Inorg. Chem. **1980**, *19*, 3244–3247

Contribution from the Departments of Chemistry, Union College, Schenectady, New York 12308,
and New Mexico State University, Las Cruces, New Mexico 88003

Kinetics of the Reduction of Hexacyanoferrate(III) Ion by Dithionite Ion[1]

CHARLES W. J. SCAIFE* and RALPH G. WILKINS

Received February 20, 1980

Kinetics of the reduction of hexacyanoferrate(III) ion, $Fe(CN)_6^{3-}$, by dithionite ion, $S_2O_4^{2-}$, have been investigated by stopped-flow spectrophotometry as a function of $S_2O_4^{2-}$ concentration, temperature, ionic strength, and pH. With stoichiometric amounts, $[S_2O_4^{2-}]:[Fe(CN)_6^{3-}] = 1:2$, or with a small excess of $S_2O_4^{2-}$ varying from 3:1 to 9:1, dissociation of $S_2O_4^{2-}$ is rate determining and is followed by rapid reduction of $Fe(CN)_6^{3-}$ by $SO_2^{-}\cdot$. The rate law is $-d[Fe(CN)_6^{3-}]/dt = 2k_1[S_2O_4^{2-}]$ with $k_1 = 1.7 \pm 0.3\ s^{-1}$ (pH 6.8, $\mu = 0.10$ M, 25.0 °C), $\Delta H^{\ddagger}_1 = 11.2 \pm 0.5$ kcal mol^{-1}, and $\Delta S^{\ddagger}_1 = -20 \pm 4$ cal K^{-1} mol^{-1}. With a large excess of $S_2O_4^{2-}$ greater than 30:1, parallel reactions involving reduction of $Fe(CN)_6^{3-}$ by $SO_2^{-}\cdot$ and $S_2O_4^{2-}$ are rate determining. The rate law is $-d[Fe(CN)_6^{3-}]/dt = \{k_2K_1^{1/2}[S_2O_4^{2-}]^{1/2} + k_3[S_2O_4^{2-}]\}[Fe(CN)_6^{3-}]$ with $k_2 = (2.0 \pm 0.6) \times 10^8$ M^{-1} s^{-1} (pH 6.8, $\mu = 0.10$ M, 25.0 °C), $\Delta H^{\ddagger}_2 = 4 \pm 2$ kcal mol^{-1}, and $\Delta S^{\ddagger}_2 = -8 \pm 20$ cal K^{-1} mol^{-1} for reduction by $SO_2^{-}\cdot$ and with $k_3 = (1.0 \pm 0.4) \times 10^5$ M^{-1} s^{-1} (pH 6.8, $\mu = 0.10$ M, 25.0 °C), $\Delta H^{\ddagger}_3 = 8 \pm 1$ kcal mol^{-1}, and $\Delta S^{\ddagger}_3 = -8 \pm 13$ cal K^{-1} mol^{-1} for reduction by $S_2O_4^{2-}$. k_1 is independent of ionic strength whereas k_2 and k_3 increase with increasing ionic strength. k_1 decreases slightly with increasing pH from 6.8 to 13.2 whereas k_2 and/or k_3 increase slightly over the same range.

Introduction

Dithionite ion, $S_2O_4^{2-}$, is a strong and versatile, but also mechanistically complex, two-electron reducing agent.[2] It is used for vat dyeing, for bleaching, for manufacture of various chemicals, for elimination of dissolved oxygen from solutions, and as a biochemical reductant. Hexacyanoferrate(III) ion, $Fe(CN)_6^{3-}$, is about as strong and as versatile a one-electron oxidizing agent.[3] Just as $S_2O_4^{2-}$ is useful for preparing and maintaining the reduced states of proteins, enzymes, and cofactors, so is $Fe(CN)_6^{3-}$ useful for preparing and maintaining the oxidized states of such species.

Kinetic studies of reductions by $S_2O_4^{2-}$ have been interpreted in terms of one or more rate-determining steps involving (1) $S_2O_4^{2-}$ dissociation, (2) attack on an oxidant by $SO_2^{-}\cdot$, and (3) attack on an oxidant by $S_2O_4^{2-}$.[2,4] The $SO_2^{-}\cdot$ radical is a stronger reducing agent than $S_2O_4^{2-}$[5] and is the dominant reductant at typical concentrations used for studying reductions of biochemical species.[2,4,6]

