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LIGATIONAL EFFECTS ON REDUCTION OF MYOGLOBIN AND HORSERADISH PEROXIDASE BY INORGANIC REAGENTS

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Previous studies of the reduction of metmyoglobin and adducts by dithionite have been extended to horseradish peroxidase and its complexes. In addition, the reduction of metmyoglobin, horseradish peroxidase and adducts by a much bulkier reactant, cobalt(II) sepulchrate has been studied. Similar patterns of kinetic behavior were observed, namely, direct reduction of cyanide and imidazole adducts of the iron(III) proteins and indirect (via dissociation) reduction of the fluoride adduct. In the reduction of horseradish ferriperoxidase by cobalt(II) sepulchrate, three steps are observed and the spectral properties of the intermediate(s) and their kinetic behavior delineated. The final product is ferroperoxidase confirmed by spectral properties and its behavior on oxygenation. Reduction of cytochrome $c^{(III)}$ and Hipip by cobalt(II) sepulchrate appears to be a uniphasic reaction and second-order rate constants have been determined.

Introduction

The kinetics of reactions of metalloproteins with oxidizing and reducing agents continue to be a much examined subject. This interest is prompted by the theoretical advances which have been made in understanding electron-transfer processes involving simple [1,2] and complicated [3] molecules. In addition, the electron-transport proteins which have been largely studied represent an important group of metalloproteins.

We and others have previously examined the reduction by dithionite ion of metmyoglobin and of a number of its adducts with anions and neutral ligands [4–6]. Dithionite ion is an important reducing agent in biochemistry and interesting kinetically in being able to react either as $S_2O_4^{2-}$ ion or as the dissociated fragment, SO_2^- radical [7]. Several different kinds of kinetic behavior for the reduction of metmyoglobin have been observed [4–6]. We now extend these studies to another heme-containing protein, namely, horseradish peroxidase which is an enzyme catalyzing the oxidation of a variety of compounds by hydrogen peroxide or related compounds [8,9]. Horseradish peroxidase, like myoglobin, exists in an iron(III) form, ferriperoxidase, which can bind to certain ligands. The reduction by dithionite of ferriperoxidase and several ligand adducts (chosen to represent some general behavior observed with metmyoglobin) has therefore been studied. In addition, we use a previously unexamined reductant cobalt(II) sepulchrate (see structure I), first prepared by Sargeson and co-workers [10]. This extremely stable compact molecule is a good reducing agent ($E^0 = -0.30$ V [10]) and can be resolved into optical forms which

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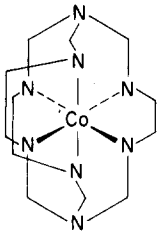
Abbreviations: Mb^+, metmyoglobin; Mb^0, deoxymyoglobin; cytochrome $c^{(III)}$, the oxidized form of cytochrome *c*; Hipip, the oxidised form of the high-potential iron-sulfur protein; adducts are denoted Mb^+X for the oxidised form and Mb°X for the reduced form. Co(II) sepulchrate is the trivial name for (I), the cobalt(II) complex of 1,3,6,8,10,13,16,19-octaazobicyclo-6,6,6-eicosane; CDTA, 1,2-cyclohexanediaminetetraacetate ion; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

do not racemize. The importance of sterochemical interactions can thus be assessed. We have studied the reduction by (I) of metmyoglobin, ferriperoxidase and a number of their adducts, as well as cytochrome $c^{(III)}$ and Hipip, so as to compare with the behavior of the small reductant radical, SO_2^-.



(I)

Materials and Methods

Horse heart myoglobin, horseradish peroxidase and horse heart cytochrome *c* were purchased from Sigma.

