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Kinetics and Mechanisms of Reactions of Water-Soluble Ferriporphyrins. 3. Ascorbic Acid Reduction of the [5,10,15,20-Tetrakis(4-*N*-methylpyridyl)porphine]iron(III)–Histidine System

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Received November 23, 1977

Spectrophotometric titrations were used to determine the composite equilibrium quotient 4×10^4 M^{-2} in neutral and slightly basic media for axial histidine binding to the water-soluble ferriporphyrin [5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphine]iron(III). The coordination number of the axial site of hemin iron was determined to be 2.0, indicating that the resulting major complexes are six-coordinate bis(histidine) hemichromes. The magnetic moment in solution (Evans' method) was found to be 2.18 ± 0.04 μ_B at 34 °C. The kinetics of the ascorbic acid reduction of the bis(histidine)ferriporphyrin has been studied by stopped-flow spectrophotometry. At 25.0 °C, $\mu = 0.10$ (NaCl), and pH ranging from 6.6 to 8.8, the reduction of the bis(histidine)ferriporphyrin follows a pseudo-first-order rate expression in excess ascorbic acid. The pseudo-first-order rate constants are found to be independent of [ascorbic acid] and related to $[H^+]$ in accordance with the following expression: $k_{obsd} = k_{diss}(1 + K_a[H^+]^{-1})^{-1}$, where K_a and k_{diss} represent the respective equilibrium constants for the deprotonation of the bis(histidine)ferriporphyrin and the rate constant for dissociation of the histidine ligand. K_a and k_{diss} are determined to be $(1.38 \pm 0.05) \times 10^{-8}$ M and 25.2 ± 0.93 s^{-1}, respectively. Possible mechanisms for electron transfer are discussed, and comparisons are made with other systems, including kinetics of axial ligand dissociations in oxyhemoglobin and oxymyoglobin.

Introduction

The biological importance of the heme proteins has led to a variety of studies on metalloporphyrins. Much of the early work was done in nonaqueous media, but with the synthesis of water-soluble porphyrins these studies have been extended into the aqueous environment. Previous publications[1,2] from this laboratory have dealt with the acid–base and association behavior of the water-soluble porphyrin [5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphine]iron(III), hereafter referred to simply as $Fe^{III}Por$, and with its reduction by ascorbic acid. The study of the reduction of $Fe^{III}Por$ to $Fe^{II}Por$ by ascorbic acid was found to follow a pseudo-second-order rate expression in excess ascorbic acid and in the absence of oxygen. The mechanism involves iron(III) porphyrin dimerization prior to electron transfer. The rate constants were shown to be pH dependent, and the reactions were slow enough to be followed by traditional spectrophotometric techniques. The exact site of electron transfer was not determined in the previous experiments with $Fe^{III}Por$ though it seemed likely that the dimer bridge was not directly involved.

In contrast, ferricytochrome *c* has been found to exhibit a pseudo-first-order rate expression with excess ascorbic acid.[3] The difference in the rate laws in the two systems has been attributed to the different environments of the iron center in the iron(III) porphyrin and in cytochrome *c* (III). Whereas dimeric $Fe^{III}Por$ has a μ-hydroxo or μ-oxo bridge, depending on pH, cytochrome *c* binds histidine in one axial position and methionine in the other. Furthermore, the rate of ascorbate reduction of modified cyt *c* (III) has been shown to follow a rate expression which is independent of the concentration of reductant, suggesting that a heme protein modification must occur prior to electron transfer.[4]

This report describes the results of a study designed to probe the mechanism of electron transfer of $Fe^{III}Por$ with axial accessibility limited by histidine. Three important features have emerged from this study: (i) the reduction of $Fe^{III}Por(his)_2$ follows a pseudo-first-order rate expression, (ii) the observed rate constants are independent of ascorbic acid concentration, and (iii) the observed pH-dependent rate constant for electron transfer attains limiting values at relatively high $[H^+]$. The results of these experiments, taken with the results of other studies, cast considerable light on the mechanisms of axial ligand interactions not only in ferriporphyrin electron-transfer systems but in dioxygen-transport systems as well.

