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Electron transfer reaction between cytochrome *c* and trisoxalatocobalt(III) †

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The irreversible outer-sphere electron transfer reaction between the oxidant $[Co(ox)_3]^{3-}$ and the redox protein horse heart cytochrome c^{II} according to the reaction,

$$[Co^{III}(ox)_3]^{3-} + \text{cytochrome } c^{II}\rceil^{6+} \rightarrow [Co^{II}(ox)_3]^{4-} + \text{cytochrome } c^{III}\rceil^{7+}$$

was studied as a function of pH, concentration, temperature and pressure using UV–Vis and stopped-flow techniques. The concentration dependence of the observed rate constant showed a saturation of the rate constant at high concentrations of $[Co(ox)_3]^{3-}$, indicative of effective precursor formation between the reactants. The temperature (6–35 °C) and pressure (up to 1300 atm) dependencies at low and high complex concentrations were carried out and activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, $\Delta V^{\ddagger}$) were determined. The results are discussed in reference to earlier studies performed in our laboratories and elsewhere, and the importance of efficient precursor formation in such reactions is highlighted.

Introduction

Redox reactions play an important role, particularly in biological systems.[1,2] Of special interest and therefore widely studied are systems involving cytochrome *c*.[3–7] In the past we have concentrated on the construction of volume profiles for intermolecular and intramolecular electron transfer reactions of cytochrome *c*.[8–18] More recently, we have focussed on intermolecular electron transfer reactions that involve negatively charged redox partners for cytochrome *c*, such that the redox partners will effectively form precursor complexes in order that the electron transfer rate constant can be separated kinetically from the overall second order rate constant.[19] For instance, in the case of the reaction of trisoxalatocobalt(III) and cytochrome c^{II} the mechanism can then be summarized as outlined in eqn. (1).

$$[Co^{III}(ox)_3]^{3-} + \text{cyt } c^{II} \underset{}{\overset{K}{\rightleftharpoons}} \{[Co^{III}(ox)_3]^{3-} \bullet \text{cyt } c^{II}\} \xrightarrow{k_{et}} \{[Co^{II}(ox)_3]^{4-} \bullet \text{cyt } c^{III}\} \xrightarrow{\text{fast}} [Co^{II}(ox)_3]^{4-} + \text{cyt } c^{III} \quad (1)$$

For this mechanism the rate equation is given by $k_{obs} = k_{et}K[Co^{III}]/(1 + K[Co^{III}])$ in the presence of an excess of Co(III). In the case of effective ion-pair formation, k_{obs} reaches a limiting value at high $[Co^{III}]$ and the rate equation simplifies to $k_{obs} = k_{et}$. Thus activation parameters for the electron transfer reaction can be obtained directly under such conditions, something that has not been possible for intermolecular electron transfer reactions involving cytochrome *c* before.

Experimental

Preparation of solutions

All chemicals were of analytical grade and used without further purification. Ultrapure water was used for the preparation of all solutions. Buffer solutions containing 0.05 M Tris [2-amino-2-(hydroxymethyl)propane-1,3-diol, Sigma–Aldrich Chemicals] were used, and the ionic strength ($I = 0.1$ M) was adjusted by the addition of $LiNO_3$. The pH was adjusted in the range 6.5–8.0 with HNO_3. Pure nitrogen gas was bubbled through the reaction solutions to remove dissolved oxygen. Oxidant solutions were shielded from light with aluminium foil.

Materials

Horse heart cytochrome *c* (Sigma–Aldrich, Type VI) was first reduced by the addition of an excess of sodium dithionite (50 mM), then hexacyanoferrate(II) (below 50 mM) was added. The reduced cytochrome *c* was then purified and isolated by repeated ultrafiltration through a 3000 Da molecular weight cut-off membrane (Amicon) with degassed ultrapure water. The reduced form of cytochrome *c* was analysed spectrophotometrically.

Potassium trisoxalatocobalt(III) ($K_3[Co(C_2O_4)_3]\cdot 3H_2O$) was prepared according to the procedures of Bailar and Jones [20] and Sörensen.[21] Deep-green needles were obtained, which were washed with ethanol and recrystallized two times. The complex is sensitive to heat and light, and was stored in the dark to protect against photo or thermal decomposition. The IR spectrum,[22,23] elemental analysis (Found: C, 14.47; H, 1.04. Calc. for $C_6H_6CoK_3O_{15}$: C, 14.75; H, 1.21%), and UV–Vis spectrum [24,25] were used to check the purity of the complex.