Most of the studies used type II (*RZ* 1.0–1.5) peroxidase, but a number of experiments with type VI (*RZ* approx. 3.0) gave similar results. *RZ* is the ratio of maximum absorbance in the Soret region to the absorbance of aromatic amino acids at 280 nm. An $RZ \geq 3.0$ indicates high purity [8,9]. Hipip was a gift from Dr. P.C. Harrington, prepared from cells of *Chromatium vinosum* D by a modification of the method of Bartsch [11]. Cobalt(III) sepulchrate and the optical forms were prepared as described by Sargeson et al. [10]. Reduction to the cobalt(II) form was effected by zinc dust in buffer, and the pH of the solution was checked just prior to use. All other chemicals were C.P. grade. The Fe-EDTA^{2-} complex was formed in situ using Fe^{2+} and a slight excess of EDTA. Since reductants and reduced proteins were all very oxygen sensitive, studies were carried out using N_2- or Ar-flushed solutions. Transfer of solutions to the serum-capped spectrophotometer cells or to the syringes of the stopped-flow apparatus were made using gas-tight syringes. Metmyoglobin adducts were formed in solution using sufficient ligand (0.5–50 mM CN^-; 100 mM imidazole; 100 mM F^- [5,12]) to effect greater than 95% complexing of the protein (10–20 μM). Ferriperoxidase similarly required 50–200 mM F^- or 25–50 mM CN^- [13–15] for substantial (greater than 90%) complexing of the protein, which was usually approx. 5 μM. The spectra of the various adducts agreed with those reported previously [5,12,16–18]. The kinetic measurements were made using a Gibson-Dionex stopped-flow apparatus interfaced with an OLIS data collecting system. Spectral measurements employed a Beckman 24 spectrophotometer. The reduction of ferriperoxidase was monitored at 432 nm (production of the iron(II) form, ferroperoxidase), 404 nm (disappearance of oxidized horseradish peroxidase), and, as a check, at several wavelengths between 500 and 650 nm. Consistent results were obtained. Reductions of Mb^+, Hipip and cytochrome $c^{(III)}$ were mainly followed at 552, 480 and 530 nm, respectively. All measurements were at 25°C, $I = 0.15$ M and pH 6.3, using Mes buffers, except for Hipip when pH 7, using Hepes buffer was employed.

Results

Myoglobin

The spectra of the products showed that all the metmyoglobin species, Mb^+, Mb^+CN^-, Mb^+ imidazole and Mb^+F^- were completely reduced to Mb^0 by excess cobalt(II) sepulchrate. The reaction of the first three species was multiphasic, although the bulk (greater than 90%) of the absorbance change at 552, 566 and 555 nm for Mb^+, Mb^+CN^- and Mb^+ imidazole, respectively, attended the fast phase. The predominant absorbance changes corresponded to first-order reactions and the associated rate constants were directly proportional to the concentration of reductant (Fig. 1). From the slope, second-order rate constants were obtained and these are collected in Table I. The reactions of Mb^+F^- with excess cobalt(II) sepulchrate and FeEDTA^{2-} were also first order, but the derived first-order rate constant was independent of the concentration of reductants and fluoride (greater than 100 mM) and a similar value resulted from both reductions (Table I).

The absorbance change which occurred after the main (reduction) reaction of Mb^+ with cobalt(II) sepulchrate was very small and it was therefore difficult to obtain accurate kinetic data for this process. At 608 nm, an isobestic point for

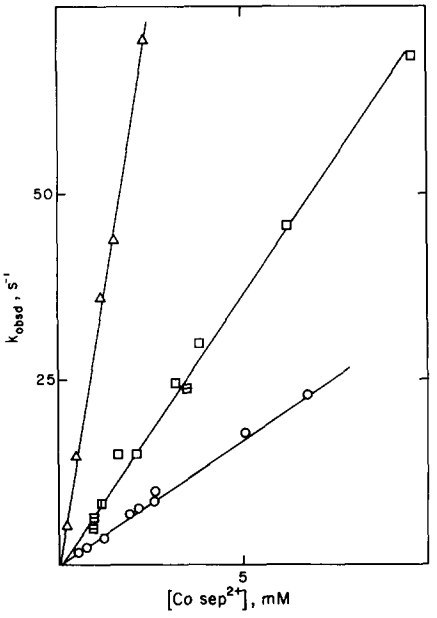


Fig. 1. Pseudo-first-order rate constant, k_{obsd} vs. [cobalt(II) sepulchrate], ([Co-sep^{2+}]), for reduction of Mb^+ (○), Mb^+CN^- (▥, 0.5 mM CN^-; □, 1.0 mM CN^-; ⊟, 50 mM CN^-) and Mb^+ imidazole (△, 100 mM imidazole), at 25°C, I = 0.15 M and pH 6.3.

