Kinetic Studies of the Oxidation of Ferrocytochrome *c* from Horse Heart and *Candida krusei* by Tris(1,10-phenanthroline)cobalt(III)

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Abstract: The oxidation of ferrocytochrome *c* by tris(1,10-phenanthroline)cobalt(III) follows a second-order rate law. For horse heart ferrocytochrome *c*, $k = 1.50 \times 10^3\ M^{-1}\ \text{sec}^{-1}$ (25°, $\mu = 0.1\ M$ (NaCl), pH 7.0 (phosphate)). The value of $\Delta H^{\pm}$ is 11.3 kcal mol^{-1} and $\Delta S^{\pm}$ is −6.2 cal $\text{deg}^{-1}\ \text{mol}^{-1}$. The oxidation rate does not change significantly in the range $6 \leqslant \text{pH} \leqslant 9$. The second-order rate constant for the oxidation of ferrocytochrome *c* from *Candida krusei* (a yeast) is $2.72 \times 10^3\ M^{-1}\ \text{sec}^{-1}$ (25°, $\mu = 0.1\ M$ (NaCl), pH 7.2 (phosphate)). Excellent agreement between experiment and Marcus theory is found, suggesting that electron transfer from ferrocytochrome *c* occurs by the same mechanism as employed in the protein self-exchange reaction. The exposed heme edge is proposed as the probable site of electron transfer from the metalloprotein.

The cytochromes are a series of heme proteins which function in the respiratory chain of all aerobic organisms. In each of the cytochromes the heme iron may exist in either the ferrous or ferric form, thus enabling reducing equivalents to pass down the chain. Through the terminal enzyme cytochrome oxidase, the system of cytochromes is able to reduce dioxygen directly.[2]

Mechanistic studies of ferricytochrome *c* reduction reactions have implicated attack at the heme and suggested that electron transfer may proceed either at the exposed edge of the heme or *via* the heme crevice, depending on the properties of the reducing agent. Evidence for the latter reaction mode has come from recent kinetic and mechanistic investigations of the chromous ion reduction of ferricytochrome *c*.[3-5] Chromium(II) becomes bound to the cytochrome molecule[4] in the area of the heme crevice, and electron transfer involving tyrosine-67 has been suggested.[5] Chromium(II) attack at the crevice is consistent with the results of ligand binding studies which have emphasized the lability of the iron–methionine-80 bond.[6] Outer-sphere reduction of ferricytochrome *c* has been accomplished using ferrocyanide[7] and the kinetics of the ferrocyanide–ferricytochrome *c* reaction have been interpreted as support for a heme edge electron transfer mechanism.[8] In the dithionite reduction, parallel pathways are operative.[9,10] One pathway involves direct "outer-sphere" or "remote" attack by $S_2O_4^{2-}$; the other pathway (which is less than first order in dithionite) is consistent with either "inner-sphere" or "adjacent" attack by dithionite[9] or with reduction by the SO_2^- radical.[9,10] Ferrous ethylenediaminetetraacetate[11,12] and hexaammineruthenium(II)[13] have also been employed as outer-sphere reductants for cytochrome *c*(III). In both cases, support was again found[12,13] for heme edge electron transfer, although the possibility of attack at some site removed from this edge could not be excluded.

For the reduction of ferricytochrome *c*, attack from the heme crevice is rendered possible by the relative lability of the iron–sulfur bond and the accessibility of the crevice to the solution; in sharp contrast is the inertness of the Fe(II)–S bond in native ferrocytochrome *c*.[14] As a consequence, rapid oxidation of the reduced heme protein *via* direct adjacent attack on the iron(II) is unlikely and only heme-edge attack or electron transfer at another site removed from the iron remain as pathways for rapid oxidation.[15] To date these predictions have not been verified to any great extent (an exception is the study of the ferricyanide oxidation[7]) owing in part to the dearth of oxidants which are tractable in the neutral pH range. Thus the need for kinetic studies of the oxidation of ferrocytochrome *c* is apparent. Among

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(14) That substitution on the heme iron of ferrocytochrome *c* is inherently slow is apparent from several studies. For example, cyanide does not directly bind the native protein at even high cyanide concentrations and dissociation of the cyano derivative is relatively slow at neutral pH: P. George and A. Schejter, *J. Biol. Chem.*, **239**, 1504 (1964). To the extent that cyanide release is slow, cyanide binding must be even slower, otherwise the equilibrium constant for cyanide binding would be large and binding would be readily observed.