Experimental Section

Materials. The [5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphine]iron(III) pentaperchlorate, $Fe^{III}Por(ClO_4)_5$, was prepared and purified as previously described.[1,5,6] The pentachloride salt was obtained and analyzed by the procedure reported by Pasternack and co-workers.[7] Anal. Calcd C:N mole ratio for $C_{44}H_{36}N_8FeCl_5$: 5.50. Found: 5.39.

All experiments were carried out in buffered media, 0.010 M phosphate, or 0.100 M histidine, adjusted to the desired pH with 0.10 M NaOH or 0.10 M HCl, at $\mu = 0.10$ (NaCl). Deionized water (>1 MΩ) was used exclusively. L-Ascorbic acid and sodium ascorbate (Sigma) were used without additional purification, and solutions were prepared fresh daily. Cr^{2+}-scrubbed N_2 was used for deaeration of all solutions.

Spectral Studies. A series of spectrophotometric titrations were conducted in which the histidine concentration was increased from 0.00 M to 5.77×10^{-2} M by stepwise addition of 0.100 M histidine solution to phosphate-buffered solutions of $Fe^{III}Por(ClO_4)_5$ of identical concentration and ionic strength. Sufficient time was allowed for the solutions to equilibrate, and the visible spectra were recorded over the region of 700–400 nm. The most convenient wavelength at which to study the spectral change proved to be 550 nm. A Cary 14 spectrophotometer was used.

Five spectrophotometric titration experiments were conducted, and three pH ranges were studied. Determination of pH was accomplished with a Krueger and Eckels 133 digital pH meter equipped with a microelectrode and standardized against Beckman reference buffers. $[H^+]$ was computed from pH using a value of 0.83 ($\mu = 0.10$) for the activity coefficient of H^+.[8]

Kinetic Studies. Solutions of $Fe^{III}PorCl_5$, buffered in histidine of appropriate pH, were deaerated with oxygen-free N_2 for 30 min. Reductant solutions were prepared by dissolving weighed quantities of sodium ascorbate in distilled water to obtain 0.1001–0.1037 M solutions. Small aliquots of these solutions (0.05–1.00 mL) were removed by syringe and added to 25.0 mL of histidine adjusted to an appropriate pH. The histidine buffer was deaerated before and after ascorbic acid addition with oxygen-free N_2 for at least 15 min.

Final concentrations of ascorbic acid were at least 66-fold greater than the $Fe^{III}Por$ concentrations. The effects of $[H^+]$ were studied over the pH range of 6.46–8.80.

The kinetics studies were run on a stopped-flow spectrophotometer consisting of a two-jet Lucite mixer with quartz windows and a 2.5-cm light path, a Beckman DU monochromator, a 1P28V1 photomultiplier, a Biomation 610B transient recorder, a Fluidyne 722 digital interface, and a Wang 220S 8K×8 microcomputer equipped with a Wang 2250

0020-1669/78/1317-3119$01.00/0

Table I. Results of Spectrophotometric Titrations (25.0 °C, $\mu = 0.10$ (NaCl))

10^5[Fe^{III}Por], M	λ, nm	pH	slope[a]
2.11	600	6.97	1.95 ± 0.14
2.11	560	6.97	1.94 ± 0.12
1.68	550	6.98	1.95 ± 0.08
2.02	550	6.98	2.00 ± 0.10
2.04	550	7.56	1.97 ± 0.19

[a] Slope of log R vs. log [his] where $R = (A_{obsd} - A_0)/(A_\infty - A_{obsd})$ (see text).

parallel interface. Nominal mixing time for this spectrophotometer is 5 ms.

Values of k_{obsd}, the pseudo-first-order rate constants for reduction of $Fe^{III}Por(his)_2$ by ascorbic acid, were obtained by least-squares analysis of absorbance vs. time data in accordance with the function $-\ln [(A_t - A_\infty)/(A_0 - A_\infty)] = k_{obsd}t$ immediately after acquisition of data. (A_t, A_0, and A_∞ represent absorbance at time t, initial absorbance, and absorbance at effective infinite time, respectively.) Of the 255 values of absorbance vs. time obtained in the course of an individual experiment, the last 15 were used to compute A_∞. Weights for each data point at time t were proportional to the excess absorbance $(A_t - A_\infty)$ at each respective point. An average of ten individual kinetics experiments was carried out under each set of specified conditions; in no case were less than five replicate runs performed.