Mb^+ and Mb^0, the data indicated the formation of an $[Mb^0]^*$ species, i.e., different from Mb^0, which under the influence of reductant changed to Mb^0. The rate constant for the reduction of Mb^+ at 608 nm is, however, the same as that at other wavelengths. Two reactions followed the main reduction of Mb^+CN^-. The first of these had a very small absorbance change with $k \approx 3\ s^{-1}$, independent of reductant concentration. The second, final reaction had also a reductant-independent rate, was first order ($k = 0.15$–$0.20\ s^{-1}$) and could be ascribed to release of CN^- from the Mb^0CN^- adduct produced in the reduction [4,5]. With similar conditions, this phase could also be obtained in dithionite reductions ($k = 0.19\ s^{-1}$) [4,5].

Horseradish peroxidase

The reductions of ferriperoxidase and the cyanide and fluoride adducts by dithionite concentrations larger than 2 mM are single first-order reactions. The first-order rate constants, k_{obsd}, are independent of the examination wavelength and linearly dependent on the square root of the dithionite concentration (Fig. 2). Changing the free cyanide concentration has no effect on k_{obsd}, but increasing the free fluoride concentration reduces the value of k_{obsd} (Fig. 2). The product of the dithionite reduction of ferriperoxidase and the fluoride adduct is ferroperoxidase characterised spectrally and by its reaction with oxygen (see next paragraph). The product of the dithionite reduction of ferriperoxidase cyanide is ferroperoxidase cyanide characterized spectrally (λ_{max} (ϵ_{max}): 565

TABLE I

RATE CONSTANTS FOR REDUCTION OF HORSE HEART MYOGLOBIN, HORSERADISH PEROXIDASE, AND HORSE HEART CYTOCHROME *c* BY Co(II) SEPULCHRATE, $FeEDTA^{2-}$ AND SO_2^- AT 25°C, pH 6.3, I = 0.15 M

Protein	Reductant	$k(M^{-1}\cdot s^{-1})$
Mb^+	Co-sepulchrate^{2+}	$3.5\cdot10^3$
	SO_2^-	$4.5\cdot10^6$ [a]
	$FeEDTA^{2-}$	31 [b]
Mb^+CN^-	Co-sepulchrate^{2+}	$7.4\cdot10^3$
	SO_2^-	$1.9\cdot10^6$ [a]
	$FeCDTA^{2-}$	$4.0\cdot10^{-2}$ [b]
Mb^+ imidazole	Co-sepulchrate^{2+}	$3.1\cdot10^4$
	SO_2^-	$8.8\cdot10^7$ [a]
Mb^+F^-	Co-sepulchrate^{2+}	0.27 [c]
	SO_2^-	0.01 [a,c]
	$FeEDTA^{2-}$	0.30 [c]
Ferriperoxidase	Co-sepulchrate^{2+}	[d]
	SO_2^-	$5.0\cdot10^5$
Ferriperoxidase cyanide	SO_2^-	$2.9\cdot10^5$
Hipip	Co-sepulchrate^{2+}	$2.1\cdot10^5$
	SO_2^-	$2.1\cdot10^6$ [f]
Cytochrome $c^{(III)}$	Co-sepulchrate^{2+}	$3\cdot10^5$
	SO_2^-	$3.9\cdot10^7$
	$FeEDTA^{2-}$	$2.6\cdot10^4$ [g]
Cytochrome $c^{(III)}$-CN^-	SO_2^-	$6.9\cdot10^5$ [h]

[a] I = 0.47 M, pH 8.2 [4,5]. k is sensitive to pH and I.
[b] Ref. 21.
[c] s^{-1}, representing dissociation of adduct.
[d] Biphasic via formation of adduct.
[e] Some evidence for direct reduction in addition to adduct dissociative path.
[f] Ref. 29, pH 7.3.
[g] Ref. 30, pH 7.0.
[h] Ref. 28.

