pH (eq 21). Moreover, the substitution of eq 14-18 and 21

$$[L] = \frac{C_L}{1 + K_{a1}[H^+] + K_{a1}K_{a2}[H^+]^2} \quad (21)$$

into eq 19 gives the distribution of $Cu^{2+}(aq)$ with solution's pH, eq 22. The fractions of the other species were obtained

$$\alpha = [Cu^{2+}]/C_{Cu} = 1/\{1 + (K_{14} + K_{16}K_{a1}[H^+]) \times [L] + (K_{15}K_{14} + K_{17}K_{21}[H^+] + K_{18}K_{a1}^2[H^+]^2)[L]^2\} \quad (22)$$

by combining eq 22 and eq 14-18.

The Cu(II)-glycine and Cu(II)-glutamic acid equilibria were treated by similar procedures.

Appendix II

The mechanism described by eq 1-10 predicts different formation rates for CO_2 and Cu(I) (eq 23 and 24). Such rates

$$\frac{\partial[CO_2]}{\partial t} = \phi I_0 \quad (23)$$

$$\frac{\partial[Cu(I)]}{\partial t} = k_2[I] + (k_9 + k'_{10})[V] \quad (24)$$

are determined by the light intensity, I_0, and the concentrations of intermediates II and V (see eq 1-5). The dependence of the copper(I) rate law on irradiation time, eq 25, was obtained

$$\frac{\partial[Cu(I)]}{\partial t} = \phi I_0\left(\frac{k_2}{k_2 + k_3}\right) + \left(\frac{K}{K + [H^+]}\right)\left\{\left[\left(\frac{k_8(K + [H^+])[Cu(II)]}{4Kk_7}\right)^2 - \frac{\phi I_0(K + [H^+])^2 k_2}{2K^2 k_7(k_2 + k_3)}\right]^{1/2} - \frac{k_8(K + [H^+])[Cu(II)]}{4Kk_7}\right\} k_8[Cu(II)] \quad (25)$$

by introducing the steady-state concentrations of the intermediates I, V, and aminoalkyl radicals, e.g., $\dot{C}H_2CH_2NH_2$, in eq 24. Such a rate law can be simplified if the concentration of copper(II) is large or the light intensities are small. The rate of copper(I) formation is given by eq 26 under these two

$$\frac{\partial[Cu(I)]}{\partial t} = 2\phi\frac{k_2}{k_2 + k_3}I_0 \quad (26)$$

limiting conditions. Moreover, the yields of carbon dioxide and copper(I) (eq 23 and 26) give an indication of the relative reactivity of intermediate I (eq 27).

$$\frac{\phi_{Cu(I)}}{\phi_{CO_2}} = 2\frac{k_2}{k_2 + k_3} \quad (27)$$

Appendix III

The decay of the copper-alkyl intermediate in neutral to basic solutions exhibited a linear dependence on acid concentration. Such a behavior is explained by means of eq 3-5 in the proposed mechanism, eq 1-10. Indeed, the rate of decay of the transient absorbance, ΔA, can be described by using the rate of decay of intermediates II, III, and IV, eq 28, where

$$\frac{\partial\Delta A}{\partial t} = \epsilon_1\frac{\partial[II]}{\partial t} + \epsilon_2\frac{\partial[III]}{\partial t} + \epsilon_3\frac{\partial[IV]}{\partial t} \quad (28)$$

ϵ_1, ϵ_2, and ϵ_3 are the extinctions of the transients. Equation 28 can be reduced to eq 29 by assuming that the equilibrium

$$\frac{\partial\Delta A}{\partial t} = \epsilon_1(k_4 + k_5)[II] + \left(\frac{\epsilon_2 + \epsilon_3 K[H^+]}{1 + K[H^+]}\right)\frac{\partial C}{\partial t} \quad (29)$$

$$C = [III] + [IV]$$

between III and IV is rapid. A further reorganization of eq 29 is obtained by assuming that species III and IV have nearly the same extinctions at the monitoring wavelength (eq 30).

$$\frac{\partial\Delta A}{\partial t} = \epsilon_1(k_4 + k_5)[II] + \epsilon_2 k_4' K[H^+]C \quad (30)$$

The different contributions to the rate of the absorbance decay can be separated under appropriate experimental conditions.

