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Kinetics and Mechanism of the Reduction of Horse Heart Ferricytochrome c by Chromium(II) at Low pH

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The rate constant for the reduction of ferricytochrome c by chromium(II) at 25° is $(40/[H^+] + 3.6 \times 10^2 + 4.7 \times 10^3[H^+])$ M^{-1} sec^{-1} in 1.0 M chloride at $[H^+] = 0.003$–0.20 M and $(3.2/[H^+] + 10)$ M^{-1} sec^{-1} in 0.1 M perchlorate at $[H^+] = 0.006$–0.08 M. The addition of thiocyanate ions to the reactant solutions markedly increases the reaction rate, and the third-order rate constant for the thiocyanate-assisted reaction at $[H^+] = 0.006$–0.05 M is $(1.1 \pm 0.2) \times 10^6$ M^{-2} sec^{-1} in 1.0 M chloride and $(2.3 \pm 0.4) \times 10^6$ M^{-2} sec^{-1} in 0.1 M chloride. The reaction in chloride media is postulated to occur by an inner-sphere mechanism with a transition state [Fe–Cl–Cr]*. The thiocyanate-assisted reaction is thought to occur mainly by an outer-sphere mechanism, with electron transfer taking place through the porphyrin ring system. However, the detection of an unstable chromium(III) product containing thiocyanate bound to the chromium through the sulfur indicates that part of the thiocyanate-assisted reaction proceeds through an inner-sphere mechanism with a transition state [Fe–NCS–Cr]*. The same unstable chromium(III) product is formed in the reaction of chromium(II) with tetrakis(4-pyridyl)porphineiron(III) chloride in chloride or perchlorate media containing added thiocyanate.

Introduction

At neutral pH, the heme group of horse heart ferricytochrome c lies in a crevice of the globular protein. The iron atom is in the plane of a porphyrin ring, and its fifth and sixth coordination positions are occupied by the nitrogen atom from the imidazole ring of histidine-18 and the sulfur atom of methionine-80.[1] We have previously studied the kinetics of the reduction of ferricytochrome c by chromium(II) in this pH region and found that the kinetic behavior in 1.0 M chloride could be explained on the basis of a mechanism which involves rate-limiting cleavage of the iron–sulfur bond and adjacent attack by the chromium(II).[2] This rate-limiting step (about 60 sec^{-1} at 25°) has also been found in reactions of ferricytochrome c with ligands such as azide, imidazole, and pyridine[3] and may also obtain in its reduction by dithionite.[4,5] Upon the addition of anions such as iodide, azide, or thiocyanate, the ferricytochrome c–chromium(II) reaction proceeds faster than the iron–methionine sulfur bond rupture and the mechanism changes to one involving remote or outer-sphere attack.[2] Outer-sphere mechanisms have also been proposed for a number of other cytochrome c reactions, and there is now firm evidence for this type of pathway.[6]

At low pH, the environment about the iron atom changes. Theorell and Akesson[7a] and later Boeri et al.[7b] have shown that both the iron–sulfur and the iron–nitrogen bonds are broken at low pH and low ionic strength, and the ferricytochrome c changes from a low-spin to a high-spin state with five unpaired electrons. If the pH is then kept constant but the ionic strength is increased, spectral changes indicative of a new form of ferricytochrome c appear. It was proposed that this form was of the "mixed-spin" type, as it appeared to possess three unpaired electrons. Aviram has recently shown that an increase in absorbance at 695 nm occurs concurrently with the appearance of other bands which are characteristic of the "mixed-spin" form;[8] since the 695-nm band has been shown to be the result of coordination of the methionine sulfur to iron,[9] Aviram concluded that formation of this bond was an important process in making the "mixed-spin" complex. She also stated that the iron–histidine nitrogen bond remained broken in this spin state of ferricytochrome c.

In view of the different environment about the iron at low pH, we decided to extend the study of the rate of reduction of ferricytochrome c by chromium(II) to the low-pH region. Some experiments done in the previous work indicated that the reduction rate below pH 3 could be described as $40/[H^+]$ M^{-1} sec^{-1} at 25° in 1.0 M chloride,[2] and these experiments have been expanded and refined. In addition, the effect of added thiocyanate on the reaction rate has been studied, along with a few measurements in perchlorate media to ascertain the effect of chloride ion. Specifically, it was hoped that answers or at least clues to the solution of the following questions would result from this work.

(1) If ferricytochrome c in acid solution existed in several spin-state or acid-dependent forms, to what extent would these forms react differently with chromium(II)?

(2) At neutral pH, the thiocyanate-assisted chromium(II) reduction proceeds by a mechanism involving remote attack. In acidic media, with at least one site on the iron free, would the mechanism of the thiocyanate-assisted reaction remain the same or would it change to one involving adjacent attack?

(3) The lability of the chromium(III) products at neutral pH precluded any direct means of establishing their identity. These chromium(III) products would be relatively inert in acidic media, so that analysis of the reaction products could yield information about the intimate mechanism of the reaction.

(4) Having at least one site free, the local environment about the iron atom in ferricytochrome c is similar to that for some iron porphyrin complexes which have been proposed as models. Since most oxidation–reduction and substitution or equilibration reaction data for these porphyrins and other proposed model complexes have been obtained at low pH, a study of the ferricytochrome c–chromium(II) reaction would allow a more direct evaluation of the usefulness of these models.

Experimental Section

Materials. Sigma horse heart cytochrome c (type III), tetrakis(4-pyridyl)porphine (Mad River Chemical Co.), and tetrakis-(4-pyridyl)porphineiron(III) chloride (Mad River Chemical Co.) were used without further purification. Chromium(II) chloride solutions were prepared by dissolving high-purity chromium metal (Varlacoid Chemical Co.) in hydrochloric acid under an argon atmosphere. Chromium(II) perchlorate solutions were made by the reduction of chromium(III) perchlorate with zinc amalgam in an argon atmosphere. Both the chloride and perchlorate solutions were analyzed for chromium(II) content by the addition of excess iron(III) and titration of the iron(II) produced with cerium(IV) using ferroin as an indicator. Thiocyanatopentaamminecobalt(III) chloride ($[(NH_3)_5CoSCN]Cl_2 \cdot 3/2H_2O$) was made using the procedure of Sargeson et al.[10a] The molar absorptivities and absorption maxima were in excellent agreement with those reported previously. Solutions containing thiocyanatopentaaquochromium(III) ($(H_2O)_5CrSCN^{2+}$) were made in situ following the procedure of Haim and Sutin.[10b,11] The solutions were stored at 0° and used within 6 hr of preparation. Sodium perchlorate was made by the reaction of sodium carbonate with perchloric acid. The solution was boiled to expel carbon dioxide, and after cooling, the pH was adjusted to 4–5 by the addition of extra

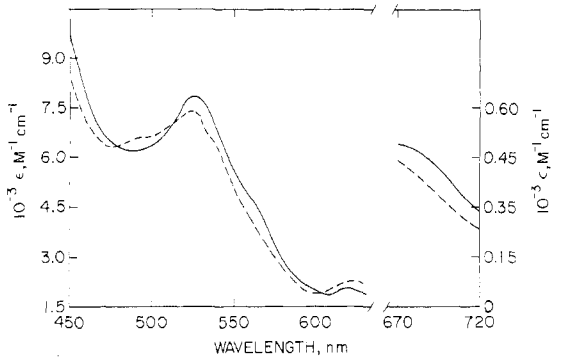

Figure 1. Spectra of ferricytochrome *c* in 1.0 *M* chloride at 25°: full curve, $[H^+] = 0.006\ M$; dashed curve, $[H^+] = 0.10\ M$.

