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Kinetics of Reduction of Hemin and the Hemin Bis(pyridine) Complex by Dithionite

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In order to delineate the important redox reactions of heme proteins, several investigators have studied the kinetics of the redox reactions of various water-soluble metalloporphyrins.[1-6] In this study we report the kinetics of reduction of monomeric hemin, (protoporphyrin IX)iron(III), and the pyridine complex of hemin by dithionite. Since hemin exists as a dimer at neutral pH, it was dissolved in sodium dodecyl sulfate micelles. This procedure had previously been shown to produce monodisperse hemin under the conditions of our experiments.[7]

Experimental Section

Hemin chloride was obtained from Nutritional Biochemicals, sodium dodecyl sulfate from Sigma, and sodium dithionite from Fisher. Dithionite solutions were standardized by reduction of excess potassium ferricyanide under anaerobic conditions. The amount of ferricyanide reduced was determined spectrophotometrically.[3] The samples were found to be 96% pure. All other chemicals were reagent grade or better.

All spectral measurements were carried out on a Beckman Acta CV spectrophotometer. The kinetic experiments were carried out as follows. Solutions of hemin (2×10^{-5} M) were prepared by diluting a stock solution (prepared fresh each day by dissolving hemin in 0.01 M NaOH) into a buffered solution containing 2% sodium dodecyl sulfate. Spectral analysis showed that under these conditions hemin exists as a monomer. Prior to reaction on the stopped-flow apparatus, the solution was deaerated with prepurified N_2. Dithionite solutions were prepared by dissolving the appropriate amount of solid sodium dithionite in a previously deaerated buffered solution also containing 2% sodium dodecyl sulfate. In the case of the reduction of the bis(pyridine) complex, both reactant solutions were brought to 0.05 M pyridine. In general, the solutions were buffered with 0.01 M phosphate or 0.01 M Tris. (At intermediate pHs both buffers were used and identical results were obtained.) Solutions were brought to an ionic strength of 0.1 M with added NaCl. The reactant solutions were then mixed on an Aminco-Morrow stopped-flow apparatus with the temperature controlled to 25 ± 0.1 °C with a circulating water bath.

In the case of the reduction of hemin, the reaction was followed at 570 nm and in the case of the bis(pyridine) complex at 555 nm. All reactions were carried out under pseudo-first-order conditions. Plots of log $|A - A_\infty|$ as a function of time, from which values for the observed rate constant, k_{obsd}, were calculated, were linear for 3 half-lives. All values given are the average of at lease three runs. The average deviation obtained for these runs was less than 10%.

Results

For the reduction of hemin by dithionite, the observed rate constant was determined as a function of dithionite concentration at pH 7 and 9. The results are shown in Figure 1. As is common with a number of dithionite reductions, the observed rate constant is proportional to the square root of the dithionite
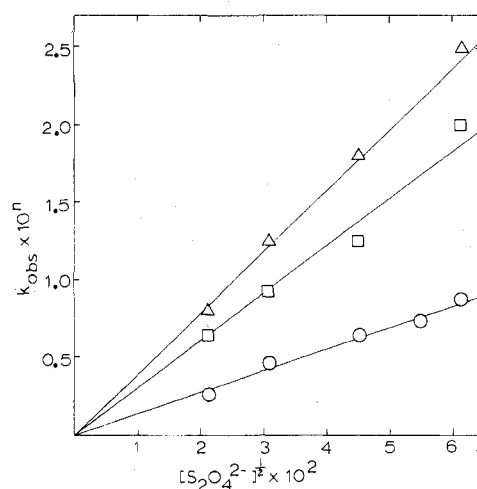

Figure 1. Plot of $k_{obsd} \times 10^{-2}$ as a function of $[S_2O_4^{2-}]^{1/2}$ for the reduction of a bis(pyridine) hemin complex by dithionite, □ (pH 7, [pyridine] = 0.05 M), and $k_{obsd} \times 10^{-1}$ and 10 as a function of $[S_2O_4^{2-}]^{1/2}$ for the reduction of hemin by dithionite at pH 7, O, and 9, △, respectively. The temperature was 25 °C and ionic strength 0.1 M.