Magnetic Susceptibility Studies. Magnetic susceptibility was determined by Evans' NMR technique.[9] A Perkin-Elmer R-20 NMR instrument, concentric NMR tubes, and 2% *tert*-butyl alcohol standard in 0.100 M buffered histidine solutions (pH 7.06) were employed. At ferriporphyrin concentrations of 0.034, 0.025, 0.016, and 0.0066 M, magnetic moments of 2.17, 2.12, 2.22, and 2.19 μ_B were obtained, respectively (34 °C). Pascal's corrections were used to calculate these magnetic moment values.[10] No effect of variation in ferriporphyrin concentration was observed, suggesting the absence of dimeric interactions, even at such high concentrations. The mean magnetic moment was found to be 2.18 ± 0.04 μ_B, characteristic of a predominantly low-spin system.

Results and Discussion

The interaction of histidine with $Fe^{III}Por$ was studied in dilute media to ensure that monomeric forms of the ferriporphyrin would be the predominant species.[1,2] The spectrum of $Fe^{III}Por$ alone exhibits an absorbance maximum at 645 nm with a 600-nm shoulder and the Soret band at 422 nm (single maximum at pH ~7), while histidine–porphyrin solutions exhibit a single band at 550 nm and the Soret band at 424 nm ($\epsilon = 1.11 \times 10^5$ M^{-1} cm^{-1}).

Evaluations of the equilibrium constants for complexations of $Fe^{III}Por$ by histidine were carried out by spectrophotometric titration (550, 560, or 600 nm, pH 7.0 or 7.6, Table I). By use of the mathematical expressions described by other workers,[12,13] the number of axial ligands (L) binding the iron and the product of the equilibrium constants were obtained.

$$Fe^{III}Por + nL \rightleftharpoons Fe^{III}PL_n \qquad K_{in} \tag{1}$$

$$K_{in} = [Fe^{III}PorL_n]/[Fe^{III}Por][L]^n = R(1/[L]^n) \tag{2}$$

$$R = (A_{obsd} - A_0)/(A_\infty - A_{obsd}) \tag{3}$$

$$\log R = \log K_{in} + n \log [L] \tag{4}$$

A_{obsd} is the absorbance of an equilibrium mixture at some given total metalloporphyrin concentration, and A_0 and A_∞ are the absorbances which the total metalloporphyrin concentration would exhibit if complexing were nonexistent or complete, respectively. K_{in} is the product of $K_1 \times K_2 \times K_3 \times \ldots \times K_n$, the equilibrium constants for the binding of one, two, three, ..., n histidines to the metalloporphyrin. Absorbance vs. [histidine] was plotted to verify that A_∞ was attained.

From eq 4 it is seen that a plot of log R vs. log [L] should be linear with slope = n and intercept = log K_{in}, provided that no more than two absorbing species are present in substantial concentrations. Linear plots were obtained from experimental data. The values of n computed by least-squares analysis are shown in Table I; the average is 1.96 ± 0.02.

Table II. Kinetics Data for the Reduction of $Fe^{III}Por(his)_2$ by Ascorbic Acid (25.0 °C, $\mu = 0.10$, λ 540 nm)

pH	10^4[ascorbic acid], M	k_{obsd},[a] s^{-1}	av k_{obsd},[b] s^{-1}	av k_{calcd},[c] s^{-1}
6.57	1.04	23.2	24.3 ± 1.0	24.2
	2.07	24.8		
	4.15	25.0		
7.20	2.07	22.4	22.4 ± 0.1	21.3
	4.15	22.5		
8.10	1.01	9.7	10.0 ± 0.4	10.4
	4.02	10.3		
8.50	1.03	5.49	5.53 ± 0.07	5.47
	2.06	5.50		
	4.12	5.61		
8.78	1.00	3.32	3.19 ± 0.18	3.19
	2.00	3.07		

[a] Each k_{obsd} represents the mean of at least five individual kinetics runs. [b] Computed by determination of k_{obsd} as $A_\infty - A_t = (A_\infty - A_0)\exp(-k_{obsd}t)$, where t, A_∞, A_0, and A_t represent time, absorbance at effective infinite time, absorbance at time 0, and absorbance at time t, respectively. Error is 1σ. [c] Calculated by least-squares analysis of k_{obsd}^{-1} vs. $[H^+]^{-1}$.