Lambeth and Palmer previously studied the kinetics of reduction of $Fe(CN)_6^{3-}$ by $S_2O_4^{2-}$.[2] With excess $Fe(CN)_6^{3-}$ the reaction was first order in both reactants, and the rate law was $-d[Fe(CN)_6^{3-}]/dt = k_3[S_2O_4^{2-}][Fe(CN)_6^{3-}]$ with $k_3 = 1.4 \times 10^5$ M^{-1} s^{-1} (pH 8.0, $\mu = 0.41$ M, 25 °C). They also found that at approximately stoichiometric concentrations the reaction was first order overall, and with excess $S_2O_4^{2-}$ the reaction was between pseudo zero and pseudo first order. The exhaustive study that forms the subject of this article was undertaken because preliminary experiments in the stoichiometric and excess $S_2O_4^{2-}$ regions indicated the possibility of investigating all three of the common rate-determining steps for $S_2O_4^{2-}$ reductions with this single pair of reactants.

Experimental Section

Materials. Mallinckrodt analytical reagent grade potassium hexacyanoferrate(III), $K_3Fe(CN)_6$, was used without additional purification. After weighed samples were dissolved in appropriate buffer solutions, concentrations were checked spectrophotometrically before final dilution ($\epsilon_{418} = 1012$ M^{-1} cm^{-1},[7] independent of pH[7] and ionic strength[3] over the ranges of our investigations). Solutions were then deoxygenated by flushing for a minimum of 20 min with oxygen-free nitrogen. The purity of J. T. Baker purified grade sodium dithionite, $Na_2S_2O_4$, was determined by spectrophotometric titration with excess $Fe(CN)_6^{3-}$ at 418 nm, by reading the absorbance immediately after mixing to avoid additional slow reduction of $Fe(CN)_6^{3-}$ by HSO_3^- or SO_3^{2-}.[6b,8] It is important for this titration and for the stopped-flow reactions that hexacyanoferrate(II) ion, $Fe(CN)_6^{4-}$,[4a,7] and also $S_2O_4^{2-}$, HSO_3^-, and SO_3^{2-}[8] do not absorb at 418 nm and $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$[3] as well as $S_2O_4^{2-}$[9] are not protonated in the pH range of our investigations. Both weighed samples of sodium dithionite and appropriate buffer solutions were deoxygenated by flushing for a minimum of 20 min with oxygen-free nitrogen. Buffer solutions were then injected via serum caps to dissolve $Na_2S_2O_4$ samples, and concentrations of some solutions were checked spectrophotometrically ($\epsilon_{315} = 6900$ M^{-1} cm^{-1} at pH 6.5 and $\mu = 0.15$ M[10]). Solutions of $S_2O_4^{2-}$ were used within 1 h to avoid very slow disproportionation or hydrolysis.[9] Fairly rapid acid hydrolysis occurred when $S_2O_4^{2-}$ solutions were prepared below about pH 4, consistent with the results of earlier studies.[4a,9] Solutions of oxygen were prepared by flushing buffer solutions with either air or O_2 for a minimum of 30 min. Concentrations of O_2 were determined by taking into account the prevailing atmospheric pressure and by using tables of solubility of O_2 at different salinities.[11] Solutions with intermediate O_2 concentrations were prepared by quantitative mixing of these solutions.

Buffer solutions were prepared as follows: pH 6.8, 0.0045 M sodium dihydrogen phosphate, 0.0018 M sodium hydrogen phosphate; pH 9.3, 0.0050 M sodium tetraborate; pH 10.4, 0.0025 M sodium hydrogen carbonate, 0.0025 M sodium carbonate; pH 12.3, 0.010 M sodium hydroxide; pH 13.2, 0.10 M sodium hydroxide. The first four were brought to 0.10 M ionic strength by addition of 0.090 M sodium chloride. Additional NaCl was added for higher ionic strength experiments. A 0.000 45 M sodium dihydrogen phosphate–0.000 18 M sodium hydrogen phosphate buffer was used for very low ionic strength experiments at pH 6.8.

Apparatus. A Beckman 24 spectrophotometer was used for checking concentrations of solutions at 418 and 315 nm and for obtaining absorption spectra of various species. A Durrum Model D-130 stopped-flow spectrophotometer in conjunction with a Tektronix Model 5103N oscilloscope was used to obtain kinetic data. Both instruments were thermostated within ±0.2 °C. In most cases the absorbance of $Fe(CN)_6^{3-}$ at 418 nm was followed. The absorbance of $S_2O_4^{2-}$ at

---

(1) Presented in part at the 178th National Meeting of the American Chemical Society, Washington, D.C., Sept 1979; see "Abstracts of Papers", INOR 112.

(2) Lambeth, D. O.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 6095.

(3) Chadwick, B. M.; Sharpe, A. G. *Adv. Inorg. Chem. Radiochem.* **1966**, *8*, 83.

