increase electron density on sulfur thereby destabilizing Ic. This would result in a lower barrier to C⋯N bond rotation. $Fe(dtc)_3$ complexes must possess less back-bonding and the "Fe(IV)" compounds must have the least. Indeed, the $\Delta F^{\ddagger}$ values of the "Fe(IV)" complexes are nearly identical with the dtc esters and $Me_4tds$.

The above argument is essentially based on electronic inductive effects. The more oxidized metal will inductively stabilize Ic while the electron rich reduced complex will destabilize this resonance form. Electronic effects of this nature have often been qualitatively observed. Chatt, Duncanson, and Venanzi[21] found that resonance form Ic is important in transition metal dtc complexes and that effects which increase sulfurs ability to accept electrons increase $\nu$(C⋯N). Cotton and McCleverty[44] showed that $Fe(MeMe(dtc))_2(CO)_2$ has a much larger $\nu$(C⋯N) than $Fe(MeMe(dtc))_3$ because the CO ligand acts as an electron acceptor relative to dtc. Indeed, this is reflected in $\nu$(C≡O) which is lowered by *ca.* 15 $cm^{-1}$ from the analogous $[FeI_2(CO)_2]_n$ compound. Coucouvanis and Fackler[19] found a direct correlation between the ability of $Ni(dtc)_2$ compounds to form base adducts and the degree to which Ic is important. This provides good evidence for metal–sulfur $\pi$ inductive effects.

More striking inductive effects can be seen in the following series of $Sn(MeMe(dtc))_2L_2$ compounds[23] where LL, $\nu$(C⋯N) are the following: ClCl, 1546; MeCl, 1536; MeBr, 1524; MeI, 1504; and MeMe, 1490 $cm^{-1}$. This trend clearly illustrates that as L is more electron withdrawing, the C⋯N bond order increases. A similar trend was reported by Blaauw, *et al.*,[22] for $(MeMe(dtc))AuL_2$ complexes where LL, $\nu$(C⋯N) are the following: MeMe, 1592 and BrBr, 1553 $cm^{-1}$. These trends clearly show a relation between C⋯N bond order[45] and electron inductive effects from other ligands.

Barriers to C⋯N bond rotation should also show an effect of N substituent. The data in Table III show no trends with RR′ for any complex type. Such dependence is presumably within experimental error. It would be expected that the more basic the parent amine the more important Ic will be. However, uniform trends in basicity may be masked by steric effects. A recent publication[43] describes the importance of amine basicity and steric effect for a series of $Fe(RR'(dtc))_3$ complexes.

**Acknowledgments.** This research was supported in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society, the Research Corporation, and the University of Minnesota Graduate School.

(45) Fackler and Coucouvanis showed that a smooth curve relationship exists between bond order and $[\nu(C\cdots N)]^2$ for a wide variety of dtc compounds: J.P. Fackler, Jr., and D. Coucouvanis, *Inorg. Chem.*, 7, 181 (1968).

# Mechanisms of the Reactions of Cytochrome *c*. II. The Rate of Reduction of Horse-Heart Ferricytochrome *c* by Chromium(II)[1,2]

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**Abstract:** The rate of reduction of ferricytochrome *c* by chromium(II) in the presence of various anions has been studied by a flow technique. Measurements in chloride media over the pH range 1–7 revealed a maximum rate at pH ~3.7. Below pH 3, the second-order rate constants ($M^{-1}$ $sec^{-1}$) are equal to $40/[H^+]$ while above pH 4.4 the rate constants are given by $3.0 \times 10^3 + 1.0 \times 10^9[H^+]$ at 25.0° and 1.0 *M* ionic strength. These reactions are interpreted in terms of an electron transfer pathway involving adjacent attack on the iron(III) center. The reduction of ferricytochrome *c* is catalyzed by iodide, azide, and thiocyanate ions. The third-order rate constants ($M^{-2}$ $sec^{-1}$) for these reactions are the following: iodide, $1.1 \times 10^4$ (pH 7.0); azide, $4.1 \times 10^5$ (pH 6.1); thiocyanate, $3.7 \times 10^5$ (pH 6.1) and $4.7 \times 10^5$ (pH 6.5 and 7.0). It is proposed that these reactions feature a remote electron transfer pathway. Similar reactivity patterns ($Cl^- < I^- < N_3^- \sim SCN^-$) in this and model systems are consistent with this hypothesis.

Cytochrome *c* is a small (molecular weight ~12,400), relatively stable heme protein found in the mitochondria of all aerobic organisms. It is a member of the respiratory chain that affects the oxidation of foodstuffs and the synthesis of adenosine triphosphate. During this process the iron atom of cytochrome *c* is alternately oxidized and reduced; consequently, the electron transfer properties of this protein are intimately related to its function.

Despite extensive studies of oxidation–reduction reactions involving cytochrome *c*,[3–5] the nature of the sites for electron transfer to and from this hemeprotein have

(1) Research performed under the auspices and the U. S. Atomic Energy Commission.

(2) For part I of this series see N. Sutin and J. K. Yandell, *J. Biol. Chem.*, 247, 6932 (1972).

(3) C. Greenwood and G. Palmer, *ibid.*, **240**, 3660 (1965).

(4) K. G. Brandt, P. C. Parks, G. Czerlinski, and G. P. Hess, *ibid.*, **241**, 4180 (1966).

(5) A. Kowalsky, *ibid.*, **244**, 6619 (1969).

yet to be characterized. A recent crystallographic study of ferricytochrome *c* has shown that the heme group lies in a crevice of the essentially globular protein with an edge of the porphyrin ring located at its surface.[6] The iron atom lies in the plane of the porphyrin ring with its fifth- and sixth-coordination sites occupied by a ring nitrogen atom of histidine-18 and the sulfur atom of methionine-80. Based on this structure it has been proposed that the electron transfer to the iron atom of ferricytochrome *c* may occur *via* the edge of the porphyrin ring system, *via* the polypeptide chain, or directly to the iron atom.[2,7-9] In an attempt to distinguish between these pathways we are studying the kinetics of the reduction of ferricytochrome *c* by various transition metal ions and complexes. This paper deals with the rate of reduction of ferricytochrome *c* by chromium(II) in the presence of various anions.