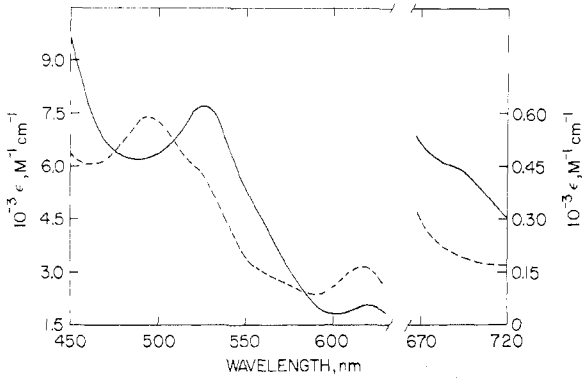

Figure 2. Spectra of ferricytochrome *c* in 0.1 *M* chloride at 25°: full curve, $[H^+] = 0.006\ M$; dashed curve, $[H^+] = 0.10\ M$.

carbonate or acid. The concentration of cytochrome *c* was determined by dissolving known weights of heme protein in given volumes of solution. All other chemicals were reagent grade; triply distilled water was used throughout.

Kinetic Measurements. The kinetic data were obtained at 25° by monitoring the absorbance changes at 550, 450, and 260 nm using a Durrum Model 110 stopped-flow spectrophotometer. The chromium(II) was always present in at least 50-fold excess, so the absorbance change measurements were analyzed by a least-squares program as a first-order process. Except for those experiments expressly designed to demonstrate the necessity of such procedures, two rules were followed. Tsong has recently shown that ferricytochrome *c* undergoes a triphasic reaction as the acidity of the solution is changed in the pH range 2–4;[12] consequently, both reactant solutions in the stopped-flow apparatus were adjusted to the same pH in order to minimize the interference of such reactions. Also, the work of Aviram[8] and Fung and Vinogradov[13] among others has shown that the ferricytochrome *c* species in solution depend not only upon the pH but upon the ionic strength as well, so the ionic strength of both reactant solutions was always kept equal.

Results

Spectral Data for Cytochrome *c*. The absorption spectra of ferricytochrome *c* in 1.0 *M* chloride, 0.1 *M* chloride, and 0.1 *M* perchlorate at the extremes of the acidity range used in this study (0.006 and 0.10 *M* H^+) are shown in Figures 1–3, respectively. It can be seen that for all three media the spectra at low acid concentrations are similar and closely resemble that of the "mixed-spin" complex reported by Aviram;[8] thus the low-spin band at 530 nm, the high-spin band at 620 nm, and the characteristic iron–methionine-80 sulfur band at 695 nm are all present.

At the higher acidity there is no significant shift in the band maxima in 1.0 *M* chloride or 0.1 *M* perchlorate. There is, however, a decrease in molar absorptivity at 695 and 530 nm and an increase at 620 and 490 nm, indicating partial conversion from the "mixed-spin" to the high-spin state. These changes occur to a slightly greater extent in perchlorate than

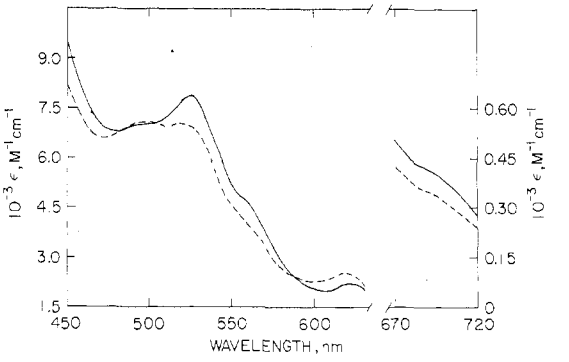

Figure 3. Spectra of ferricytochrome *c* in 0.1 *M* perchlorate at 25°: full curve, $[H^+] = 0.006\ M$; dashed curve, $[H^+] = 0.10\ M$.

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

$[H^+], M$	$10^3[Cr^{II}], M$	k_{obsd}, sec^{-1}	$[H^+], M$	$10^3[Cr^{II}], M$	k_{obsd}, sec^{-1}
0.003	0.841	11.5	0.030	1.00	1.8
0.006	1.63	11.6	0.030	1.53	2.6
0.010	0.841	3.0	0.030	5.00	7.6
0.010	2.77	11	0.040	1.03	1.9
0.020	0.530	1.2	0.050	1.53	2.2
0.020	3.33	7.2	0.050	10.0	11
0.020	5.45	11	0.050	19.8	21
0.020	10.0	21	0.100	1.04	1.4
0.020	19.8	38	0.100	3.07	3.0
0.023	1.82	4.1	0.100	10.0	7.9
			0.100	19.8	13
			0.200	1.40	2.0
			0.200	1.82	2.2

[a] All of the measurements were made at 550 nm and an ionic strength of 1.0 *M* maintained with sodium chloride; the cytochrome *c* was about 5×10^{-6} *M*. Measurements made at 290, 410, 450, and 620 nm agreed with these rates to within 5%.

chloride. Since they are not very large, one may conclude that the *dominant* species in 1.0 *M* chloride and 0.1 *M* perchlorate over the acidity range of 0.006–0.10 *M* H^+ is the "mixed-spin" complex. The data obtained in 0.1 *M* chloride, on the other hand, reveal that at least two species are present over the acidity range used. There is a significant decrease in molar absorptivity at 695 nm and a large increase at 620 nm; the low-spin band at 530 nm is replaced by the high-spin band at 490 nm. Thus, the dominant species in 0.1 *M* chloride shifts from the "mixed-spin" complex at 0.006 *M* H^+ to the high-spin complex in 0.10 *M* H^+.

To characterize the spectra more fully, the molar absorptivities at the Soret maxima (which are not shown in the figures) will be given here. In 1.0 *M* chloride, the maximum is $9.7 \times 10^4\ M^{-1}\ cm^{-1}$ at 401 nm in 0.006 *M* H^+ and is $9.0 \times 10^4\ M^{-1}\ cm^{-1}$ at 399 nm in 0.10 *M* H^+. In 0.1 *M* chloride, the maximum is $9.4 \times 10^4\ M^{-1}\ cm^{-1}$ at 398 nm in 0.006 *M* H^+ and is $1.3 \times 10^5\ M^{-1}\ cm^{-1}$ at 393 nm in 0.10 *M* H^+. Finally, in 0.1 *M* perchlorate, the maximum is $1.3 \times 10^5\ M^{-1}\ cm^{-1}$ at 394 nm in 0.006 *M* H^+ and is $1.0 \times 10^5\ M^{-1}\ cm^{-1}$ at 396 nm in 0.10 *M* H^+.