(4) (a) Pasternack, R. F.; Cobb, M. A.; Sutin, N. *Inorg. Chem.* **1975**, *14*, 866. (b) Wood, F. E.; Post, C. B.; Cusanovich, M. A. *Arch. Biochem. Biophys.* **1977**, *184*, 586. (c) Chien, J. C. W.; Dickinson, L. C. *J. Biol. Chem.* **1978**, *253*, 6965. Maim, A. *J. Phys. Chem.* **1979**, *83*, 2553.

(5) Mayhew, S. G. *Eur. J. Biochem.* **1978**, *85*, 535.

(6) (a) Hambright, P.; Chock, P. B. *Inorg. Chem.* **1974**, *13*, 3029. (b) Cox, R. P.; Holloway, M. R. *Eur. J. Biochem.* **1977**, *74*, 575. (c) Eaton, D. R.; Wilkins, R. G. *J. Biol. Chem.* **1978**, *253*, 908. (d) Harrington, P. C.; deWaal, D. J. A.; Wilkins, R. G. *Arch. Biochem. Biophys.* **1978**, *191*, 444.

(7) Aziz, F.; Mirza, G. A. *Talanta* **1964**, *11*, 889.

(8) Swinehart, J. H. *J. Inorg. Nucl. Chem.* **1967**, *29*, 2313.

(9) Senning, A. "Sulfur in Organic and Inorganic Chemistry"; Marcel Dekker: New York, 1972; Vol. 2, pp 88, 89.

(10) Creutz, C.; Sutin, N. *Inorg. Chem.* **1974**, *13*, 2041.

(11) Beckman Instructions 015-082155-B for oxygen sensor for Beckman Model 0260 oxygen analyzer.

0020-1669/80/1319-3244$01.00/0

320 nm was also followed in the regions of stoichiometric concentrations and of low excess $S_2O_4^{2-}$. At 320 nm there is slight interference from overlapping absorbances of $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ that only partially cancel as the reaction proceeds.[7] Nevertheless, results at 320 nm agreed within 5% with results at 418 nm. All kinetic traces in the pseudo-zero-order reactions with low excess $S_2O_4^{2-}$ were calibrated with known concentrations of $Fe(CN)_6^{3-}$ at 418 nm or $S_2O_4^{2-}$ at 320 nm. The absorbance of $S_2O_4^{2-}$ at 315 nm was followed in reactions with excess O_2. Neither O_2 nor the products caused interfering absorption at 315 nm. A Corning Digital 112 pH meter was used for pH measurements.

Results

Conditions for Which Dissociation of $S_2O_4^{2-}$ Is Rate Determining. Data from reduction of $Fe(CN)_6^{3-}$ by a stoichiometric amount of $S_2O_4^{2-}$, $[S_2O_4^{2-}]/[Fe(CN)_6^{3-}] = 1:2$, or by a small excess of $S_2O_4^{2-}$ from 3:1 to 9:1 are consistent with reactions 1 and 2 in which dissociation of $S_2O_4^{2-}$ is rate determining and is followed by rapid reduction of $Fe(CN)_6^{3-}$ by $SO_2^{-}\cdot$. Each $S_2O_4^{2-}$ consumes two $Fe(CN)_6^{3-}$'s by this mechanism. For the limit of slow step 1 followed by rapid step 2, the reactions are zero order in $[Fe(CN)_6^{3-}]$ and first order in $[S_2O_4^{2-}]$, and the rate law is

$$S_2O_4^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2SO_2^{-}\cdot \qquad K_1 = k_1/k_{-1} \tag{1}$$

$$SO_2^{-}\cdot + Fe(CN)_6^{3-} + H_2O \xrightarrow{k_2} HSO_3^{-} + Fe(CN)_6^{4-} + H^+ \tag{2}$$

$$-d[Fe(CN)_6^{3-}]/dt = 2k_1[S_2O_4^{2-}] \tag{3}$$

Reasons for these conclusions are described below.