† Electronic supplementary information (ESI) available: Table S1 summarizing all kinetic data. Figs. S1–S3 reporting spectral changes observed during the reaction and rate constants as a function of pH. See http://www.rsc.org/suppdata/dt/b1/b101366l/

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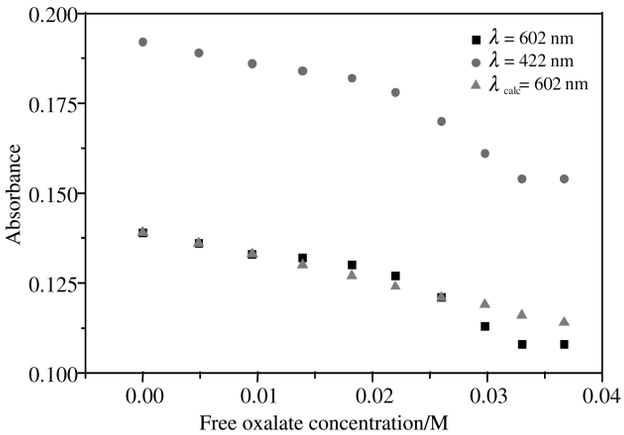

Fig. 1 The effect of free oxalate on the stability of trisoxalatocobalt(III). Experimental conditions: $[Co(ox)_3^{3-}] = 8 \times 10^{-4}$ M, pH = 7.2, Tris buffer, $T = 25$ °C.

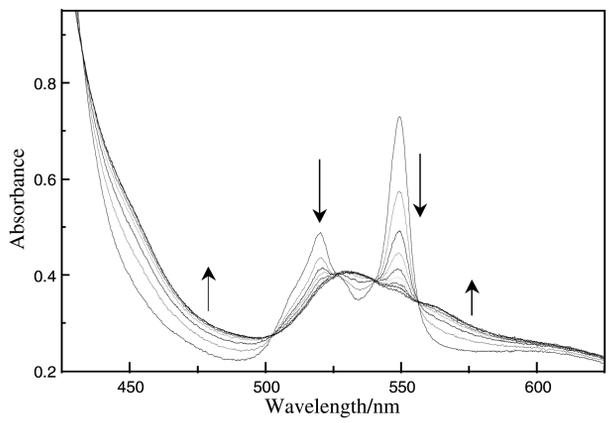

Fig. 2 Absorption spectra recorded during the oxidation of cyt c^{II} by trisoxalatocobalt(III). Experimental conditions: [cyt c^{II}] = 2.5×10^{-5} M, $[Co(ox)_3^{3-}] = 8 \times 10^{-4}$ M, $I = 0.1$ M (Tris–$LiNO_3$), pH = 7.2 (HNO_3), $T = 25$ °C, spectra recorded over a time period of 180 s.

UV–Vis spectral measurements

The reduced and oxidized forms of cytochrome *c* were analysed spectrophotometrically. Characteristic bands: for cyt c^{II} at 550 nm ($\varepsilon = 27600$ dm^3 mol^{-1} cm^{-1}) and 420 nm ($\varepsilon = 129000$ dm^3 mol^{-1} cm^{-1}); for cyt c^{III} at 521 nm ($\varepsilon = 9100$ dm^3 mol^{-1} cm^{-1}) and 416 nm ($\varepsilon = 89000$ dm^3 mol^{-1} cm^{-1}).[26,27] The absorption spectrum of the trisoxalatocobalt(III) complex shows two bands at 605 nm ($\varepsilon = 175$ dm^3 mol^{-1} cm^{-1}) and 425 nm ($\varepsilon = 230$ dm^3 mol^{-1} cm^{-1}), whereas the trisoxalatocobalt(II) complex $[Co(ox)_3]^{4-}$ is characterized by a single maximum at 534 nm. The comparison of absorbance data with literature values was used to determine the concentrations of the species $[Co(ox)_3]^{3-}$ and cytochrome *c* in solution. All spectra were monitored on Shimadzu UV-2100 and Cary G-5 spectrophotometers (see Figs. S1 and S2 in the electronic supplementary information).