Reductions in Chloride and Perchlorate Media. The kinetic data for the reaction of chromium(II) and ferricytochrome *c* at 25° and 1.0 *M* chloride are given in Table I. All the rate constants reported in the tables are the average of four to six replicate runs, and in each case every individual run is within 10% of the mean of the runs. At higher acidities, the calculated second-order rate constant appears to decrease with increasing chromium(II) concentrations; the fact that this decrease is wavelength independent suggests that it is due to a limiting rate attained at higher chromium(II) concentrations rather than to two separate reactions occurring on the same time scale

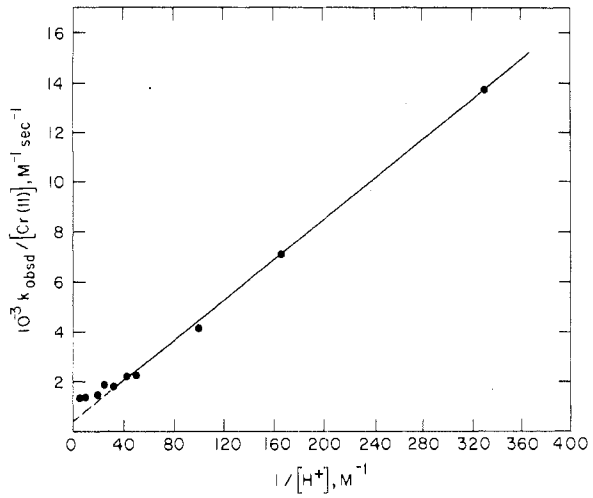

Figure 4. Acid dependence of the second-order rate constants for the reduction of ferricytochrome *c* by chromium(II) in 1.0 *M* chloride at 25°; the chromium(II) concentration used in this work was relatively low ($\leq 1.8 \times 10^{-3}$ *M*).

Table II. Rate Constants for the Reaction of Ferricytochrome *c* with Chromium(II) in 0.1 *M* Perchlorate at 25°[a]

$[H^+]$, *M*	$10^3[Cr^{II}]$, *M*	k_{obsd}, sec^{-1}	$[H^+]$, *M*	$10^3[Cr^{II}]$, *M*	k_{obsd}, sec^{-1}
0.0064	2.19	1.1	0.060	2.08	0.12
0.010	2.19	0.71	0.060	10.4	0.66
0.020	1.10	0.18	0.080	2.19	0.12
0.020	6.56	1.1			

[a] The ionic strength was maintained with sodium perchlorate and the ferricytochrome *c* concentration was about 5×10^{-6} *M*.

since these would tend to interfere more at one wavelength than another.

The second-order rate constants obtained at low ($\sim 10^{-3}$ *M*) chromium(II) concentrations are plotted vs. $1/[H^+]$ in Figure 4. By use of a nonlinear least-squares analysis the low chromium(II) data can be fitted to the equation

$$-d[Cytc^{III}]/dt = (40/[H^+] + 3.6 \times 10^2 + 4.7 \times 10^3[H^+])[Cr^{II}][Cytc^{III}] \quad (1)$$

It is also evident from the data that only the last two terms decrease at higher ($\sim 10^{-2}$ *M*) chromium(II) concentrations, and a mechanism accounting for all of these observations is given in the Discussion.

In contrast to those seen for 1.0 *M* chloride, the kinetic data for the reaction of ferricytochrome *c* in 0.1 *M* chloride are quite complex. At low acidity (≤ 0.01 *M* H^+) and low chromium(II) concentrations, the reaction rate is first order with respect to chromium(II) concentration and the second-order rate constant is $18/[H^+]$ M^{-1} sec^{-1}. However, it is again not first order with respect to chromium(II) at high acid, but this non-first-order behavior now extends to lower acidities (0.02 *M* H^+) than in 1.0 *M* chloride. Also, a definite wavelength dependence is observed here; the plots of log (absorbance) vs. time are curved, and the rates seen at 550, 450, and 620 nm vary by a substantial amount, especially at the higher acidities. Since the spectra revealed that the dominant species in 0.1 *M* chloride media changed as the acidity increased, it is not altogether surprising that the kinetic data are complex. Cohen et al.[14] have recently shown, using PMR techniques, that several proton-associated processes occur between pH 3.5 and pH 1.0 at 0.1 *M* ionic strength. No further analysis of the data for this system will be given at this time.

The chromium(II)–ferricytochrome *c* reaction is well behaved in 0.1 *M* perchlorate media; the reaction is wavelength independent and also first order with respect to the chromium(II) concentration over the range studied. The data are presented in Table II. A plot of k_{obsd} vs. $1/[H^+]$ is similar to the plot obtained in 1.0 *M* chloride in the region 0.006–0.06 *M* H^+. By use of a nonlinear least-squares program the rate law for the reaction in 0.1 *M* perchlorate is found to be

$$-d[Cytc^{III}]/dt = 10 + 3.2/[H^+])[Cr^{II}][Cytc^{III}] \quad (2)$$

It should be mentioned at this point that several experiments were performed in 1.0 *M* perchlorate media. The plots of log (absorbance) vs. time were curved and wavelength dependent. In addition, there was a severe precipitation–denaturation problem. About 20 min after the introduction of cytochrome *c* into the reaction flask, the solutions became almost colorless as dark brown globules formed on the sides of the flask. This phenomenon was not observed in the 0.1 *M* perchlorate solution for at least a period of many hours; the rate of this precipitation–denaturation was evidently some function of perchlorate concentration and acidity. However, since no meaningful information was obtainable, no further work in 1.0 *M* perchlorate was attempted.

Table III. Rate Constants for the Thiocyanate-Assisted Ferricytochrome *c*–Chromium(II) Reaction at 25°[a]

$10^3[SCN^-]$, *M*	$10^3[Cr^{II}]$, *M*	k_{obsd}, sec^{-1} [b]	k_{obsd}, sec^{-1} [c]
0.50	1.00		2.6
0.50	1.53	3.2	
1.0	0.52	1.5	
1.0	1.00		4.7
1.0	1.53	4.2	
1.0	3.00	8.1	
1.0	5.00	13	
2.0	1.00		6.7
2.0	1.53	5.6	
3.0	0.50		5.9
3.0	1.00		11
3.0	1.53	6.9	
3.0	3.35		28
4.0	1.00		14
4.0	1.53	9.2	
5.0	1.00		18
5.0	1.53	12	
10.0	1.00		44

[a] The ionic strength was maintained by the addition of sodium chloride. All of the measurements were made at 550 nm; rate constants measured at 450 nm agreed with those reported to within 5%. The cytochrome *c* concentration was about 5×10^{-6} *M*, and the thiocyanate was present initially in only the chromium(II) solution. [b] In 1.0 *M* Cl^-; 0.030 *M* H^+. [c] In 0.10 *M* Cl^-, 0.020 *M* H^+.

Thiocyanate-Assisted Reactions. Table III shows the variation in rate with added thiocyanate for the ferricytochrome *c*–chromium(II) reaction in 0.1 and 1.0 *M* chloride. The rate constants are independent of wavelength and first order with respect to the chromium(II) concentrations. A plot of $k_{obsd}/[Cr^{II}]$ vs. $[SCN^-]$ yields a straight line with a slope of 9.8×10^5 M^{-2} sec^{-1} for the data at 1.0 *M* chloride and 0.03 *M* H^+. This rate variation with added thiocyanate was repeated at several different acidities. The resulting third-order rate constants from similar plots of $k_{obsd}/[Cr^{II}]$ vs. $[SCN^-]$ are 1.3×10^6, 1.1×10^6, 8.4×10^5, and 6.5×10^5 M^{-2} sec^{-1} at 0.006, 0.012, 0.05, and 0.10 *M* H^+, respectively. There is a definite trend as the third-order rate constant decreases with increasing acidity. However, the data at 0.10 *M* H^+ exhibited curvature at the higher thiocyanate concentrations and are not as reliable. Therefore the value for the thiocyanate-assisted rate constant in 1.0 *M* chloride can be given as $(1.1 \pm 0.2) \times 10^6$ M^{-2} sec^{-1} in the range 0.006–0.05 *M* H^+, and there are

Table IV. Rate Constants for the Thiocyanate-Assisted Ferricytochrome *c*-Chromium(II) Reaction at 25°[a]