Registry No. $Cu(\beta\text{-Al})_2$, 14040-31-4; $Cu(Glu)_2$, 15169-63-8; $Cu(Gly)^+$, 15841-47-1.

Contribution No. 6115 from the Arthur Amos Noyes Laboratory, California Institute of Technology, Pasadena, California 91125

Kinetic Studies of the Oxidation of Horse Heart Ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ at Low pH

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The kinetics of the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ have been studied over the pH range 1.25-6.8 at 25 °C. An increase in the rate of oxidation was observed between pH values of 4.0 and 3.0. Very little change in the rate was found below pH 2.5 and above 4.5. Analysis of this pH-rate profile yields second-order rate constants of 6.0×10^3 and 3.77×10^4 M^{-1} s^{-1} for the oxidation of ferrocytochrome *c* and a monoprotonated species (H^+-ferrocytochrome *c*), respectively, with the equilibrium constant for protonation being 1.40×10^3 M^{-1}. It is suggested that a small structural change is induced in the region of the protein redox center in H^+-ferrocytochrome *c*, thereby allowing easier reagent access to the heme *c* group. Comparison of NMR titration data and the kinetic results indicates that H^+-ferrocytochrome *c* is protonated at His-26.

Introduction

Analysis of the kinetics of oxidation of horse heart ferrocytochrome *c* by inorganic complexes such as $[Co(phen)_3]^{3+}$, $[Co(dipic)_2]^-$, and $[Ru(NH_3)_5py]^{3+}$ suggests that electron transfer occurs at a site near the partially exposed edge of the heme *c* group.[1-5] This interpretation of the electron-transfer pathway is appropriate for the protein in its native confor-

(1) Mauk, A. G.; Scott, R. A.; Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 4360.

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mation, which is the predominant species in solution under the conditions of pH and ionic strength employed in our studies.

The present investigation was undertaken to elucidate the electron-transfer reactivity of ferrocytochrome c at low pH. It is well established[6-8] that the reduced protein is oxidized more rapidly by inorganic and organic oxidants in solutions below pH 4, and it is likely that this enhanced reactivity is related to an acid-induced protein structural change. With the goal of probing this matter further, we have extended our investigation[4] of the kinetics of oxidation of ferrocytochrome c by $[Ru(NH_3)_5py]^{3+}$ to include a wide variation in pH (6.8–1.25). The results of our extended study are reported herein.

Experimental Section

Reagent grade chemicals and distilled water were used throughout. Nitrogen gas passed through two chromous scrubbing towers, to remove oxidizing impurities, was used to deoxygenate kinetic solutions, and argon gas, purified in an identical manner, was used in the preparation of $[Ru(NH_3)_5py](ClO_4)_3$.[4]

Horse heart cytochrome c (type VI) was obtained from Sigma Chemical Co. Solutions of ferrocytochrome c were prepared by three different methods. For the pH range 6.8–3.0, oxidized protein was dissolved in 2–3 mL of the appropriate buffer and reduced with a 20-fold excess of $Fe(HEDTA)^-$. Excess $Fe(HEDTA)^-$ and Fe(HEDTA) were removed with the use of a Sephadex G-25 gel filtration column (2 × 15 cm, Sigma Chemical Co.) equilibrated with the appropriate deoxygenated buffer. The reduced protein was loaded onto the column, eluted with deoxygenated buffer, and diluted to the desired volume. Owing to the autooxidizability of the reduced protein, two preparative methods were employed for experiments at pH 3.0 and below. For experiments at pH values of 2.5 and 2.0, the oxidized protein was dissolved in the appropriate buffer, degassed in a serum-capped bottle by gently bubbling with nitrogen, and reduced with a 2-fold excess of sodium dithionite. No attempt was made to remove the oxidized product. For the buffer jump experiments, reduced protein solutions were prepared in distilled water, with use of $Fe(HEDTA)^-$ as reductant and the gel filtration method described above.

