

Reduction of Ferricytochrome-c by Co(II)-Sepulchrate

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Electron transfer reactions between inorganic complexes and metalloproteins provide an interesting way of characterizing the redox active sites of biological molecules [1–4]. To probe metalloprotein reactivity it is required, however, that the complexes present suitable structural, kinetic, electrochemical and solubility properties. The cobalt-sepulchrate complex (sep = 1,3,6,8,10,13,16,19-octaazabicyclo[6.6.6]icosane) seems to be very promising in this sense. The cage compound undergoes reversible electron transfer [5–7] in aqueous solution, in contrast with typical cobalt-ammines. The self exchange rate of $[\text{Co}(\text{sep})]^{3+/2+}$ is comparable to that of $[\text{Co}(\text{phen})_3]^{3+/2+}$; however, its redox potential is 600 mV more negative. In this work, we have investigated the reduction of horse heart ferricytochrome-c by cobalt(II)-sepulchrate, including a detailed Marcus theory analysis of the kinetics and activation parameters of the electron transfer reaction.

Experimental

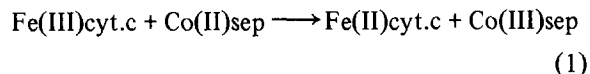
Horse heart cytochrome-c was obtained from Sigma, type VI. The macrobicyclic complex, $[\text{Co}(\text{sep})]\text{Cl}_3$ was prepared as described in the literature [5], by condensing the $[\text{Co}(\text{en})_3]^{3+}$ ion with formaldehyde and ammonia. The $\Lambda(\text{S})$ and $\Delta(\text{R})$ forms of the complex were synthesized, respectively, from Λ - and Δ - $[\text{Co}(\text{en})_3]\text{Cl}_3$. *Anal.* Calcd. for $\text{CoC}_{12}\text{N}_8\text{H}_{30}\text{Cl}_3$: C, 31.9; N, 24.8; H, 6.7. Found: $\Lambda(\text{S})$ isomer, C, 31.1; N, 24.0; H, 7.0; $\Delta(\text{R})$ isomer, C, 31.8; N, 24.9; H, 7.1.

All the measurements in this work were carried out under argon atmosphere. Cyclic voltammetry was performed with a Princeton Applied Research Corporation instrument, consisting of a 173 potentiostat and a 175 universal programmer. A gold disk electrode was used for the measurements, with Ag/AgCl (1 M KCl) as the reference electrode, using the conventional Luggin capillary arrangement to minimize the ohmic drop. A platinum wire was used as the auxiliary electrode. Temperature dependence studies were performed using a non-isothermic

arrangement, as described in the literature [8]. Electronic spectra in the visible and near-UV region were recorded on a Cary 17 spectrophotometer, fitted with thermostatted cell compartments. Stopped-flow kinetics were carried out with a Durrum D-150 instrument, equipped with a Kel-F flow system. Co(II)-sepulchrate was freshly prepared by reducing the cobalt(III) complex with zinc amalgam. The cytochrome-c was treated with an equimolar amount of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 0.025 M phosphate buffer (pH 6.8) and purified by passing through a 1×10 cm DEAE-25 Sephadex column. The concentration of cytochrome-c was monitored by the absorption band at 549 nm ($\epsilon = 29,500 \text{ M}^{-1} \text{ cm}^{-1}$) of the reduced form, generated in the presence of ascorbate or cobalt(II)-sepulchrate. After mixing in the stopped-flow instrument, the final pH varied between 6.8–7.1. The calculation of the rate constants and of Marcus theory equations were carried out with a microdigital TK 85 computer.

Results and Discussion

The reduction of horse heart ferricytochrome-c by cobalt(II)-sepulchrate proceeds according to a first order kinetics for at least three half lives, in the presence of an excess of the cobalt complex.



$$\frac{d[\text{Fe(II)cyt.c}]}{dt} = k_{12} [\text{Fe(III)cyt.c}] [\text{Co(II)sep}] \quad (2)$$

Second order rate constants for the electron transfer kinetics with the $\Lambda(\text{S})$ and $\Delta(\text{R})$ -Co(II) sepulchrate isomers, at several temperatures, can be seen in Table I. The activation parameters were respectively, $\Delta H^\ddagger = 0$, $\Delta S^\ddagger = -33.6$ and $\Delta H^\ddagger = 0$ kcal mol^{-1} , $\Delta S^\ddagger = -33.7 \text{ cal mol}^{-1} \text{ deg}^{-1}$. No difference was observed between the two series of measurements, indicating negligible influence of chirality in the electron transfer reaction.