(15) Conformity with the principle of microscopic reversibility requires that if reduction of ferricytochrome *c* by M

$$M + \text{cyt } c(III) = M^+ + \text{cyt } c(II)$$

is accomplished by an adjacent attack mechanism, then oxidation of ferrocytochrome *c* by M^+ must also feature an adjacent attack pathway (requiring crevice opening). This path may, however, be difficult to observe experimentally. Since the equilibrium constants are large for those reduction reactions for which adjacent attack has been proposed and the reverse rates must consequently be small, other processes (*e.g.*, denaturation or autoxidation of the protein or reaction with M^+ to yield different products) are likely to predominate.

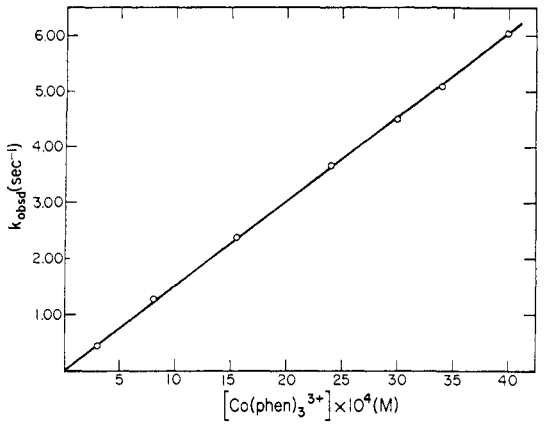

Figure 1. Plot of k_{obsd} *vs.* [Co(phen)$_3^{3+}$] for the oxidation of horse heart type VI ferrocytochrome *c* (25°, pH 7.0 (phosphate), μ = 0.1 *M* (NaCl)). The reaction was followed at 550 nm. Points plotted in all figures represent averages of at least four data points.

the more important questions is whether or not there exist separate pathways of electron transport for the reduction and oxidation of cytochrome *c in vivo*. Based on the results of experiments with antibodies produced in rabbits against human cytochrome *c*[16,17] and with the chemically modified protein,[18-20] it has been proposed that the oxidase and reductase binding sites differ. Further, the upper heme crevice region of cytochrome *c* has been associated with binding to the oxidase,[20] whereas the left side has been suggested as the reductase binding site.[21] Separate binding sites have been taken as evidence that two distinct electron transfer pathways do indeed exist.[19]

We have begun a study of the kinetics of the oxidation of ferrocytochrome *c* by tris complexes of 1,10-phenanthroline and its derivatives[22] with cobalt(III). By modifying the outer edges of the tris(1,10-phenanthroline)cobalt(III) ion, we hope to be able to learn through kinetic studies something about the steric requirements and the mechanism of the oxidation of ferrocytochrome *c*. The present paper deals with the kinetics of oxidation of ferrocytochrome *c* from horse heart and *Candida krusei* (a yeast) by Co(phen)$_3^{3+}$. Rate constants are reported as functions of pH, ionic strength, and temperature; and the results obtained are compared to predictions of the Marcus theory.[23]

Experimental Section

Reagent grade chemicals were used throughout. Deionized distilled water was used in the preparation of all solutions used for synthetic or kinetic experiments. Nitrogen gas was passed through two chromous scrubbing towers to remove oxidizing impurities. Horse heart cytochrome *c* (type VI and type III) obtained from Sigma Chemical Co. and *Candida krusei* cytochrome *c* (Calbiochem) were used without further purification.

Type VI ferrocytochrome *c* solutions were prepared by adding a 20-fold excess of Fe(EDTA)$^{2-}$ to nitrogen-saturated, buffered solutions of ferricytochrome *c*. Excess Fe(EDTA)$^{2-}$ and Fe(EDTA)$^{-}$ were removed from the ferrocytochrome solution using the hollow fiber Dow Beaker Dialyzer obtained from Bio-Rad Laboratories. Protein solutions of 75–100 ml were dialyzed under a constant flow of nitrogen against approximately 1.75 l. of nitrogen-saturated buffer solution of ionic strength 0.1 *M*.