The $[Ru(NH_3)_5py]^{3+}$ solutions for kinetic experiments with buffered ferrocytochrome c were prepared in buffer of the same composition. Those for use with distilled water solutions of ferrocytochrome c were prepared in buffer of twice the required concentration so that the required pH and ionic strength were attained on mixing the two solutions in the stopped-flow apparatus. Acetate and phosphate buffers were used throughout, as they gave solutions of $[Ru(NH_3)_5py]^{3+}$ which could be stored for the duration of an experiment without observable decomposition.

Nitrogen was carefully bubbled through all solutions for 20 min prior to kinetic measurements. Most solutions were stored in serum-capped round-bottom flasks, each with a nitrogen inlet tube and a glass Luer-lock fitting. They were then transferred to the stopped-flow apparatus through Teflon tubing connected to the inlet port. The use of an all-glass system is particularly important for transferring solutions of $[Ru(NH_3)_5py]^{3+}$, as the complex is reduced if solutions are passed through stainless-steel needles.

Kinetic Measurements. Kinetic measurements were made on a Durrum Model D-110 stopped-flow spectrophotometer. The ferrocytochrome c solutions reduced with dithionite had to be used immediately, owing to autooxidation in the stopped-flow drive syringes. All other solutions to be mixed were allowed to temperature equilibrate (25 °C) for at least 15 min. Data collection was performed as described previously.[9] An absorbance change of 0.1 at 550 nm was usually measured, except in the buffer (pH/ionic strength) jump experiments where a change of 0.2 was followed. The oxidant concentration was always in pseudo-first-order excess over the reductant and was usually varied over a 15-fold range.

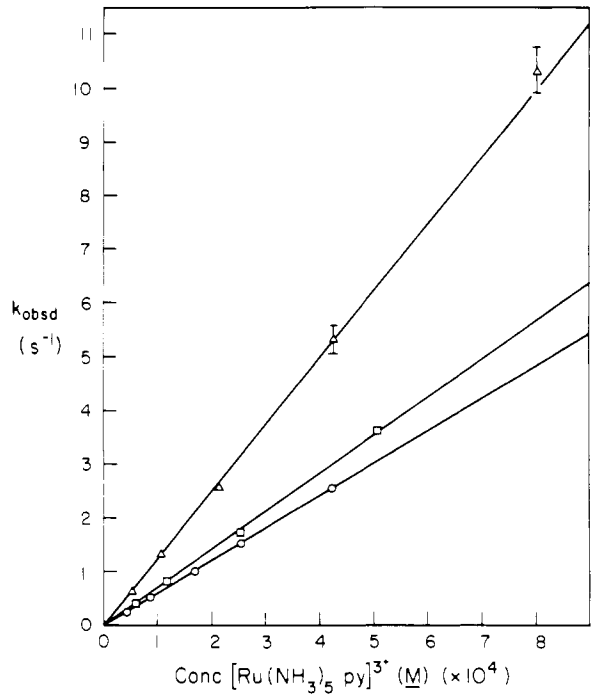

Figure 1. Dependences of the observed rate constants for ferrocytochrome c oxidation on the concentration of $[Ru(NH_3)_5py]^{3+}$ at 25 °C, μ = 0.1 M (acetate): pH 5.0, ○; pH 4.2, □; pH 3.8, △.

Data Analysis. For each reaction that did not involve a buffer jump, the pseudo-first-order rate constant (k_{obsd}) was obtained from a plot of log ($A_t - A_\infty$) vs. t (line was determined by a linear least-squares method). The data from the buffer jump experiments were fitted by a least-squares procedure to the parallel first-order equation (1).

$$A_{tot} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad (1)$$

The concentration dependence for each reaction, except where specifically stated otherwise, was analyzed with the use of weighted least squares. In each case the weighting factor was the square of the inverse of the standard deviation from the mean of the multiple determinations (usually four to eight) done from one filling of the drive syringes.

Results

Absorbance–time data for the oxidation of ferrocytochrome c by $[Ru(NH_3)_5py]^{3+}$ in the pH range 2–6.8, where a buffer jump was not employed, show first-order behavior for at least three reaction half-lives. The dependences of the observed rate constants on $[Ru(NH_3)_5py]^{3+}$ concentration are illustrated in Figure 1. In all cases, the rate law is first order in each reactant over the range of oxidant concentrations employed.