$[H^+], M$	$[SCN^-], M$	$10^3[Cr^{II}], M$	k_{obsd}, sec^{-1}
0.0064	0.0010	2.19	5.0
0.010	0.0010	2.19	3.8
0.020	0.0010	1.10	1.6
0.020	0.0010	6.56	7.3
0.020	0.0020	1.10	3.3
0.020	0.0020	6.56	14
0.020	0.0030	2.19	11
0.020	0.0030	2.19	11
0.060	0.0010	2.08	2.4
0.060	0.0010	2.08	2.1[b]
0.060	0.0010	10.4	8.0
0.060	0.0010	10.4	8.3[b]
0.080	0.0010	2.19	2.5
0.080	0.0010	4.38	3.7

[a] The measurements were made in 0.1 *M* perchlorate. The cytochrome *c* was about 5×10^{-6} *M*, and the thiocyanate was present initially in the chromium(II) solution unless noted. All of the measurements were made at 550 nm; rate constants at 450 nm agreed with those reported to within 5%. [b] The thiocyanate is in the cytochrome *c* solution.

processes occurring at higher acidity which would lower this value.

At 0.1 *M* chloride, the decrease in chloride concentration apparently results in a greater contribution from a path which is proportional to the square of the thiocyanate concentration.[15] If the reaction rate is given by

$$-d[Cytc^{III}]/dt = (k_1 + k_2[SCN^-] + k_3[SCN^-]^2)[Cr^{II}][Cytc^{III}] \quad (3)$$

where k_1 is the rate constant seen in the absence of thiocyanate, a plot of $((k_{obsd}/[Cr^{II}]) - k_1)/[SCN^-]$ vs. $[SCN^-]$ should be linear with intercept k_2 and slope k_3. When the data for 0.1 *M* chloride and 0.02 *M* H^+ are plotted in such a fashion, a straight line does result with an intercept of 2.3×10^6 M^{-2} sec^{-1} and slope of 1.7×10^8 M^{-3} sec^{-1}. The corresponding values at 0.006 *M* H^+ are $k_2 = 2.7 \times 10^6$ M^{-2} sec^{-1} and $k_3 = 3.1 \times 10^8$ M^{-3} sec^{-1}, while at 0.05 *M* H^+ they are 1.7×10^6 M^{-2} sec^{-1} and 1.2×10^8 M^{-3} sec^{-1}. Thus, a change in the ionic strength from 1.0 to 0.1 *M* chloride roughly doubles the thiocyanate-assisted rate constant, and the path second order with respect to the thiocyanate concentration is more affected by the acidity at 0.1 *M* chloride than is the first-order path.

Rate constants for the thiocyanate-assisted reaction of ferricytochrome *c* with chromium(II) in 0.1 *M* perchlorate are given in Table IV. The reactions are wavelength independent, but it can be seen that the second-order rate constant decreases with increasing chromium(II) concentration even at relatively low (0.02 *M*) acid. The data reported do not include a large variation in thiocyanate concentration; it was found that contributions from higher order thiocyanate paths dominated even at low thiocyanate concentrations (as shown by the data at 0.02 *M* H^+). It is not altogether surprising that higher order paths would occur, as replacing chloride (which is a potential binding ligand) by perchlorate (which is not) should result in more participation by the potentially binding thiocyanate ion. However, it is difficult to separate the various paths accurately, especially in view of the non-first-order behavior with respect to the chromium(II) concentration. Hence, as an aid in estimating the effect of thiocyanate, several runs were done with low thiocyanate and low chromium(II) concentrations. The results give rate constants of 1.8×10^6, 1.4×10^6, 7.0×10^5, 1.1×10^6, and 1.1×10^6 M^{-2} sec^{-1} at 0.0064, 0.01, 0.02, 0.06, and 0.08 *M* H^+, respectively. The value of the third-order rate constant does not appear to change greatly from 0.1 *M* Cl^- to 0.1 *M* ClO_4^-. (It must be emphasized that these numbers are only estimates. The rate of isomerization of the chromium(III) product, discussed next, indicates that even at this low thiocyanate concentration a good part of the reaction may occur by the $[SCN^-]^2$ path.) Two runs shown in Table IV indicate that the oxidation–reduction reaction is independent of whether the thiocyanate is placed in the chromium(II) or cytochrome *c* solution; this is a general result which holds true for all the reactions in every medium reported here.

Table V. Rate Constants for the Reaction of Chromium(II) with the Product of the Ferricytochrome *c*-Chromium(II)-Thiocyanate Reaction at 25°[a]

$[H^+], M$	$10^3[Cr^{II}], M$	k_{obsd}, sec^{-1}	$[H^+], M$	$10^3[Cr^{II}], M$	k_{obsd}, sec^{-1}
0.0064	2.19	0.35	0.050	2.19	0.13
0.010	2.19	0.26	0.050	4.38	0.28
0.020	1.10	0.068	0.080	2.19	0.10
0.020	6.56	0.50	0.080	4.38	0.19

[a] All of the measurements were made at 260 nm and an ionic strength of 0.1 *M* maintained by sodium perchlorate. The cytochrome *c* was about 2×10^{-5} *M*, and the thiocyanate was 0.001 *M* and present initially in the chromium(II) solution.

Chromium(III) Products. Besides the oxidation–reduction reactions just discussed, a slower reaction at 260 nm was observed in the thiocyanate-assisted studies. This slow reaction was initially postulated to be the chromium(II)-catalyzed linkage isomerization of $CrSCN^{2+}$, i.e.

$$CrSCN^{2+} + Cr^{2+} \rightarrow CrNCS^{2+} + Cr^{2+} \quad (4)$$

The equilibrium constant for the above reaction has been estimated by Haim and Sutin[11] to be about 10^5. This wrong-bonded chromium(III) thiocyanate has been found[16] to be the product of the reaction of $(NH_3)_5CoSCN^{2+}$ and Cr^{2+} and, perhaps closer to the present study, the reaction of $FeNCS^{2+}$ and Cr^{2+}.[11] Haim and Sutin have found that the rate law for the linkage isomerization is

$$-d[CrSCN^{2+}]/dt = (40 + 2.0/[H^+])[Cr^{II}][CrSCN^{2+}] \quad (5)$$

at 25° and 1.0 *M* perchlorate. Since our experimental conditions were different, the rate expression was not directly applicable. Therefore, the rate of the chromium(II)-catalyzed isomerization of the chromium(III) product of the $Co(NH_3)_5SCN^{2+}$–Cr^{2+} reaction was measured and compared to the rate of the 260-nm slow reaction seen in the thiocyanate-assisted chromium(II)–ferricytochrome *c* reaction under the same conditions. A typical set of data obtained is shown in Table V for the ferricytochrome *c* reaction at 25°, 0.1 *M* perchlorate, and 0.001 *M* thiocyanate placed in the chromium(II) solution. The reaction is first-order with respect to the chromium(II) concentration, and a plot of $k_{obsd}/[Cr^{II}]$ vs. $1/[H^+]$ leads to a second-order rate constant of $(38 + 0.79/[H^+])$ M^{-1} sec^{-1}.

A summary of the results of similar experiments appears in Table VI. Along with data for ferricytochrome *c* and $Co(NH_3)_5SCN^{2+}$, some data for the reduction of tetrakis-(4-pyridyl)porphineiron(III) chloride (FeTpyP) are included. The rate of reaction of this compound with chromium(II) in the presence of chloride, perchlorate, and thiocyanate has been previously measured by Hambright and Fleischer,[17] but no analysis of the chromium(III)–thiocyanate product was made.