Over a small range of concentrations around 2.0×10^{-5} M, reactions of stoichiometric concentrations of $Fe(CN)_6^{3-}$ and $S_2O_4^{2-}$ give first-order traces for which plots of log (absorbance) vs. time are linear for 4 or 5 half-lives. Calibration of the traces with known concentrations of $Fe(CN)_6^{3-}$ at 418 nm or $S_2O_4^{2-}$ at 320 nm shows that $S_2O_4^{2-}$ and $Fe(CN)_6^{3-}$ react in a 1:2 mole ratio. Nevertheless, one cannot determine experimentally with stoichiometric concentrations whether the reaction is first order in $[Fe(CN)_6^{3-}]$, is first order in $[S_2O_4^{2-}]$, or has a more complex dependence that gives rise to first-order traces. However, if the reaction is assumed to be zero order in $[Fe(CN)_6^{3-}]$ and first order in $[S_2O_4^{2-}]$ in accordance with eq 3, slopes of plots of log (absorbance) vs. time, calculated as 3.5 s^{-1}, equal $2k_1$, and k_1 is 1.7 ± 0.2 s^{-1} (pH 6.8, $\mu = 0.10$ M, 25.0 °C). This value was relatively independent of $S_2O_4^{2-}$ concentrations varying between $[S_2O_4^{2-}]/[Fe(CN)_6^{3-}] = 1:2$ and 1:1. All of the $S_2O_4^{2-}$ was not used as its concentration was increased above stoichiometric amounts. Correspondence of this value of k_1 with that obtained from the next concentration range to be presented indicates that this is a reasonable interpretation.

Reactions of $Fe(CN)_6^{3-}$ with a small excess of $S_2O_4^{2-}$ varying from 3:1 to 9:1 give pseudo-zero-order traces over the middle 60% of the traces, and plots of absorbance vs. time are linear for at least 3 half-lives, indicating that the reactions are zero order with respect to the deficient reactant, $Fe(CN)_6^{3-}$. On the assumption that the reactions are first order with respect to $S_2O_4^{2-}$ in accordance with eq 3, slopes of plots of absorbance vs. time give k_{obsd} values that equal $2k_1[S_2O_4^{2-}]$. Therefore, a plot of k_{obsd} vs. $[S_2O_4^{2-}]$ should give a straight line passing through the origin and having a slope equal to $2k_1$. This is shown to be the case in Figure 1. The upper concentration limit in Figure 1 is the point above which mixed pseudo-zero- and pseudo-first-order traces are obtained. At 418 nm $2k_1$ equals 3.2 s^{-1}, and k_1 is 1.6 ± 0.3 s^{-1} (pH 6.8, $\mu = 0.10$ M, 25.0 °C), in agreement with the results obtained by using stoichiometric amounts of reactants. The error for k_1 by this pseudo-zero-order method is somewhat larger than that from reactions of stoichiometric amounts because of the necessity of calibrating concentrations for all pseudo-zero-order traces. Data in Figure 1 at 320 nm show that $S_2O_4^{2-}$ is used only half as rapidly as $Fe(CN)_6^{3-}$, in agreement with the 1:2 mole ratio found by reacting stoichiometric concentrations. These data are much more difficult to obtain because it is hard to calibrate kinetic traces with the reactant in excess particularly since there is slight overlapping absorption from $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$.[7]

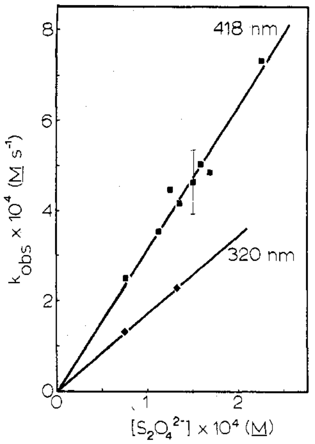

Figure 1. Plot of k_{obsd} vs. $[S_2O_4^{2-}]$ for pseudo-zero-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by small excess $S_2O_4^{2-}$ (pH 6.8, $\mu = 0.10$ M, 25.0 °C): ■, 418 nm; ◆, 320 nm.[12]

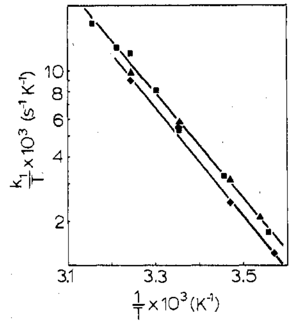

Figure 2. Plots of k_1/T vs. $1/T$ for first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by 1.30×10^{-5} M $S_2O_4^{2-}$ (▲), for pseudo-zero-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by small excess $S_2O_4^{2-}$ (■), and for first-order oxidation of 2.0×10^{-5} M $S_2O_4^{2-}$ by excess O_2 (◆) (pH 6.8, $\mu = 0.10$ M).

It has thus been shown that first-order reduction of $Fe(CN)_6^{3-}$ by a stoichiometric amount of $S_2O_4^{2-}$ and pseudo-zero-order reduction of $Fe(CN)_6^{3-}$ by a small excess of $S_2O_4^{2-}$ are both consistent with the same mechanism. Therefore, they will be treated concurrently in the following paragraphs.