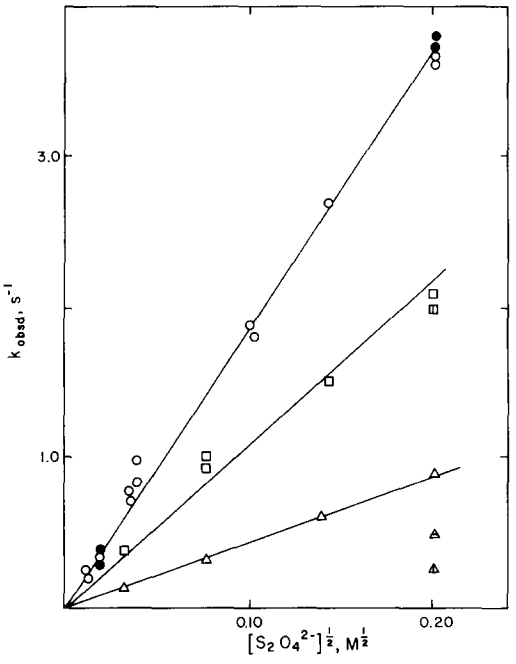


Fig. 2. Pseudo-first-order rate constant, k_{obsd} vs. $[S_2O_4^{2-}]^{1/2}$ for reduction of ferriperoxidase (○; ●, highly purified protein), and adducts (▣, 25 mM CN^-; □, 50 mM CN^-; △, 50 mM F^-; ▲, 100 mM F^-; ▲, 200 mM F^-). All at 25°C, $I = 0.15$ M and pH 6.3.

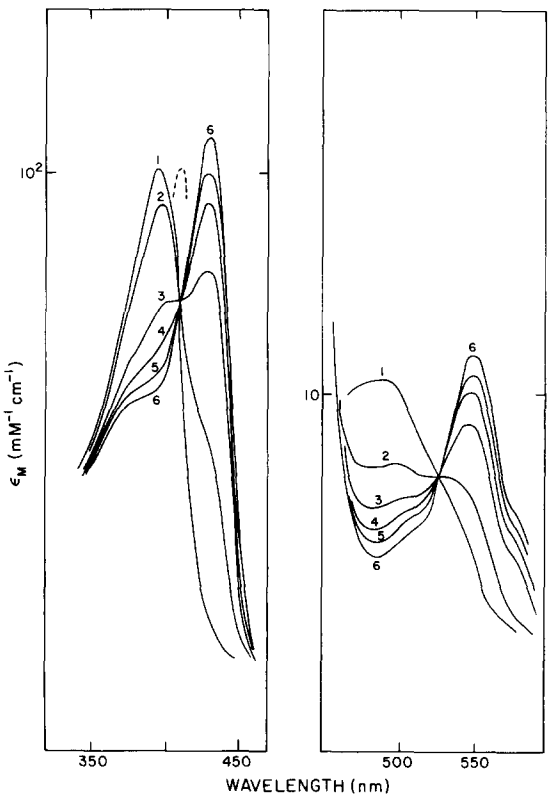


Fig. 3. Interaction of oxidized horseradish peroxidase with Co sepulchrate^{2+}. Right: 80 μM horseradish peroxidase, 330 μM Co sepulchrate^{2+} at 6°C. Spectra: (1) ferriperoxidase alone, (2) approx. 20 s after mixing, (3) 110 s, (4) 220 s, (5) 330 s and (6) 15, 25 min (ferroperoxidase). A number of such experiments showed an isosbestic point at 530 nm. Left: 13 μM horseradish peroxidase, 195 μM Co sepulchrate^{2+} at 25°C. Spectra: (1) ferriperoxidase alone, (2) approx. 10 s after mixing, (3) 120 s, (4) 240 s, (5) 360 s, (6) 14, 20 min (ferroperoxidase). An isosbestic point at 410 nm was maintained throughout this part of the reaction. Broken line peak at 412 nm after adding O_2 to No. 6 (oxyferroperoxidase).

(18), 535 nm (14 $mM^{-1} \cdot cm^{-1}$); literature: 566 (19.0), 536 nm (14.5 $mM^{-1} \cdot cm^{-1}$) [16,17]). Unlike the myoglobin system, no dissociation of CN^- from the product occurred under our conditions. When concentrations less than about 1.5 mM dithionite are used, there is a small absorbance change at 432 nm (about 5% of the total change) which precedes the major reaction. The data for the slower major reaction still conform to the plots of Fig. 2, and the faster change, accurate kinetics for which are difficult to obtain, is ascribed to a conformational change which cannot be observed when the reduction is made sufficiently rapid at high reductant concentrations.