The ferrocytochrome c oxidations that were studied by the buffer jump technique in the pH range 1.25–3.0 gave observed rate constants for the fast reaction (k_1) that are linearly dependent on the oxidant concentration. Figure 2 shows that the fit for pH 3.0 data gives observed rate constants (k_1 and k_2) with low standard deviations. The second-order rate constants from these concentration dependences at pH values of 3.0, 2.5, and 2.0 are in good agreement with those obtained from the experiments without a buffer jump, confirming that the fast redox pathway in the parallel first-order reaction is equivalent to the redox reaction measured by the more conventional technique.

(2) Mauk, A. G.; Coyle, C.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5054.
(3) Wherland, S.; Gray, H. B. In "Biological Aspects of Inorganic Chemistry"; Addison, A. W., Cullen, W. R., Dolphin, D. W., James, B. R., Eds.; Wiley: New York, 1977.
(4) Cummins, D.; Gray, H. B. *J. Am. Chem. Soc.* **1977**, *99*, 5158.
(5) McArdle, J. V.; Gray, H. B.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1974**, *96*, 5737.
(6) Castro, C. E.; Bartnicki, E. W. *Biochemistry* **1975**, *14*, 498.
(7) Brunschwig, B. S.; Sutin, N. *Inorg. Chem.* **1976**, *15*, 631.
(8) Dickerson, R. E.; Timkovich, R. In "The Enzymes", 3rd ed.; Boyer, P., Ed.; Academic Press: New York, 1975; Vol. 11, Chapter 7.
(9) Wherland, S.; Holwerda, R. A.; Rosenberg, R. C.; Gray, H. B. *J. Am. Chem. Soc.* **1975**, *97*, 5260.

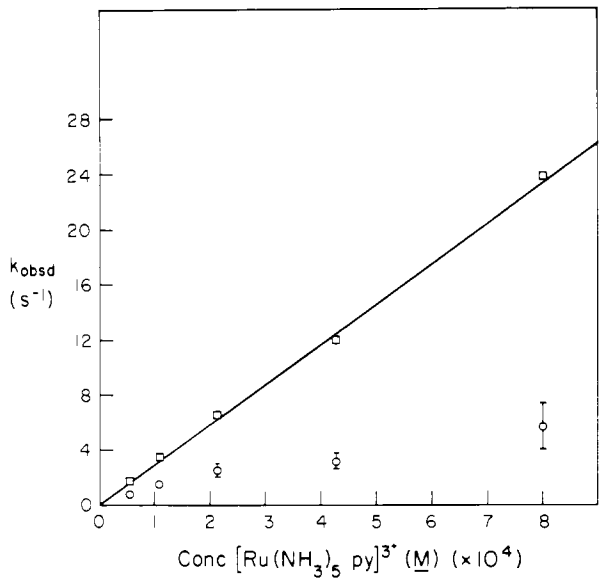

Figure 2. Dependences of the observed rate constants from the parallel first-order fit on the concentration of $[Ru(NH_3)_5py]^{3+}$ at 25 °C, pH 3.0, μ = 0.1 M (phosphate): k_1, □; k_2, ○.

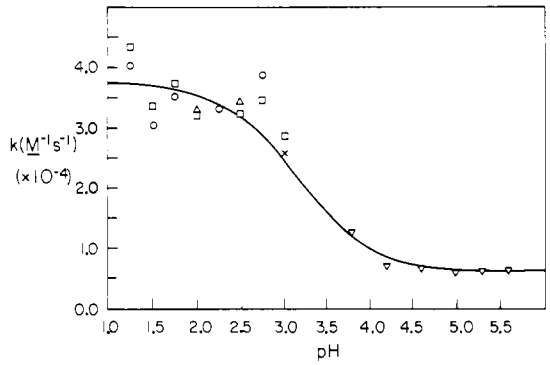

Figure 3. pH dependence of the second-order rate constants for the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ at 25 °C: μ = 0.1 M (acetate), ▽; μ = 0.1 M (phosphate), ×; μ = 0.1 M (phosphate), buffer jump (ΔA = 0.2), □; μ = 0.1 M (phosphate), buffer jump (ΔA = 0.1), ○; μ = 0.1 M (phosphate), cytochrome *c* reduced with excess dithionite, △.