We propose the following model in which $K_2 >> K_1$ and $Fe^{III}Por(his)$ never attains appreciable concentration:

$$Fe^{III}Por + his \rightleftharpoons Fe^{III}Por(his) \quad K_1$$

$$Fe^{III}Por(his) + his \rightleftharpoons (his)Fe^{III}Por(his) \quad K_2$$

$$Fe^{III}Por + 2his \rightleftharpoons (his)Fe^{III}Por(his) \quad K_{12} = K_{in}$$

K_{in} as defined by eq 4 is the antilog of the intercept of the plots of log R vs. log [his], for which an average value of (4.0 ± 0.8) × 10^3 M^{-2} was obtained.[14,15]

$Fe^{III}Por$ is known to dimerize in neutral and alkaline media.[1] To ensure that dimerization was not involved in the spectrophotometric titrations of $Fe^{III}Por$ with histidine, Beer's law experiments were conducted at pH 7.7. [$Fe^{III}Por(his)_2$] was increased from 1.65×10^{-6} to 1.65×10^{-4} M. The plot of absorbance (λ 550 nm) vs. [$Fe^{III}Por(his)_2$] was linear.

Attempts to carry out spectrophotometric titrations at pH 3.94 proved futile, as no absorbance changes were observed. Protonation of the imidazole nitrogen ($pK = 6.08$)[11] is probably responsible for failure to observe complexation in mildly acidic media. Indeed, addition of imidazole to $Fe^{III}Por$ solutions produces spectral changes which are very similar to those observed with histidine, whereas attempts to observe spectrophotometric evidence for binding of acetate, alanine, or glycine were unsuccessful, even in neutral media. We conclude that histidine binds the ferriporphyrin at the imidazole nitrogen.

The series of experiments designed to examine possible pathways for electron transfer from ascorbic acid to $Fe^{III}Por(his)_2$ were carried out at 25.0 °C. In the experiments previously conducted without the histidine ligands,[2] the course of the reaction between $Fe^{III}Por$ and ascorbic acid is characterized by the relatively slow disappearance of the $Fe^{III}Por$ Soret band (λ 422 nm) with the concomitant appearance of a new Soret band (λ 445 nm). Unlike the case of the previous experiments, the rate of reaction of the histidine-complexed ferriporphyrin was so rapid that the Cary 14 spectrophotometer was not adequate to monitor it, and a stopped-flow spectrophotometer was used. With this device it was convenient to watch the appearance of a new visible peak at 540 nm which was accompanied by the simultaneous disappearance of the 550 nm peak. The results of the kinetics experiments are presented in Table II.

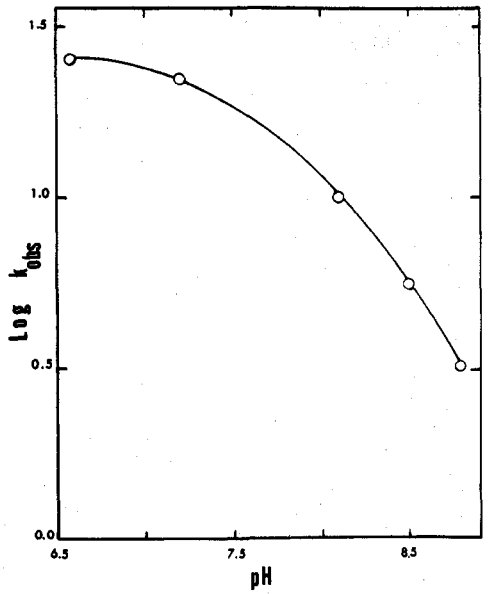

Figure 1. Plot of log k_{obsd} vs. pH for reduction of $Fe^{III}Por(his)_2$ by ascorbic acid (μ = 0.10, 25.0 °C).

Linear kinetics plots of $-\ln (A_\infty - A_t)$ vs. time were obtained, implying a rate law with first-order dependence on $Fe^{III}Por(his)_2$, with the reactant in limiting concentration. In spite of a wide variation in [ascorbic acid], observed rate constants k_{obsd} appeared independent of [ascorbic acid]. The observed pseudo-first-order rate constants were found to increase with increasing $[H^+]$.

