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]eicosane) 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 $[Co(sep)]^{3+/2+}$ is comparable to that of $[Co(phen)_3]^{3+/2+}$; 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 macrobicycle complex, $[Co(sep)]Cl_3$ was prepared as described in the literature [5], by condensing the $[Co(en)_3]^{3+}$ ion with formaldehyde and ammonia. The Λ(S) and Δ(R) forms of the complex were synthesized, respectively, from Λ- and Δ-$[Co(en)_3]Cl_3$. *Anal.* Calcd. for $CoC_{12}N_8H_{30}Cl_3$: C, 31.9; N, 24.8; H, 6.7. Found: Λ(S) isomer, C, 31.1; N, 24.0; H, 7.0; Δ(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 progammer. 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 $K_3[Fe(CN)_6]$ 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 (ϵ = 29,500 M^{-1} 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.

$$Fe(III)cyt.c + Co(II)sep \longrightarrow Fe(II)cyt.c + Co(III)sep \quad (1)$$

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

Second order rate constants for the electron transfer kinetics with the Λ(S) and Δ(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}, $\Delta S^{\ddagger} = -33.7$ cal mol^{-1} 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 $[Co(sep)]^{3+/2+}$ complex has been measured by Sargeson *et al.* [5] as 5.1 M^{-1} s^{-1} (25 °C, I = 0.2 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

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TABLE I Kinetic Results for the Λ(S) and Δ (R)-Co(II)sep–Fe(III)cyt c Reactions.[a]

T °C	[Λ(S)Cosep] 10^{-4} M	k_{obs} s^{-1}	k_{12} 10^5 M^{-1} s^{-1}	[Δ (R)Cosep] 10^{-4} M	k_{obs} s^{-1}	k_{12} 10^5 M^{-1} 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] = 4×10^{-6} M, I = 0 10 M (KCl), pH 6 8 (0.025 M phosphate buffer), 549 nm

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

and

$$\Delta G_{12}^{**} = \Delta G_{12}^{*} - W_{12} \tag{5}$$

$$\Delta G_{11}^{**} = \Delta G_{11}^{*} - W_{11} \tag{6}$$

$$\Delta G_{22}^{**} = \Delta G_{22}^{*} - W_{22} \tag{7}$$

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

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

$$k = \kappa_{el}\Gamma_n V \nu_n \cdot \exp - (\Delta G^*/RT) \tag{9}$$

where the product of the electronic and nuclear tunneling factors, $\kappa_{el}\Gamma_n$ is close to unity, $V = 4\pi Nr^3/3000$; ν_n is the nuclear vibration frequency, and $V \cdot \nu_n \cong kT/h$. 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 M^{-1} s^{-1}. The reported values derived from the analogous reactions with $[FeEDTA]^{2-}$ and $[Ru(NH_3)_6]^{3+}$ are 6.2 and 16 M^{-1} s^{-1}, respectively [4]. On the other hand, k_{11} derived from electron transfer reactions of Fe(II)-cyt.c with the aromatic $[Co(phen)_3]^{3+}$ [4] and ferricinium complexes [10] were 7.1×10^2 and 1.0×10^3 M^{-1} s^{-1}, respectively. For negatively charged complexes such as $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_5L]^{n-}$, k_{11} vary from 10^4 to 10^6 M^{-1} s^{-1} [4, 11].

A recent study has located the preferred sites for electron transfer between cytochrome-c and inorganic 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 $[Co(phen)_3]^{3+}$ 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.