Reproducible data were not obtained for the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ at pH values lower than 1.25. This is probably attributable to the very high autooxidizability of the reduced protein in such media and the difficulty both of removing the last trace of oxygen from the stopped-flow apparatus and of keeping it anaerobic.

The second-order rate constants for the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ are collected in Table I, and the values for the pH range 1.25–5.6 are plotted in Figure 3. There is a substantial increase in rate between pH values of 4.0 and 3.0, whereas very small rate changes were observed below pH 2.5 and above pH 4.5. The second-order rate constants were fitted to eq 2 with use of a least-squares procedure.[10] The line in Figure 3 is the best fit of eq 2 to the experimental data points in the pH range 1.5–5.6. The parameters obtained from this fit are $k_3 = 6.0 \times 10^3$ M^{-1} s^{-1}, $k_4 = 3.77 \times 10^4$ M^{-1} s^{-1}, and $K = 1.40 \times 10^3$ M^{-1}.

$$k = (k_3 + k_4[H^+])/(1 + K[H^+]) \quad (2)$$

Table I. pH Dependence of the Second-Order Rate Constants for the Oxidation of Ferrocytochrome *c* by $[Ru(NH_3)_5py](ClO_4)_3$ at 25 °C

pH[a]	$10^{-4}k$, M^{-1} s^{-1}	$10^{-3}[\sigma, M^{-1} s^{-1}]$[b]
6.8	0.926	[0.013]
6.4	0.908	[0.004]
6.0	0.876	[0.004]
5.6[c]	0.642	[0.011]
5.3[c]	0.616	[0.014]
5.0[c]	0.596	[0.003]
4.6[c]	0.661	[0.006]
4.2[c]	0.713	[0.035]
3.8[c]	1.266	[0.046]
3.0	2.567	[0.196]
3.0	2.874[d]	[0.084]
2.75	3.870[e]	[2.96]
2.75	3.453[d]	[1.49]
2.5	3.402[f]	[0.56]
2.5	3.219[d]	[0.66]
2.25	3.296[e]	[3.63]
2.0	3.292[f]	[1.32]
2.0	3.210[d]	[1.043]
1.75	3.530[e]	[0.44]
1.75	3.745[d]	[1.795]
1.5	3.032[e]	[1.747]
1.5	3.353[d]	[5.699]
1.25	4.345[e]	[4.313]
1.25	4.042[d]	[9.403]

[a] μ = 0.1 M (phosphate) except where noted otherwise. [b] Standard deviation from the mean of multiple determinations done from one filling of the drive syringe. [c] μ = 0.1 M (acetate). [d] k_1 value from buffer jump, ΔA(550 nm) = 0.2. [e] k_1 value from buffer jump, ΔA(550 nm) = 0.1. [f] Cytochrome *c* reduced with excess dithionite; rate constant was taken from nonweighted least-squares analysis of concentration dependence data.

The k_2 values from the parallel first-order fits of data from the buffer jump experiments were fitted for each pH to eq 3

$$1/k_2 = a + b/[Ru(NH_3)_5py]^{3+} \quad (3)$$

by a weighted least-squares method.[11] The *a* and *b* values are essentially pH independent: a = 8.06 (2.72) × 10^{-2} s; b = 5.08 (1.61) × 10^{-5} M s.[12]

Discussion

We can account for the kinetics of oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ in the pH range 1.25–6.8 by the scheme given in eq 4–6. The principal species present

$$\text{ferrocytochrome } c + [Ru(NH_3)_5py]^{3+} \xrightarrow{k_3} \text{ferricytochrome } c + [Ru(NH_3)_5py]^{2+} \quad (4)$$

$$H^+\text{-ferrocytochrome } c + [Ru(NH_3)_5py]^{3+} \xrightarrow{k_4} H^+\text{-ferricytochrome } c + [Ru(NH_3)_5py]^{2+} \quad (5)$$

$$H^+ + \text{ferrocytochrome } c \overset{K}{\rightleftharpoons} H^+\text{-ferrocytochrome } c \quad (6)$$

above pH 4 is oxidized to ferricytochrome *c* by an outer-sphere electron transfer mechanism characterized by the rate constant k_3. Both the reduced and oxidized forms of the protein in the

