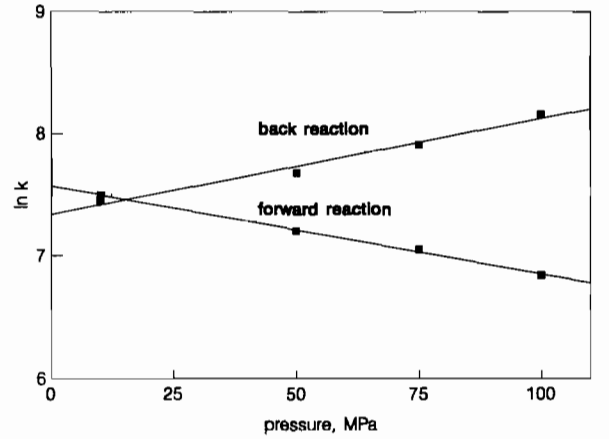

Fig. 3. Plot of ln k vs. pressure for the forward and reverse reaction. For experimental conditions see Tables 1 and 2.

more negative than for the forward reaction. A similar trend is observed in the reported $\Delta V^{\neq}$ values.

The overall equilibrium constant for this redox system was determined using different methods. In the first, it was measured by the systematic addition of the Co(II) complex to cytochrome c^{III}. An increase in absorbance at 550 nm as a function of [Co(II)] was observed and the absorbance change from the reactants to products was used to estimate the equilibrium constant. This resulted in a K value of 0.7 ± 0.2. This value is in close agreement with that obtained from the kinetic measurements ($K=k_f/k_b=1427/1704=0.8\pm0.1$) and with the value calculated from the available potential data ($K=0.9$). We used the following potential data in our calculations, $Co(terpy)_2^{3+/2+}$: $E_{11}^{\circ}=0.270$ V; cytochrome $c^{III/II}$: $E_{22}^{\circ}=0.273$ V [9,17].

From the pressure dependence of K we could determine the overall reaction volume in the usual way [9]. We recorded spectra of an equilibrium mixture of cytochrome c and the cobalt complex as a function of

pressure (Fig. 4). The spectra show that a systematic increase in pressure causes an increase in the concentration of cytochrome c^{II}, i.e. equilibrium (1) is shifted to the left side on increasing the pressure. In addition, this corresponds to a volume collapse on reducing the protein by the cobalt complex. The spectral changes as a function of pressure can be used to calculate the equilibrium constant at different pressures. From the plot of ln K versus pressure (Fig. 5) we could calculate a reaction volume of -33 ± 3 cm^3 mol^{-1} for the reduction of cytochrome c^{III}. This value is in good agreement with the value estimated from the kinetic data, viz. $\Delta\bar{V}=\Delta V_f^{\neq}-\Delta V_b^{\neq}=-36\pm2$ cm^3 mol^{-1}. Similar to our earlier work on the $Ru(NH_3)_5isn^{2+/3+}$ system [9], we again observe a very good agreement between

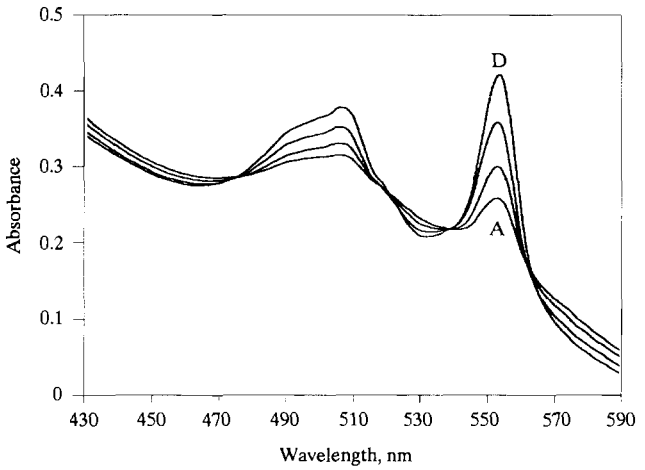

Fig. 4. UV–Vis spectra of an equilibrium mixture of Cyt $c^{II/III}$ and $Co(terpy)_2^{2+/3+}$ as a function of pressure: $A=5$, $B=50$, $C=100$, $D=150$ MPa. Experimental conditions: [Cyt c] $=3\times10^{-6}$ M, [Co] $=2.8\times10^{-3}$ M, pH = 7.2, ionic strength = 0.1 M, [Tris] = 0.05 M, [$LiNO_3$] = 0.05 M, temp. = 25.0 °C.