The spectral changes which accompany the reaction of ferriperoxidase with cobalt(II) sepulchrae are shown in Fig. 3. There is an intermediate (curve 2) which is rapidly formed within 15–20 s, and this slowly transforms with clean isosbestic points at 410 and 530 nm to the final product (curve 6). This has spectral characteristics consistent with those reported for ferroperoxidase, with peaks at 435 nm ($\epsilon = 100$ $mM^{-1} \cdot cm^{-1}$) and 550 nm ($\epsilon = 11.0$ $mM^{-1} \cdot cm^{-1}$) and shoulders at approx. 510 and 580 nm [17–19]. This product was identical with that produced by dithionite reduction of ferriperoxidase and both, treated with saturated O_2 solution, gave oxyferroperoxidase with peaks at 412, 535 and 575 nm. This rapidly transforms to ferriperoxidase (peaks at 395 and 485 nm) via isosbestic points at 400, 455 and 520 nm [19,20]. The nature of the intermediate represented by curve 2 was probed in some qualitative spectral experiments. Addition of F^- to the inter-

mediate gave the spectrum of ferriperoxidase fluoride. Since F^- does not bind to ferroperoxidase (checked spectrally), this experiment strongly suggests that the intermediate represented by curve 2 (Fig. 3) contains Fe(III) and not Fe(II). Further, when ferriperoxidase is reduced with a mixture of $S_2O_4^{2-}$ and cobalt(II) sepulchrate the adduct rapidly formed is reduced by dithionite at the same rate as ferriperoxidase. This is both evidence for retention of the original oxidation states in the adduct and different sites of attack for the two reductants.

The formation of the intermediate is nicely first order and the dependence of k_{obsd} on reactant concentration, as measured by stopped-flow, is shown in Fig. 4. At the completion of this reaction, most easily monitored at 432 nm, there is a very small absorbance decrease ($k_{obsd} \approx 1.5\ s^{-1}$), and then a major increase to the final reduced product represented by curve 6 (Fig. 3). This slow final step is an excellent first-order reaction, the rate constant for which is basically independent of the concentrations of reactants but does vary, inexplicably and randomly, from 0.008 to 0.014 s^{-1} (with occasionally wider variants). One run using ferriperoxidase (3.3 μM) and (+)-cobalt(II) sepulchrate (2.0 mM) gave phases and rates identical

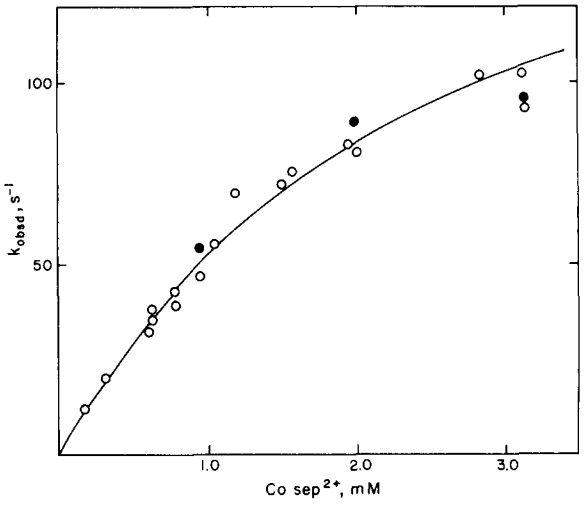


Fig. 4. Pseudo first-order rate constant, k_{obsd} vs. [cobalt(II) sepulchrate], (Co sep^{2+}) for first fast phase of reaction. Curved line represents Eqn. 11 with $K_8 = 350\ M^{-1}$ and $k_9 = 200\ s^{-1}$. (●) Highly purified protein. Temperature 25°C, $I = 0.15$ M and pH 6.3.

to that using the racemic form. Finally, we checked that no obvious (spectral) changes occurred when ferriperoxidase was treated with SO_3^{2-}, $S_2O_6^{2-}$ or cobalt(III) sepulchrate, or when ferroperoxidase was added to cobalt(II) or (III) sepulchrate. Ferriperoxidase was not reduced by $FeEDTA^{2-}$ but ferroperoxidase was oxidized by $FeEDTA^-$.

Hipip and cytochrome $c^{(III)}$

The reduction of these two proteins by cobalt(II) sepulchrate was examined. In both cases only single first-order reactions were observed using the reductant in excess. The associated first-order rate constants were in both cases linearly dependent on the concentration of reductant. The slopes of the k_{obs}/[reductant] plots gave values of the second-order rate constants (Table I).