Some aspects of the reaction of ferricytochrome *c* with chromium(II) have been previously investigated.[5] In a pioneering study, Kowalsky showed that the product of the reduction of ferricytochrome *c* by chromium(II) in the presence of phosphate (pH 4.00) is a complex containing 1 mol each of chromium(III), phosphate, and cytochrome *c*. More recently, Dawson, *et al.*,[10] reported that the activation energy for the chromium(II) reduction of ferricytochrome *c* in a chloride medium (pH 4.2) is 17.4 kcal mol$^{-1}$. However, because of the possibility of rearrangements and other ligand replacement reactions, the results of these studies are difficult to interpret.

## Experimental Section

**Materials.** Chromium(II) chloride solutions were prepared by dissolving chromium metal (purity 99.99%) in hydrochloric acid in an oxygen-free atmosphere. These solutions were filtered, stored under argon, and the chromium(II) concentration determined by titration with iron(III). The hydrochloric acid concentration in these solutions was determined by pH titration after removal of the chromium through the following procedure. The chromium(II) was oxidized to $CrBr^{2+}$ with bromine, the excess bromine was removed by bubbling with argon, and the $CrBr^{2+}$ was allowed to aquate overnight. The resulting $Cr^{3+}$ was adsorbed on a Dowex 50W-X8 cation-exchange resin in the $Li^+$ form, the acid eluted with 4 *M* lithium chloride, and the eluate titrated with standard sodium hydroxide. Sigma horse-heart ferricytochrome *c* (type III) was used without further purification. The cytochrome *c* concentration of the reactant solutions was determined from the absorbance of ferrocytochrome *c* at 550 nm following reduction with sodium dithionite. A value of $1.76 \times 10^4\ M^{-1}\ cm^{-1}$ was used for the molar absorptivity of ferrocytochrome *c* at 550 nm.[11] The other chemicals used were of the highest quality commercially available, and all solutions were prepared with triply distilled water.

**Kinetic Measurements.** The acid dependence of the reduction rate was studied in the pH range 1–7. Unbuffered solutions were used below pH 3.1, 20–100 m*M* acetate buffers in the pH range 3.1–5.1, a 20 m*M* cacodylate buffer at pH 6.1, and a 40 m*M* lutidine buffer at pH 7.0. The reaction mixtures contained 10–40 $\mu M$ of ferricytochrome *c* and at least a tenfold excess of chromium(II); these solutions were maintained at ionic strengths of 1.0 or 0.15 *M* with sodium chloride.

With the exception of the measurements in the presence of azide, the reactions were studied with the stopped-flow apparatus previously described.[12] Azide data were determined with a modification of this apparatus equipped with four driving syringes. This arrangement allowed the azide to be kept separate from both the chromium(II) and the ferricytochrome *c* until mixed in the mixing chamber of the flow apparatus. This was necessary because azide reacts with both chromium(II) and ferricytochrome *c* (note, however, that the chromium(II)–azide reaction was much slower than the chromium(II)–ferricytochrome *c* reaction under the conditions used). The iodide and thiocyanate were initially present in only the chromium(II) solution. Cytochrome *c* solutions containing the buffer mixture were prepared by dissolving cytochrome *c* in a degassed solution of the required ionic strength and pH and were used within 3 hr of preparation.

The rate of reduction of ferricytochrome *c* was usually determined by following the formation of ferrocytochrome *c* at 550 nm; no dependence of the rate constants on the wavelength used to observe the reaction was found when the disappearance of ferricytochrome *c* was also followed at 450 and 695 nm. All of the measurements were performed at 25.0 ± 0.2°.

## Results

Complete reduction of the ferricytochrome *c* by chromium(II) was established spectrally in this study and in the stoichiometric study of Kowalsky.[5] Pseudo-first-order rate constants for the reduction of ferricytochrome *c* at pH 6.1 are presented in Table I. It is apparent that the rate constants are not directly proportional to the chromium(II) concentration, but tend to a limiting value at high chromium(II) concentrations. This is further illustrated in Figure 1 where $1/k_{obsd}$ is plotted *vs.* 1/[Cr(II)]. There are two mechanisms which lead to rate constants which become independent of the concentration of chromium(II) at high concentrations. The first is illustrated by eq 1 and 2 and predicts that, at

**Table I.** First-Order Rate Constants for the Reaction of Ferricytochrome *c* with Chromium(II) at 25°[a,b]

| $10^4$[Cr(II)], *M* | $k_{obsd}$, sec$^{-1}$ | $10^4$[Cr(II)], *M* | $k_{obsd}$, sec$^{-1}$ |
|---|---|---|---|
| 2.56 | 1.05 | 38.1 | 12.1 |
| 5.10 | 2.10 | 71.0 | 20.9 |
| 10.1 | 3.74 | 99.7 | 26.8 |
| 19.8 | 6.77 | 125 | 28.3 |
| 29.2 | 9.99 | | |

[a] Ionic strength of 1.0 *M* maintained with sodium chloride [b] Solutions buffered at pH 6.1 with sodium cacodylate.

$$\text{ferricyt } c \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ferricyt* } c \qquad (1)$$

$$\text{ferricyt* } c + \text{Cr(II)} \xrightarrow{k_2} \text{ferrocyt } c + \text{Cr(III)} \qquad (2)$$

high concentrations of chromium(II), a limiting rate constant, $k_1$, should be reached which is independent of the concentration of chromium(II). This mechanism yields eq 3 for the observed pseudo-first-order rate constants when the usual steady-state approximation for the concentration of the intermediate is made. Equation 3

$$k_{obsd} = \frac{k_1 k_2[\text{Cr(II)}]}{k_{-1} + k_2[\text{Cr(II)}]} \qquad (3)$$