Stopped-flow measurements

The oxidation of cyt c^{II} by $[Co(ox)_3]^{3-}$ was monitored at 550 nm. The kinetic measurements were performed on an Applied Photophysics stopped-flow instrument at ambient pressure and on a home-made high pressure stopped-flow instrument at pressures up to 130 MPa.[28] Pseudo-first order conditions were achieved by using an excess of oxidant over the metalloprotein. The reactants were thermostatted before mixing (± 0.1 °C). Rate constants were calculated as the mean of at least six reproducible kinetic runs.

Results and discussion

Behaviour of $[Co(ox)_3]^{3-}$ in solution

During the spectral measurements, a small decrease in the concentration of the complex was observed due to photochemical decomposition. Longer exposure of solutions to light resulted in a change in the solution colour (from green to pink) and an overall decomposition of the compound, characterized by the fading of the absorption bands at 605 and 425 nm, and formation of a new band at 534 nm, similar to that shown in Fig. S2. This is in line with the photochemical reduction of the complex.

In order to increase the stability of the cobalt(III) complex, an excess of the free oxalate ligand was added to the reaction medium, and the influence of the free ligand concentration on the stability of the complex is shown in Fig. 1. The observed decrease in absorbance as a function of oxalate concentration is due to dilution of the complex solution during addition of oxalate. The changes in the absorption spectrum and therefore the apparent aquation of the complex are negligible.

We did, however, find that an excess of free oxalate does decelerate the oxidation rate of cyt c^{II} by trisoxalatocobalt(III). This can be the result of an interaction between the negatively charged oxalate ions and the positively charged amino acid sites on the cytochrome surface, thus interfering with ion-pair formation with the trisoxalatocobalt(III) complex. Free oxalate was therefore not added to the reaction solution in the kinetic experiments.

Reaction of $[Co(ox)_3]^{3-}$ with cyt c^{II}

The reaction progress was followed by UV–Vis spectroscopy within the range 300–700 nm. Typical spectral changes observed during reaction (1) are accompanied by a characteristic absorbance decrease at 550 nm. The spectra shown in Fig. 2 show five isosbestic points at 436, 505, 526, 537 and 557 nm. The reaction could only be followed in one direction, and no evidence for a reverse process was found. This is in agreement with the reduction potential, *viz.* $E = 0.26$ V for the protein and $E = 0.57$ V for the cobalt complex.[29] All kinetic measurements were performed under pseudo-first order conditions as a function of pH, concentration, temperature and pressure. All kinetic data are summarized in Table S1.

In terms of the outer-sphere type of electron transfer mechanism outlined in eqn. (1), three fundamental steps can be distinguished. The first step involves the rapid formation of a precursor complex due to electrostatic interaction between the reactants, characterized by a precursor formation constant (K). The subsequent step involves rate-determining electron transfer (k_{et}). Less significant for our measurements is the third step that leads to the formation of the products through the rapid dissociation of the successor complex. In the case of weak precursor complex-formation, saturation of the observed rate constant at high complex concentrations is not observed, and the concentration dependence has a linear character for which $k_{obs} = k_{et}K[Co(III)]$.

The oxidation of cytochrome c^{II} by $[Co(ox)_3]^{3-}$ shows a limiting value of the observed rate constant at high concentrations of the cobalt complex as seen from the data in Fig. 3. This is a result of electrostatic interactions between the oppositely charged ions (net charge on the reduced form of cyt *c* is +6.5, originating from the hydrophobic side-chains, and the charge on the cobalt complex is −3). For the studied reaction it was possible to separate the constants K and k_{et}, and the obtained values are $K = 253 \pm 34$ dm^3 mol^{-1}, $k_{et} = 0.158 \pm 0.035$ s^{-1} and $k_{12} = k_{et}K = 40 \pm 15$ dm^3 mol^{-1} s^{-1} at 25 °C.

From theoretical calculations based on Marcus–Hush theory [30–35] [eqns. (2)–(4)] we were also able to predict values for k_{12}, K and k_{et}:

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

Table 1 Activation parameters for electron transfer between cyt c^{II} and trisoxalatocobalt(III). Experimental conditions: [cyt c^{II}] = 1×10^{-5} M, $I = 0.1$ M (Tris–$LiNO_3$), pH = 7.2 (HNO_3), $T = 25$ °C, $\lambda = 550$ nm