The Fe(EDTA)$^{2-}$ solutions used to prepare ferrocytochrome *c* were made by combining aliquots of ferrous chloride solution with buffered solutions containing a 20% excess over the stoichiometric amount of Na_2H_2EDTA.[12] The ferrous chloride solutions were prepared by dissolving high purity iron wire (Allied Chemical) in excess HCl under a stream of nitrogen. The Fe(II) concentration of the ferrous chloride solutions was determined by transferring aliquots into excess Ce(IV) and back titrating to a ferroin end point with As(III).[24]

Horse heart type III and *Candida krusei* ferrocytochrome *c* solutions were prepared within 2 hr of use for the kinetic runs by sodium dithionite reduction of the ferric protein. The reduced solution was loaded onto Bio-Rex 70 cation exchange resin, washed with water, eluted with a few milliliters of 1.00 *M* sodium chloride, and diluted to the desired volume and composition with an appropriate buffer–sodium chloride solution and water. For the studies made on these proteins, no effort was made to exclude air.

Tris(1,10-phenanthroline)cobalt(III) chloride and perchlorate were prepared according to the method of Pfeiffer and Werdelmann,[25] and characterized spectrally in the region 380–220 nm.[26] Various amounts of stock solution of Co(phen)$_3^{3+}$ were diluted with sodium chloride and water to obtain a suitable range of Co(phen)$_3^{3+}$ concentrations with μ = 0.1 *M*. The concentrations of the Co(phen)$_3^{3+}$ solutions were determined spectrophotometrically in the region 380–320 nm (ϵ_{350} 3700, ϵ_{320} 4680 M^{-1} cm^{-1} [27]).

Buffered solutions in the range pH 6–9 were used for the kinetic measurements. Type VI cytochrome *c* solutions were stored in nitrogen-purged, serum-capped bottles. Nitrogen was passed only slowly through the cytochrome *c* solutions or above them to prevent protein denaturation. The Co(phen)$_3^{3+}$ solutions were stored in serum-capped, round-bottom flasks fitted with a nitrogen inlet tube and a glass luer-lock fitting, thus allowing introduction of the Co(phen)$_3^{3+}$ solution into the stopped-flow apparatus through an all-glass and Kel-F Teflon system.[3a] Absorbance-time data were accumulated as digital output from an analog to digital converter.

The kinetics of oxidation of ferrocytochrome *c* were followed at 550 nm ($\Delta\epsilon_{550}$ 18.5 × 10^3 M^{-1} cm^{-1} [28]). The concentration of Co(phen)$_3^{3+}$ was varied between 9.0 × 10^{-5} and 3.6 × 10^{-3} *M* and was always kept in large excess over cytochrome *c*.

Results and Discussion

First-order plots of the absorbance–time data observed at 550 nm are linear for greater than 90% of the reaction. The first-order dependence of observed rate constants of the Co(phen)$_3^{3+}$ concentration is illustrated in Figure 1. The least-squares slope of the data of Figure 1 gives a second-order rate constant k = (1.50 ± 0.05) × 10^3 M^{-1} sec^{-1} for the oxidation of type VI horse heart ferrocytochrome *c* in 0.05 *M* pH 7.0 phosphate buffer of ionic strength 0.1 *M* at 25°. In Table I are reported the observed pseudo-first-order rate constants for the reactions of horse heart type III and *Candida krusei* ferrocytochromes *c* with Co(phen)$_3^{3+}$. Under all conditions reported in this work, the rate law obtained for the oxidation of ferrocytochrome *c* is

$$\frac{-\mathrm{d[cyt}\ c\mathrm{(II)}]}{\mathrm{d}t} = k[\mathrm{Co(phen)_3^{3+}}][\mathrm{cyt}\ c\mathrm{(II)}] \qquad (1)$$

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Table I. Rate Constants (k_{obsd}) for the Reaction of Horse Heart Type III and *Candida krusei* Ferrocytochrome *c* with Tris(1,10-phenanthroline)cobalt (III) at 25°[a]

10^3[Co(phen)$_3^{3+}$], *M*	k_{obsd} Horse heart (sec^{-1}, 550 nm)[b]	k_{obsd} *Candida krusei* (sec^{-1}, 550 nm)[c]
0.289[d]	0.473	0.708
0.406[e]	0.687	
0.562[d]	0.914	
0.609[d]		1.55
1.02[e]	1.71	
1.13[d]	1.96	2.93
1.53[d]	2.71	
1.58[d]		4.51
2.03[e]	3.30	
4.06[e]	6.37	

[a] The solutions were buffered at pH 7.2 with 2 m*M* phosphate, maintained at μ = 0.10 *M* with sodium chloride, and were ~30 μM in cytochrome *c*. [b] The same rates were observed at 695 nm, while those for 450 nm were ~15% higher. [c] Rates determined at 695 nm were 10–20% higher. [d] Perchlorate salt. [e] Chloride salt.