To evaluate the self-exchange rate constants of cytochrome-c, we have employed Marcus theory with the Wherland–Gray formalism [4] for the work terms, W_{11} and W_{11} . The self-exchange rate constant, k_{22} for the $[\text{Co}(\text{sep})]^{3+/2+}$ complex has been measured by Sargeson *et al.* [5] as $5.1 \text{ M}^{-1} \text{ s}^{-1}$ (25 °C, $I = 0.2 \text{ M}$). The reorganization free energies, ΔG_{11}^{**} , are given by the equation

$$\Delta G_{11}^{**} = 2\Delta G_{12}^{**} - \Delta G_{22}^{**} - \Delta G_r^\circ(1 - \alpha_{12}) \quad (3)$$

where

TABLE I Kinetic Results for the $\Lambda(S)$ and $\Delta(R)$ -Co(II)sep-Fe(III)cyt c Reactions.^a

T °C	$[\Lambda(S)\text{Cosep}]$ 10^{-4} M	k_{obs} s^{-1}	k_{12} $10^5 \text{ M}^{-1} \text{ s}^{-1}$	$[\Delta(R)\text{Cosep}]$ 10^{-4} M	k_{obs} s^{-1}	k_{12} $10^5 \text{ M}^{-1} \text{ s}^{-1}$
14.4	0.610	17.0	2.78	1.19	32.6	2.73
20.6				1.19	30.3	2.54
21.2	0.610	17.8	2.90			
25.0	0.610	16.0	2.62	0.610	17.0	2.78
25.0	1.19	31.8	2.67	1.19	31.9	2.68
25.0	1.74	42.1	2.42	2.27	66.0	2.90
25.0	2.27	60.0	2.64			
27.9	0.61	17.7	2.90			
30.2				1.19	31.8	2.67
34.2	0.61	17.2	2.82			
34.7				1.19	30.3	2.54

^a[cyt.c] = $4 \times 10^{-6} \text{ M}$, $I = 0.10 \text{ M}$ (KCl), pH 6.8 (0.025 M phosphate buffer), 549 nm

$$\alpha_{12} = \frac{\Delta G_r^\circ}{4(\Delta G_{11}^{**} + \Delta G_{22}^{**})} \quad (4)$$

and

$$\Delta G_{12}^{**} = \Delta G_{12}^* - W_{12} \quad (5)$$

$$\Delta G_{11}^{**} = \Delta G_{11}^* - W_{11} \quad (6)$$

$$\Delta G_{22}^{**} = \Delta G_{22}^* - W_{22} \quad (7)$$

$$\Delta G_r^\circ = \Delta G_{12}^\circ - W_{12} + W_{21} \quad (8)$$

Analogously to Wherland and Gray [4], we have used a pre-exponential factor of $6 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$ in Marcus equation, as in the semiclassical formalism [9],

$$k = \kappa_{\text{el}} \Gamma_{\text{n}} V \nu_{\text{n}} \exp - (\Delta G^*/RT) \quad (9)$$

where the product of the electronic and nuclear tunneling factors, $\kappa_{\text{el}} \Gamma_{\text{n}}$ is close to unity, $V = 4\pi N r^3/3000$; ν_{n} is the nuclear vibration frequency, and $V \nu_{\text{n}} \cong kT/h$. The input and output parameters are given in Table II.

