Contribution from the College of Oceanography,
Oregon State University, Corvallis, Oregon 97331

Rates of Nitrous Oxide Production in the Oxidation of Hydroxylamine by Iron(III)

James H. Butler*† and Louis I. Gordon

Received February 12, 1986

The dependence of the rate of N_2O formation on the reactants and on pH in the oxidation of NH_2OH by Fe(III) has been investigated over a range of reactant concentrations, with emphasis on low concentrations of hydroxylamine. The production rate of N_2O was pseudo first order with respect to total iron, pseudo first order or less with respect to total hydroxylamine, and inversely proportional to $[H^+]^{2.5}$. The reaction is highly sensitive to temperature. Of the competing reactions producing nitrogenous products other than N_2O, at least one is catalyzed by light. An empirical rate law, based on the total concentrations of the reactants, is presented, and a more general rate law, based on the concentrations of presumed reactive species, is derived.

Introduction

Hydroxylamine is reactive and unstable in oxygenated water.[1] In aqueous solution, it is oxidized readily by oxygen, peroxide, and an array of transition-metal cations;[2-4] it also will condense with aldehydes and ketones to form oximes.[5] These properties of hydroxylamine make it particularly valuable for industrial and synthetic applications, but also render it labile and, historically, difficult to analyze with good precision.[6,7] In natural waters, where hydroxylamine is produced and consumed by nitrifying bacteria, both its biochemical activity and its abiotic lability result in nanomolar to submicromolar concentrations that, until recently, have eluded detection and measurement by aquatic chemists and biologists.[8,9]

Recently, we and our colleagues have developed and improved a gas chromatographic technique for the analysis of nanomolar levels of hydroxylamine in natural waters.[10,11] As for many earlier techniques,[7] this method relies on the quantitative oxidation of hydroxylamine by Fe(III), where

$$4Fe(III) + 2NH_2OH = 4Fe(II) + N_2O + H_2O + 4H^+ \quad (1)$$

Our technique differs from earlier ones in that, rather than measuring the residual Fe(II), we analyzed for N_2O by electron-capture gas chromatography.[12] This has led to a far greater sensitivity than reported before and, for the first time, permits the measurement of hydroxylamine at ambient concentrations in aquatic systems.

However, in developing the technique, we found that the amount of N_2O produced was not stoichiometric with hydroxylamine as indicated by eq 1, that the change in N_2O concentration depended systematically on the pH and salinity of the solution, and that cations, notably Cu(II) and Hg(II), negatively interfered.[11] Because the effect of salinity on the production of N_2O could not be attributed alone to ionic strength, we concluded that the difference must be a result of the kinetics of competing reactions. These competing reactions, however, must provide for the oxidation of hydroxylamine to a +1 state by Fe(III) if Fe(II) is to be produced quantitatively. These considerations led us to investigate further the kinetics of this reaction under conditions that are near those of the analytical method applied to natural waters. We present here our findings on the effects of iron, hydroxylamine, and hydrogen ion concentrations on the kinetics of the oxidation of hydroxylamine by Fe(III) in acid solution.

Experimental Section

Apparatus and Reagents. The apparatus and reagents are described in detail in an earlier paper.[11] All chemicals used were analytical reagent grade. Hydroxylammonium ($pK_a = 5.97$) stock solutions were acidified to pH 3 and stored at 2 °C between experiments to retard degradation, and working standards were prepared fresh daily. Nitrous oxide was measured by electron-capture gas chromatography with a technique that involves stripping the N_2O from solution onto a liquid-nitrogen-cooled trap for subsequent injection into the gas chromatograph.[12] The coefficient of variation ($s/x \times 100$) for a single analysis of N_2O was about 2%.

Procedure. Our approach involved primarily the measurement of initial rates of N_2O production following the injection of hydroxylammonium standard into a suitable reaction medium in a gastight container. Fe(III) concentrations were varied from 80 nM to 1.2 mM, hydroxylammonium concentrations from 80 nM to 4 μM, and pH from 1.0 to 2.6. Because of the pronounced effect of pH on reaction rate, the most reliable measurements of the reaction rate were attained below pH 1.7. Tests of incremental amounts of iron and hydroxylammonium were conducted in solutions of pH 1.40. N_2O was measured when the reaction was between 5% and 10% complete, usually about 1.5 h, but much shorter at higher pH.