Table II. Rate Constants (*k*) for the Oxidation of Horse Heart Ferrocytochrome *c* by Tris(1,10-phenanthroline)cobalt(III) at 25° and μ = 0.1 *M*

pH	Buffer	$10^{-3}k$, M^{-1} sec^{-1} (550 nm)
6.0	41 m*M* phosphate	1.01[a]
7.0	24 m*M* phosphate	1.50[a]
7.0	54 m*M* Tris	1.28[a]
7.2	2 m*M* phosphate	1.60[b]
7.8	18 m*M* phosphate	1.65[a]
9.0	414 m*M* Tris	1.82[a]
9.0	414 m*M* Tris	1.77[a,c]

[a] Type VI cytochrome *c*, 3×10^{-6} *M*. [b] Type III cytochrome *c*, 3×10^{-5} *M*. [c] Reaction followed at 695 nm.

Table II gives the second-order rate constants found at various pH values in phosphate and tris buffers for horse heart cytochrome *c*. The activation parameters at pH 7.0 in phosphate buffer obtained from the plot of log (k/T) *vs.* $1/T$ shown in Figure 2 are $\Delta H^{\ddagger}$ = 11.3 kcal mol^{-1} and $\Delta S^{\ddagger}$ = −6.2 cal deg^{-1} mol^{-1}. The second-order rate constant for the *Candida krusei* ferrocytochrome *c* oxidation (2 m*M* phosphate, pH 7.2, μ = 0.1 *M*, 25°) is $2.72 \times 10^3 M^{-1}$ sec^{-1}.

The agreement between the rate constants for type VI and type III (reduced with Fe(EDTA)$^{2-}$ and dithionite, respectively) horse heart cytochrome *c* is noteworthy (Table II). The rate constants for the horse heart and *Candida krusei* proteins are also remarkably similar (the values differ by less than a factor of 2), considering that these cytochromes differ in ~50% of their amino acid residues.[29] The same similarity has been observed for the reactions of these heme proteins with other reagents.[30]

The second-order rate constants obtained for the oxidation of horse heart ferrocytochrome *c* by Co(phen)$_3^{3+}$ are seen to be nearly independent of pH. No evidence was found for biphasic kinetics at pH 9 (550 nm), as has been observed in the reduction of ferricytochrome *c*.[9,12,31,32] The kinetic behavior of the reduction reaction at high pH has been interpreted[9,12,31-33] in terms of the presence of a high pH isomer,[34] possibly containing a lysine-79 as sixth ligand,[35,36] in addition to the native, methionine-80 ligated protein. The failure to observe biphasic kinetics at 550 nm is not inconsistent with previous observations, as at this wavelength the absorbance difference between the lysine- and methionine-bound forms of ferricytochrome *c* is minimal.[33] In fact, our kinetic runs utilizing 695 nm as monitoring wavelength clearly show both the oxidation and isomerization steps. After an initial increase in absorbance at 695 nm owing to oxidation of ferrocytochrome *c* to native ferricytochrome *c*, spectral changes corresponding to isomerization of the protein were observed. The initial phase of the reaction was analyzed by the Guggenheim method,[37] yielding values for k_{obsd} at 695 nm. A plot of k_{obsd} (695 nm) *vs.* [Co(phen)$_3$]$^{3+}$ gave a second-order rate constant of 1.77×10^3 M^{-1} sec^{-1}, in excellent agreement with the value found at 550 nm (Table II). The isomerization reaction is independent of the concentration of Co(phen)$_3^{3+}$ ($0.75–4.0 \times 10^{-3}$ *M*) and is characterized by a rate constant *k*(native → high pH) of 0.071 sec^{-1}. Our value of 0.071 sec^{-1} accords well with the first-order rate constant for isomerization obtained by Wilson and Greenwood at pH 9 (20°).[32]