To test whether the product is really $(H_2O)_5CrSCN^{2+}$, samples of this complex were made in situ by the method of Haim and Sutin[11] and were allowed to react under the same experimental conditions as the rest of complexes. The results in Table VI tend to indicate that the same product is formed in the reaction of chromium(II) with cytochrome *c*, FeTpyP, and $Co(NH_3)_5SCN^{2+}$; however, it does not appear that the product is $(H_2O)_5CrSCN^{2+}$. From the increased value of the

Table VI. Rate Constants for the Reactions of Chromium(II) with the Products of Various Reactions at 25°[a]

Conditions	Oxidant[b]	$k_{obsd}/[Cr(II)]$, M^{-1} sec^{-1}
1.0 *M* ClO_4^-	$(H_2O)_5CrSCN^{2+}$	40 + 2.0/[H^+][c]
0.1 *M* ClO_4^-	$(NH_3)_5CoSCN^{2+}$	16 + 1.2/[H^+]
0.1 *M* ClO_4^-, 0.001 *M* SCN^-	$(H_2O)_5CrSCN^{2+}$	20 + 1.3/[H^+]
0.1 *M* ClO_4^-, 0.001 *M* SCN^-	$(NH_3)_5CoSCN^{2+}$	37 + 0.78/[H^+]
0.1 *M* ClO_4^-, 0.001 *M* SCN^-	Cytc	38 + 0.79/[H^+]
0.1 *M* ClO_4^-, 0.001 *M* SCN^-	FeTpyP	40 + 0.45/[H^+]
0.1 *M* ClO_4^-, 0.003 *M* SCN^-	$(NH_3)_5CoSCN^{2+}$	40 + 0.54/[H^+]
1.0 *M* Cl^-	$(NH_3)_5CoSCN^{2+}$	40 + 1.4/[H^+]
1.0 *M* Cl^-, 0.03 *M* SCN^-	$(H_2O)_5CrSCN^{2+}$	44 + 1.8/[H^+]
1.0 *M* Cl^-, 0.003 *M* SCN^-	$(NH_3)_5CoSCN^{2+}$	63 + 0.73/[H^+]
1.0 *M* Cl^-, 0.003 *M* SCN^-	Cytc	60 + 0.65/[H^+]
0.1 *M* Cl^-	$(NH_3)_5CoSCN^{2+}$	22 + 1.3/[H^+]
0.1 *M* Cl^-, 0.003 *M* SCN^-	$(NH_3)_5CoSCN^{2+}$	32 + 0.72/[H^+]
0.1 *M* Cl^-, 0.003 *M* SCN^-	Cytc	40 + 0.84/[H^+]
0.1 *M* Cl^-, 0.003 *M* SCN^-	FeTpyP	36 + 0.53/[H^+]

[a] All measurements were made at 260 nm under the conditions specified. The concentration of the oxidizing agent was about 2×10^{-5} *M*, and the thiocyanate was present initially in the chromium(II) solution. [b] Except for $(H_2O)_5CrSCN^{2+}$, what is measured is the rate of reaction of chromium(II) with the chromium(III) product of the Cr^{2+}–(Cytc, tetrakis(pyridyl)porphineiron(III), or $(NH_3)_5CoSCN^{2+}$) reaction. [c] Data from ref 11.

acid-independent term of the rate expression for the former three complexes, it appears that the product is probably a 1+-charged species, possibly $ClCrSCN^+$ in chloride and $SCNCrSCN^+$ in perchlorate. (The latter complex has been synthesized by Brown and Pennington[18] by the reaction of Cr^{2+} with $FeNCS^{2+}$ in the presence of free thiocyanate ion.)

Until the proposed unstable chromium(III) product is independently synthesized and identified, no quantitative information about the details of the reaction mechanism can be given. However, a useful internal calibration can be performed to determine the percentage yield of the unstable chromium(III) product. For the reaction of $Co(NH_3)_5SCN^{2+}$ with chromium(II), the slow absorbance change seen at 260 nm was measured as a function of known cobalt(III) reactant concentration, and a molar absorptivity change of 2.1×10^3 M^{-1} cm^{-1} was determined for the isomerization reaction both in 0.1 *M* chloride and in 0.1 *M* perchlorate. Applying this number to the absorbance changes seen for the slow reaction in the ferricytochrome *c*–chromium(II) system, one finds that the unstable chromium(III) product accounts for 10% of the reaction at 0.006 *M* H^+ and up to about 35% of the reaction at 0.10 *M* H^+. Thus the thiocyanate-assisted reaction appears to proceed by two parallel pathways.

Two remarks should be made about the FeTpyP results. First, the agreement with the cobalt(III) system is not as good as that found for cytochrome *c*. A second slow reaction, especially evident at lower acidity, tended to interfere with the "infinity" value for the first reaction. This interference is the probable cause of the smaller value for the acid-dependent term. Second, no work was done in 1.0 *M* chloride. Hambright and Fleischer[17] reported that the rate constant for the Cr^{2+}–Cl^-–FeTpyP reaction is 9.1×10^5 M^{-1} sec^{-1}, while the value for the Cr^{2+}–SCN^-–FeTpyP reaction is 2.3×10^7 M^{-1} sec^{-1} at μ = 1.0 *M*, 25°. If the chloride is 1.0 *M* and the thiocyanate is 0.003 *M*, then the percentage of reaction occurring by the thiocyanate path is about 8%.

We have performed several experiments to determine whether $CrSCN^{2+}$ can be formed in an outer-sphere reaction. We do not find any slow reaction at 260 nm indicative of $CrSCN^{2+}$ formation in the reactions of chromium(II) with $Fe(bipy)_3^{3+}$ or $Fe(phen)_3^{3+}$ in chloride media or in the inner-sphere reaction of $Co(NH_3)_5Cl^{2+}$ with Cr^{2+} in perchlorate medium in the presence of added thiocyanate. Thus $CrSCN^{2+}$ is not the product of an outer-sphere thiocyanate-assisted reaction or of an inner-sphere reaction with chloride as the bridging ligand.

One more important comment should be made about the slow reactions at 260 nm. It was found that a "blank" correction was needed; that is, a solution of chromium(II) and thiocyanate mixed with a solution of chloride or perchlorate of identical ionic strength showed a signal at 260 nm comparable in rate to those seen for the $CrSCN^{2+}$ isomerizations. We have previously found it extremely difficult to remove traces ($(0.5–1) \times 10^{-5}$ *M*) of oxygen from the Durrum flow system.[4] Apparently, the reaction of chromium(II) with oxygen in the presence of thiocyanate produces a chromium(III) product which can react with the excess chromium(II) present. An interesting aspect of this reaction is that it depends on the position of the thiocyanate. If thiocyanate is introduced via the chromium(II) solution, an absorbance decrease at 290 nm much like a $CrSCN^{2+}$ isomerization is seen. The latter is first order with respect to Cr^{2+} and is enhanced by the deliberate introduction of oxygen into the solution. If the thiocyanate is not in the same solution as the chromium(II), an absorbance increase occurs at 260 nm which is possibly indicative of $CrNCS^{2+}$ formation. This "blank" correction is very small in perchlorate media, and the results here are unambiguous. In 0.1 *M* chloride, the signal derived from the $Co(NH_3)_5SCN^{2+}$, cytochrome *c*, or FeTpyP was always at least 5–10 times larger than the "blank" correction, so the results are again unambiguous. However, in 1.0 *M* chloride media, at the higher acidities only, the "blank" is 30–40% of the total absorbance change. Consequently, while the signal resulting from the isomerization of the oxidation–reduction product always predominates, the results in 1.0 *M* chloride must be viewed with caution, as the interference from the chromium(II)–oxygen reaction was substantial. Because of this interference, oxidizing agent concentrations greater than 2×10^{-5} *M* were needed to study accurately the slow isomerization reactions at 260 nm.