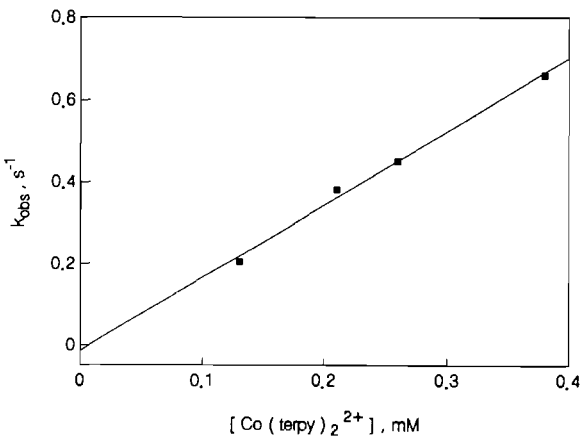

Fig. 5. Plot of ln K vs. pressure for reaction (1). For experimental condition see Fig. 4.

the thermodynamically and kinetically determined reaction volumes.

From the available volume data we are now able to construct a volume profile for the overall reaction which displays the volume changes along the reaction coordinate (Fig. 6). It shows that the oxidation of the protein is accompanied by a large volume increase of 36 cm^3 mol^{-1} (kinetic data) and the transition state is located exactly halfway between reactant and product states on a volume basis. If we compare the literature data in Table 3 with our present results we can see some interesting trends. The reduction of the positively charged metal complexes from the oxidation state +3 to +2 is always accompanied by a positive volume of activation and vice versa for the oxidation from +2 to +3. The reaction volume for the oxidation of cytochrome c by $Ru(NH_3)_5isn^{3+}$ is also very close to the value determined for the electrochemical reduction of $Ru(NH_3)_6^{3+}$, viz. $+29\pm1$ cm^3 mol^{-1} [19]. For the $Co(terpy)_2^{3+/2+}$ system the reaction volume for the reduction of Co(III) can be calculated from the partial molar volume of these complexes, viz. 362.4–325.8 = 36.6 cm^3 mol^{-1} [12]. This value is once again very close to the values obtained for the reduction of $Co(terpy)_2^{3+}$ by cytochrome c. The apparent difference between the data for the phen and terpy systems is unclear and calls for further investigation. All these results suggest that the main volume changes observed during the redox reactions of cytochrome c occur on the redox partner, i.e. the metal complex, and not on the cytochrome c molecule itself. This conclusion is supported by electrochemical measurements on cytochrome c at elevated pressure, which resulted in a reaction volume of ~2 cm^3 mol^{-1} for the oxidation of cytochrome c [20].

In the case of the ruthenium complexes it is known that the observed volume changes are not due to changes in the metal–ligand bond length, since the redox reaction

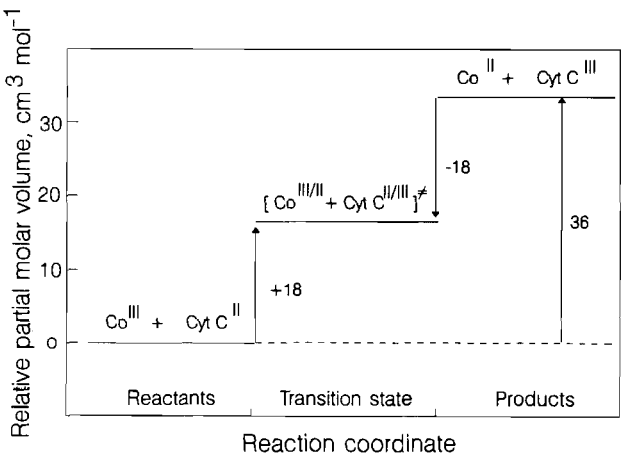

Fig. 6. Volume profile for the overall reaction $Co(terpy)_2^{3+}$ + Cyt $c^{II} \rightleftharpoons Co(terpy)_2^{2+}$ + Cyt c^{III}.

Table 3
Summary of rate and activation parameters for some intra- and intermolecular electron-transfer reactions