The rate of oxidation of horse heart ferrocytochrome *c* by Co(phen)$_3^{3+}$ increases with increasing ionic strength at pH 7, as would be expected for a reaction between positively charged species. Theory predicts a linear relationship between log *k* and $\mu^{1/2}$ for ionic strengths below 0.01 *M*.[37] Although the interval $0.06 \leq \mu \leq 0.20$ *M* we have examined is well above the upper limit demanded by theory, a plot of log *k* *vs.* $\mu^{1/2}$ nevertheless yields an excellent straight line (Figure 3). In earlier work we obtained a linear log *k* *vs.* $\mu^{1/2}$ plot for the reduction of ferricytochrome *c* by Fe(EDTA)$^{2-}$ and estimated an "active site charge" of +1.7 for the oxidized

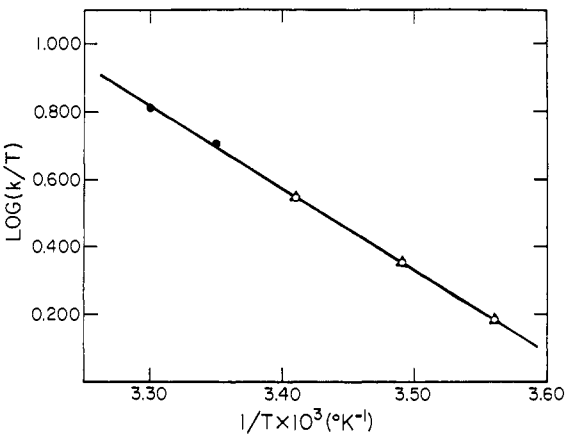

Figure 2. Eyring plot of rate data for the oxidation of horse heart type VI ferrocytochrome *c* by Co(phen)$_3^{3+}$ (pH 7.0 (phosphate), μ = 0.1 *M* (NaCl)): ○, [Co(phen)$_3^{3+}$] = 2.4×10^{-3} *M*; △, [Co(phen)$_3^{3+}$] = 3.0×10^{-3} *M*; ●, [Co(phen)$_3^{3+}$] = 3.4×10^{-3} *M*.

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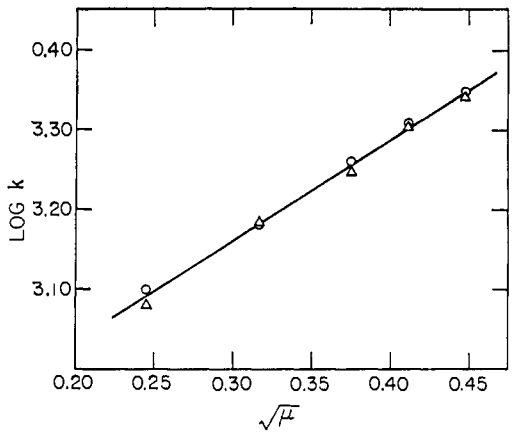

Figure 3. Plot of log k *vs.* $\mu^{1/2}$ for the oxidation of horse heart type VI ferrocytochrome *c* by $Co(phen)_3^{3+}$ (25°, pH 7.0 (phosphate)): O, $[Co(phen)_3^{3+}] = 1.5 \times 10^{-3}$ *M*; Δ, $[Co(phen)_3^{3+}] = 3.0 \times 10^{-3}$ *M*.

protein.[12] An analogous calculation based on the data presented in Figure 3 gives an "active site charge" of +0.4 for ferrocytochrome *c*. The exact values cannot of course be taken seriously, but it is clear that they are much smaller than the overall charge of about +7[12] on the protein in neutral solution. It is also interesting that the "active site charge" is approximately one unit less positive for ferrocytochrome *c*.

The oxidant $Co(phen)_3^{3+}$ is known to behave according to the Marcus theory for outer-sphere electron transfer in some,[27,38] but not all,[17,39-43] of its redox reactions. Typical examples of good behavior include the oxidation of $Co(terpy)_2^{2+}$ [38] and V_{aq}^{2+},[27] where theory and experiment are in reasonably close agreement. In addition, the calculated rate constant for the reaction between Fe_{aq}^{3+} and $Co(phen)_3^{2+}$ is within a factor of 3 of the experimental value.[27] The theoretical expression for the rate constant (k_{12}) of the ferrocytochrome *c*–$Co(phen)_3^{3+}$ reaction is

$$\log k_{12} = 0.5\,[\log k_{11} + \log k_{22} + 16.9\,\Delta E_{12}^{0}] \quad (2)$$