Discussion

Oxidation–Reduction Reactions. The kinetic data for the reaction of ferricytochrome *c* with chromium(II) in 1.0 *M* chloride indicate that the reaction rate varies inversely with the acid concentration at low acidity but contains a complex acid-dependent term at higher acidity. The reaction is first order with respect to chromium(II) at lower acidity but tends to decrease with increasing chromium(II) at higher acidity. Reaction 6, which involves four different cytochrome *c* species,

$$\begin{array}{ccccc} \mathrm{Cytc^1} & \overset{H^+}{\rightleftharpoons} & \mathrm{Cytc^2} & \overset{k_2}{\rightleftharpoons} & \mathrm{Cytc^3} \overset{H^+,\,K_4}{\rightleftharpoons} \mathrm{Cytc^4} \\ k_1 \downarrow \mathrm{Cr^{II}} & \overset{K_1}{} & k_0 \downarrow \mathrm{Cr^{II}} & \overset{k_{-2}}{} & k_3 \downarrow \mathrm{Cr^{II}} \qquad k_4 \downarrow \mathrm{Cr^{II}} \\ \text{products} & & \text{products} & & \text{products} \qquad \text{products} \end{array} \tag{6}$$

can account for the kinetic behavior in 1.0 *M* chloride. Assuming steady-state kinetics for $Cytc^3$, one can derive expression 7. Comparison with the parameters of eq 1 shows

$$\frac{-d[Cytc^{III}]}{dt} = \frac{K_1 k_1}{[H^+]} + k_0 + \frac{k_2(k_3 + k_4K_4[H^+])}{k_{-2} + (k_3 + k_4K_4[H^+])[Cr^{II}]}[Cytc^{III}][Cr(II)] \tag{7}$$

that $K_1k_1 = 40$ sec^{-1}, $k_0 + k_2k_3/k_{-2} = 360$ M^{-1} sec^{-1}, and $k_2k_4K_4/k_{-2} = 4.7 \times 10^3$ M^{-2} sec^{-1}. Note that this mechanism assumes that the dominant cytochrome *c* species in 1.0 *M* chloride solution is $Cytc^2$. The spectral data in Figure 1 show that the mixed-spin complex is the dominant species under these conditions; the kinetic data indicate that it is relatively unreactive toward chromium(II). The oxidation–reduction process at low acid (0.003–0.020 *M* H^+) is accomplished by

Cytc[1], a species which has one redox-linked proton less than Cytc[2]. Since K_1 has previously[2] been estimated to be $\leq 1 \times 10^{-4}$ M, we calculate that the rate constant for the reduction of Cytc[1] is $\geq 4 \times 10^5$ M^{-1} sec^{-1}.

It would be useful to be able to discuss the possible structures of Cytc[1], Cytc[2], etc., with respect to the experimental evidence obtained by previous workers. Unfortunately not enough work has been done on ferricytochrome c at this low pH and high ionic strength to do so. Fung and Vinogradov[13] have shown that ferricytochrome has two pK_a values in 1.0 M chloride and acid media, one at 3.5 and the other at 1.0. The results of Yandell, Fay, and Sutin[2] on the reduction of ferricytochrome c by chromium(II) in 1.0 M chloride implicate two redox-linked pK's, one at (or below) 3.4 and the other at (or above) 4.0. On the basis of the absorbance at 620 nm, Aviram has concluded[8] that the iron–methionine sulfur is at least partially intact at pH 1.5 in 1.0 M chloride. Recent NMR measurements show that at low pH there are two water molecules in the inner coordination sphere of the iron(III).[19] To summarize, the above observations suggest that in the mixed-spin species the bond between the iron and histidine-18 is broken while the iron–methionine-80 bond is intact and that none of the iron–protein bonds is intact in the high-spin species.

In eq 6 it appears that the oxidation–reduction process at high acidity (>0.02 M H^+) utilizes the Cytc[3] and Cytc[4] species. Since the spectrum at 0.10 M H^+ reveals the presence of some of the high-spin form, it will be assumed that Cytc[4] is the high-spin form. One interpretation consistent with the kinetic scheme is that k_2 and k_{-2} are the rates of dissociation and formation of the iron–sulfur bond and K_4 is the protonation constant for the free methionine group. A combination of iron–sulfur bond breaking and tertiary structural rearrangement is also possible for the k_2, k_{-2} steps; there is simply not enough information available to be specific about these points.

An unusual feature of the above scheme is that the dominant iron(III) species reacts relatively slowly with chromium(II). One possible explanation for this behavior is provided by the nature of the ferrocytochrome c species in acid solution. It is known that ferrocytochrome c is readily autooxidizable and binds carbon monoxide below pH ~3.[6,20] The PMR spectrum of ferrocytochrome c also undergoes a major change as the pH is decreased below 4.5.[21] At pH 3 the resonances assigned to the histidine-18 and methionine-80 have disappeared and ferrocytochrome c changes from a low-spin to a high-spin form. Despite the above evidence for substantial changes in the ferrocytochrome c as the pH is lowered, no major changes are observed in the visible spectrum in the pH range 2–6.[22] Moreover, no evidence for a mixed-spin form of ferrocytochrome c has, as yet, been discovered, suggesting that a mixed-spin ferrocytochrome c species is not particularly stable with respect to the high- or low-spin forms. With this in mind it is reasonable to speculate that the conversions of high-spin iron(III) to high-spin iron(II) or low-spin iron(III) to low-spin iron(II) are more favorable than that of mixed-spin iron(III) to the unstable mixed-spin iron(II). In terms of this interpretation the relatively slow rate of (inner-sphere) reduction of the mixed-spin iron(III) complex by chromium(II) need not be a consequence so much of the spin change per se as of the conformation change accompanying the spin change. An alternative, perhaps more attractive, cause for the nonreactivity of the mixed-spin form by an inner-sphere path may be steric hindrance. In this explanation, the nonreactivity might be a result of the conformation of the protein rendering the iron atom inaccessible to adjacent attack by chromium(II).

The data in 0.1 M perchlorate media can also be described by eq 6 since the dominant species over the acidity range is again the mixed-spin complex. Although there are not enough data at high acid concentrations to assign values for all parameters, the results do nevertheless show that K_1k_1 is 3.0 sec^{-1} and $k_0 + k_2k_3/k_{-2} \approx 10$ M^{-1} sec^{-1}. Since these values may be dependent upon ionic strength and chloride binding, there is no a priori requirement that they be similar to the values obtained in 1.0 M chloride.

Since the spectral data for 0.1 M chloride show that different species are present at high and low acidity, the mechanism shown in eq 6 cannot be applied directly. The same ferricytochrome species can be postulated to exist in 1.0 M chloride and 0.1 M chloride with the relative amounts of the various forms changing. At low acid, where Cytc[2] predominates, the kinetics follow a simple $1/[H^+]$ dependence with the K_1k_1 term having a value of ~18 sec^{-1}. At higher acid two rates are seen, each with a complex chromium(II) dependence. These two rates are presumably the reduction of the Cytc[1] and Cytc[3]–Cytc[4] species with some possible concurrent interconversion as well.