(10) The weights used were $1/\sigma$, as this gave a better balance between the high and low pH data points. The use of $1/\sigma^2$ weighting factors resulted in a fit that virtually ignored the low pH data points. This was because the standard deviations on these low pH points, obtained from the parallel-first-order treatment, were so much higher than those on the higher pH data points. Unweighted least-squares analysis gave a fit that ignored the higher pH points because there are so many less points here than there are in the low pH region.

(11) Each weighting factor was the inverse of the square of the standard deviation of $1/k_2$, where $\sigma(1/k_2) = \sigma(k_2)/(k_2)^2$.

(12) The mechanistic interpretation of this minor pathway in the buffer jump experiments is not clear. Apparently, a new species of the reduced protein is produced in the buffer jump, and this species is not readily oxidized by $[Ru(NH_3)_5py]^{3+}$. The observed saturation kinetic behavior could be explained by binding of the oxidant to the new species prior to electron transfer, by dead-end complex formation, or by a pathway in which the new species is converted to a more reactive form with a first-order rate constant k_5 (the rate constant for the reverse process is k_{-5}), followed by oxidation (k_6). For the latter mechanism, $a = 1/k_5$ and $b = k_{-5}/k_5k_6$ (k_5 = 12.4 ± 6.3 s^{-1}; k_{-5}/k_6 = (6.3 ± 2.0) × 10^{-4} M.

pH range 4–6.8 contain low-spin heme *c* groups that are axially ligated by an imidazole nitrogen atom of His-18 and the sulfur atom of Met-80.[8] Although the native protein conformation isolates the heme *c* group from solvent molecules, it is probable that reagents with hydrophobic, π-conjugated ligands can penetrate the hydrophobic region surrounding the protein redox center, thereby facilitating electron transfer. More specifically, in recent work we have estimated[1] that the edge of the coordinated pyridine comes within 4 Å of the heme *c* edge at the instant of electron transfer from the protein redox center to $[Ru(NH_3)_5py]^{3+}$.

Addition of one proton to ferrocytochrome *c* with $K = 1.40 \times 10^3$ M^{-1} produces a species that is able to transfer an electron to $[Ru(NH_3)_5py]^{3+}$ more rapidly. Neither the spin state nor the axial ligation of this species, H^+-ferrocytochrome *c*, is known for certain. A few studies have been published that bear on this point, but they are not definitive. It is known, for example, that CO binds slowly to ferrocytochrome *c* just below pH 3, but no binding is observed between pH 3 and 4.[13] Changes in ^1H NMR spectra are observed[14] between pD 4.5 and 3 upon addition of DCl to D_2O solutions of ferrocytochrome *c*; these changes are substantial between pD 4.5 and 3, and possible explanations include the presence of a high-spin heme *c* or axial ligand loss (or both). The evidence for a spin-state change in this pH range is not compelling, however, and the CO binding experiments[13] are more easily reconciled by assuming that the heme *c* group is still axially ligated and low spin. The latter assumption also is supported by the observation that the electronic absorption spectrum of ferrocytochrome *c* does not vary significantly in the pH range 1–6.[7]

Whatever the structural change may be, it is clear that the protonated protein is oxidized by a variety of reagents more rapidly than is ferrocytochrome *c*.[6-8] The higher reactivity must be related to some type of structural perturbation because the analogous protonated form of the oxidized protein, H^+-ferricytochrome *c*, is reduced by $[Ru(NH_3)_6]^{2+}$ more rapidly than is native ferricytochrome *c*.[15] Based on the value of *K* for ferrocytochrome *c*, it is reasonable to suggest that the site of protonation is the imidazole ring of His-26. This site is protonated below pH 3.5 in ferricytochrome *c*,[16] and it would be expected[17] to exhibit similar behavior in the reduced protein. A small conformational change induced in the heme *c* region by protonation of His-26 could easily account for the increased outer-sphere electron-transfer reactivity of H^+-ferrocytochrome *c*, with the assumption that reagent access to the protein redox center was somewhat greater in the perturbed structure. Other factors being equal, it would require only a small change in the percentage of the heme *c* surface available for reagent contact to account for the observed increase in the rate constant for oxidation of the protonated form of the protein by $[Ru(NH_3)_5py]^{3+}$ ($k_4/k_3 \approx 6$).