(6) R. E. Dickerson, T. Takano, D. Eisenberg, O. B. Kallai, L. Samson, A. Cooper, and E. Margoliash, *J. Biol. Chem.*, **246**, 1511 (1971).
(7) A. Schejter and I. Aviram, *Biochemistry*, **8**, 149 (1969).
(8) C. E. Castro and H. F. Davis, *J. Amer. Chem. Soc.*, **91**, 5405 (1969).
(9) R. E. Dickerson, T. Takano, D. Eisenberg, O. B. Kallai, and L. Samson, Proceedings of the Wenner-Gren Symposium on Oxidative Enzymes, Aug 1970.
(10) J. W. Dawson, H. B. Gray, R. A. Holwerda, and E. W. Westhead, *Proc. Nat. Acad. Sci. U. S.*, **69**, 30 (1972).
(11) E. Margoliash, N. Frohwirt, and E. Wiener, *Biochem. J.*, **71**, 559 (1959). Note, however, that a value of $2.99 \times 10^4\ M^{-1}\ cm^{-1}$ has also been reported for the molar absorptivity of ferrocytochrome *c* at 550 nm (V. Massey, *Biochem. Biophys. Acta*, **34**, 255 (1959)). The rate constants determined in the present work do not depend on the molar absorptivity of cytochrome *c* because the reactions were run under pseudo-first-order conditions with the chromium(II) present in large excess.
(12) G. Dulz and N. Sutin, *Inorg. Chem.*, **2**, 917 (1963).

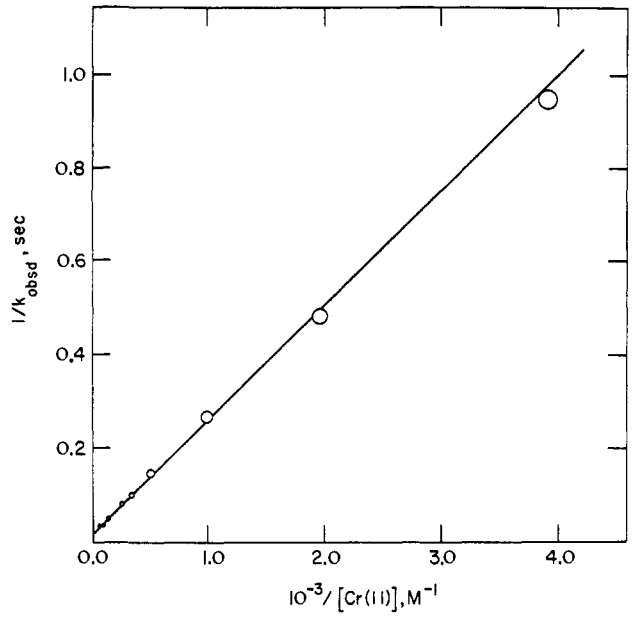



Figure 1. Plot, suggested by eq 4, of $1/k_{obsd}$ *vs.* 1/[Cr(II)]: pH 6.1, ionic strength 1.0 *M*.

may be rearranged to give eq 4, which is seen to be

$$\frac{1}{k_{obsd}} = \frac{1}{k_1} + \frac{k_{-1}}{k_1 k_2 [Cr(II)]} \quad (4)$$

consistent with the data plotted in Figure 1. The values of $k_1$ and of $k_1k_2/k_{-1}$ calculated from the intercept and slope of this figure are 60 ± 20 $sec^{-1}$ and 4.0 × $10^3$ $M^{-1}$ $sec^{-1}$, respectively, at pH 6.1.

The second mechanism which leads to a limiting rate at high chromium(II) concentrations involves the rapid preequilibrium formation of a complex between ferricytochrome *c* and chromium(II). This is illustrated in eq 5 and 6.

$$\text{ferricyt } c + Cr(II) \rightleftharpoons \text{ferricyt } c\text{–}Cr(II) \quad (5)$$

$$\text{ferricyt } c\text{–}Cr(II) \xrightarrow{k_3} \text{ferrocyt } c + Cr(III) \quad (6)$$

This mechanism leads to the following expression for the observed rate constants

$$k_{obsd} = \frac{K_c k_3 [Cr(II)]}{1 + K_c[Cr(II)]} \quad (7)$$

which is seen to be of the same form as eq 3. In the above expression, $K_c$ is the equilibrium constant for the formation of the complex between ferricytochrome *c* and chromium(II). The first mechanism is preferred for reasons that will be discussed later.

The effect of pH on the rate of reduction of ferricytochrome *c* by chromium(II) is shown in Figure 2. The rate constants were either determined at relatively low chromium(II) concentrations (1.54 × $10^{-4}$ *M*) or from the initial slopes of plots of $k_{obsd}$ *vs.* [Cr(II)]. It is evident from Figure 2 that the rate of reduction of ferricytochrome *c* goes through a maximum in the vicinity of pH 3.7. The kinetic data ($\mu$ = 1.0 *M*) below pH 3 are given by $k = a/[H^+]$, with $a$ = 40 $sec^{-1}$, while above pH 4.4 the kinetic data are given by $k = c + d[H^+]$, with $c$ = 3.0 × $10^3$ $M^{-1}$ $sec^{-1}$ and $d$ = 1.0 × $10^9$ $M^{-2}$ $sec^{-1}$. The curve drawn in Figure 2 was calculated from eq 8 (which has the correct limiting

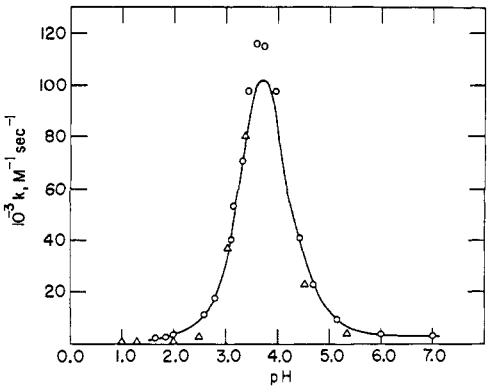



Figure 2. Plot of *k vs.* pH for the reaction of ferricytochrome *c* with chromium(II) in chloride media: circles, ionic strength 1.0 *M*; triangles, ionic strength 0.15 *M*.