Interpretation of the observed rate law must take into account not only the first-order dependence in $Fe^{III}Por(his)_2$ but also the insensitivity of the observed rate constant to reductant concentration. One mechanism consistent with the observed behavior consists of initial dissociation of one axial histidine, the rate-determining step, followed by subsequent transfer of electrons from the reductant (H_2A) to the monohistidine substituted ferriporphyrin.

$$Fe^{III}Por(his)_2 \xrightarrow{k_{diss}} Fe^{III}Por(his) + his \quad \text{rate limiting}$$

$$Fe^{III}Por(his) + H_2A \rightarrow Fe^{II} \text{ products}$$

The observed rate constants were found to vary only slightly over the pH range 6.57–7.20 decreasing by only a factor of 1.1 for a 4.3-fold decrease in $[H^+]$. Above pH 7.20, however, a dramatic decrease in rate constant with increasing pH is seen. A plot of log k_{obsd} vs. pH is presented in Figure 1. Thus the rate expression for the reduction of $Fe^{III}Por(his)_2$ exhibits a distinct $[H^+]$ dependence at low $[H^+]$, decreasing to low order in $[H^+]$ at higher $[H^+]$, corresponding to a saturation of the observed rate constant for reduction at higher acidities.

One mechanism which is consistent with the experimental data consists of rapid H^+ attack on the porphyrin–ligand complex, followed by the rate-limiting step, the loss of one histidine. The monosubstituted ferriporphyrin is then reduced by ascorbic acid to the Fe(II) state.

$$Fe^{III}Por(his)_2 + H^+ \rightleftharpoons Fe^{III}Por(his)_2H \quad K_a^{-1} \text{ rapid}$$

$$Fe^{III}Por(his)_2H \rightarrow Fe^{III}Por(his) + Hhis \quad k_{diss} \text{ rate determining}$$

$$Fe^{III}Por(his) + H_2A \rightarrow Fe^{II} \text{ products}$$

For such a mechanism the rate expression for the reduction of the ferriporphyrin can be expressed as follows: M_T, ML_2, and ML_2H are used to represent total metalloporphyrin, $Fe^{III}Por(his)_2$, and the protonated porphyrin–bis(histidine) complex, respectively.

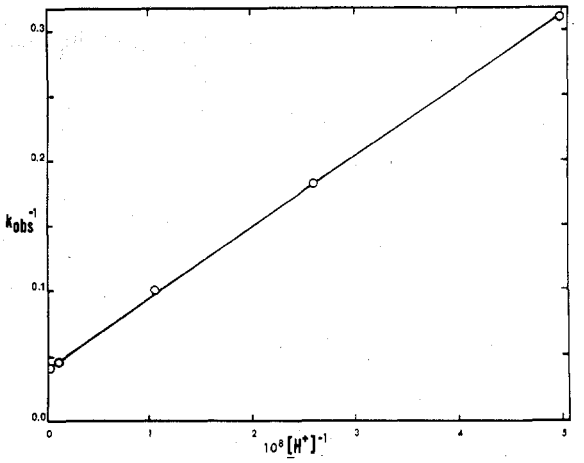

Figure 2. Plot of k_{obsd}^{-1} vs. $[H^+]^{-1}$ for reduction of $Fe^{III}Por(his)_2$ by ascorbic acid (μ = 0.10, 25.0 °C).

The equilibrium expression

$$K_a = [H^+][ML_2]/[ML_2H]$$

rearranges to give

$$[ML_2] = [ML_2H]/[H^+]K_a^{-1}$$

Since

$$[M_T] = [ML_2] + [ML_2H]$$

then

$$[M_T] = [ML_2H]/[H^+]K_a^{-1} + [ML_2H] = [ML_2H](1 + K_a[H^+]^{-1})$$

If the rate-determining step is the loss of a histidine ligand, we obtain

$$\text{rate} = -d[M_T]/dt = k_{diss}[ML_2H] = k_{diss}(1 + K_a[H^+]^{-1})^{-1}[M_T]$$