Reaction[a]	$-\Delta G°$ (eV)	k_{298}	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})	$\Delta V^{\neq}$ (cm^3 mol^{-1})	$\Delta \bar{V}$ (cm^3 mol^{-1})	Reference
Intramolecular							
A_5Ru^{II}–His(33)hh^{III}	0.11	39 s^{-1}	14.6	−163	−17.7		[8]
A_5Ru^{II}–His(39)ck^{III}	0.11	87 s^{-1}	9.6	−171	−18.3		[8]
Intermolecular							
$RuA_6^{2+} + hh^{III}$	0.27	6.3×10^{-4} M^{-1} s^{-1}	3.8	−142	−15.6		[8]
$hh^{II} + RuA_5isn^{3+}$	0.11	1.1×10^{5} M^{-1} s^{-1}	22	−75	+16.0	+33[b], +31[c]	[9]
$RuA_5isn^{2+} + hh^{III}$	−0.11	1.5×10^{3} M^{-1} s^{-1}	28	−87	−17.2		
$hh^{II} + Co(phen)_3^{3+}$	0.12	1.9×10^{3} M^{-1} s^{-1}	47	−26	+8.5	+20	[7,18]
$Co(phen)_3^{2+} + hh^{III}$	−0.12				−11.5[b]		
$hh^{II} + Co(terpy)_2^{3+}$	−0.003	1.4×10^{3} M^{-1} s^{-1}	40.5	−47	+18.4	+36[b], +33[c]	this work
$Co(terpy)_2^{2+} + hh^{III}$	0.003	1.7×10^{3} M^{-1} s^{-1}	14	−136	−18.0		

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

is only accompanied by a change of 0.02 Å in the metal–ligand bond length [21]. The cobalt complexes however show a different behavior. The metal–ligand bond length changes about 0.2 Å during the redox reaction [21]. For $Co(terpy)_2^{3+/2+}$ radii of 505 and 524 pm were reported, respectively [12]. In this system there is a mixture between high spin and low spin d^7 configuration for the Co(II) complex [22]. This means that oxidation of Co(II) to Co(III) will result in volume decreases due to an increase in electrostriction and an intrinsic volume collapse caused by the high spin to low spin changeover. In addition, the contribution from the changes in electrostriction is expected to be smaller due to the shielding effect of the pyridine rings as compared to the corresponding ammine complexes. Thus the overall effect seems to be very similar.

The electron-transfer reactions of the $Co(terpy)_2^{3+/2+}$ complexes with the cytochrome *c* molecule are outer-sphere electron-transfer processes as outlined in Eqs. (2) and (3) [21,23]. This mechanism includes precursor formation, K_{OS}, the rate-determining electron-transfer step, k_{ET}, and successor dissociation.

$$Co(terpy)_2^{3+} + Cyt\ c^{II} \overset{K_{OS}}{\rightleftharpoons} \{Co(terpy)_2^{3+} \cdot Cyt\ c^{II}\}$$

$$\{Co(terpy)_2^{3+} \cdot Cyt\ c^{II}\} \overset{k_{ET}}{\rightleftharpoons} \{Co(terpy)_2^{2+} \cdot Cyt\ c^{III}\} \quad (2)$$

$$\{Co(terpy)_2^{2+} \cdot Cyt\ c^{III}\} \rightleftharpoons Co(terpy)_2^{2+} + Cyt\ c^{III}$$

$$k_f = K_{OS}k_{ET} \quad (3)$$

$$k_{12} = (k_{11}k_{22}K_{12})^{1/2} \quad (4)$$

According to Marcus, the rate constant of the cross reaction can be calculated using the Marcus cross relationship [24–26] as outlined in Eq. (4) (assuming that the work terms and the factor f_{12} can be neglected [26]). In Eq. (4), K_{12} is the overall equilibrium constant and k_{11} and k_{22} are the self-exchange rate constants of the Co complex and cytochrome *c*, respectively. For the reaction of $Co(terpy)_2^{3+/2+}$ with cytochrome $c^{II/III}$, the following values were employed: $Co(terpy)_2^{3+/2+}$: $E_{11}° = 0.270$ V [27,28], $k_{11} = (1.94\text{–}3.38)\times10^3$ M^{-1} s^{-1} [5,29], radii: 505/524 pm, respectively [27]; Cyt $c^{III/II}$: $E_{22}° = 0.273$ V [9,17], $k_{22} = 350$ M^{-1} s^{-1}[1], radius 1.66 nm [31] and charges +7.5/+6.5, respectively [31]. $\Delta G_{12}° = 0.29$, $K = 0.9$.

Using the Marcus cross relation we obtained $k_f = 782\text{–}1032$ M^{-1} s^{-1} and $k_b = 869\text{–}1147$ M^{-1} s^{-1}. These calculated values are in reasonable agreement with the experimentally determined values. This is consistent with previous findings for the oxidation of cytochrome c^{II} by $Ru(NH_3)_5isn^{3+}$, $Co(phen)_3^{2+}$ and other complexes [5,6,9,25]. Furthermore, this agreement has not only been reported for cytochrome *c*, but also for other redox proteins. Rates reported for the oxidation of blue copper proteins (for example plastocyanin, stellacyanin and azurin) by several inorganic complexes are at least comparable with those predicted from the Marcus cross relation.