$$k = \frac{a/[H^+] + ac/d[H^+]^2}{1 + a/d[H^+]^2} \quad (8)$$

forms) using the above values of *a*, *b*, and *c*. As expected, the curve fits the data well at the high- and low-pH limits; however, it is evident that the fit is rather poor in the intermediate pH range. Although the fit in this range can be improved at the expense of the fit at the pH extremes (for example, by increasing the values of *a* and *d* to 44 $sec^{-1}$ and 1.4 × $10^9$ $M^{-2}$ $sec^{-1}$ and decreasing the value of *c* to 2.8 × $10^3$ $M^{-1}$ $sec^{-1}$) or by introducing additional terms, these procedures do not seem justified at this time since the pH–rate profile is almost certainly a composite curve representing the combined effect of pH and anions.

**The Effect of Anions.** It is evident from Figure 2 that the rate of reduction of ferricytochrome *c* by chromium(II) is relatively slow and insensitive to the acidity in the pH range 6–7. This pH region was therefore selected for the study of the effect of anions on the reduction rate. It was found that replacing chloride by perchlorate in this pH range resulted in a modest increase in the reduction rate. However, no systematic study of this effect was made. The effect of replacing chloride by iodide, azide, and thiocyanate ions at a constant ionic strength of 1.0 *M* is shown in Tables II–IV,

**Table II.** First-Order Rate Constants for the Reaction of Ferricytochrome *c* with Chromium(II) in the Presence of Iodide Ions at 25° [a,b]

| $10^2[I^-]$, *M* | $10^4[Cr(II)]$, *M* | $k_{obsd}$, $sec^{-1}$ |
|---|---|---|
| 1.98 | 4.93 | 2.13 |
| 3.96 | 4.93 | 2.23 |
| 7.20 | 4.93 | 2.35 |
| 14.9 | 4.93 | 2.80 |
| 24.8 | 4.93 | 3.27 |

[a] Ionic strength of 1.0 *M* maintained with sodium chloride. [b] Solutions buffered at pH 7.0 with lutidine.

respectively. The observed rate constants are consistent with eq 9 which may be rearranged to eq 10. In

$$k_{obsd} = \left[\frac{k_0 + k_1'[X^-] + k_2'[X^-]^2}{1 + K_1[X^-]}\right][Cr(II)] \quad (9)$$

$$\frac{k_{obsd}(1 + K_1[X^-]) - k_0}{[Cr(II)][X^-]} = k_1' + k_2'[X^-] \quad (10)$$

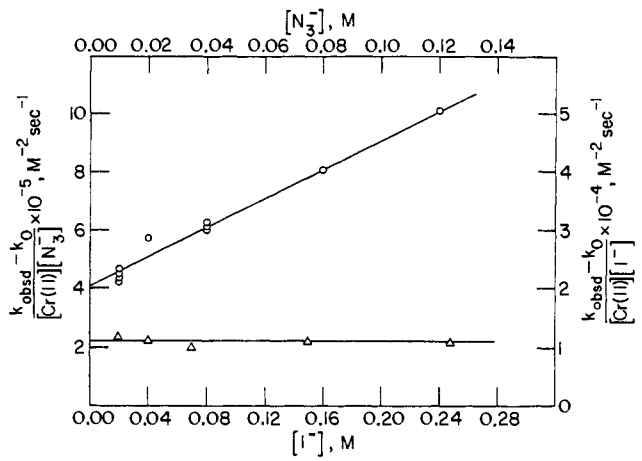



Figure 3. Plot, suggested by eq 7, of the kinetic data in the presence of added iodide and azide ions: triangles, iodide data, pH 7.0; circles, azide data, pH 6.1.

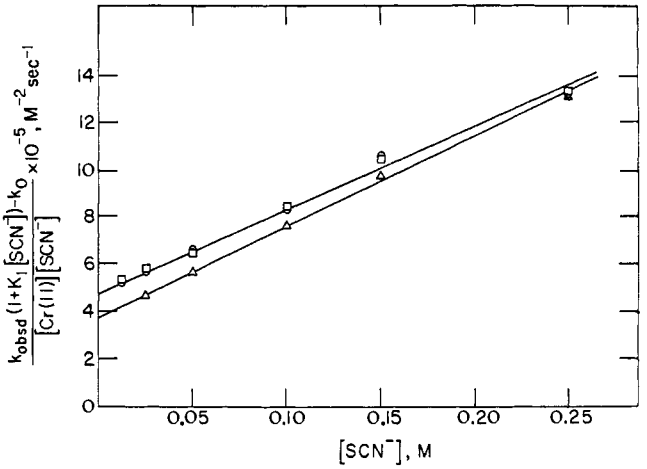



Figure 4. Plot, suggested by eq 7, of the kinetic data in the presence of added thiocyanate ions: triangles, pH 6.1; squares, pH 6.5; circles, pH 7.0.

Table III. First-Order Rate Constants for the Reaction of Ferricytochrome *c* with Chromium(II) in the Presence of Azide Ions at 25°[a,b]

| $10^2[N_3^-]$, *M* | $10^4$[Cr(II)], *M* | $k_{obsd}$, $sec^{-1}$ |
|---|---|---|
| 1.0 | 2.50 | 2.1 |
| 1.0 | 5.10 | 4.4 |
| 1.0 | 10.1 | 8.8 |
| 1.0 | 19.1 | 14.8 |
| 1.0 | 2.50 | 2.1 |
| 4.0 | 2.50 | 6.5 |
| 8.0 | 2.50 | 17 |
| 12.0 | 2.50 | 36 |
| 2.0 | 10.1 | 15.3 |
| 4.0 | 5.1 | 15.5 |
| 6.0 | 10.1 | 28.2 |

[a] Ionic strength maintained at 1.0 *M* with sodium chloride. [b] Solution buffered at pH 6.1 with sodium cacodylate.