$[K_3[Co(C_2O_4)_3]]$/M	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G^{\ddagger}_{calc}$ (at 25 °C)/kJ mol^{-1}	$\Delta V^{\ddagger}$/cm^3 mol^{-1}
7×10^{-3}	—	—	—	-6.5 ± 1.0
6.25×10^{-3}	$+73 \pm 4$	-15 ± 11	78	-4.6 ± 0.5
1.25×10^{-3}	$+81 \pm 4$	$+2 \pm 13$	80	—
0.8×10^{-3}	$+86 \pm 18$	$+18 \pm 61$	81	-11.7 ± 0.6
0.2×10^{-3}	—	—	—	-10.0 ± 1.2

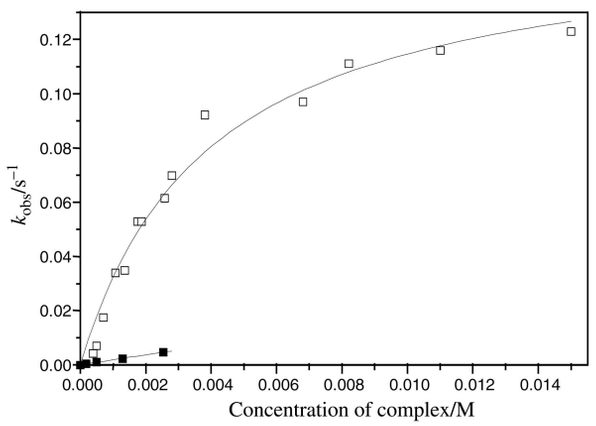

Fig. 3 Dependence of k_{obs} on the concentration of trisoxalatocobalt(III). Experimental conditions: [cyt c^{II}] = (1–2.5) $\times 10^{-5}$ M, (□) $I = 0.1$ M (Tris–$LiNO_3$), pH = 7.2 (HNO_3), $T = 25$ °C, $\lambda = 550$ nm, (■) $I = 0.5$ M (phosphate).[40]

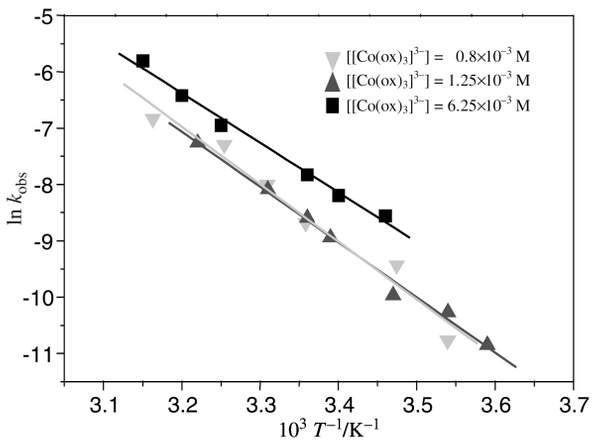

Fig. 4 Temperature dependence of the oxidation of cyt c^{II} by trisoxalatocobalt(III) as a function of complex concentration. Experimental conditions: [cyt c^{II}] = (1.5–2.0) $\times 10^{-5}$ M, $I = 0.1$ M (Tris–$LiNO_3$), pH = 7.2 (HNO_3), $\lambda = 550$ nm.

where $k_{11} = 3.5 \times 10^2$ M^{-1} s^{-1},[36–38] $k_{22} = 2.8 \times 10^{-7}$ M^{-1} s^{-1},[39] and $\ln f = (\ln K_{12})^2/4\ln(k_{11}k_{22}/Z^2)$, $\ln K_{12} = nF\Delta E/RT$,

$$K = \tfrac{4}{3}\pi N_A \delta^3 \exp(-w_{12}/RT),$$
$$w_{12} = \frac{z_1 z_2 e_o^2 N_A}{4\pi\varepsilon_0\varepsilon_1\delta(1+\kappa\delta)} \quad (3)$$

$\kappa = 3.29\ I^{0.5}$ nm^{-1}, $I = 0.1$ M (for our conditions: $\varepsilon_r = 78.5$, $\kappa = 1.04$ nm^{-1}) z_1 (cyt $c^{II/III}$) = +6.5/+7.5, $z_2(Co(ox)_3^{3-/4-}) = -3/-4$, $\delta = r_1 + r_2$, r_1(cyt $c^{II/III}$) = 1.66 nm, $r_2(Co(ox)_3^{3-/4-}) = 0.37$ (calculated with Hyperchem 5.1, ChemSketch 3.5) to 0.45 nm,[39]

$$k_{et} = k_{12}/K \quad (4)$$

from which it follows that $k_{12(calc)} = 3.04$ dm^3 mol^{-1} s^{-1}, $K_{(calc)} = 187$ M^{-1} and $k_{et} = 0.02$ s^{-1}.