Variable-temperature data have been obtained for both first-order and pseudo-zero-order reductions. A plot of log k_1/T vs. $1/T$ (Figure 2) yields $\Delta H^{\ddagger}_1 = 11.2 \pm 0.5$ kcal mol^{-1} and $\Delta S^{\ddagger}_1 = -20 \pm 4$ cal K^{-1} mol^{-1}. This temperature dependence for k_1 differs significantly from that obtained by Lambeth and Palmer using the reaction of $S_2O_4^{2-}$ with excess O_2.[2] Therefore, reaction of $S_2O_4^{2-}$ with excess O_2 as a function of temperature was repeated as a check on our results. These reactions have been shown to be first order with respect to $S_2O_4^{2-}$ and nearly zero order with respect to O_2.[2,10] Therefore, slopes of plots of log (absorbance) vs. time (linear for 3–4 half-lives) give k_{obsd} values equal to k_1 that are independent of O_2 concentration within experimental error. The temper-

---

[12] Error bars indicated are standard deviations calculated from at least eight different oscilloscope traces resulting from stopped-flow reactions of at least two sets of independently prepared solutions of the same concentrations.

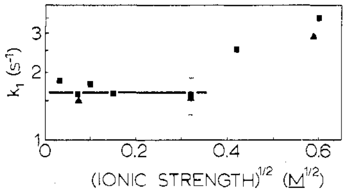

Figure 3. Plots of k_1 vs. $\mu^{1/2}$ for first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by 1.30×10^{-5} M $S_2O_4^{2-}$ (▲) and for pseudo-zero-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by 1.50×10^{-4} M $S_2O_4^{2-}$ (■) (pH 6.8, 25.0 °C).[12]

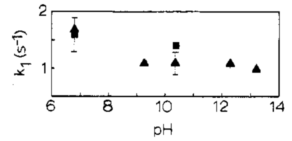

Figure 4. Plots of k_1 vs. pH for first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by 1.30×10^{-5} M $S_2O_4^{2-}$ (▲) and for pseudo-zero-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by small excess $S_2O_4^{2-}$ (■) (μ = 0.10 M, 25.0 °C).[12]

ature dependence of k_1 from reaction of $S_2O_4^{2-}$ with $Fe(CN)_6^{3-}$ or with O_2 is essentially the same although absolute values from the O_2 reaction are slightly smaller than those for the $Fe(CN)_6^{3-}$ reaction except at 25 °C. A plot of log k_1/T vs. $1/T$ for the O_2 reaction (Figure 2) yields $\Delta H^{\ddagger}_1 = 11.6 \pm 0.8$ kcal mol^{-1} and $\Delta S^{\ddagger}_1 = -19 \pm 6$ cal K^{-1} mol^{-1}, comparable to values obtained for the $Fe(CN)_6^{3-}$ reaction.

A plot of log k_1 vs. $\mu^{1/2}$ (Figure 3) indicates that, within experimental error, k_1 is independent of ionic strength at low ionic strengths but increases significantly at high ionic strengths. It should be noted that the pseudo-zero-order traces begin to take on first-order character at high ionic strengths, and at $\mu^{1/2}$ equal to 1.8, good first-order traces are obtained for the same reactant concentrations. A plot of k_1 vs. pH (Figure 4) indicates that k_1 is nearly constant but decreases slightly at higher pH. Just as in Figures 1 and 2, there is reasonable correspondence between first-order data and pseudo-zero-order data in Figures 3 and 4.

Conditions for Which $SO_2^{-}\cdot$ or $S_2O_4^{2-}$ Attack Is Rate Determining. Data from the reduction of $Fe(CN)_6^{3-}$ by a large excess of $S_2O_4^{2-}$ > 30:1 are consistent with reactions 1, 2, and

$$S_2O_4^{2-} + Fe(CN)_6^{3-} \xrightarrow{k_3} S_2O_4^{-} + Fe(CN)_6^{4-} \qquad (4)$$

4 in which parallel reactions 2 and 4 involving $SO_2^{-}\cdot$ and $S_2O_4^{2-}$ are rate determining. Each $S_2O_4^{2-}$ consumes two $Fe(CN)_6^{3-}$'s by the path involving steps 1 and 2. For the limit of rapid step 1 and slow steps 2 and 4, the reactions are first order in $[Fe(CN)_6^{3-}]$ and half order and first order in $[S_2O_4^{2-}]$ for (2) and (4), respectively. The rate law can be expressed as the sum of two terms; see eq 5.