For each series of experiments, deionized, distilled water (DDW) was acidified to the appropriate pH with HCl and distributed into acid-washed, DDW-rinsed, volume-calibrated, 150-mL bottles with greased, ground-glass stoppers. A few glass beads (approximate volume, 1.0 mL) were added to each bottle to enhance mixing of the reagents. Ferric ammonium sulfate (FAS) was injected into each bottle to obtain the desired concentration, and the bottle was restoppered and shaken to distribute the Fe(III). A hydroxylammonium standard was then injected into each bottle, which was shaken again and allowed to stand for 1.5 h before analysis for N_2O. Analysis of N_2O involved drawing a portion of the sample into a syringe and injecting it via a septum into the stripping flask of the gas chromatograph; the pH of the solution in the stripping flask was kept below 0.5 to stop the reaction during stripping, which took about 7 min. Corrections for N_2O originally in the reaction medium and for the effects of reagent and standard additions on pH were included in the calculations.

In addition to the measurements of initial reaction rate, the reaction in the presence of excess Fe(III) was followed to completion at pH 1.0 and 1.4. Potential surface effects were evaluated by measuring the reaction rates in bottles of different volume, and the role of a potentially interfering free-radical mechanism, the decomposition of hyponitrous acid to N_2 and NO_3^-, was investigated by conducting the reaction at pH 3 in the presence of 0.1 mol % of ethanol.[13] The effect of temperature was observed by conducting the reaction at 1.8 and 20.4 °C.

Results

Effect of Total Fe(III) on the Reaction Rate. With Fe(III) added in considerable excess of hydroxylamine, the production

† Present address: Cooperative Institute for Research in Environmental Sciences, University of Colorado/NOAA, Boulder, CO 80309.

(1) Moews, P. C.; Audrieth, L. F. *J. Inorg. Nucl. Chem.* **1959**, *11*, 242.
(2) Szilard, I. *Acta Chem. Scand., Ser. A* **1963**, *A17*, 2674.
(3) Erlenmeyer, H.; Flierl, C.; Sigel, H. *J. Am. Chem. Soc.* **1969**, *91*, 1065.
(4) Hughes, M. N.; Nicklin, H. G. *J. Chem. Soc. A* **1971**, 164.
(5) Sharon, N.; Katchalsky, A. *Anal. Chem.* **1952**, *24*, 1509.
(6) Bray, W. C.; Simpson, M. E.; Mackenzie, A. A. *J. Am. Chem. Soc.* **1919**, *41*, 1363.
(7) Kolasa, T.; Wardenki, W. *Talanta* **1974**, *21*, 845.
(8) Baxter, R. M.; Wood, R. B.; Prosser, M. V. *Limnol. Oceanogr.* **1953**, *18*, 470.
(9) Fiadero, M.; Solorzano, L.; Strickland, J. D. H. *Limnol. Oceanogr.* **1967**, *12*, 555.
(10) von Breymann, M. T.; deAngelis, M. A.; Gordon, L. I. *Anal. Chem.* **1982**, *54*, 1209.
(11) Butler, J. H.; Gordon, L. I. *Mar. Chem.* **1986**, *19*, 229.
(12) Cohen, Y. *Anal. Chem.* **1977**, *49*, 1238.
(13) Buchholz, J. R.; Powell, R. E. *J. Am. Chem. Soc.* **1963**, *85*, 509.
(14) Kester, D. R.; Byrne, R. H.; Liang, Y. *ACS Symp. Ser.* **1975**, *No. 18*, 56.
(15) Stumm, W.; Morgan, J. J. *Aquatic Chemistry*; Wiley: New York, 1981.
(16) Willix, R. L. S. *Trans. Faraday Soc.* **1963**, *59*, 1315.