where k_{11} and k_{22} are the appropriate self-exchange rate constants. The horse heart cytochrome *c* self-exchange rate is in the range $(0.2\text{–}1.0) \times 10^3\ M^{-1}\ \text{sec}^{-1}$ at pH 7.0, $\mu = 0.1\ M$, and 25°,[44-46] and the $Co(phen)_3^{2+/3+}$ self-exchange rate, extrapolated to 25° from the data of Baker, *et al.*,[47] is $2.1 \times 10^1\ M^{-1}\ \text{sec}^{-1}$. Taking reduction potentials for the $Co(phen)_3^{2+/3+}$ and cytochrome *c*(II)/(III) couples as +0.42[48] and +0.261 V,[49] respectively, we calculate k_{12} to fall in the range $(1.2\text{–}2.7) \times 10^3\ M^{-1}\ \text{sec}^{-1}$ for the horse heart reaction, which is in rather good agreement with experiment. Using the same data for the cytochrome *c*(II)/(III) self-exchange rate and redox couple, Ewall and Bennett[13] have reported an equally successful Marcus calculation of the rate of *reduction* of ferricytochrome *c* by $Ru(NH_3)_6^{2+}$ (calculated, $(3\text{–}6) \times 10^4\ M^{-1}\ \text{sec}^{-1}$; found, $3.78 \times 10^4\ M^{-1}\ \text{sec}^{-1}$). As a further check on the interpretation, it should be noted that the rate of oxidation of $Ru(NH_3)_6^{2+}$ by $Co(phen)_3^{3+}$ is also in fair agreement with the Marcus relation (calculated, $1 \times 10^5\ M^{-1}\ \text{sec}^{-1}$; found, $(1\text{–}2) \times 10^4\ M^{-1}\ \text{sec}^{-1}$).[50]

It is important to note that neither reduction nor oxidation of cytochrome *c* by outer-sphere reagents would be expected to yield rate constants which are predictable by the Marcus theory if the electron transfer mechanism in question were radically different from that employed in the self-exchange reaction. It should also be noted that, since $\log k_{12} - \log k_{21} = 16.9\Delta E_{12}^{0}$, reversibility of the cross-reaction is implicit in eq 2. Thus, if the forward rate satisfies eq 2, then the reverse rate must also, and *vice versa*. An apparent breakdown of reversibility can occur if certain equilibria or conformation changes take place that are not sufficiently rapid, in which case conformity to eq 2 must to some extent have been accidental in the first place. The fact that excellent agreement with eq 2 has been obtained for both a reduction and an oxidation reaction lead to the conclusion that, in these cytochrome *c* systems, electron transfer to and from the heme iron of the protein follows the same pathway as in the self-exchange reaction. Conformity with eq 2, however, does not necessarily require that this pathway be simple. Consider, for example, that the reactive forms of ferri- and ferrocytochrome *c* are not the native proteins but are instead two conformers that exist in rapid equilibrium with the native forms (eq 3–6). In terms of this scheme, the

$$\text{cyt } c(\text{III}) \rightleftharpoons \text{cyt } c(\text{III})^* \quad K_{\text{III}} \quad (3)$$

$$\text{cyt } c(\text{II}) \rightleftharpoons \text{cyt } c(\text{II})^* \quad K_{\text{II}} \quad (4)$$

$$\text{cyt } c(\text{III})^* + \text{cyt } c(\text{II})^* \rightleftharpoons \text{cyt } c(\text{II})^* + \text{cyt } c(\text{III})^* \quad k_{11}^* \quad (5)$$

$$Co(phen)_3^{3+} + \text{cyt c(II)}^* \rightleftharpoons Co(phen)_3^{2+} + \text{cyt c(III)}^* \quad k_{12}^*,\ K_{12}^* \quad (6)$$

observed rate constant for the oxidation of ferrocytochrome *c* by $Co(phen)_3^{3+}$ is given by the following expressions

$$k_{12} = K_{\text{II}} k_{12}^* \quad (7)$$

$$= K_{\text{II}}(k_{11}^* k_{22} K_{12}^*)^{1/2} \quad (8)$$

The rate and equilibrium constants k_{11}^* and K_{12}^* are related to the observed constants by

$$k_{11} = K_{\text{II}} K_{\text{III}} k_{11}^* \quad (9)$$

$$K_{12} = K_{\text{II}} K_{12}^* / K_{\text{III}} \quad (10)$$

Substitution in eq 8 gives eq 11, which is identical with

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

eq 2. Thus agreement with eq 2 does not preclude more complex mechanisms of the type discussed here. However, we consider a rapid crevice-opening conformation change for ferrocytochrome *c* highly unlikely for the reasons discussed earlier.[14]