for which the observed rate constant, k_{obsd}, is

$$k_{obsd} = k_{diss}(1 + K_a[H^+]^{-1})^{-1}$$

Rearrangement to

$$k_{obsd}^{-1} = k_{diss}^{-1} + k_{diss}^{-1}K_a[H^+]^{-1}$$

indicates that k_{obsd}^{-1} should be a linear function of $[H^+]^{-1}$ with slope = $k_{diss}^{-1}K_a$ and intercept = k_{diss}^{-1}. Such a plot is indeed linear, Figure 2, least-squares analysis giving intercept k_{diss}^{-1} = (3.96 ± 0.15) × 10^{-2} s and slope = (5.47 ± 0.059) × 10^{-10} M s, leading to values of K_a and k_{diss} of (1.38 ± 0.05) × 10^{-8} M and 25.2 ± 0.93 s^{-1}, respectively (corresponding to a pK_a of 7.86).

The exact site of protonation is unknown. It is possible that the influence of the highly positively charged ferriporphyrin enhances the acidity of the α-NH_3 group of histidine (normal pK_a = 9.20).[11]

The most striking feature of the present study is the observation that the rate of electron transfer to the $Fe^{III}Por(his)_2$ is limited by the rate of dissociation of an axial ligand. Numerous studies of electron-transfer reactions of metalloporphyrin systems have been undertaken. For example, the reduction of analogous Co(III) complexes by both SO_2^- (from dissociation of $S_2O_4^{2-}$) and Cr^{2+} appear to proceed via bridged intermediates in which axial ligands such as SCN^- and pyridine act as a direct bridge for transfer of the electron from the reducing agent to the metal center.[16,17] Reduction of (tetraphenylporphine)iron(III) chloride in nonaqueous media produces $CrCl^{2+}$ as the principal product.[18] Electron transfer in these systems is not limited by axial dissociation kinetics

Table III. Kinetics Parameters for Axial Ligand Dissociation in Low-Spin Iron Porphyrin Systems

	iron porphyrin system	leaving group	k, s^{-1}	ref
1	ferricytochrome *c*	methionine-80-*S*	60	19
2	$Fe^{III}Por(his)_2$	histidine-*N*	25	this work
3	pyrroheme-(2-imid)[a]	O_2	35	31
4	myoglobin (sperm whale)	O_2	10	32
5	myoglobin (aplysia)	O_2	70	33
6	myoglobin (horse heart)	O_2	37	34
7	myoglobin (lamprey)	O_2	59	35
8	isolated α chains	O_2	31	32
9	hemoglobin	first O_2	48	36
10	hemoglobin	second O_2	28	36
11	hemoglobin	third O_2	244	36
12	hemoglobin	fourth O_2	1080	36
13	myoglobin	CO	1.7×10^{-2}	37
14	hemoglobin	CO	4.0×10^{-3}	34
15	hemoglobin	ethyl isocyanide	0.23	38

[a] Pyrroheme(2-imid) represents pyrroheme-*N*-[3-(1-imidazolyl)-propyl]amide.

since the reductant apparently utilizes the axial group as a bridging ligand.

Ascorbic acid is not able to reduce the ferriporphyrin via the bridging ligand mechanism discussed above, nor does it appear to attack the porphyrin periphery. The course of electron transfer is undoubtedly direct attack at the iron center. Perhaps the most useful comparison that can be made involves the ascorbic acid reduction of ferricytochrome *c*. Access to the iron center in cytochrome *c* (III) is limited by the rate of the heme-crevice opening. Sutin and co-workers have established that the rate constant for direct electron attack is limited by the dissociation of the axial S atom of methionine-80, for which the rate constant of 60 s^{-1} has been determined.[19] Pseudo-first-order rate constants for ascorbic acid reduction of modified cytochrome *c* (III) to cytochrome *c* (II) do not exceed this value.[4] The observation that axial dissociation occurs with the limiting rate constant of 25 s^{-1} in the present system suggests that the ascorbic acid reduction of both $Fe^{III}Por(his)_2$ and cyt *c* (III) may occur via very similar mechanisms. Indeed, the reduction of the $Fe^{III}Por(his)_2$ system by ascorbic acid resembles the similar cytochrome *c* (III) reduction more closely than it does the reduction of its parent compound $Fe^{III}Por$. The course of ascorbic acid reduction of modified cyt *c* (III) proceeds at a rate which is uninfluenced by the concentration of the reductant.[4] Similarly, the rate of reduction of $Fe^{III}Por(his)_2$ is limited only by the rate of axial ligand dissociation. Furthermore, as in the cyt *c* (III) system, $Fe^{III}Por(his)_2$ exhibits first-order dependence on the concentration of the metalloporphyrin, while $Fe^{III}Por$ exhibits a rate law with second-order dependence on [$Fe^{III}Por$]. Addition of an axial histidine ligand alters the metalloporphyrin in such a way that direct bimolecular electron transfer becomes possible. Since alterations of the axial ligand field can produce spin state changes in these ferriporphyrin systems[7,20] and since both $Fe^{III}Por$ dimer and $Fe^{III}Por(his)_2$ are low spin, whereas $Fe^{III}Por$ monomer is in the high-spin state, it appears likely that the low-spin configuration is necessary for relatively facile ferriporphyrin reduction by ascorbic acid.