0020-1669/86/1325-4573$01.50/0

Table I. Distribution of Fe(III) Species in Aqueous Solution as a Fraction of Total Fe(III) Concentration at pH 1.40[a]

	fraction of total [Fe(III)]						
[FAS][e]	Fe^{3+}	$FeOH^{2+}$ $pK = 2.37$[b]	$Fe(OH)_2^{+}$ $pK = 7.13$[b]	$Fe_2(OH)_2^{4+}$ $pK = 2.33$[c]	$FeCl^{2+}$ $pK = -1.1$[b]	$FeCl_2^{+}$ $pK = -0.28$[b]	$FeSO_4^{+}$ $pK = -3.16$[d]
6.8	0.616 158	0.065 956	0.000 029	0.000 008	0.309 966	0.001 877	0.006 008
27.0	0.605 584	0.064 823	0.000 028	0.000 029	0.304 642	0.001 844	0.023 050
54.2	0.592 413	0.063 412	0.000 028	0.000 056	0.298 010	0.001 804	0.044 277
169.0	0.546 894	0.058 535	0.000 026	0.000 149	0.275 092	0.001 665	0.117 640
1018.0	0.393 057	0.042 029	0.000 018	0.000 462	0.197 518	0.001 196	0.365 720

[a] Calculated for $[Cl^-] = 0.04$ M and ionic strength $I = 0.04$ M and with ferric ammonium sulfate (FAS) as the source of Fe(III). [b] Reference 14. [c] Reference 15. [d] Reference 16. [e] Units for [FAS] are μM.

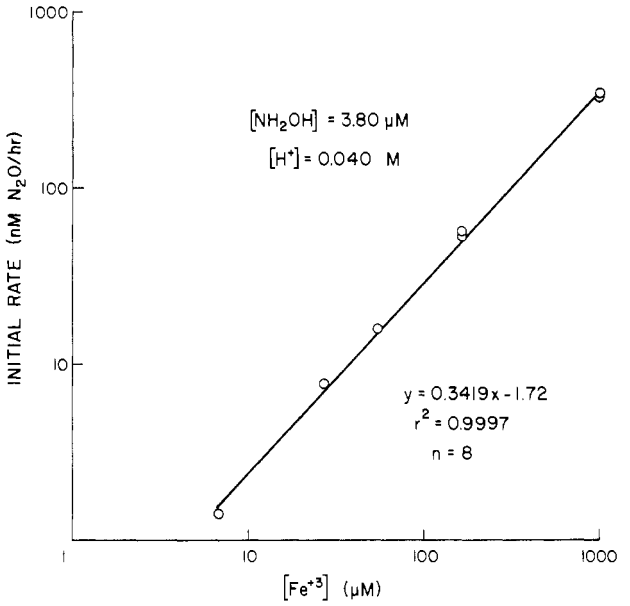

Figure 1. Initial N_2O production rate vs. total Fe(III) concentration. Although the curve is shown as a log–log plot, the regression equation is based on the linear data.

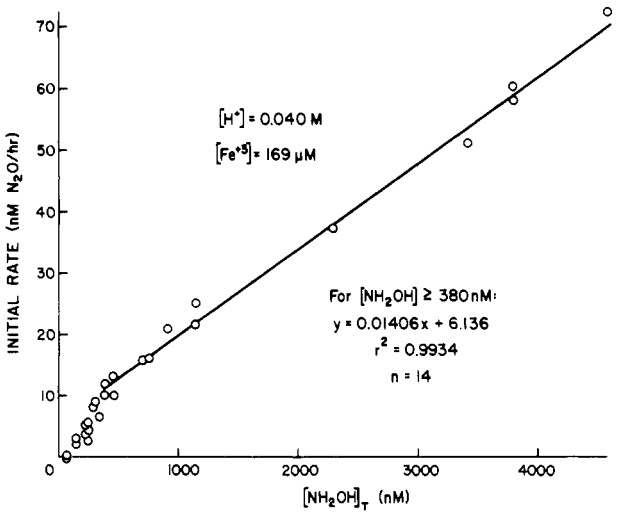

Figure 2. Initial N_2O production rate vs. total NH_2OH concentration. The regression equation shown is for those points where $[NH_2OH]_T$ is 380 nM or greater. These tests were conducted in clear bottles, exposing the samples to ambient laboratory light.