The experimental values for k_{et}, K and k_{12} are in reasonable agreement with the calculated values. The calculated second-order rate constant ($k_{12\ (calc)} = 3.04$ dm^3 mol^{-1} s^{-1}) is smaller than the experimental value ($k_{12\ (exp)} = 40 \pm 15$ M^{-1} s^{-1}). The difference presumably results from the fact that the reactants are of opposite charge. The ion-pair formation constant is large enough to cause a significant deviation from linearity in the plot of k_{obs} *vs.* Co(III) concentration at a low ionic strength (see Fig. 3).

A comparison of these results with an earlier studied system in our laboratory reveals some interesting trends. The ion-pair formation constant for the reaction of cyt c^{II} with *trans*-bis(2-ethyl-2-hydroxybutanoato(2−))oxochromate(v), is much smaller, *viz.* $K = 37 \pm 5$ M^{-1} ($T = 288$ K).[19] The k_{et} is larger and equal to 1510 ± 180 s^{-1}. The chromium(v) complex has negatively charged donor centres. There are three possible positions of negative charges on the Cr^V complex, *viz.* the oxo group, the ring with carboxyl oxygen, and the carboxyl oxygen, which are all accessible for water. The pre-equilibrium constant, K, and the electron transfer rate constant, k_{et}, were calculated varying the possible charge on the cytochrome c^{II} centre. Since the chromium reactant carries an overall charge of −1, and the cytochrome c^{II} has a net charge of +6.5, the product of charges can adapt theoretical values between −1 and −6.5. This product depends on the charge neutralized in the presence of counter ions and the influence of local charge effects on the increasing radius of the chromium complex. The values calculated from the Fuoss equation are, within 75%, in agreement with the measured results for the possible contact radii. The most probable position for negative charge localization on the chromium complex is the Cr···C=O group, which is more likely than the Cr–O and Cr=O groups. For the reaction cited above the overall value k_{12} is equal to 55870 M^{-1} s^{-1}.[19]

Our results for the reaction with trisoxalatocobalt(III) differ from published data.[40] Holwerda *et al.* did not observe saturation kinetics over the investigated concentration range (see data included in Fig. 3), such that the observed dependence of the rate constant on the concentration of the cobalt complex was found to be linear. This is presumably due to the higher ionic strength selected by these authors, which will result in a lower ion-pair formation constant. For this system the rate of the reaction decreases with increasing ionic strength,[18,40,41] as confirmed in the present investigation.

A series of kinetic experiments were performed to investigate the pH dependence of the electron transfer process. The rate constant of the reaction of cyt c^{II} with $[Co(ox)_3]^{3-}$ remained unchanged in the pH range 6.5–8.0 (see Fig. S3).

The activation parameters obtained from the Eyring plot shown in Fig. 4, are summarized in Table 1. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were determined for three concentrations of the complex, *i.e.* to cover the non-linear dependence of k_{obs} on the cobalt concentration. The selected concentrations were limited by the high absorbance of the large excess of $[Co(ox)_3]^{3-}$. The slow electron transfer reaction is characterized by a high activation enthalpy, whereas the $\Delta S^{\ddagger}$ values are close to zero. The activation volume was determined for four concentrations of the cobalt complex, the results are illustrated in Fig. 5 and summarized in Table 1. The activation volume for the combined ion-pair formation and electron transfer processes is revealed by the data measured

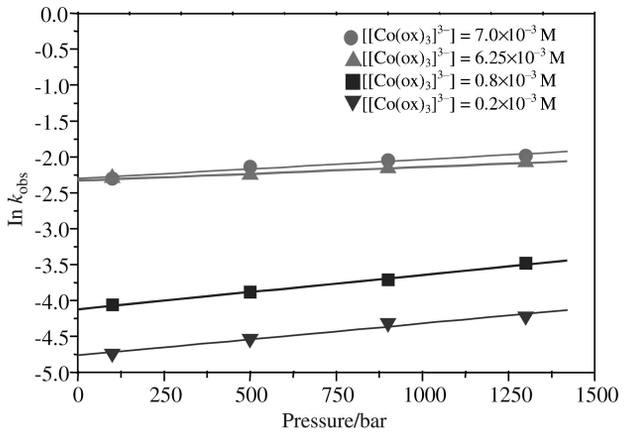

Fig. 5 Pressure dependence of the oxidation of cyt c^{II} by trisoxalatocobalt(III) as a function of complex concentration. Experimental conditions: [cyt c^{II}] = 1.5×10^{-5} M, $I = 0.1$ M (Tris–$LiNO_3$), pH = 7.2 (HNO_3), $T = 25$ °C, $\lambda = 550$ nm.