Since actual electron transfer is to the mono(histidine)- rather than to the bis(histidine)ferriporphyrin complex, it is probable that the mono(histidine)ferriporphyrin has its iron center in a low-spin configuration. Indeed, the rapid addition of a second histidine to the mono(histidine) complex, observed in the spectrophotometric titrations, is best explained by the accessibility of a planar, low-spin iron.

The similarity in dissociation rate constants for axial histidine dissociation in the present study (25 s^{-1}) and for Fe–(met S) dissociation in cyt *c* (III) (60 s^{-1})[19] suggests the possibility that the exact nature of the leaving group may have little effect on the dissociation rate constant and that axial ligand dissociation proceeds by a predominantly D-type mechanism[21] (at least in low-spin ferriporphyrin systems with an imidazole-type ligand trans to the leaving group). This premise is reinforced by consideration of axial ligand dissociation rate constants for the other iron–porphyrin systems summarized in Table III. Entries 3–12 represent the first-order rate constants for dissociation of O_2 from a series of ferrohemes and ferroheme models. With the exception of the rate constants for the last two O_2 molecules to leave hemoglobin,[22] all fall within the narrow range of 10–70 s^{-1}, in surprising agreement with the values of 25 and 60 s^{-1} cited above. But these are rate constants for presumably Fe(II)–axial ligand dissociations, and comparisons are being drawn to Fe(III) dissociation rate constants. We propose that such comparisons may prove useful. Indeed, few subjects in chemistry have elicited such controversial attention as the "oxidation state" of iron in oxyhemoglobin.[23-29] The debate surrounding the geometrical aspects of $Fe–O_2$ interaction has abated since the elucidation of the angular end-on structure of $Fe–O_2$ in Collman's synthetic "picket-fence porphyrin",[30] but the questions regarding the electronic configuration continue. Essentially, the questions distill to whether $Fe–O_2$ is to be considered as (1) Fe(II) with a spin-paired dioxygen ligand or (2) $Fe^{III}(O_2^-)$ with one electron on low-spin Fe(III) antiferromagnetically coupled to one of the bound superoxide ligands. The former possibility is inferred from the facile reversibility of oxygenation and diamagnetism of the system[23] and the latter from the results of a host of spectroscopic probes.[28]

We propose that (1) axial ligand dissociation from low-spin Fe(III) porphyrins with imidazole ligands trans to the leaving group proceed via essentially dissociative mechanisms with minimal kinetic leaving-group effects and (2) oxyhemoglobin, oxymyoglobin, and ferroheme model compounds with low-spin configuration and "proximal" imidazole ligands trans to O_2 are actually best represented as having considerable $Fe^{III}(O_2^-)$ contribution. Indeed, this last proposal is further justified when one considers the rate constants for axial ligand dissociation from an "authentic" Fe(II) heme bound by a nonoxidizing ligand. Rate constants for CO[34,37] and ethyl isocyanate[38] dissociation from (carbon monoxide)myoglobin, (carbon monoxide)hemoglobin and (ethyl isocyanato)hemoglobin are 1.7×10^{-2}, 4.0×10^{-3}, and 2.3×10^{-1} s^{-1}, respectively, considerably lower than any of the other values given in Table III. We propose that these exceptionally low values are not solely attributable to the nature of the leaving group (e.g., to extensive M→L back-bonding by CO) but that they reflect an actual difference in the effective oxidation state of the central metal ion.