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

where

$$\Delta S_{11}^{**} = \Delta S_{11}^{*} + \frac{\partial W_{11}}{\partial T} \tag{11}$$

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

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]
$\frac{\partial W_{jj}}{\partial T}$ (cal mol^{-1} deg^{-1})	2 456[a] 2.241[b]		
ΔE°, E°_{rc} (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}, ΔS°_{rc} (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]
$\Delta S^{\ddagger}_{ij}$, $\Delta S^{\ddagger}_{ii}$ (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]
$\Delta H^{\ddagger}_{ij}$, $\Delta H^{\ddagger}_{ii}$ (kcal mol^{-1})	0	9 6[e]	8.4[a] 8.6[b]

[a]Based on eqn. 16. [b]Based on eqn 17. [c]Ref. 16. [d]Refs. 17, 18 [e]Ref. 5.

$$\Delta S^{**}_{12} = \Delta S^{*}_{12} + \frac{\partial W_{12}}{\partial T} \tag{13}$$

$$\Delta S^\circ_r = \Delta S^\circ_{12} + \frac{\partial W_{12}}{\partial T} - \frac{\partial W_{21}}{\partial T} \tag{14}$$

The reaction entropy ΔS°_{12} can be obtained from the difference of the experimental redox couple entropies:

$$\Delta S^\circ_{12} = \Delta S^\circ_{rc[cyt.c]} - \Delta S^\circ_{rc[Cosep]} \tag{15}$$

Derivation of the work terms is given [9] by

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

or alternatively [13], by

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

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],

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

and

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

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, as expressed by

$$\Delta H^{**}_{12} = \left(\frac{\Delta H^{**}_{11}}{2} + \frac{\Delta H^{**}_{22}}{2}\right)(1 - 4\alpha_{12}{}^2) + \frac{\Delta H^\circ_r}{2}(1 + 2\alpha_{12}) \tag{20}$$

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

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References

1 S Wherland and H B Gray, *Proc Nat Acad Sci. U.S.A., 73,* 2950 (1976)
2 J. V. McArdle, H. B. Gray, C. Creutz and N. Sutin, *J. Am. Chem. Soc , 96,* 5737 (1974).
3 A Grant Mauk, C L Coyle, E. Bordignon and H. B. Gray, *J. Am. Chem Soc , 101,* 5054 (1979)
4 S. Wherland and H B Gray, in D. Dolphin *et al.* (eds.), 'Biological Aspects of Inorganic Chemistry', Wiley, New York, 1977, pp. 289–368.
5 I. I. Creaser, R. J. Geue, J. M. Harrowfield, A. J. Herlt, A. M. Sargeson, M. R. Snow and J Springborg, *J Am Chem. Soc , 104,* 6016 (1982)
6 I. I. Creaser, J. M. Harrowfield, A. J. Herlt, A M. Sargeson, J. Springborg, R J. Geue and M R Snow, *J. Am. Chem Soc., 99,* 3182 (1977)
7 J. F Endicott, G. R Brubaker, T. Ramasami, K. Kumar, K Dwarakanath, J. Cassel and D. Johnson, *Inorg Chem , 22,* 3754 (1983).
8 S. Sahami and M. J. Weaver, *J Electroanal. Chem., 122,* 155 (1981).
9 N. Sutin, in J J. Zuckerman (Ed.), 'Inorganic Reactions and Methods', Springer Verlag, West Berlin, in press.
10 J. R. Pladziewicz and M. J. Carney, *J Am. Chem. Soc , 104,* 3544 (1982).
11 H E. Toma and A A. Batista, *J Inorg Biochem., 20,* 53 (1984).
12 J. Butler, S. K Chapman, D. M. Davies, A. G. Sykes, S. H. Speck, N. Osheroff and E. Margoliash, *J. Biol. Chem., 258,* 6400 (1983).
13 F Moattar, J. R Walton and L. R. Bennett, *Inorg Chem., 22,* 550 (1983)
14 R K. Gupta, S. H. Koenig and A. G Redfield, *J Magn. Reson , 7,* 66 (1972).
15 R K Gupta, *Biochim. Biophys. Acta, 292,* 291 (1973)
16 S. Sahami and M. J. Weaver, *J Electroanal Chem , 122,* 171 (1981)
17 V T. Taniguchi, N. S. Scott, F. C Anson and H. B. Gray, *Pure Appl Chem , 52,* 2275 (1980)
18 P. George, W A. Eaton and M Trachman, *Fed Proc., 27,* 526 (1968)