Table IV. Effect of Thiocyanate Ions on the Rate of Reaction of Ferricytochrome *c* with Chromium(II) at 25° [a,b]

| pH | $10^2$[$SCN^-$], *M* | $10^4$[Cr(II)], *M* | $k_{obsd}$, $sec^{-1}$ |
|---|---|---|---|
| 6.1 | 10.0 | 3.07 | 16.8 |
| 6.1 | 10.0 | 5.10 | 27.3 |
| 6.1 | 10.0 | 10.1 | 54.4 |
| 6.1 | 10.0 | 19.8 | 96.6 |
| 6.1 | 10.0 | 29.2 | 138 |
| 6.1 | 10.0 | 38.1 | 174 |
| 6.1 | 2.50 | 5.10 | 7.30 |
| 6.1 | 5.00 | 5.10 | 13.7 |
| 6.1 | 10.0 | 5.10 | 28.9 |
| 6.1 | 15.0 | 5.10 | 46.3 |
| 6.1 | 20.0 | 5.10 | 67.1 |
| 6.1 | 25.0 | 5.10 | 85.0 |
| 6.5 | 1.25 | 4.93 | 5.3 |
| 6.5 | 2.50 | 4.93 | 8.4 |
| 6.5 | 5.00 | 4.93 | 15.2 |
| 6.5 | 9.90 | 4.93 | 29.5 |
| 6.5 | 15.0 | 4.93 | 49.7 |
| 6.5 | 25.0 | 4.93 | 82.9 |
| 7.0 | 10.0 | 2.47 | 15.5 |
| 7.0 | 10.0 | 4.93 | 31.4 |
| 7.0 | 10.0 | 8.54 | 58.2 |
| 7.0 | 10.0 | 12.1 | 77.3 |
| 7.0 | 1.25 | 4.93 | 5.2 |
| 7.0 | 2.50 | 4.93 | 8.5 |
| 7.0 | 5.00 | 4.93 | 15.7 |
| 7.0 | 10.0 | 4.93 | 31.6 |
| 7.0 | 15.0 | 4.93 | 51.3 |
| 7.0 | 25.0 | 4.93 | 84.0 |

[a] Ionic strength maintained at 1.0 *M* with sodium chloride. [b] Solution buffered at pH 6.1 with sodium cacodylate and at pH 6.5 and 7.0 with lutidine.

these expressions, $k_0$ is the rate constant in the absence of added $X^-$ and $K_1$ is the equilibrium constant for the formation of the complex between chromium(II) and the added anion. The kinetic data are plotted in Figures 3 and 4. Under the conditions used $K_1[X^-] \ll 1$ for iodide and azide, and $K_1 = 4.0\ M^{-1}$ for thiocyanate. The values of $k_1'$ ($M^{-2}$ $sec^{-1}$) obtained from the intercepts of the plots are the following: iodide, $1.1 \times 10^4$ (pH 7.0); azide, $4.1 \times 10^5$ (pH 6.1); thiocyanate, $3.7 \times 10^5$ (pH 6.1) and $4.7 \times 10^5$ (pH 6.5 and 7.0). The values of $k_2'$ ($M^{-3}$ $sec^{-1}$) calculated from the slopes are the following: iodide, $\leq 2 \times 10^4$; azide, $5 \times 10^6$; thiocyanate, $4 \times 10^6$. The apparent pH dependence of the intercept in the case of thiocyanate is within the probable experimental error of the measurements. Finally, it is also evident from Figure 2 that increasing the chloride ion concentration from 0.1 to 1.0 *M* increases the rate by almost a factor 5 at pH 2. Evidently the chloride ion concentration (and ionic strength) has a relatively large effect on the rate at low pH.

## Discussion

The kinetic data show that the rate of reduction of ferricytochrome *c* by chromium(II) approaches a limiting value at high chromium(II) concentrations. An analogous type of kinetic behavior has been previously observed in the reactions of ferricytochrome *c* with various anions.[2] The latter reactions were interpreted in terms of a model in which the added anions bind to the iron by displacing the methionine-80 sulfur from its coordination site, and a value of 60 $sec^{-1}$ was estimated for the rate constant for the opening of the heme crevice and/or the rupture of the iron–sulfur bond in the pH range 6.1–7.0. The limiting rate constant for the chromium(II) reduction of ferricytochrome *c* in chloride media at pH 6.1 is remarkably similar to this value, and it is therefore tempting to propose that the two reactions involve the same initial step. In terms of this interpretation, the "uncatalyzed" oxidation–reduction reaction involves the opening of the heme crevice

and the (chloride mediated) attack of the chromium(II) on the iron(III) center. More specifically, the species denoted as ferricyt* *c* in reactions 1 and 2 could be an intermediate formed by the rupture of the iron–sulfur bond; this intermediate then reacts with chromium(II) *via* a –FeClCr– transition state.