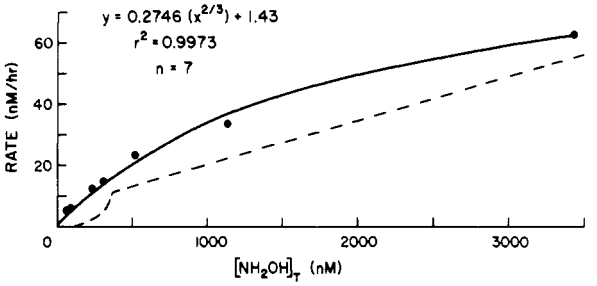

Figure 3. Initial N_2O production rate vs. $[NH_2OH]$ in opaque bottles. For comparison, the dashed line represents N_2O production in clear bottles (Figure 2).

of N_2O appeared pseudo first order with respect to Fe(III) over nearly 3 orders of magnitude (Figure 1). This was unexpected because the stoichiometry of the reaction according to eq 1 requires the consumption of four Fe(III) ions for every N_2O produced. However, there is no reason to expect that the reaction would be as simple as written in eq 1; recent investigations indicate the production of nitrogenous compounds other than N_2O, requiring a sequence of reactions.[10,11]

The pseudo-first-order dependence on total Fe(III) is not explained by iron speciation alone. The proportions of all species change with increasing [FAS] (Table I), the concentrations of the hydroxylated monomers and chlorides dropping in response to significant increases in $[FeSO_4^+]$. The fraction of $Fe_2(OH)_2^{4+}$, a particularly appealing form in that the reaction requires the transfer of two electrons to each hydroxylamine, increases linearly with total Fe(III). Thus, doubling the concentration of total Fe(III) results in a quadrupling of $Fe_2(OH)_2^{4+}$ concentration. Were the reaction rate dependent on the dimer, it therefore would have to be a square-root dependence, an unlikely circumstance on inspection. $FeSO_4^+$ also could be ruled out on similar grounds. If the rate-limiting step does involve a particular Fe(III) species, then one of the other monomeric forms, for which the concentration changes much less with Fe(III), would be more likely.

Effect of Total Hydroxylamine on the Reaction Rate. The relationship of the N_2O production rate to total hydroxylamine was somewhat complicated. Above a hydroxylamine concentration of 380 nM the reaction appeared pseudo first order; however, the line generated by those points missed the origin significantly and, below 380 nM hydroxylamine, the data, although scattered, defined a steeper curve (Figure 2). Below 80 nM, the production of N_2O was insignificant. This pattern implied the involvement of other reactions that may have predominated at lower concentrations. We found no apparent bottle effect at micro- or nanomolar concentrations of hydroxylamine, indicating that neither the production of N_2O nor any major competing reactions were catalyzed by glass surfaces. However, we did find that ambient light in the laboratory negatively affected the production rate of N_2O (Figure 3). This phenomenon was virtually zero order with respect to hydroxylamine concentration; hence, its relative effect was more pronounced at lower hydroxylamine concentrations. Hydroxylamine is photooxidized, both as a vapor[17,18] and in aqueous solution,[19] yielding products other than N_2O. There is no evidence, however, that the same mechanism governs this reaction.

By allowing the reaction to go to completion in both light and dark bottles at pH 1.4, we found that the total production of N_2O also was reduced by light (Figure 4). The curves in Figure 4 are pseudo first order according to eq 2, where $[N_2O]_t$ represents

$$[N_2O]_t = [N_2O]_{max}(1 - e^{-k_{N2O}t}) \quad (2)$$

(17) Smith, R. N.; Leighton, P. A. *J. Am. Chem. Soc.* **1944**, *66*, 172.
(18) Betts, J.; Back, R. A. *Can. J. Chem.* **1965**, *43*, 2678.
(19) Behar, D.; Shapira, D.; Treinin, A. *J. Phys. Chem.* **1972**, *76*, 180.