The k_{12} values determined in this study for the oxidation of cytochrome *c* are a factor of 1.6 larger than the values reported by Drake et al. [17], viz. 9.0×10^2 M^{-1} s^{-1} at 0.1 M ionic strength (NaCl). For the reduction of cytochrome *c*, they [17] reported a value of 1.0×10^3 M^{-1} s^{-1}, which is again a factor of 1.7 smaller than our value. We repeated these measurements in a similar medium and were able to confirm these values. The reason for these deviations is probably

[1]The self exchange rate constant was extrapolated to 25 °C using the kinetic data and activation energy value published in Ref. [30].

a secondary ion effect that involves some specific interaction of chloride with cytochrome *c*. In order to check this, it would be essential to repeat the measurements in a perchlorate medium. Unfortunately, due to solubility limitations of the cobalt complex in perchlorate medium, such measurements were not possible.

According to procedures developed by Stranks, Swaddle and Wherland [32–38], it is possible to calculate the activation volume for an electron-transfer process, according to Eq. (5).

$$\Delta V^{*} = \Delta V_{IR}^{*} + \Delta V_{COUL}^{*} + \Delta V_{DH}^{*} + \Delta V_{SR}^{*} + \beta RT + \lambda^{*}\Delta\bar{V} \quad (5)$$

The activation volume consists of six components; ΔV_{IR}^{*} is the inner-sphere rearrangement, which is normally neglected, ΔV_{COUL}^{*} is the coulombic term, ΔV_{DH}^{*} includes Debye Hückel or other electrolyte effects, ΔV_{SR}^{*} is the contribution from the rearrangement of the surrounding solvent molecules and βRT the contribution from the preexponential part of the work terms ($=1.3$ cm^3 mol^{-1} [35]). For a non-symmetrical electron-transfer reaction the last term in Eq. (5) represents the contribution due to the overall volume change. The λ^{*} parameter gives the location of the transition state relative to products and reactants, and can be calculated according to the Marcus theory by Eq. (6) [24]. In Eq. (6) λ is the reorganization energy, and w_{12} and w_{21} are the work terms required to bring the reactants or products in the transition state. For the low driving force system $Co(terpy)_2^{3+/2+}$ we calculated a λ^{*} value of 0.5, which is in excellent agreement with the experimental data.

$$\lambda^{*} = \frac{1}{2}\left(1 + \frac{\Delta G_{12}^{\circ} + w_{21} - w_{12}}{\lambda}\right) \quad (6)$$

For the forward reaction we calculated the following volume contributions (cm^3 mol^{-1}): $\Delta V_{COUL}^{*} = -5.1$; $\Delta V_{DH}^{*} = +6.3$; $\Delta V_{SR}^{*} = -4.7$; $\lambda^{*}\Delta\bar{V} = 0.5 \times 35 = 17.5$; and for the reverse reaction: $\Delta V_{COUL}^{*} = -3.9$; $\Delta V_{DH}^{*} = +4.9$; $\Delta V_{SR}^{*} = -4.6$; $\lambda^{*}\Delta\bar{V} = -0.5 \times 35 = -17.5$ cm^3 mol^{-1}. These values result in a ΔV_f^{*} value of 15.3 and a ΔV_b^{*} value of -19.8 cm^3 mol^{-1}, respectivcly. Similar to our work on the $Ru(NH_3)_5$-$isn^{2+/3+}$ complexes [9] we can see that the calculated activation volume for the forward reaction is slightly smaller than the experimental one. For the reverse reaction the calculated activation volume is more negative than that obtained from the kinetic measurements. In our previous paper [9] we suggested that these differences may partly arise from the smaller contribution from the solvent reorganization around the cytochrome *c* molecule as compared to the model complexes. In the case of the $Co(terpy)_2^{2+/3+}$ complexes the ratio of the radii for the cobalt complex and the precursor complex is about 0.23. This means that the ΔV_{SR}^{*} term should only be ~23% of the calculated value if the main volume changes are determined by the cobalt complex only. This results in a ΔV_{SR}^{*} value of -1.1 cm^3 mol^{-1}. Following this correction we obtain: $\Delta V_f^{*} = 18.9$ cm^3 mol^{-1} and $\Delta V_b^{*} = -16.3$ cm^3 mol^{-1}.

If 2σ error limits are used the measured ΔV^{*} values are -18.0 ± 2.8 and 18.4 ± 2.6 cm^3 mol^{-1}, which requires no further correction of ΔV_{SR}^{*}. In general we can conclude that there is a close agreement between the calculated and experimental results independent of the use of the ΔV_{SR}^{*} correction.

These calculations support our suggestion that the observed volume changes occur mainly on the cobalt center and not on the protein.

Acknowledgements

The authors gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft, Fond der chemischen Industrie, Volkswagen-Stiftung and the Daniela and Jürgen Westphal Stiftung.

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