The simplest and most straightforward interpretation of the kinetic studies both of the protein self-exchange

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and the reaction of ferricytochrome *c* with model outer-sphere reductants is that electron transfer occurs *via* the exposed heme edge.[8,12,13] The present experiments on the oxidation of ferrocytochrome *c* from both horse heart and *Candida krusei* by tris(1,10-phenanthroline)-cobalt(III) are also most easily reconciled with a mechanistic model featuring outer-sphere electron transfer utilizing contact between the heme edge and one of the phenanthroline rings. Indeed, given an edge–edge mechanism for the self-exchange reaction, edge transfer to $Co(phen)_3^{3+}$ must be the path of choice in order to understand the excellent Marcus-theory correlation. It is also important to note that the measured activation parameters and the ionic strength dependence of the protein reaction with $Co(phen)_3^{3+}$ provide very little evidence of any unusual features and therefore are entirely consistent with an ordinary adiabatic electron transfer process. In view of all of the results on model systems, then, it is reasonable to propose that the remote attack reactions utilize a common heme-edge site for both oxidation and reduction of cytochrome *c*. This model is in contrast to the proposal that electron transfer takes place *in vivo* at separate oxidase and reductase binding sites.[16–20] However, it is difficult to understand why a common heme-edge pathway should not also be employed *in vivo*, unless access to the edge is blocked as a consequence of binding to the membrane or to the oxidase or reductase.

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Mechanism for Chiral Recognition of a Prochiral Center and for Amino Acid Complexation to a Cobalt(III) Tetramine. The Crystal Structure, Absolute Configuration, and Circular Dichroism of $\Lambda(-)_{436}$-β_2-[(2*S*,9*S*)-2,9-Diamino-4,7-diazadecanecobalt(III) aminomethylmalonate] Perchlorate Monohydrate

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Abstract: The crystal structure of $\Lambda(-)_{436}$-β_2-[(2*S*,9*S*)-2,9-diamino-4,7-diazadecanecobalt(III) aminomethylmalonate] perchlorate monohydrate, cell dimensions $a = 13.001$ (2), $b = 14.656$ (3), and $c = 10.601$ (1) Å, space group $P2_12_12_1$, has been determined and refined, with all hydrogen atoms included, to $R = 0.075$. The results of the X-ray determination have shown that the complex has the conformation Λ-β-*R* with the *pro-S* carboxyl group of the malonate coordinated to Co(III). All of the Λ-β-complex is in the *R* conformation, presumably because in this conformation three-point attachment of the malonate *via* an internal hydrogen bond occurs. On decarboxylation of the pure Λ-β- complex, 65% (*S*)-alanine and 35% (*R*)-alanine are formed. The CD spectra of these complexes are analyzed as Gaussian sums as an aid in spectral determination of absolute configuration. Since Λ-α-, trans-, or Δ-β- starting complexes all give similar yields of Λ-β- and Δ-β- products on treatment with amino acids, we suggest a common trans intermediate which gives β_1 complexes at high pH when the amino group attacks first and β_2 complexes at low pH when the carboxylate group attacks first.

The crystal structure of the Λ-β- complex formed between α,α-aminomethylmalonate and $\Lambda(-)_{436}$-α-dichloro-(2*S*,9*S*)-2,9-diamino-4,7-diazadecanecobalt-(III) chloride (**1**) is described here. The α,α-aminomethylmalonate dianion is an example of a prochiral ion since it has two equivalent carboxyl groups. When it interacts with the cobalt complex (**1**) the primary product was shown to be the Λ-β- complex with some Δ-β- product but no Λ-α- product. A comparison of the circular dichroism curve of the Λ-β- complex, with those of corresponding Λ-β- complexes with (*R*)- or (*S*)-alanine, suggested that the malonate moiety was bound in a fixed configuration so that only the *pro-S* carboxyl group (and not the *pro-R* group) was directly coordinated to the Co(III) ion[2] (eq 1). This differentiation between the carboxyl groups occurs in spite of the fact that the binding of an asymmetric tetradentate ligand on the Co(III) ion leaves only two octahedral positions available for coordination of the malonate ion instead of the three positions which have been proposed as a condition[3] for significant chiral recognition of a prochiral center. A specific differentiation between prochiral functional groups is common to enzyme reactions (eq 2)[3–5] but has not been previously observed

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