Table II. Fe(III) and Hydroxylamine Speciation and Measured N_2O Production Rates from pH 1.0 to 1.7[a]

pH	N_2O prod rate	$[Fe(OH)^{2+}]$	$[Fe_2(OH)_2^{4+}]$	$[Fe(OH)_2^{+}]$	$[NH_2OH]$	$k(o)'$	$k(o)''$
0.99	−8.52	−5.40	−8.39	−9.17	−10.40	9.99	6.56
1.09	−8.26	−5.30	−8.19	−8.97	−10.30	9.95	6.55
1.22	−7.81	−5.18	−7.94	−8.72	−10.17	10.01	6.65
1.31	−7.57	−5.09	−7.77	−8.54	−10.18	9.99	6.66
1.39	−7.37	−5.01	−7.62	−8.38	−10.00	9.94	6.64
1.49	−7.12	−4.92	−7.43	−8.19	−9.90	9.90	6.63
1.61	−6.82	−4.81	−7.21	−7.96	−9.78	9.84	6.62
1.69	−6.63	−4.74	−7.07	−7.81	−9.70	9.80	6.59

[a] Fe^{3+}, $FeCl^{2+}$, $FeCl_2^{+}$, and $FeSO_4^{+}$ have been omitted as they are essentially constant. The N_2O production rates ($M\ h^{-1}$) are expressed as log values; hydroxylated Fe(III) species and un-ionized hydroxylamine are molar concentrations expressed as log values. Total Fe(III) concentration is 169 μM; total hydroxylamine concentration is 3.8 μM. $k(o)'$ and $k(o)''$ are as described in the text. Formation constants are as noted in Table I.

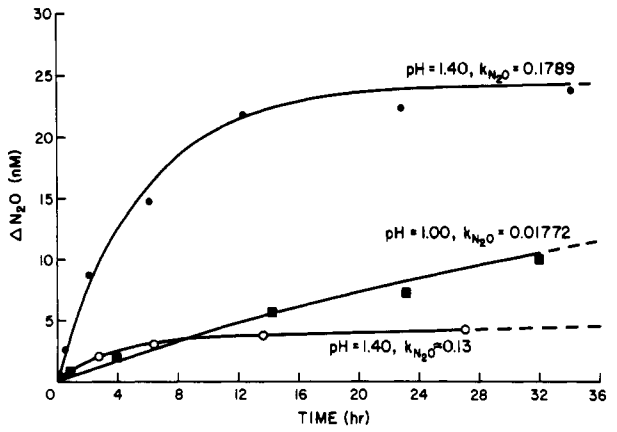

Figure 4. N_2O production vs. time: (●) opaque bottles at pH 1.40; (■) opaque bottles at pH 1.00; (○) clear bottles at pH 1.4. Initial NH_2OH concentration in all bottles was 76 nM. $[N_2O]_{max}$ for the opaque bottles was 65% of theoretical, or 24.7 nM, and for the clear bottles was 8% of theoretical, or 3 nM. k_{N_2O} values are expressed as h^{-1}.

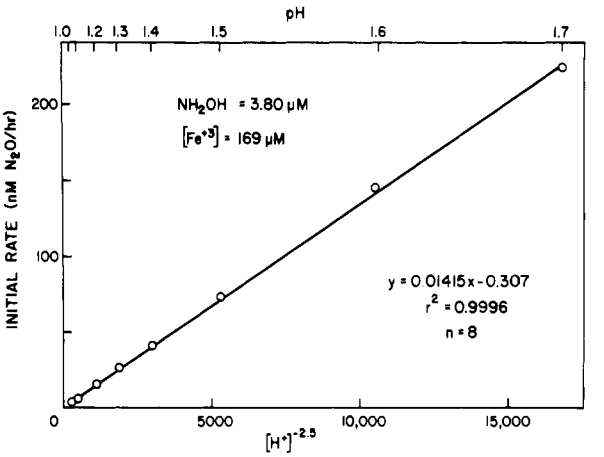

Figure 5. Initial N_2O production rate vs. $1/[H^+]^{2.5}$.

the amount of N_2O produced at time t, $[N_2O]_{max}$ represents the amount of N_2O produced by the reaction at completion, and k_{N_2O} is the first-order rate constant. Because the maximum amount of N_2O produced dropped considerably in the presence of light, yet the apparent rate constant changed very little, it is likely that the light catalyzed a competing reaction, rather than retarding the reaction that gave rise to N_2O.