at low concentrations of the cobalt complex where $\Delta V^{\ddagger} = \Delta V(K) + \Delta V^{\ddagger}(k_{et}) = -11 \pm 1$ cm^3 mol^{-1}, since $k_{obs} = Kk_{et}[Co^{III}]$. At high Co(III) concentrations we could determine $\Delta V^{\ddagger}(k_{et})$, since $k_{obs} = k_{et}$ under these conditions, and $\Delta V^{\ddagger}(k_{et})$ has a small negative value of -5.6 ± 0.6 cm^3 mol^{-1}. It follows that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are larger and $\Delta V^{\ddagger}$ is more negative for the lower concentrations of $[Co(ox)_3]^{3-}$, *i.e.* where two steps of the reaction are responsible for these values, namely the formation of the precursor complex and electron transfer process. At the high concentration limit only the electron transfer process determines the activation parameters.

A comparison with the thermal activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ determined in the low concentration range (second-order rate constant, *i.e.* Kk_{et}) for the chromium(V) system, shows that the activation enthalpy is lower, $\Delta H^{\ddagger} = 21 \pm 1$ kJ mol^{-1}, and activation entropy is more negative, $\Delta S^{\ddagger} = -80 \pm 2$ J K^{-1} mol^{-1} as compared to the trisoxalatocobalt(III) system. The observed significantly negative activation entropy suggests a highly-structured transition state.[19]

The difference in the $\Delta V^{\ddagger}$ data reported for the reaction at high and low trisoxalatocobalt(III) concentrations, is an approximate indication of the value for $\Delta V(K)$ and equals -5.4 ± 1.6 cm^3 mol^{-1}. Thus ion-pair formation between cyt c^{II} and $[Co(ox)_3]^{3-}$ must involve a significant overlap of the van der Waals radii of the reactants without significant charge neutralization which would induce desolvation. The ion-pair can then be depicted as solvent separated. Such a conclusion was also reached for a quite different outer-sphere electron transfer reaction, for which $\Delta V(K)$ was also measured, between $[Fe(CN)_6]^{4-}$ and $[Co(NH_3)_5X]^{3+}$ (X = H_2O, py, Me_2SO).[42] The value for $\Delta V^{\ddagger}(k_{et})$ of -5.5 ± 1.0 cm^3 mol^{-1} indicates that the transition state for the electron transfer reaction within the precursor (ion-pair) complex is more compact, which is most probably related to a volume decrease on the $[Co^{III}(ox)_3]^{3-}$ partner, since it is known that oxidation of cyt c^{II} to cyt c^{III} is accompanied by a volume increase of 5 cm^3 mol^{-1}.[43] When $[Co^{III}(ox)_3]^{3-}$ is reduced to $[Co^{II}(ox)_3]^{4-}$, the increase in overall negative charge will cause an increase in electrostriction which could account for the observed negative volume of activation.

$\Delta V^{\ddagger}$ values for the chromium complex (results only for the low concentration range) are -9.2 ± 0.6 for pH = 5.0 and -11.1 ± 0.8 cm^3 mol^{-1} for pH = 4.8.[19] The overall reaction volumes are -11.8 ± 0.5 (pH = 5.0) and -14.7 ± 0.7 cm^3 mol^{-1} (pH = 4.8) and reflect the overall volume changes arising from the reduction of Cr^{V} to Cr^{IV}, as well as that caused by a change in the overall charge from -1 to -2. The solvational effects arising from the increase in electrostriction account for the overall negative reaction volumes. The oxidation of cyt c^{II} by the Cr^{V} oxo species is therefore associated with an overall volume decrease of 7 and 10 cm^3 mol^{-1}. The localization of the transition state can be described as "late" referring to the overall volume change and is close to the product state. At this stage it can only be related to the efficient precursor formation occurring in the system.[19]