Effects of Anions. We have previously shown that anions may have a dramatic effect on the reaction rates of reducing agents with inorganic complexes[23] and with cytochrome c at neutral pH.[2] As more information about anion effects is obtained, it becomes necessary to place these effects into three categories and analyze these categories separately at first and then together later. The three types of anion effects that can obtain in the reduction (oxidation) of cytochrome c by a metal center are as follows: (1) bulk—interaction primarily with protein or water; (2) peripheral—interaction primarily with porphyrin; (3) inner sphere—interaction primarily with the iron center. The actual means of classification here is the number of bonds the anion forms in the transition state with the metals which are oxidized or reduced. The bulk anion effect is one in which no bonds are formed between the metals and the anion in the transition state. The anion changes the rate of reaction by binding to a reactant at a site remote from the electron-transfer site (as perhaps chloride to some part of cytochrome c other than the iron atom) or by merely changing the form of the reactant due to an ionic strength effect. Examples of the latter are an ion-pair complex for a simple inorganic molecule or the chaotropic effect proposed by Aviram for the cytochrome c molecule.[8] (A chaotropic effect occurs when anions such as perchlorate or trichloroacetate "break up" the structure of water and allow nonpolar molecules such as proteins to utilize the decreased polarity to make them more soluble in water.[24]) Note, however, our comment[25] on some of the other conclusions reached by Aviram.

The second type of anion effect is termed peripheral. This effect will occur when the anion is bound to one of the metals in the transition state. The best example of this type is the reaction of vanadium(II) with $Co(phen)_3^{3+}$.[23] The marked increase in reduction rate observed in this system upon the addition of thiocyanate was shown to be due to the formation of the relatively reactive $(H_2O)_5VNCS^+$, which reduces the cobalt(III) complex by an outer-sphere mechanism. This type of outer-sphere anion catalysis varies with the type of oxidizing and reducing agent used. For both vanadium(II) and chromium(II) reactions with $Co(phen)_3^{3+}$ and $Co(NH_3)_6^{3+}$, the ratio of k_{NCS}/k_{Cl} (the third-order rate constant for the thiocyanate and chloride rate enhancement) is 10 times greater for $Co(phen)_3^{3+}$ than for $Co(NH_3)_6^{3+}$.[23] Since the phenanthroline complex has a π system capable of interacting with thiocyanate while the hexaamminecobalt(III) does not, it was postulated that the magnitude of this outer-sphere rate enhancement might yield clues to the nature of the actual electron-transfer site.

The third type of anion effect, the "inner-sphere" type, occurs when the anion is bound to both metals in the transition state. Detection of this type of effect is accomplished usually

Table VII. Rate Constants for the Anion-Assisted Oxidation-Reduction Reactions of Chromium(II) with Several Oxidizing Agents at 25°[a]

Oxidant	k_{NCS}/k_{Cl}	Oxidant	k_{NCS}/k_{Cl}
Ferricytochrome *c*	3×10^3	$CoTMP^{3+}$ [d]	55
FeTpyP[b]	30	$Co(phen)_3^{3+}$	1.3×10^3
$Fe(H_2O)_6^{3+}$ [c]	91	$Co(NH_3)_6^{3+}$	66

[a] The measurements are at pH 1-3, $\mu = 1.0$ *M*, unless specified. The ratios are of the respective third-order rate constants. [b] Data from ref 17; FeTpyP is tetrakis(pyridyl)porphineiron(III). [c] Data from G. Dulz and N. Sutin, *J. Am. Chem. Soc.*, **86**, 229 (1964); M. Orhanovic and N. Sutin, *ibid.*, **90**, 4286 (1968). [d] Data from ref 26; $\mu = 0.5$ *M*. CoTMP is tetrakis(4-*N*-methylpyridyl)porphinecobalt(III).

by analysis of the reaction products. If one of the products has an anion bound to it in more than the expected equilibrium distribution, then an inner-sphere electron-transfer path is indicated. Sometimes the product is unstable, as for example $CrSCN^{2+}$,[11] and the decay of the unstable product can be used as evidence for such an inner-sphere path.

With these three types of anion effects involved, the anion-assisted oxidation–reduction rates of chromium(II) with cytochrome *c* and two porphyrin complexes are shown in Table VII. The rates of reaction with hexaaquoiron(III) (inner sphere) and tris(1,10-phenanthroline)cobalt(III) and hexaamminecobalt(III) (the latter two both outer sphere) are included in the table to show the magnitude of the anion effects on reactions with known mechanisms. On the basis of the similarity of the anion effects of the reactions of chromium(II) with tetrakis(4-*N*-methylpyridyl)porphinecobalt(III) and $Co(NH_3)_6^{3+}$, Pasternack and Sutin[26] have concluded that reduction of the cobalt(III) porphyrin proceeds through an outer-sphere mechanism not involving the porphyrin ring system. Detection of an unstable $XCrSCN^+$ complex ($X^- = Cl^-$, SCN^-) in the present work establishes that the chromium(II)–tetrakis(pyridyl)porphineiron(III) reaction proceeds by an inner-sphere mechanism, and the observed anion effects are much like those seen for the reaction of hexaaquoiron(III) with chromium(II).

This leaves only the effect of thiocyanate on the cytochrome *c* reaction to be discussed. The value of the (acid-independent) k_{NCS}/k_{Cl} ratio at $\mu = 1.0$ *M*, 3×10^3, is similar to that seen in the $Co(phen)_3^{3+}$–Cr^{2+} system, where it was postulated that the electron-transfer reaction was outer sphere and was proceeding through the phenanthroline ring π system. On this basis one may conclude that electron transfer takes place primarily through the heme ring of cytochrome *c*. Additional evidence supporting a peripheral mechanism is the lack of acid dependence of the k_{NCS} term whereas the reaction in chloride media is acid dependent. Also of interest is the fact that the rate constant for the chloride-assisted acid-dependent path increases with increasing ionic strength, while that for the thiocyanate-assisted path decreases with increasing ionic strength. These data are consistent with the hypothesis that the chloride path involves reaction of two positively charged species (chromium(II) and the iron(III) center) and that the thiocyanate path involves attack of the chromium(II) at a different part of the protein (possibly the π-electron cloud of the heme ring).

The detection of a $XCrSCN^+$ complex as a reaction product, while at first seemingly at odds with the conclusion that the thiocyanate-catalyzed reaction is outer sphere, can be rationalized in terms of the proposed scheme. The yield of the unstable product increases with increasing acidity; this means that the oxidation–reduction proceeds by parallel inner- and outer-sphere reactions with a greater contribution from an inner-sphere path at higher acidity (where Cytc[4] predominates). The question of the identity of the unstable chromium(III) product thus arises. Since the rate law for the decay of the product was not the same as that seen for $(H_2O)_5CrSCN^{2+}$, it was postulated that the product was $ClCrSCN^+$ or $SCNCrSCN^+$. The former complex has not been independently synthesized, while the latter has.[18] However, the details of the mechanism of decay might be difficult to solve, as one is faced with the several possibilities

$$\begin{matrix} ClCrSCN^+ \\ \text{or} \\ SCNCrSCN^+ \end{matrix} + \begin{matrix} Cr^{2+} \\ CrNCS^+ \\ CrCl^+ \end{matrix} \rightarrow \begin{matrix} CrNCS^{2+} \\ Cr(NCS)_2^+ \\ ClCrNCS^+ \end{matrix}$$

In addition, hydrolyzed chromium species must be considered where the $1/[H^+]$ path predominates. Though the detailed mechanism and stoichiometry of the reactions have not been ascertained, the similarity of the rate laws for the decomposition of the chromium(III) species produced in the reduction of cytochrome *c* and $Co(NH_3)_5SCN^{2+}$ (known to produce $CrSCN^{2+}$) is very strong evidence for the operation of an inner-sphere thiocyanate-bridged path in both of the reductions.