The effect of hydroxylamine on the reaction rate in these experiments would seem defined by three concentration ranges. However, a more plausible explanation, and one that is consistent with the results from dark-bottle experiments (Figure 3), is that the reaction is less than first order with respect to hydroxylamine over the entire range tested and that the apparent, higher order relationship between 80 and 380 nM is an artifact produced by the competing, light-catalyzed reaction. The relationship to hydroxylamine concentrations then would appear first order over a limited range, as was observed. The less than first-order fit could be the result of either inhibition of an activated complex or competition with yet another reaction.

Effect of Hydrogen Ion on the Reaction Rate. The rate of N_2O production was inversely proportional to $[H^+]^{2.5}$ within the pH range 1.0–1.7 (Figures 4 and 5). This nonintegral, higher order effect implies a complex involvement of hydrogen ion with the reactants, indicating that the speciation of the reactants is important in the production of N_2O.

At low pH, hydroxylamine is present predominantly as the hydroxylammonium ion (pK_a = 5.97). However, the reactive species is most likely the un-ionized form. Investigators working with other cations have suspected this,[4,20,21] and the rapid disappearance of hydroxylamine from alkaline solutions supports this contention.[1] Futher, a molecule with a free electron pair would have a greater affinity for a cation than would another positively charged ion. The effect of H^+ on the concentration of the hydroxylammonium ion at low pH is insignificant; however, an increase in pH from 1.0 to 1.7 results in a 5-fold increase in the concentration of un-ionized hydroxylamine (Table II).

Similarly, the effect of pH on the concentrations of the chlorides, sulfate, and unassociated Fe(III) is nil, but the hydroxylated forms vary significantly with changing pH (Table II). If we assume that the only effect of the hydrogen ion in the reaction is that of altering the relative composition of the reactive species, we can use these data to deduce which of these species are reacting. For the reaction

$$4Fe(OH)_2^{+} + 2NH_2OH = 4Fe(OH)^{+} + N_2O + 5H_2O \quad (3a)$$

where NH_2OH is the un-ionized hydroxylamine in solution, the second-order rate law would be

$$\frac{d[N_2O]}{dt} = k(o)'[Fe(OH)_2^{+}][NH_2OH] \quad (3b)$$

$k(o)'$ is almost constant over the pH range tested, although a systematic trend is present (Table II). Since the concentrations of $Fe(OH)_2^{+}$ and NH_2OH are nearly proportional to the concentrations of their total counterparts, a reaction such as this would be consistent with the apparent first-order dependencies on Fe(III) and total hydroxylamine.

Other Observed Effects. In our previous work,[11] we noted that the maximum amount of N_2O produced in the oxidation of hydroxylamine by Fe(III) was around 80% of that expected for a complete reaction consistent with eq 1. This maximum was attained at pH 3. We postulated a reaction sequence, based on our work and the findings of other investigators, that involved *cis*- or *trans*-hyponitrite as an intermediate. Buchholz and Powell,[13] working with *trans*-hyponitrite, found that a free radical reaction of hyponitrite interfered with the production of N_2O below pH 1.4 and that the interfering reaction could be inhibited completely by addition of excess ethanol as an OH radical trap. In this study, we added ethanol to a concentration of 0.1 mol % in our reaction

(20) Anderson, J. H. *Analyst (London)* **1966**, *91*, 532.
(21) Jindal, V. K.; Agrawal, M. C.; Mushran, S. P. *J. Chem. Soc. A* **1970**, 2060.

Table III. Effect of Temperature on the Production Rate of N_2O[a]

temp, °C	rate, nM/h	n	s
1.8	6.7	4	0.5
20.4	134.1	4	4.2

[a] [Fe(III)] = 1.69 μM; $[NH_2OH]_T$ = 3.8 μM; pH 1.4.

bottles and found that the maximum production of N_2O from hydroxylamine at pH 3 was still 80% of theoretical. This result indicates that *trans*-hyponitrite is not an intermediate in the reaction sequence and that the production of N_2O is not a chain reaction involving OH radicals. *cis*-Hyponitrite could be involved, but it also is possible that the reaction does not involve any free nitrogenous intermediates and that N_2O is formed directly from an iron–nitrogen complex.