Analysis of kinetic data

The reductions of the proteins P and their adducts P·X by the reducing agent R can be accommodated by the scheme:

$S_2O_4^{2-} \rightleftarrows 2SO_2^-$	K_1,	(1)
$P \cdot X \rightleftarrows P + X$	k_2, k_{-2}, K_2	(2)
$P + R \rightarrow$ products	k_3	(3)
$P \cdot X + R \rightarrow$ products	k_4	(4)

If the protein is substantially complexed (i.e., $K_2 < [X]$), then it is readily shown that Eqn. 5 holds:

$$k_{obsd} = \frac{d[\text{products}]}{dt} \cdot \frac{1}{[P \cdot X]} = \frac{k_2 k_3 [R]}{k_{-2}[X] + k_3[R]} + k_4[R] \quad (5)$$

For P = myoglobin, X = F^- and R = Co(II) sepulchrate or $FeEDTA^{2-}$, it is easily demonstrated that $k_3[R] > k_{-2}[X]$, in agreement with the observed independence of k_{obsd} on [R]. This requires that $k_{obsd} = k_2$. Our value of k_2, 0.27 s^{-1}, is in excellent agreement with that obtained in anation studies (0.29 s^{-1} at pH 6.1 [22]). For P = myoglobin, X = CN^- or imidazole and R = Co(II) sepulchrate, k_{obsd} is independent of [X] but linearly dependent on [R] (Fig. 1). This requires only that $k_4[R] > k_2$ and therefore $k_{obsd} = k_4$[Co(II) sepulchrate].

For reduction of ferriperoxidase and adducts by dithionite, R = SO_2^- since a dependence of the rate on the square root of the concentration of

dithionite is observed [4–7] (Fig. 2). For $X = CN^-$, k_{obsd} is independent of [X] and therefore:

$$k_{obsd} = k_2 + k_4 K_1^{1/2} [S_2O_4^{2-}]^{1/2} \quad (6)$$

Since $k_2 = 0.2\ s^{-1}$ at pH 6.5 [14,15], $k_{obsd} > k_2$ at higher dithionite concentrations (Fig. 2) and from the known value of K_1 ($1.4 \cdot 10^{-9}$ M [7]), the derived value of k_4 is $2.9 \cdot 10^5\ M^{-1} \cdot s^{-1}$. For $X = F^-$, it is easily shown that $k_{-2}[F^-] \gg k_3[SO_2^-]$ and that therefore:

$$k_{obsd} = K_2 k_3 K_1^{1/2} [S_2O_4^{2-}]^{1/2} [F]^{-1} + k_4 K_1^{1/2} [S_2O_4^{2-}]^{1/2} \quad (7)$$

The plot of k_{obsd} vs. $[F]^{-1}$ at constant $[S_2O_4^{2-}]$ using the data in Fig. 2 was linear and yielded values of $k_4 \approx 10^4\ M^{-1} \cdot s^{-1}$ and $K_2 k_3 = 5.3 \cdot 10^3\ s^{-1}$. The latter product can be estimated as $1.6 \cdot 10^{-2}$ M [13] times $5 \cdot 10^5\ M^{-1} \cdot s^{-1}$ (Table I) $= 8.0 \cdot 10^3\ s^{-1}$, in reasonable agreement with the value measured by us.

The form of the k_{obsd}/[Co(II) sepulchrate] plot associated with the fast phase of the reduction of ferriperoxidase (Fig. 4) suggests a mechanism of the form:

$$\text{ferriperoxidase} + \text{Co(II) sepulchrate} \rightleftarrows (\text{adduct})_A \qquad K_8 \quad (8)$$

$$(\text{adduct})_A \rightarrow (\text{adduct})_B \qquad k_9 \quad (9)$$

$$(\text{adduct})_B \rightarrow \text{ferroperoxidase} + \text{Co(III) sepulchrate} \qquad k_{10} \quad (10)$$

The associated rate law for the formation of $(\text{adduct})_B$ (Eq. 11):

$$k_{obsd} = \frac{k_9 K_8 [\text{Co(II) sepulchrate}]}{1 + K_8 [\text{Co(II) sepulchrate}]} \quad (11)$$

fits the experimental values well with $K_8 = 350\ M^{-1}$ and $k_9 = 200\ s^{-1}$ (Fig. 4).