concentration.[8] These data (at a single pH) are consistent with the mechanism previously proposed for dithionite reductions[8]
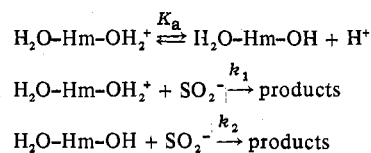
$$S_2O_4^{2-} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} 2SO_2^-$$

$$SO_2^- + \text{hemin} \xrightarrow{k'} SO_2 + \text{heme}$$
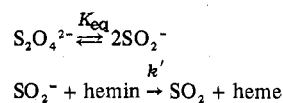
The rate law consistent with this mechanism is

$$\text{rate} = K_{eq}^{1/2}k'[S_2O_4^{2-}]^{1/2}[\text{hemin}] \tag{1}$$

or

$$k_{obsd} = K_{eq}^{1/2}k'[S_2O_4^{2-}]^{1/2} \tag{2}$$

Values for for k' (where $k' = k_{obsd}/K_{eq}^{1/2}[S_2O_4^{2-}]^{1/2}$) were determined as a function of pH at a constant $[S_2O_4^{2-}]$ of 1.0×10^{-3} M and using a K_{eq} of 1.4×10^{-9} M.[8] The rate constant k' was found to be approximately proportional to $[H^+]$. This behavior is consistent with the mechanism

$$H_2O-Hm-OH_2^+ \underset{}{\overset{K_a}{\rightleftharpoons}} H_2O-Hm-OH + H^+$$

$$H_2O-Hm-OH_2^+ + SO_2^- \xrightarrow{k_1} \text{products}$$

$$H_2O-Hm-OH + SO_2^- \xrightarrow{k_2} \text{products}$$

The rate law consistent with this mechanism is

$$\text{rate} = \frac{[H^+]k_1 + K_ak_2}{K_a + [H^+]}[SO_2^-][\text{Hm}]_T \tag{3}$$

where $[\text{Hm}]_T$ is the total hemin concentration and $H_2O-Hm-OH_2^+$ refers to hemin with two water molecules coordinated in the axial positions.[9] Thus

$$\frac{k'(K_a + [H^+])}{K_a} = \frac{k_1[H^+]}{K_a} + k_2 \tag{4}$$

A spectrophotometric titration of hemin under the conditions of our experiments yielded a value for pK_a of 5.89 (in reasonable agreement with a value of 5.5 determined under somewhat different conditions[10]). A plot of $k'(K_a + [H^+])/K_a$ as a function of $[H^+]$ is shown in Figure 2. From the slope k_1 is equal to $(6.0 \pm 0.7) \times 10^6$ M^{-1} s^{-1}, and from the intercept k_2 is about $(5 \pm 2) \times 10^3$ M^{-1} s^{-1}.

In the case of the reduction of the bis(pyridine) complex, the spectrum of hemin as a function of pyridine concentration was determined prior to the kinetic experiments under the
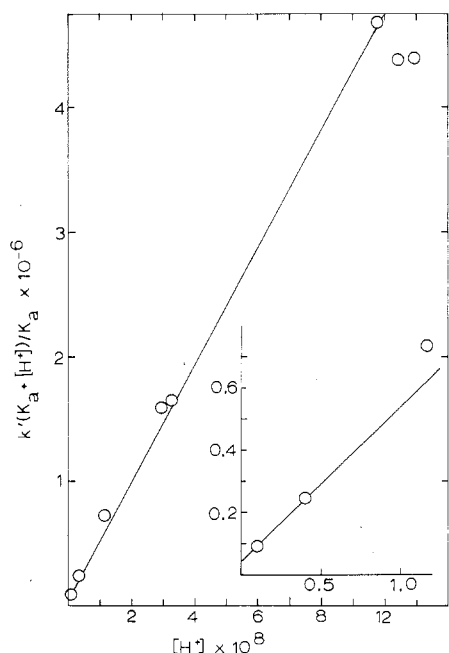
Figure 2. Plot of $[k'(K_a + [H^+])/K_a] \times 10^{-6}$ as a function of $[H^+] \times 10^8$ for the reduction of hemin by dithionite where $k' = k_{obsd}/(1.4 \times 10^{-9})^{1/2}[S_2O_4^{2-}]^{1/2}$ and pK_a is 5.89. The inset shows in more detail the region near the origin.