$$-d[Fe(CN)_6^{3-}]/dt = k_2K_1^{1/2}[S_2O_4^{2-}]^{1/2}[Fe(CN)_6^{3-}] + k_3[S_2O_4^{2-}][Fe(CN)_6^{3-}] \qquad (5)$$

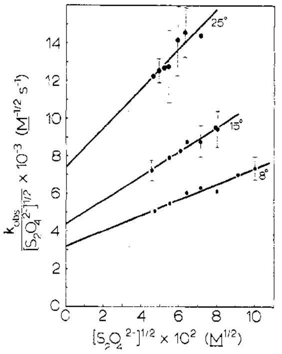

Figure 5. Plots of $k_{obsd}/[S_2O_4^{2-}]^{1/2}$ vs. $[S_2O_4^{2-}]^{1/2}$ for pseudo-first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by large excess $S_2O_4^{2-}$ (pH 6.8, μ = 0.10 M).[12]

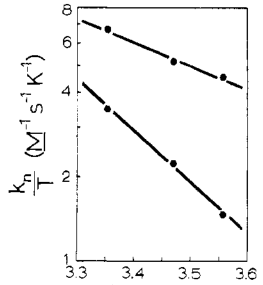

Figure 6. Plots of k_2/T vs. $1/T$ (●) and k_3/T vs. $1/T$ (●) for pseudo-first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by large excess $S_2O_4^{2-}$ (pH 6.8, μ = 0.10 M).

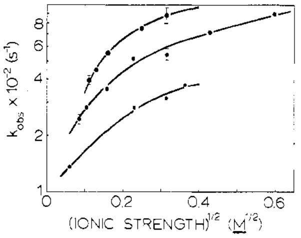

Figure 7. Plots of k_{obsd} vs. $\mu^{1/2}$ for pseudo-first-order reduction of 2.60×10^{-5} M $Fe(CN)_6^{3-}$ by 4.00×10^{-3} M $S_2O_4^{2-}$ (upper curve), 2.00×10^{-3} M $S_2O_4^{2-}$ (middle curve), and 1.00×10^{-3} M $S_2O_4^{2-}$ (lower curve) (pH 6.8, 25.0 °C).[12]

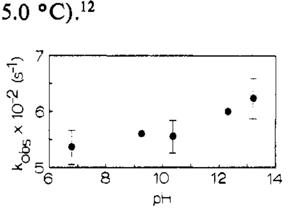

Figure 8. Plot of k_{obsd} vs. pH for pseudo-first-order reduction of 2.60 $\times 10^{-5}$ M $Fe(CN)_6^{3-}$ by 2.00×10^{-3} M $S_2O_4^{2-}$ (μ = 0.10 M, 25.0 °C).[12]

Reactions of $Fe(CN)_6^{3-}$ with a large excess of $S_2O_4^{2-}$ give pseudo-first-order kinetic traces. The associated rate constant k_{obsd} is given by (6). Combination of (5) and (6) gives (7)

$$-d[Fe(CN)_6^{3-}]/dt = k_{obsd}[Fe(CN)_6^{3-}] \qquad (6)$$

and (8). Thus, a plot of $k_{obsd}/[S_2O_4^{2-}]^{1/2}$ vs. $[S_2O_4^{2-}]^{1/2}$ should

$$k_{obsd} = k_2K_1^{1/2}[S_2O_4^{2-}]^{1/2} + k_3[S_2O_4^{2-}] \qquad (7)$$

$$k_{obsd}/[S_2O_4^{2-}]^{1/2} = k_2K_1^{1/2} + k_3[S_2O_4^{2-}]^{1/2} \qquad (8)$$

be linear, with a slope equal to k_3 and an intercept equal to $k_2K_1^{1/2}$. Figure 5 shows that data at several temperatures can be fitted to (8) within the large experimental errors inherent in the very rapid rates. The lower concentration limits in Figure 5 are points below which mixed pseudo-zero- and pseudo-first-order traces are obtained. The upper concentration limits are governed by the maximum rate of reaction that can be observed given the dead time of the stopped-flow spectrophotometer. From Figures 5 and 6, $k_3 = (1.0 \pm 0.4) \times 10^5$ M^{-1} s^{-1}, $\Delta H^{\ddagger}_3 = 8 \pm 1$ kcal mol^{-1}, and $\Delta S^{\ddagger}_3 = -8 \pm 7$ cal K^{-1} mol^{-1}. From the intercept and K_1 values[2,4c,13] at the three temperatures, it was possible to calculate $k_2 = (2.0 \pm 0.6) \times 10^8$ M^{-1} s^{-1}, $\Delta H^{\ddagger}_2 = 4 \pm 2$ kcal mol^{-1}, and $\Delta S^{\ddagger}_2 = -8$

---

(13) Wood, F. E. J. Ph.D. Thesis, University of Arizona, Tucson, 1974, p 69; *Diss. Abstr. Int. B* **1975**, *35*, 3809.