The overall $\Delta V^{\ddagger}(k_{12})$ value of -11 ± 1 cm^3 mol^{-1} found in this study can also be compared to values found in other systems where it was not possible to separate K and k_{et}. The reduction of our Co(III) complex is accompanied by a volume decrease *i.e.* exactly opposite to that observed in the case of the pentaammineruthenium(III) and diiminecobalt(III) complexes which are positively charged. These $\Delta V^{\ddagger}$ values are positive and range from +12.5 to +17.9 cm^3 mol^{-1} for the series of complexes.[11,12] Ruthenium complexes exhibit negligible intrinsic volume changes and therefore mainly solvation effects account for the observed volume changes.[11] In the case of the diimine cobalt complexes, the volume increases due to a decrease in the solvent electostriction and an intrinsic contribution.[12] The electron transfer processes are controlled by electrostriction effects on the redox partner of the protein. These systems are characterized by large volume changes, and the transition state lies almost halfway between the reactant and product states on a volume basis. This means that the reorganization in the transition state is very similar for both forward and back reactions in the reversible electron-transfer process.

We were not able to construct a volume profile for the investigated system due to unsuccessful efforts to obtain either volume data for the reverse reaction or high pressure electrochemical data on the $[Co^{III}(ox)_3]^{3-}/[Co^{II}(ox)_3]^{4-}$ redox system. Earlier studies demonstrated that volume profile analyses are very useful to locate the position of the transition state along the reaction coordinate.[9,10,12,19,44–50]

Conclusions

The outer-sphere electron transfer reaction between cytochrome c^{II} and trisoxalatocobalt(III) clearly showed saturation kinetics as a result of effective ion-pair formation. This enabled a separation of the ion-pair formation constant and the electron transfer rate constant in terms of all thermodynamic and kinetic parameters. Under kinetic saturation conditions, activation parameters for the electron transfer reaction could be determined. These reveal detailed information on structural and solvational changes associated with the electron transfer process. More systems will have to be studied along the same lines in order to analyse in more detail the nature of the precursor complex (for instance the site where the anionic reactant binds to the protein surface) and the effect that precursor formation has on the subsequent rate-determining electron transfer process.

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References

1 H. B. Gray and J. R. Winkler, *Annu. Rev. Biochem.*, 1996, **65**, 537.
2 R. D. Cannon, *Electron Transfer Reactions*, Butterworths, London, 1980.
3 S. S. Isied, G. Worosila and S. J. Artherton, *J. Am. Chem. Soc.*, 1982, **104**, 7659.
4 S. S. Isied, in *Electron transfer in Inorganic, Organic, and Biological Systems*, ed. J. R. Bolton, N. Mataga and G. McLendon, ACS, Washington, DC, 1991.
5 S. S. Isied, *Adv. Chem. Ser.*, 1997, **253**, 331.
6 J. V. McArdle, H. B. Gray, C. Creutz and N. Sutin, *J. Am. Chem. Soc.*, 1974, **96**, 5737.