Some support for the above interpretation is provided by the pH dependence of the oxidation–reduction reaction. The rate maximum at pH 3.7 can be rationalized in terms of the following scheme.

$$H_2(\text{ferricyt } c) \rightleftharpoons H^+ + H(\text{ferricyt } c) \qquad K_1'$$

$$H(\text{ferricyt } c) \rightleftharpoons H^+ + \text{ferricyt } c \qquad K_2'$$

$$H_2(\text{ferricyt } c) + Cr(II) \xrightarrow{k_a}$$

$$H(\text{ferricyt } c) + Cr(II) \xrightarrow{k_b}$$

$$\text{ferricyt } c + Cr(II) \xrightarrow{k_c}$$

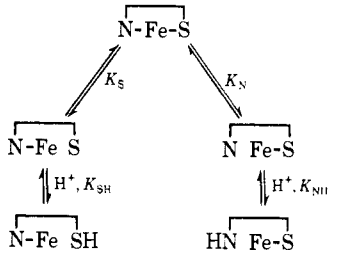
Provided that the acid-dependent equilibria are established rapidly compared to the oxidation–reduction reactions, the above scheme leads to the following expression for the observed second-order rate constants

$$k = \frac{k_a + k_b K_1'/[H^+] + k_c K_1' K_2'/[H^+]^2}{1 + K_1'/[H^+] + K_1' K_2'/[H^+]^2} \qquad (11)$$

Equation 11 is identical with eq 8 provided that the first term in the numerator and the second term in the denominator of eq 8 may be neglected. In terms of this interpretation, $K_1'K_2' = 4 \times 10^{-8}\ M^2$, $K_1' \leq 1 \times 10^{-4}\ M$, $K_2' \geq 4 \times 10^{-4}\ M$, $k_a \leq 2 \times 10^2\ M^{-1}\ \text{sec}^{-1}$, $k_b \geq 4 \times 10^5\ M^{-1}\ \text{sec}^{-1}$, and $k_c = 3 \times 10^3\ M^{-1}\ \text{sec}^{-1}$. Note that since $K_2' > K_1'$, the concentration of the singly protonated species never builds up to significant levels. This species is, however, the important one kinetically since $k_b \gg k_c \gg k_a$. Evidently addition of the first proton results in a conformation change which increases the ease of protonation of the second group and which also makes the heme group more susceptible to attack by chromium(II).

Since it is known that the iron–protein bonds are broken at low pH,[13] it seems reasonable to identify the acid-dependent terms in the rate law with the rupture of these bonds (Scheme I). The iron–sulfur bond is

**Scheme I**




probably much weaker than the iron–nitrogen bond (as has been proposed above), and it is therefore likely that $K_S \gg K_N$. Moreover, since the imidazole nitrogen is almost certainly much more basic than the methionine sulfur, it is also likely that $K_{NH} \gg K_{SH}$. It is more difficult to decide whether $K_S K_{SH} \gg K_N K_{NH}$. Although eq 11 can be readily generalized to allow for the formation of both of the protonated species, it does not seen profitable to pursue this line of analysis in the absence of the relevant equilibrium data.[14] It is nevertheless important to note that the kinetic data require that the species in which one iron–protein bond has been broken be considerably more reactive (at least in chloride media) than the species in which both of the iron–protein bonds are intact (pH >5.5) or broken (pH <1.0).

In the above discussion of the pH dependence it has been tacitly assumed that the pH–rate profile is a consequence of the pH-dependent equilibria of ferricytochrome *c* rather than of the reductant. This assumption is strongly supported by the observation of a similar pH–rate profile in the reduction of ferricytochrome *c* in other systems. Thus the ferricytochrome *c* catalyzed oxidation of pyrogallol to purpurogallin by hydrogen peroxide has a pH optimum of about 3.5.[15] Neither hydrogen peroxide nor pyrogallol has a p*K* in the pH range studied. An optimum pH between 3 and 4 has also been observed in the reaction of the carboxyl radical with ferricytochrome *c*.[16] Although the authors interpreted this optimum in terms of the protonation of $COO^-$ to form COOH with the latter being the more reactive, they offered no explanation for the decrease in rate below pH 3.

The position and height of the rate maximum in the peroxide reaction depend upon the composition of the medium, and for this reason the pH optimum has been interpreted in terms of the enhanced reactivity of complexes formed between ferricytochrome species in which one of the iron–protein bonds has been broken and the anions (acetate, citrate, or chloride) present in the solution. In a similar manner the rate maximum observed in this work could also be affected by the anions (chloride and acetate) present, and this could account for our difficulty in providing a quantitative description of the rate changes in the vicinity of the pH optimum. Finally, the observation of a complex pH and anion dependence indicates the need for considerable caution in interpreting the activation parameters for the reduction of ferricytochrome *c*. Thus the activation energy reported[10] for the reduction of ferricytochrome *c* by chromium(II) at pH 4.2 is almost certainly a complex composite of rate and equilibrium parameters.

**Bridging and Other Anion Effects.** We have seen that the nature of the medium has a relatively large effect on the reactivity of ferricytochrome *c* below a pH of about 5.5 and that the medium effects can be attributed to the formation of reactive complexes between the anions and ferricytochrome *c* species in which one of the iron–protein bonds has been broken. These complexes presumably contain the anion directly bonded to the iron atom, and it is very likely that they react with chromium(II) by bridging mechanisms in which the anion is also bonded to the chromium atom. *In other words, it is proposed that these reactions proceed via –FeXCr– transition states similar to the one proposed above for the chloride mediated reaction in the pH range 6.1–7.0.* By analogy with simpler redox systems, we may regard these reactions as proceeding by *adjacent attack* of the chromium(II) on the heme iron. It may be noted that the adjacent attack mechanism can also

(13) E. Boeri, A. Ehrenberg, K. G. Paul, and H. Theorell, *Biochim. Biophys. Acta*, **12**, 273 (1963).

(14) It should be noted that two completely independent protonatable sites are not compatible with our results.

(15) T. Flatmark, *Acta Chem. Scand.*, **19**, 2059 (1965).

(16) E. J. Land and A. J. Swallow, *Arch. Biochem. Biophys.*, **145**, 365 (1971).

account for the formation of the cytochrome *c*–chromium–phosphate complex reported[5] to be a product of the chromium(II) reduction of ferricytochrome *c* in phosphate medium at pH 4.00.

One purpose of the present investigation was to obtain evidence for electron transfer through the edge of the porphyrin ring system of ferricytochrome *c* by studying the effect of anions on the reaction rate. To some extent this expectation has been realized. Thus it is apparent that azide, thiocyanate, and, to a lesser extent, iodide, markedly catalyze the rate of reduction of ferricytochrome *c* by chromium(II). Since these anions were not present in the ferricytochrome *c* solution prior to the mixing of the reactants on the flow machine, the reaction mixture did not initially contain any –FeX complexes. Consequently, provided the rate of complexing of the anions with the iron is sufficiently slow, the anion catalysis must be attributed to electron transfer pathways other than the one involving –FeXCr– transition states.