± 10 cal K^{-1} mol^{-1} (all data at pH 6.8, $\mu = 0.10$ M, 25 °C).

Plots of log k_{obsd} vs. $\mu^{1/2}$ for several $S_2O_4^{2-}$ concentrations are shown in Figure 7. Approximate calculations of k_2 and k_3 from data taken from the curves in Figure 7 show that k_2, and even more k_3, increases as a function of ionic strength. At the limit of low ionic strength the maximum slope for log k_2 vs. $\mu^{1/2}$ is 2.1 and for log k_3 vs. $\mu^{1/2}$ is 4.9. There is only a small change of k_{obsd} with pH (Figure 8), and separation into k_2 and k_3 values was unnecessary.

Discussion

Both first-order reduction of $Fe(CN)_6^{3-}$ by a stoichiometric amount of $S_2O_4^{2-}$ and pseudo-zero-order reduction of $Fe(CN)_6^{3-}$ by a small excess of $S_2O_4^{2-}$ (Figure 1) are interpreted in terms of (1) being the rate-determining step. The value of k_1 is independent of ionic strength at low ionic strength (Figure 3) as predicted for the single reactant $S_2O_4^{2-}$. At high ionic strength reactions tend toward first order with respect to $Fe(CN)_6^{3-}$, and a different mechanism involving attack of $S_2O_4^{2-}$ on $Fe(CN)_6^{3-}$ prevails. Thus, rate constants at high ionic strength in Figure 3 are composites of k_1 and k_3. Values of k_1 change only slightly with increasing pH (Figure 4), as would be expected for the aprotic reactant in this pH region.

Our value of k_1 is generally in good agreement with those determined for reduction of other oxidants by $S_2O_4^{2-}$ under conditions in which $S_2O_4^{2-}$ dissociation is rate determining. Thus, values of 1.75 s^{-1} (pH 7.4, $\mu = 0.05$ M, 23 °C) from reduction of oxidized iron–protein of nitrogenase,[14] 1.7 s^{-1} (pH independent, $\mu = 0.25$ M, 25 °C) from reduction of O_2, ferricytochrome *c*, and lumiflavin 3-acetate,[2] and 2.5 s^{-1} (pH 6.5, $\mu = 0.15$ M, 25 °C) from reduction of O_2[10] have been reported. A small or no pH dependence is observed. Our values of $\Delta H^{\ddagger}_1$ and $\Delta S^{\ddagger}_1$ differ seriously from those calculated from the Lambeth and Palmer data[2] ($\Delta H^{\ddagger}_1 = 23.2$ kcal mol^{-1} and $\Delta S^{\ddagger}_1 = +21$ cal K^{-1} mol^{-1}). The latter were obtained where there was a small O_2 dependence. Our examination of the same system involved conditions where there was no O_2 dependence, and activation parameters were close to those obtained with $Fe(CN)_6^{3-}$ as oxidant (Figure 2). Combination of these parameters with ΔH_1 and ΔS_1 values of 8.5 kcal mol^{-1} and −15 cal K^{-1} mol^{-1}, respectively, obtained by Wood[13] leads to values of $\Delta H^{\ddagger}_{-1}$ and $\Delta S^{\ddagger}_{-1}$ of 3.1 kcal mol^{-1} and −4 cal K^{-1} mol^{-1} for the dimerization of $SO_2^{-}\cdot$, very reasonable values for a near-diffusion-controlled radical dimerization. It should, however, be pointed out that the Lambeth and Palmer values[2] in conjunction with ΔH_1 (21.3 ± 0.4 kcal mol^{-1}) and ΔS_1 (+28.6 cal K^{-1} mol^{-1}) values from ESR studies of Burlamacchi et al.[15] lead to $\Delta H^{\ddagger}_{-1}$ and $\Delta S^{\ddagger}_{-1}$ values similar to those estimated by us.