7 J. V. McArdle, K. Yocom and H. B. Gray, *J. Am. Chem. Soc.*, 1977, **12**, 4141.
8 D. G. Nocera, J. R. Winkler, K. M. Yocom, E. Bordignon and H. Y. Gray, *J. Am. Chem. Soc.*, 1984, **106**, 5145.
9 B. Bänsch, M. Meier, P. Martinez, R. van Eldik, C. Su, J. Sun, S. S. Isied and J. F. Wishart, *Inorg. Chem.*, 1994, **33**, 4744.
10 M. Meier and R. van Eldik, *Inorg. Chim. Acta*, 1994, **225**, 95.
11 M. Meier, J. Sun, R. van Eldik and J. F. Wishart, *Inorg. Chem.*, 1996, **35**, 1564.
12 M. Meier and R. van Eldik, *Chem. Eur. J.*, 1997, **3**, 39.
13 V. S. Sivozhelezov, Y. E. Komarov and G. B. Postnikova, *Biofizika*, 1997, **41**, 1180.
14 J. Sun, C. Su, M. Meier, S. S. Isied, J. F. Wishart and R. van Eldik, *Inorg. Chem.*, 1998, **37**, 6129.
15 J. R. Winkler and H. B. Gray, *Chem. Rev.*, 1992, **92**, 369.
16 J. F. Wishart, J. Sun, M. Cho, C. Su and S. S. Isied, *J. Phys. Chem. B*, 1997, **101**, 687.
17 J. F. Wishart, R. van Eldik, J. Sun, C. Su and S. S. Isied, *Inorg. Chem.*, 1992, **31**, 3986.
18 J. D. Rush, J. Lan and W. H. Koppenol, *J. Am. Chem. Soc.*, 1987, **109**, 2679.
19 M. Körner and R. van Eldik, *Eur. J. Inorg. Chem.*, 1999, 1805.
20 J. C. Bailar and J. E. M. Jones, *Inorg. Synth.*, 1939, **1**.
21 S. P. L. Sörensen, *Z. Anorg. Allg. Chem.*, 1896, **11**, 355.
22 H. Siebert, *Z. Anorg. Allg. Chem.*, 1959, 52.
23 N. Tanaka and M. Nanjo, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 330.
24 J. Fujita and Y. Shimura, *Bull. Chem. Soc. Jpn.*, 1963, **36**, 1281.
25 A. Mead, *J. Phys. Chem.*, 1934, 1052.
26 G. W. Pettigrew and G. R. Moore, *Cytochromes c. Evolutionary, Structural Physicochemical Aspects*, Springer-Verlag, New York, 1990.
27 A. W. Adamson, H. Ogata, J. Grossman and R. Newbury, *J. Inorg. Nucl. Chem.*, 1958, **6**, 319.
28 D. Magde and R. van Eldik, in *High Pressure Techniques in Chemistry and Physics*, ed. W. B. Holzaplfel and N. S. Isaacs, Oxford University Press, Oxford, 1997.
29 L. Hin-Fat and W. C. E. Higgison, *J. Chem. Soc. A*, 1967, 298.
30 R. A. Marcus, *J. Phys. Chem.*, 1968, **72**, 891.
31 T. W. Swaddle, *Inorg. Chem.*, 1990, **29**, 5017.
32 M. Grace, H. Takagi and T. W. Swaddle, *Inorg. Chem.*, 1994, **33**, 1915.
33 R. A. Marcus, *J. Phys. Chem. B*, 1998, **102**, 10071.
34 G. McLendon, S. KomarPanicucci and S. Hatch, in *Electron Transfer from Isolated Molecules to Biomolecules, Pt. 2*, ed. J. Jortner and M. Bixon, 605 3Rd Ave/New York/NY 10016/USA, 1999.
35 R. A. Marcus, in *Electron Transfer from Isolated Molecules to Biomolecules, Pt. 1*, ed. J. Jortner and M. Bixon, 605 3Rd Ave/New York/NY 10016/USA, 1999.
36 R. K. Gupta, S. H. Koenig and A. G. Redfield, *J. Magn. Reson.*, 1972, **7**, 66.
37 R. K. Gupta, *Biochim. Biophys. Acta*, 1973, **292**, 291.
38 S. Wherland and H. B. Gray, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 2950.
39 R. Farina and R. G. Wilkins, *Inorg. Chem.*, 1968, **7**, 514.
40 R. A. Holwerda, D. B. Knaff, H. B. Gray, J. D. Clemmer, R. Crowley, J. M. Smith and A. G. Mauk, *J. Am. Chem. Soc.*, 1980, **102**, 1142.
41 J. T. Ficke, J. R. Pladziewicz, E. C. Sheu and A. G. Lappin, *Inorg. Chem.*, 1991, **30**, 4282.
42 I. Krack and R. van Eldik, *Inorg. Chem.*, 1986, **25**, 1743.
43 J. Sun, J. F. Wishart, R. van Eldik, R. D. Shalders and T. W. Swaddle, *J. Am. Chem. Soc.*, 1995, **117**, 2600.
44 A. Drljiaca, C. D. Hubbard, R. van Eldik, T. Asano, M. V. Basilevsky and W. J. le Noble, *Chem. Rev.*, 1998, **98**, 2167.
45 R. van Eldik, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 673.
46 R. van Eldik, *High Pressure Res.*, 1991, **6**, 251.
47 R. van Eldik, in *High Pressure Chemistry, Biochemistry and Materials Science*, ed. R. Winter and J. Jonas, Kluwer Academic Publishers, Dordrecht, 1993.
48 R. van Eldik, in *Advances in Chemistry Series 254. Photochemistry and Radiation Chemistry. Complementary Method for the Study of Electron Transfer*, ed. J. F. Wishart and D. G. Nocera, ACS, Washington, DC, 1998.
49 R. van Eldik, in *High Pressure Molecular Science*, ed. R. Winter and J. Jonas, Kluwer Academic Publishers, Dordrecht, 1999.
50 G. Stochel and R. van Eldik, *Coord. Chem. Rev.*, 1999, **187**, 329.