The maximum rate of formation of the –FeX complexes is given by eq 12, the anion-catalyzed reduction

$$F = k_f[\text{ferricyt } c][X^-] \tag{12}$$

rates are given by eq 13, and the ratio of these rates is

$$R = k_1'[\text{ferricyt } c][Cr(II)][X^-] \tag{13}$$

given by eq 14. The second-order rate constants, $k_f$,

$$\frac{R}{F} = \frac{k_1'[Cr(II)]}{k_f} \tag{14}$$

for the binding of azide and cyanide to the iron atom of ferricytochrome *c* are about 25 $M^{-1}$ sec$^{-1}$ in the pH range 6.1–7.0.[2] In the absence of any direct observation of significant binding of thiocyanate or iodide to ferricytochrome *c*, it will be assumed that the rate constants for the reactions of these anions with ferricytochrome *c* are less than 25 $M^{-1}$ sec$^{-1}$. Substitution into eq 14 shows that $R/F$ is larger than 5 for all of the azide and thiocyanate runs, and that this ratio is larger than 80 for some of the thiocyanate runs.[17] This ratio is less favorable for the iodide system. However, the fact that the kinetic plots were first-order for this system indicates that the pathway involving the formation of an iron–iodide bond does not make an important contribution to the observed rate. We may therefore conclude with some confidence that the thiocyanate- and azide-catalyzed pathways, and, with less certainty, that the iodide-catalyzed pathway, do not involve substitution on the iron center, but that these reactions proceed by *remote attack*; that is, they make use of an electron transfer site remote from the iron(III), possibly the exposed edge of the porphyrin ring system. Moreover, if the conclusion reached above that the "uncatalyzed" electron transfer reaction involves chloride bridging to the iron is correct, then it may be further concluded that azide and thiocyanate are at least 1000 times as effective as chloride in promoting the remote electron transfer pathway.

Further support for the above conclusions is provided by reactivity pattern considerations. If the first step in the reactions is the complexing of $X^-$ with $Cr^{2+}$ to form $CrX^+$ and that this complex then reduces ferricytochrome *c* (eq 15 and 16), then the reaction rates

$$Cr^{2+} + X^- \rightleftharpoons CrX^+ \quad K_1 \tag{15}$$

$$\text{ferricyt } c + CrX^+ \longrightarrow \text{ferrocyt } c + CrX^{2+} \tag{16}$$

will be affected by the different stabilities of the $CrX^+$ complexes and by differences in the driving forces for the actual electron transfer steps. The former will vary as $K_1$, while in the simplest model[18] the latter will vary as $\sqrt{K_3/K_1}$ where $K_3$ is the stability constant of $CrX^{2+}$, the chromium(III) complex formed by the oxidation of $CrX^+$. Thermodynamic considerations thus suggest that the rates of reduction of ferricytochrome *c* should increase as $K_1\sqrt{K_3/K_1} = \sqrt{K_1K_3}$. Data on the stabilities of all of the complexes are lacking but the thermodynamic factors clearly favor the azide and thiocyanate reactions and predict the reactivity order $I^- < Cl^- < SCN^- < N_3^-$. Since this is not the observed order, thermodynamic differences are not the sole and perhaps not even the major factors determining the relative reaction rates.

In a more detailed treatment it is necessary to allow for differences in the stabilities of the precursor complexes formed in the remote electron-transfer pathway.[19,20] If the electron-transfer site is the edge of the porphyrin ring system, then an attractive mechanism for these reactions is one in which the iodide, azide, or thiocyanate ion acts as a bridging group between the chromium(II) and the porphyrin ring system. Support for this view is provided by the fact that the reactivity pattern $Cl^- < I^- < N_3^- \sim SCN^-$ is strikingly similar to the order previously observed in the reaction of $Fe(phen)_3^{3+}$ with $Fe^{2+}$.[21] In those studies the relatively high reactivity of thiocyanate was ascribed to the formation of a bridged intermediate by the nucleophilic attack of the sulfur atom of $SCN^-$ on a carbon atom of the ligand ring system bearing a partial positive charge.[21] The thiocyanate-catalyzed reduction of ferricytochrome *c* may similarly proceed by nucleophilic attack of the sulfur atom on a $\beta$ carbon of the pyrrole ring. Alternatively, $\pi$ complexes may be formed between the thiocyanate and the aromatic ring systems. Similar mechanisms may also obtain in the azide- and iodide-catalyzed reactions and could account for the effectiveness of these ions in bringing about electron transfer by the remote pathway.[22,23]

To summarize, depending upon the conditions and the nature of the medium, the reaction of ferricytochrome *c* with chromium(II) can proceed by adjacent and/or remote attack. The evidence suggests that the reaction in chloride media proceeds predominantly by a pathway involving adjacent attack of the chromium(II) on the iron(III). On the other hand, when the pH is

(17) Note also that some of the pseudo-first-order rate constants in the presence of thiocyanate exceed the limiting value of 60 sec$^{-1}$.

(18) R. A. Marcus, *J. Phys. Chem.*, **67**, 853 (1963).
(19) D. P. Fay and N. Sutin, *Inorg. Chem.*, **9**, 1291 (1970).
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(22) N. Sutin, *Chem. Brit.*, **8**, 148 (1972).
(23) Although a bridging role has been ascribed to the added anions in the remote electron-transfer pathway, these anions could also function as nonbridging ligands. Nonbridging ligand effects may be quite large and while we cannot exclude the possibility that the added anions are acting as nonbridging ligands, it should be noted that such effects, at least with chromium(II) as a reductant, generally tend to parallel the changes in the driving force for the reaction.[24,25] The bridging–nonbridging uncertainty does not, of course, affect the conclusion that the catalyzed reactions are proceeding by a remote electron-transfer pathway.
(24) J. E. Earley, *Progr. Inorg. Chem.*, **13**, 243 (1970).
(25) D. E. Pennington and A. Haim, *Inorg. Chem.*, **6**, 2138 (1967).

greater than about 5.5 and the electron transfer is very rapid, as is the case, for example, in the presence of azide and thiocyanate ions (and also when ferricytochrome *c* reacts with hydrated electrons[16,26]), the reaction proceeds predominantly by a remote pathway.