Rate law 5 for pseudo-first-order reduction of $Fe(CN)_6^{3-}$ by a large excess of $S_2O_4^{2-}$ is consistent with parallel rate-determining steps 2 and 4. The larger effect of ionic strength on the value of k_3 than that of k_2 is expected for a 2− charged species vs. 1− charged species reacting with 3− charged $Fe(CN)_6^{3-}$. The more gradual increase in k_{obsd} at high ionic strengths may result from ion pairing of $Fe(CN)_6^{3-}$ with Na^+ or K^+, thus decreasing its reactivity; however, alkali metal cations have also been shown to catalyze reduction of $Fe(CN)_6^{3-}$ through ion pairing.[16] In addition, the dissociation constant K_1 decreases at high ionic strength[4c] so that $SO_2^{-}\cdot$ reduction no longer competes with $S_2O_4^{2-}$ reduction. Thus, k_{obsd} is primarily a measure of k_3 at high ionic strength. There is little effect of pH on k_{obsd}, which is a composite of k_2 and k_3, consistent with the aprotic character of $Fe(CN)_6^{3-}$, $S_2O_4^{2-}$, and $SO_2^{-}\cdot$.[3,9]

The value for k_3 [$(1.0 \pm 0.4) \times 10^5$ M^{-1} s^{-1}] compares well with the Lambeth and Palmer value (1.4×10^5 M^{-1} s^{-1} at pH 8.0, $\mu = 0.41$ M, and 25 °C).[2] Rate constants for reaction of $S_2O_4^{2-}$ with Fe(III)-containing oxidants (k_3) vary little,[2] 1.5×10^4–1.4×10^5 M^{-1} s^{-1}. Smaller values of k_3 have not been observed probably because $SO_2^{-}\cdot$ attack on the oxidant dominates for such systems. By contrast, rate constants for reaction of $SO_2^{-}\cdot$ with Fe(III)-containing oxidants (k_2) cover a wide range from about 1.3×10^4 [6b] to 2.9×10^8 M^{-1} s^{-1}.[6c] In systems where both k_2 and k_3 have been determined,[2,4a] the ratio k_2/k_3 must be approximately 10^3 since the concentration of $SO_2^{-}\cdot$ is approximately $^1/_{1000}$th of that of $S_2O_4^{2-}$ for the typical concentrations used. The enhanced reactivity of $SO_2^{-}\cdot$ over $S_2O_4^{2-}$ arises in part from the stronger reducing potential of the former.[5]

As a final check, we can use the values of k_1, k_2, and k_3 determined in this study, in conjunction with k_{-1},[2] to estimate the rates of the various steps in (1), (2), and (4). We find that the relative rate conditions assumed to hold for (a) dissociation of $S_2O_4^{2-}$ to be rate limiting $\{k_2[SO_2^{-}\cdot][Fe(CN)_6^{3-}] > 2k_1\cdot[S_2O_4^{2-}], k_3[S_2O_4^{2-}][Fe(CN)_6^{3-}]\}$ and (b) reaction of $SO_2^{-}\cdot$ and $S_2O_4^{2-}$ with $Fe(CN)_6^{3-}$ to be rate limiting $\{2k_1[S_2O_4^{2-}] > k_2[SO_2^{-}\cdot][Fe(CN)_6^{3-}] \approx k_3[S_2O_4^{2-}][Fe(CN)_6^{3-}]\}$ do, in fact, apply. It has to be considered that many of the kinetic traces refer to the very end of the reaction (because of the reaction lost in the mixer) and that therefore a much lower concentration of $Fe(CN)_6^{3-}$ than the initial concentration pertains.

In general, as the concentration of $S_2O_4^{2-}$ used increases, the $[S_2O_4^{2-}]/[SO_2^{-}\cdot]$ ratio increases. Thus, when the oxidant is weakly colored (as with many transition-metal complexes), relatively high concentrations of reactants have to be used, and $S_2O_4^{2-}$ attack may predominate. For the highly colored metalloproteins, however, both low oxidant and $S_2O_4^{2-}$ concentrations can be used, in which case $SO_2^{-}\cdot$ attack predominates.[2,4,6,14] However, in such cases, if deficient $S_2O_4^{2-}$ is used, the dissociation step may be rate limiting.[2]

Acknowledgment. Part of this work was supported by a National Science Foundation Grant. A Mellon Foundation Faculty Development Grant (C.W.J.S.) is gratefully acknowledged.

Registry No. $Fe(CN)_6^{3-}$, 13408-62-3; $S_2O_4^{2-}$, 14844-07-6; $SO_2^{-}\cdot$, 12143-17-8; O_2, 7782-44-7.

---

(14) Thorneley, R. N. F.; Yates, M. G.; Lowe, D. J. *Biochem. J.* **1976**, *155*, 137.

(15) Burlamacchi, L.; Casini, G.; Faglioli, O.; Tiezzi, E. *Ric. Sci.* **1967**, *37*, 97.

(16) Jain, D. V. S.; Nandel, F. S. *J. Chem. Soc., Dalton Trans.* **1977**, 949.