The calculated self-exchange rate constant of cytochrome-c based on the Fe(III)cyt.c-Co(II)sepulchrate reaction is $13 \text{ M}^{-1} \text{ s}^{-1}$. The reported values derived from the analogous reactions with $[\text{FeEDTA}]^{2-}$ and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ are 6.2 and $16 \text{ M}^{-1} \text{ s}^{-1}$, respectively [4]. On the other hand, k_{11} derived from electron transfer reactions of Fe(II)-cyt.c with the aromatic $[\text{Co}(\text{phen})_3]^{3+}$ [4] and ferricinium complexes [10] were 7.1×10^2 and $1.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, respectively. For negatively charged complexes such as $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_5\text{L}]^{n-}$, k_{11} vary from 10^4 to $10^6 \text{ M}^{-1} \text{ s}^{-1}$ [4, 11].

A recent study has located the preferred sites for electron transfer between cytochrome-c and inor-

ganic complexes around the solvent accessible edge of the heme group, or a closely related structure on the front surface of the molecule. As a rule, negatively charged complexes react with cytochrome-c much faster than positively charged complexes, because of the charge distribution in the protein surface. It has been pointed out [12] that positively charged complexes tend to interact non productively with the back surface of the protein at a much greater frequency than the electrostatically unfavorable, but productive encounters with the area of the heme edge. The greater efficiency of $[\text{Co}(\text{phen})_3]^{3+}$ in comparison to the Co-sepulchrate complex supports Gray's conclusion [3] regarding the importance of hydrophobic π -conducting ligands in electron transfer between proteins and small metal complexes.

The activation parameters for the cytochrome-c-Co(II)sep. reaction are rather unusual for a bimolecular electron transfer reaction. The process seems to be exclusively dependent on entropy effects, with a null activation enthalpy. To evaluate the specific contributions from the reactants we carried out a detailed analysis of the activation parameters. The reorganization entropies associated to electron transfer can be expressed in the following way.

$$\Delta S_{12}^{**} = \left(\frac{\Delta S_{11}^{**}}{2} + \frac{\Delta S_{22}^{**}}{2} \right) (1 - 4\alpha_{12}^2) + \frac{\Delta S_r^\circ}{2} (1 + 2\alpha_{12}) \quad (10)$$

where

$$\Delta S_{11}^{**} = \Delta S_{11}^* + \frac{\partial W_{11}}{\partial T} \quad (11)$$

$$\Delta S_{22}^{**} = \Delta S_{22}^* + \frac{\partial W_{22}}{\partial T} \quad (12)$$

TABLE II. Self-Exchange Rate Constants and Activation Parameters for the Co(II)sepulchrate–Fe(III)cytochrome-c Reaction at 25 °C and $I = 0.10$ M.

	Cyt.c(III)/Cosep(II)	Cosep(III)/Cosep(II)	Cyt.c(III)/Cyt.c(II)
Z	7.5/2.0	3.0/2.0	7.5/6.5
r (10^2 pm)	16.5/3.8	3.7/3.8	16.5/16.5
W_{ij} , W_{ii} (kcal mol $^{-1}$)	0.777	1.649	0.414
W_{ij} (kcal mol $^{-1}$)	0.589		
$\frac{\partial W_{ij}}{\partial T}$, $\frac{\partial W_{ii}}{\partial T}$ (cal mol $^{-1}$ deg $^{-1}$)	3.238 ^a 2.955 ^b	7.126 ^a 7.900 ^b	1.697 ^a 1.361 ^b
$\frac{\partial W_{ii}}{\partial T}$ (cal mol $^{-1}$ deg $^{-1}$)	2.456 ^a 2.241 ^b		
ΔE^0 , E_{rc}^0 (V vs. NHE)	0.588	−0.323 −0.296 ^c	0.265 ^d
k_{ij} , k_{ii} (M $^{-1}$ s $^{-1}$)	2.7×10^5	2.62 ^e	13.1
ΔG_{ij}^{**} , ΔG_{ii}^{**} (kcal mol $^{-1}$)	9.25	15.21	15.50
ΔS_{12}^0 , ΔS_{rc}^0 (cal mol $^{-1}$ deg $^{-1}$)	−33	18	−15 ^d
ΔS_{ij}^{**} , ΔS_{ii}^{**} (cal mol $^{-1}$ deg $^{-1}$)	−29.3 ^a −28.7 ^b	−13.8 ^a −13.1 ^b	−21.4 ^a −20.9 ^b
$\Delta S_{ij}^\ddagger$, $\Delta S_{ii}^\ddagger$ (cal mol $^{-1}$ deg $^{-1}$)	−33.6	−23 ^e	−25.1 ^a −24.2 ^b
ΔH_{ij}^{**} , ΔH_{ii}^{**} (kcal mol $^{-1}$)	0.53 ^a 0.71 ^b	11.1 ^a 11.3 ^b	9.1 ^a 9.2 ^b
$\Delta H_{ij}^\ddagger$, $\Delta H_{ii}^\ddagger$ (kcal mol $^{-1}$)	0	9.6 ^e	8.4 ^a 8.6 ^b