Experiments designed to explore the effects of other ligand groups in both ferri- and ferroporphyrins are in progress in these laboratories.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the Faculty Research Council, California State University, Northridge, for support of this research. We thank Professor F. L. Harris for helpful advice.

Registry No. $Fe^{III}Por(his)_2^{5+}$, 67180-34-1; ascorbic acid, 50-81-7.

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Kinetics and Mechanism of Aquation and Formation Reactions of Carbonato Complexes. 14. Base-Catalyzed Carbonato Ring Opening of Carbonato(β,β',β''-triaminotriethylamine)cobalt(III) Perchlorate and Spontaneous Decarboxylation of Aquobicarbonato(β,β',β''-triaminotriethylamine)cobalt(III) Ion[1]

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Received May 25, 1978

Base hydrolysis of the title compound enables the preparation of the uncharged dechelated species Co(tren)(OH)(CO_3). In acidic solution, the latter protonates to form Co(tren)(OH_2)(HCO_3)$^{2+}$, which undergoes rapid decarboxylation (k = 1.19 s^{-1} at 25 °C, $\Delta H^{\ddagger}$ = 14.3 ± 0.4 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −10.2 ± 1.2 cal deg^{-1} mol^{-1}) by a mechanism completely analogous to that of acid-catalyzed decarboxylation of related pentaaminecobalt(III) complex ions. The rate of base-catalyzed dechelation of the title compound is quite slow (k = 3.6 × 10^{-3} M^{-1} s^{-1} at 25 °C, $\Delta H^{\ddagger}$ = 17.2 ± 1.9 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −12 ± 6 cal deg^{-1} mol^{-1}) and is succeeded by even slower carbonate ion elimination to yield as the final product Co(tren)$(OH)_2^+$ (k = 8.2 × 10^{-6} M^{-1} s^{-1} at 25 °C, $\Delta H^{\ddagger}$ = 24.7 ± 1.2 kcal mol^{-1}, $\Delta S^{\ddagger}$ = 1.0 ± 4.0 cal deg^{-1} mol^{-1}). Comparisons with the results of related base-hydrolysis studies strongly suggest that the mechanism is one of direct dissociative substitution involving metal–oxygen fission in both steps of the carbonate release.

Introduction

Our series of studies of the decomposition reactions of chelated carbonato complexes of cobalt(III) has so far focused entirely on the proton-promoted ring-opening and decarboxylation processes, such as previously reported[2] for the title complex ion, Co(tren)$(CO_3)^+$ (tren ≡ β,β',β''-triaminotriethylamine). Furthermore, in these acidic systems, cabonato chelate ring opening is the rate-determining step except at high acid concentration, where the rate of ring opening can effectively catch up with[2,3] or even substantially exceed[4] the rate of CO_2 release. Base-catalyzed hydrolysis of chelated carbonato species is also known to occur, though at a very slow rate relative to the acid-induced process for the same complex. Furthermore, the ring-opened intermediate is known to be quite stable, in the case both of Co(tren)(OH)(CO_3) (from the base hydrolysis of Co(tren)$(CO_3)^+$)[5] and of Co$(en)_2$(OH)(CO_3) (from Co$(en)_2(CO_3)^+$, where en ≡ ethylenediamine).[5-9] So far, detailed studies of the kinetics of base hydrolysis of a chelated carbonato complex have been reported only for the Co$(en)_2(CO_3)^+$ species,[7,9] for which the two-step S_N1cB mechanism has been proposed. These studies, however, are subject to some uncertainties of interpretation, since cis/trans isomerization can occur in the case of both the intermediate and the final products, Co$(en)_2$(OH)(CO_3) and Co$(en)_2(OH)_2^+$, and may indeed play a significant role in the reaction mechanism. We feel, therefore, that an investigation of the kinetics of base hydrolysis of the geometrically rigid complex Co(tren)$(CO_3)^+$ should enable some clarification of the nature of this type of reaction. A further motivation for this work is the fact that the reported stable intermediate, Co(tren)(OH)(CO_3), can be used as the starting material with which to study the acid-catalyzed decarboxylation. This enables one to bypass the proton-promoted ring-opening reaction, which has usually been the only unequivocally observable process in carbonato chelate aquation studies.[3]

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0020-1669/78/1317-3123$01.00/0