Discussion

Both dithionite and cobalt(II) sepulchrate are effective reducing agents for converting iron(III) into iron(II) in both myoglobin and horseradish peroxidase. This is expected since although metmyoglobin ($E^0 = 0.06$ V [12]) and horseradish peroxidase ($E^0 = -0.17$ V [23]) are only weak oxidants, both reducing agents are strong ($E^0 = -0.66$ V, pH 7 [24] and $E^0 = -0.30$ V [10] for SO_2^- and cobalt(II) sepulchrate, respectively). Only in the reactions of the cyanide adducts are the immediate products of the reduction other than deoxymyoglobin or ferroperoxidase. The Mb^0CN^- produced by reduction of Mb^+CN^- dissociates to Mb^0 by a first-order process ($k = 0.20\ s^{-1}$) whereas ferroperoxidase cyanide is stable at the relatively high concentrations of CN^- used [16,17].

Although it has been suggested that 'reduction of ferriperoxidase to ferroperoxidase by dithionite is neither a rapid nor a clean reaction [8], we have found smooth, rapid reduction if larger than millimolar concentrations of dithionite are used. The reduction of ferriperoxidase by cobalt(II) sepulchrate, by contrast, is complex and relatively slow. The kinetic data for the fastest step (Fig. 4) suggest that $(\text{adduct})_A$ is very rapidly formed, and that this transforms more slowly to a different adduct (Eqns. 8 and 9). The latter changes, perhaps via another intermediate, with a rate invariant under all conditions. Studies of the properties of $(\text{adduct})_B$, the first intermediate we can handle outside the stopped-flow, indicate that the original oxidation states are preserved in this (and presumably the prior) adducts. Its spectrum (curve 2 in Fig. 3) has marked similarities to that of ferriperoxidase (although lower peak absorbances). In this respect, it resembles those of adducts of ferriperoxidase with NO_3^-, ClO_4^- and $CH_3CO_2^-$ at pH ≈ 4 or of phenols, aromatic amines and indole derivatives at neutral pH (ref. 15 and references therein). Binding in these adducts is believed to be away from the iron site [15]. The last, slow, change (1), $k \approx 0.011\ s^{-1}$, represents we believe an intramolecular electron transfer from protein-attached cobalt(II) to the iron center (and dissociation of the cobalt(III) sepulchrate from the protein). The low rate constant would suggest a considerable distance, perhaps 20–25 Å, separating the redox centers. This might be anticipated on theoretical grounds [3] as well as based on recently described redox behavior of a fericytochrome *c* adduct [25,26], and the respiratory protein hemerythrin [27]. The reaction of ferriperoxidase

with one of the optical forms of cobalt(II) sepulchrate gave identical kinetic behavior to that of the racemic mixture. Obviously sterochemical selectivity was absent in this reaction, and this was disappointing in view of the variety of interactions (adduct formation, conformational changes and redox processes) in which it may have shown up. Peripheral to the main study, we have confirmed that ferroperoxidase produced by both dithionite and cobalt(II) sepulchrate reduction of ferriperoxidase reacts with oxygen to give oxyferroperoxidase. This auto-oxidises rapidly to ferriperoxidase with spectral characteristics for the stages as described in the literature [17,19].

The basic kinetic patterns established in the reduction of metmyoglobin and its derivatives by dithionite are also observed with horseradish peroxidase. Ferriperoxidase cyanide is reduced directly by SO_2^- at comparable rates to those of Mb^+CN^- [4,5] and the horse heart cyanocytochrome *c* complex [28] (Table I). With ferriperoxidase fluoride, reduction by SO_2^- as with Mb^+F^- [4,5] occurs only after the F^- has dissociated, although a minor direct path with $k \approx 10^4$ $M^{-1} \cdot s^{-1}$ may operate. When cobalt(II) sepulchrate is used as reductant, Mb^+CN^- and Mb^+ imidazole are reduced directly and Mb^+F^- is reduced only through the dissociated entity Mb^+. From the study of the reduction of metmyoglobin adducts with a variety of imidazoles [6], it was concluded that direct attack of SO_2^- was on the ligand, and that this was followed by electron transfer through the π-electron system to the metal ion. It is difficult to envisage a bulky macrocyclic complex approaching near to the heme center and it is more likely that transfer of an electron is from a peripheral position on the protein [21]. Differences in the character of the reduction by SO_2^- and cobalt(II) sepulchrate are also supported by the values for the rate constant. For the latter, reduction of Mb^+CN is faster than for Mb^+, whereas for SO_2^- reduction, the opposite occurs (Table I).

Acknowledgement

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