^aBased on eqn. 16. ^bBased on eqn. 17. ^cRef. 16. ^dRefs. 17, 18. ^eRef. 5.

$$\Delta S_{12}^{**} = \Delta S_{12}^* + \frac{\partial W_{12}}{\partial T} \quad (13)$$

$$\Delta S_r^0 = \Delta S_{12}^0 + \frac{\partial W_{12}}{\partial T} - \frac{\partial W_{21}}{\partial T} \quad (14)$$

The reaction entropy ΔS_{12}^0 can be obtained from the difference of the experimental redox couple entropies:

$$\Delta S_{12}^0 = \Delta S_{rc}^0[\text{cyt.c}] - \Delta S_{rc}^0[\text{Cosep}] \quad (15)$$

Derivation of the work terms is given [9] by

$$\frac{\partial W}{\partial T} = -\frac{W}{2T(1 + \beta r I^{1/2})} \times \left[2 \left(\frac{\ln D_s}{\ln T} \right) + \beta r I^{1/2} \left(\frac{\ln D_s}{\ln T} \right) - \beta r I^{1/2} \right] \quad (16)$$

or alternatively [13], by

$$\frac{\partial W}{\partial T} = Z_1 Z_2 \left(0.868 I^{1/2} + \frac{19.5}{r} \right) \exp(-0.329 r I^{1/2}) \quad (17)$$

where D_s is the static dielectric constant, r is the metal–metal separation in the activated complex, and the other terms have the conventional meaning. For an adiabatic mechanism [9],

$$\Delta S^\ddagger = \Delta S^{**} - \frac{\partial W}{\partial T} - R \quad (18)$$

and

$$\Delta H^\ddagger = \Delta H^{**} + W - T \frac{\partial W}{\partial T} - RT \quad (19)$$

The calculated activation parameters for the cytochrome-c–Co(II)–sepulchrate reaction are presented in Table II. The null activation enthalpy arises from the compensation of the reorganization enthalpies ΔH_{ii}^{**} and the reaction enthalpy ΔH_r^0 , as expressed by

$$\Delta H_{12}^{**} = \left(\frac{\Delta H_{11}^{**}}{2} + \frac{\Delta H_{22}^{**}}{2} \right) (1 - 4\alpha_{12}^2) + \frac{\Delta H_r^0}{2} (1 + 2\alpha_{12}) \quad (20)$$

and

$$\Delta H_r^\circ = \Delta H_{12}^\circ - W_{12} + W_{21} + T \left(\frac{\partial W_{12}}{\partial T} - \frac{\partial W_{21}}{\partial T} \right) \quad (21)$$

For the cross reaction, $\Delta H_r^\circ = -23.5 \text{ kcal mol}^{-1}$, in comparison to $\Delta H_{11}^{**} = 11.1$ and $\Delta H_{22}^{**} = 9.1 \text{ kcal mol}^{-1}$. The activation parameters of cytochrome-c derived from the Co(II)-sepulchrate reaction are $\Delta H^\ddagger = 8.5 \text{ kcal mol}^{-1}$ and $\Delta S^\ddagger = -25 \text{ cal mol}^{-1} \text{ deg}^{-1}$. The literature values for the cytochrome-c self-exchange reaction are $\Delta H^\ddagger = 7.0 \text{ kcal mol}^{-1}$ and $\Delta S^\ddagger = -17 \text{ cal mol}^{-1} \text{ deg}^{-1}$ [14, 15]. Our results confirm the rule that saturated, positively charged complexes are less effective in electron transfer reactions with cytochrome-c.

Acknowledgements

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