If one accepts the postulate that the inner-sphere path provides the preferred mechanism for the chloride-assisted reaction of all the neutral and low-pH cytochrome *c* species, then an interesting correlation between spin state and reactivity can be developed. Previous studies[2] have shown that native low-spin cytochrome *c* is relatively unreactive toward inner-sphere reduction by chromium(II) ($k = 3.0 \times 10^3$ M^{-1} sec^{-1}, 25°, 1.0 *M* ionic strength; inner-sphere reaction of the low-spin form requires rupture of an iron–protein bond). This and the earlier study also show that Cytc[1], the cytochrome *c* species formed by the addition of one redox-linked proton to nature cytochrome *c*, undergoes rapid inner-sphere reduction by chromium(II) ($k \geq 4 \times 10^5$ M^{-1} sec^{-1}, 25°, 1.0 *M* ionic strength; presumably the added proton either labilizes an iron–protein bond by adding to an amino acid or breaks an iron–protein bond by adding to the coordinated nitrogen or sulfur). Evidence has been presented in this study that Cytc[2], the dominant mixed-spin species, is less reactive than either the low pH high-spin or the native low-spin forms. On the other hand, the low- and mixed-spin forms are of comparable reactivity toward reductions utilizing the peripheral mechanism. The high-spin form appears to be relatively unreactive toward reduction by the peripheral mechanism, as the thiocyanate-assisted reaction apparently changes to an inner-sphere mechanism as the pH is lowered below 1.[27] If these observations are generalized, then the results suggest that the low- and mixed-spin forms of cytochrome *c* will show larger reactivity differences in inner-sphere than in outer-sphere reactions.

In conclusion, we would state that the above interpretation must be regarded as tentative at this time and needs to be confirmed in studies with a large variety of oxidizing and reducing agents. This work does suggest, however, that rather unusual correlations obtain between the spin state and the reactivity of ferricytochrome *c*.

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Registry No. Tetrakis(4-pyridyl)porphineiron(III) chloride, 55621-88-0; chromium(II), 22541-79-3; thiocyanate, 302-04-5.

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(25) We disagree in part with Aviram about the effect of chloride and thiocyanate as chaotropic agents. Aviram has measured the rate of conversion of cytochrome *c* from its high- to its mixed-spin form. Since the rate of conversion did not change appreciably as the chloride concentration increased from 0.05 to 0.15 *M*, she concluded that chloride binding was not important in her system. We would suggest that chloride binding could occur but the rate of conversion would be relatively insensitive to chloride concentration in at least two possible cases, as follow.
(a) A rate-determining dissociation step: if

$$\mathrm{Cyt} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Cyt'} \xrightarrow[\mathrm{Cl^-}]{k_2} \mathrm{CytCl}$$

then

$$\frac{d[\mathrm{CytCl}]}{dt} = \frac{k_2 k_1 [\mathrm{Cl^-}]}{k_{-1} + k_2 [\mathrm{Cl^-}]}[\mathrm{Cytc}]$$

assuming Cyt' to be in steady-state concentrations; if $k_2[\mathrm{Cl^-}] >> k_{-1}$, then the rate will be independent of chloride.
(b) A reestablishment of an equilibrium

$$\mathrm{Cyt} + \mathrm{Cl^-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{CytCl}$$

$$\frac{d[\mathrm{CytCl}]}{dt} = (k_1 [\mathrm{Cl^-}] + k_{-1})[\mathrm{Cytc}]$$

If $K = k_1/k_{-1} < 0.1\ M^{-1}$, then changing the chloride concentration from 0.05 to 0.15 *M* will have only a small effect on the rate. Thus, a wider range of experiments must be performed before potential binding ligands such as thiocyanate or chloride can be proven not to be binding.
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(27) We have found that the rate of reaction of cytochrome *c* with V^{2+} or $Ru(NH_3)_6^{2+}$ at pH 1 is independent of the nature of the reducing agent or its concentration. This suggests that a rate-limiting slow conformational or spin-state change is necessary for reaction of the high-spin form of cytochrome *c* with outer-sphere reducing agents, supporting the hypothesis that the high-spin form of cytochrome *c* is relatively nonreactive by a peripheral mechanism.

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Electrochemical Studies of Oxo- and Sulfido-Bridged Molybdenum(VI), -(V), and (IV) Diethyldithiocarbamate Complexes in Aprotic Solvents

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The electrochemistry of a series of *N,N*-diethyldithiocarbamate (DTC) complexes of molybdenum(VI), -(V), and -(IV) has been studied in dimethyl sulfoxide, dimethylformamide, and acetonitrile at platinum electrodes. The specific complexes that have been investigated by cyclic voltammetry and controlled-potential coulometry are $Mo^{VI}O_2(DTC)_2$, $Mo^{V}_2O_3(DTC)_4$, $Mo^{IV}O(DTC)_2$, $Mo^{V}_2O_4(DTC)_2$, $Mo^{VI}S_2(DTC)_2$, and $Mo^{V}_2O_2S_2(DTC)_2$ and include binuclear molybdenum groups with oxo and sulfido bridges. Oxidation–reduction mechanisms are proposed on the basis of the electrochemical data, spectroscopic measurements, and analysis of the electrolysis products. In all three solvents the monooxo-bridged molybdenum(V) complex $Mo^{V}_2O_3(DTC)_2$ disproportionates to the Mo(VI) and Mo(IV) complexes. The disulfido-bridged molybdenum(V) complex $Mo^{V}_2O_2S_2(DTC)$ undergoes a one-electron reduction at −1.00 V vs. SCE in dimethylformamide to give a product species that reacts with the parent complex to form a mixed oxidation state tetramer, $[Mo_4O_4S_4(DTC)_2]^-$. The later is reduced by a second electron at −1.35 V to give the corresponding dianion.

Molybdenum is an important trace element in living organisms and is known to occur in at least five enzymes; xanthine oxidase,[1] aldehyde oxidase,[2] nitrate reductase,[3] sulfite oxidase,[4] and nitrogenase.[5] All of these enzymes catalyze oxidation–reduction processes that involve two or more electrons per substrate molecule. In each case the enzyme appears to contain two atoms of molybdenum, and in the cases of xanthine oxidase, aldehyde oxidase, and possibly nitrate reductase, two molecules of flavine also are present in the enzyme.[4]

Although some Mo(V) has been detected by ESR in four of the enzymes in the presence of a substrate,[2,3,6-8] the oxidation state(s) of the molybdenum atoms in each of the native enzymes is (are) not known; the VI, V, IV, and III states have been postulated.[4] Regardless of the direction of the electron flow in these systems, evidence indicates a close interaction between flavine and molybdenum in the molybdoflavoprotein enzymes.[9]

The importance of molybdenum in flavoproteins has been reviewed from the standpoint of possible coordination chemistry[10] and has prompted a study of the kinetics of complex formation in aqueous solution between Mo(VI) and 8-quinolinol;[11] this latter complex has been proposed as a model for xanthine oxidase. A review of oxomolybdenum(VI) and -(V) and their complexes provides a useful background for electrochemical studies of Mo(VI) and -(V) model compounds.[12]

The question of the degree of interaction or association