These conclusions must be regarded as tentative at this time and need to be confirmed with a larger variety of reducing agents. Particular attention will have to be paid to the various forms of ferricytochrome *c* and the rate at which equilibrium between these forms is established.

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# Chromium(II) Reductions of Aromatic Nitrile Complexes of Pentaamminecobalt(III)

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**Abstract:** The kinetics of the reduction of the pentaamminecobalt(III) complexes of terephthalonitrile, and 3- and 4-cyanophenol have been studied. In all cases the rate law is $-d \ln [\text{cobalt(III) complex}]/dt = k[\text{Cr(II)}]$. The rate constant ($M^{-1}$ sec$^{-1}$) at 25° in 1.0 *M* $HClO_4$–$LiClO_4$, the activation energy (kcal mol$^{-1}$), and activation entropy (cal mol$^{-1}$ deg$^{-1}$) respectively are for the terephthalonitrile complex 0.92, 5.5 ± 0.3, −40 ± 2; for the 3-cyanophenol complex $4.17 \times 10^{-2}$, 9.4 ± 1.0, −34 ± 3; and for the 4-cyanophenol complex $2.96 \times 10^{-2}$, 11.1 ± 1.0, −28 ± 3. At 25° the rate constants ($M^{-1}$ sec$^{-1}$) for reduction of the benzonitrile and 4-cyanobenzoic acid (nitrile bonded to cobalt) are found to be $4.27 \times 10^{-2}$ and 0.28 $M^{-1}$ sec$^{-1}$, respectively. The reaction proceeds with only 10–15% ligand transfer for the cyanophenol systems and with no detectable ligand transfer with terephthalonitrile. The variation in rates is considered to be inconsistent with a normal outer-sphere electron transfer mechanism and is explained by an outer-sphere nitrile ligand reduction mechanism.

Since the original observation by Taube, *et al.*,[2] that the reduction of some $(NH_3)_5Co^{III}X$ complexes by chromium(II) proceeds with transfer of the X ligand to the chromium(III) product, a wide range of X groups have been studied in this reaction. This work has been reviewed by Gould and Taube.[3] In the present investigation a series of aromatic nitrile ligands have been studied in the general reaction

$$((NH_3)_5Co^{III}N{\equiv}CC_6H_4Y)^{3+} + Cr^{2+} \xrightarrow{H^+} Co^{2+} + Cr^{3+} + N{\equiv}CC_6H_4Y + 5NH_4^+$$

It was of interest to determine if the reaction proceeded with ligand transfer and to determine how the rate and amount of ligand transfer varied with the Y substituent of the nitrile.

From the point of view of electron transfer these nitrile ligands have the advantage that the reducing agent cannot attack at the atom or group directly bonded to cobalt(III) since there are no free electron pairs available for bonding to the reducing agent. They are similar, in this respect, to the substituted pyridine systems[3] studied previously and differ from the carboxylic acid ligands.[3] The nitriles have the further advantage that their relative reducibilities have been systematized by polarographic studies.[4] The latter data may prove useful if ligand reduction is important in the electron transfer process.

## Experimental Section

Aquopentaamminecobalt(III) perchlorate was prepared by slowly adding solid carbonatopentaamminecobalt(III) nitrate[5] to warm 1 *M* perchloric acid. The product was recrystallized twice from 1 *M* perchloric acid.

**$(NH_3)_5Co$(terephthalonitrile)$(ClO_4)_3$.** Terephthalonitrile (15 g), aquopentaamminecobalt(III) perchlorate (20 g), and Linde 3A molecular sieves (~20 g) were mixed in 200 ml of trimethyl phosphate (TMP). The mixture was heated on a steam bath for 3 hr. The orange solution was cooled, filtered, and mixed with 800 ml of *sec*-butyl alcohol. The product tended to separate as an oil which could be converted to solid by stirring with *sec*-butyl alcohol. The solid was slurried in methanol for 24 hr to remove any TMP complex. The product was recrystallized from warm water with added sodium perchlorate. Ion-exchange chromatography indicated that the product contained a small amount (<5%) of higher charged impurity suspected to be the symmetrical dimeric product. *Anal.* Calcd for $(NH_3)_5CoNCC_6H_4CN(ClO_4)_3$: C, 16.8; H, 3.33; N, 17.2. Found: C, 16.9; H, 3.29; N, 17.3.

**$(NH_3)_5Co$(4-cyanophenol)$(ClO_4)_3$.** Aquopentaamminecobalt(III) perchlorate (10 g), 4-cyanophenol (18 g), and Linde 3A molecular sieve (30 g) were mixed in 120 ml of TMP and heated on a steam bath for 45 min. The yellowish brown solution was cooled, filtered, and treated with 900 ml of *sec*-butyl alcohol. Precipitate formed and was collected by filtration and recrystallized from warm water and dilute perchloric acid. The freshly recrystallized material is bright yellow. However, during 1 week at room temperature the solid becomes yellowish brown and has a phenolic odor. The product can be stored in a refrigerator without apparent decomposition for several months. *Anal.* Calcd for $(NH_3)_5Co(NCC_6H_4OH)(ClO_4)_3$: C, 15.0; H, 3.56; N, 15.0. Found: C, 15.0; H, 3.46; N, 14.8.

This compound has also been prepared and purified by the procedure given for the 3-cyanophenol complex. The product had the

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