conditions of these experiments. The results were consistent with the simultaneous binding of 2 mol of pyridine/mol of hemin. The concentration of hemin required for the reaction to reach 50% completion was 0.01 M, yielding an equilibrium constant of 1.0×10^4 M^{-2} for the reaction

$$H^+ + 2py + H_2O\text{-Hm-OH} \rightleftarrows py\text{-Hm-py}$$

The spectrum of the product after reduction of the bis(pyridine) complex was identical with that reported for the bis-(pyridine) complex of heme.[11] The observed rate constant was determined as a function of $[S_2O_4^{2-}]$ in 0.05 M pyridine as is shown in Figure 1. (Our titration results show that the complex is completely formed under these conditions.) As in the case of the reduction of hemin, the observed rate constant is proportional to $[S_2O_4^{2-}]^{1/2}$ with a similar mechanism. From the slope of the line the rate constant for reduction by SO_2^- was equal to $(8.5 \pm 0.7) \times 10^7$ M^{-1} s^{-1}. The rate constant was measured as a function of pyridine concentration and did not vary over a fourfold range (0.05–0.20 M).

Discussion

The rate constants for the reduction of the bis(pyridine), aquo, and hydroxide complexes of hemin by SO_2^- are 8.5 × 10^7, 6.0 × 10^6, and 5 × 10^3 M^{-1} s^{-1}, respectively. The reduction of the bis(pyridine) complex occurs via an outer-sphere mechanism since the observed rate constant would be inversely proportional to the pyridine concentration if the pyridine molecule were displaced by SO_2^- as would be the case in an inner-sphere mechanism. In the case of the aquo and hydroxide complexes, it is more difficult to determine whether the reaction occurs via an inner- or outer-sphere mechanism. In the case of the reduction of the water-soluble cobalt(III) porphyrin, the mechanism was determined to be outer-sphere since the rate of substitution was much slower than the rate of reduction.[3] Such is not the case here. The rate of reaction of a water-soluble iron(III) porphyrin with imidazole has been measured and rate constants on the order of 10^7 M^{-1} s^{-1} have been obtained for the reaction of both the aquo and hydroxide species.[12] Based on these data it is difficult to unambiguously assign either an inner- or outer-sphere mechanism. However, if the rate of substitution were rate limiting as might be

expected in an inner-sphere mechanism, then one would expect little difference in the rates of reduction of the aquo and hydroxide species since the rates of substitution are expected to be the same. However, the rates are markedly different and this difference is consistent with that previously observed for reduction reactions involving outer-sphere mechanisms; e.g. the reduction of a water-soluble cobalt(III) porphyrin by SO_2^{-3} and the reduction of metmyoglobin by the *trans*-1,2-di-aminocyclohexane-N,N,N',N'-tetraacetate complex of iron-(III).[13]

The bis(pyridine) complex of hemin is reduced more rapidly than the aquo complex. This is consistent with the observation that pyridine stabilizes the iron(II) oxidation state relative to the iron(III) state because of the stability associated with the fully filled t_{2g} orbitals in the low-spin d^6 electronic configuration.[11] In the case of the water-soluble cobalt(III) porphyrin, the aquo complex was reduced more rapidly than the bis-(pyridine) complex. In this case, the low-spin d^6 configuration is associated with Co(III); hence, in this case, the Co(III) oxidation state is stabilized relative to Co(II) by coordination with pyridine.

The results obtained in this study can be compared with those observed in the reduction of heme proteins by dithionite. The following rate constants have been observed for the reduction by SO_2^-: metmyoglobin,[8] 2.7 × 10^6 M^{-1} s^{-1}; ferri-cytochrome c,[6,14] 3.8 × 10^7 M^{-1} s^{-1} (there is some disagreement with respect to the mechanism here); and cyanoferri-cytochrome c,[15] 7 × 10^5 M^{-1} s^{-1}. Thus our value for the reduction of the aquo complex by SO_2^- is very similar to rate constants observed for heme proteins.

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Organonitrogen Derivatives of Metal Carbonyls. 12. Reactions of Cyclopentadienylmetal Tricarbonyl Chlorides of Molybdenum and Tungsten with Pyrazole and Imidazole[1]

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Recently we reported reactions of $C_5H_5M(CO)_3Cl$ (M = Mo and W) with the ketoximes[3] RR'C=NOH and acetone