Our scheme for the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ below pH 4 is simpler than the one proposed[7] by Brunschwig and Sutin for the oxidation of the protein by $[Co(phen)_3]^{3+}$ at low pH. In the latter case, a maximum in the pH/rate profile was found at pH 2.9, and our results do not indicate any such maximum. Therefore, we need only postulate two different reactive protein species rather than the three that are required by the kinetic data collected by Brunschwig and Sutin. It is likely that the difference in kinetic behavior is due to the presence of chloride in the ferrocytochrome *c*/$[Co(phen)_3]^{3+}$ solutions, one reasonable possibility being that binding of Cl^- to the protein produces at least one additional species at low pH. Clearly, more work will be required to determine whether chloride-binding effects are sufficient to account for the difference in pH/rate profiles for the $[Co(phen)_3]^{3+}$ and $[Ru(NH_3)_5py]^{3+}$ oxidations of ferrocytochrome *c*.

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Registry No. Ferrocytochrome *c*, 9007-43-6; $[Ru(NH_3)_5py]^{3+}$, 33291-25-7.

(13) Theorell, H.; Åkesson, Å. *J. Am. Chem. Soc.* **1941**, *63*, 1812.
(14) McDonald, C. C.; Phillips, W. D. *Biochemistry* **1973**, *12*, 3170.
(15) Adegite, A.; Okpanachi, M. I. *J. Am. Chem. Soc.* **1980**, *102*, 2832.
(16) Cohen, J. S.; Hayes, M. B. *J. Biol. Chem.* **1974**, *249*, 5472.
(17) Drew, H. R.; Dickerson, R. E. *J. Biol. Chem.* **1978**, *253*, 8420.

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Kinetic Study of Oxygen Exchange in $[Re(CO)_6][PF_6]$ with $H_2{}^{17}O$

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The exchange between ^{17}O-enriched water and $[Re(CO)_6][PF_6]$ in CH_3CN has been investigated with utilization of ^{17}O NMR spectroscopy. Oxygen-17 NMR spectroscopy offers several advantages over other techniques for following oxygen-exchange reactions. The rate of exchange follows the equation $R_O = k_{ex}[Re(CO)_6^+][H_2O]^2$. The second-order dependence of H_2O for the reaction indicates the important role of H_2O in the exchange of oxygen atoms on the carbonyl ligand and H_2O. The reaction rate has activation parameters of $\Delta H^\ddagger = 11.5 \pm 0.7$ kcal/mol and $\Delta S^\ddagger = -33.6 \pm 1.8$ cal/(deg mol). The reaction mechanism for oxygen exchange is discussed.

Introduction

Recently, we have shown useful applications of oxygen-17 NMR in the structural characterization of transition metal carbonyl compounds.[1,2] In the present paper we will demonstrate the potential of oxygen-17 NMR as an aid to deducing mechanistic pathways of oxygen enrichment. The establishment of a mechanism of enrichment for metal carbonyl compounds should lead to new methods for enriching a large range of carbonyl complexes and provide further basic information about oxygen-exchange processes.

Muetterties[3] has observed that $Re(CO)_6^+$ readily exchanges the carbonyl oxygens with the oxygen atoms of $H_2{}^{18}O$. The exchange was believed to occur by nucleophilic attack at a

(1) Kump, R. L.; Todd, L. J. *J. Chem. Soc., Chem. Commun.* **1980**, 292.
(2) Kump, R. L.; Todd, L. J. *J. Organomet. Chem.* **1980**, *194*, C43.
(3) Muetterties, E. L. *Inorg. Chem.* **1965**, *4*, 1841.

0020-1669/81/1320-3715$01.25/0