The rate of N_2O production was strongly temperature dependent (Table III). An increase of 18.6 °C accelerated the reaction rate by 20 times. This would correspond to an activation energy of 25.9 kcal/mol and a van't Hoff Q_{10} of 5.0, both of which are somewhat high but are in reasonable agreement with findings of an earlier investigation.[22] The entropy of activation at the reaction pH of 1.4 then would be 5.3 eu. These activation numbers at this time are only tentative, owing to the possible involvement of another reaction that may be more or less temperature sensitive than the production of N_2O. Another problem is that, over this temperature range, the concentration of OH^- more than doubles; were OH^- directly involved as part of the rate determining sequence, our values would be overestimates of the actual parameters. Hydroxyl ion may be involved directly in the reaction, but a more likely prospect is that discussed below, where the primary effect of pH is on the speciation of the reactants.

Discussion

Rate Laws. These results lead to two possible rate law expressions. One is empirical and based on the total concentrations of the reactants in solution. The other is a more fundamental rate law and takes into account the speciation of the reactants and the role of competing reactions. The empirical rate law for N_2O production as a function of the total hydroxylamine, total iron, and pH is

$$\frac{d[N_2O]}{dt} = k_0\frac{[Fe(III)][NH_2OH]_T}{[H^+]^{2.5}} \quad (4)$$

where [Fe(III)] and $[NH_2OH]_T$ represent the total concentrations of ferric iron and hydroxylamine. This would apply for Fe(III) concentrations of up to 1.2 mM, for hydroxylamine concentrations of 0.4-4 μM, and for a pH range 1.0–1.7. Extrapolation beyond these ranges is inadvisable since the relationship with hydroxylamine is not truly linear and we know so little of the nature of the competing reactions. Using data from the curves in Figures 1, 2, and 5, we arrive at a rate constant, k_0, of 0.025 $M^{1.5}$ h^{-1} (6.92×10^{-6} $M^{1.5}$ s^{-1}) for the reaction.

At lower concentrations of hydroxylamine, the empirical rate law still applies if the range is not too great. For reactions with concentrations of hydroxylamine of less than 80 nM (Figure 4), k_0 can be calculated from k_{N_2O} by using eq 5, where k_0, k_{N_2O},

$$k_0 = \frac{[N_2O]_{max}}{2[N_2O]_{th}}\left(\frac{[H^+]^{2.5}}{[Fe(III)]}\right)k_{N_2O} \quad (5)$$

$[N_2O]_{max}$, and [Fe(III)] are as described earlier and $[N_2O]_{th}$ is the amount of N_2O that would be produced were all hydroxylamine converted to N_2O. For the dark-bottle reaction at pH 1.4, k_0 at these low concentrations was about 0.107 $M^{1.5}$ h^{-1} (3.0×10^{-5} $M^{1.5}$ s^{-1}); for the reaction in clear bottles at the same pH, k_0 was about 0.08 $M^{1.5}$ h^{-1} (2.2×10^{-5} $M^{1.5}$ s^{-1}), although, as noted earlier, variability in the reaction with such low recoveries make the latter number less reliable.

A more theoretically based rate law must consider the lower order of the reaction with respect to hydroxylamine and must address the speciation of both reactants. The N_2O production rate in dark bottles was best described by a $^2/_3$ power function for hydroxylamine (Figure 3), implying an inhibitory role of hydroxylamine in the reaction. Considering this relation along with the speciation data (Table II), the rate law then becomes

$$\frac{d[N_2O]}{dt} = k(o)''[Fe(OH)_2^+][NH_2OH]^{0.67} \quad (6)$$

where the power of 0.67 for NH_2OH represents the empirically derived order for a greater range of hydroxylamine concentrations. This equation, which represents a reaction that does not directly involve H^+ or OH^-, gives a much better fit for the rate constant than does eq 3b (Table II).

Mechanism. A two-electron transfer from iron as an iron dimer ($Fe_2(OH)_2^{4+}$) to hydroxylamine is an attractive step in the mechanism. However, as already noted, direct involvement of $Fe_2(OH)_2^{4+}$ is unlikely. This leaves us with a rather unsatisfactory set of circumstances and any of a number of possible mechanisms. A further complication is a previous finding that Fe(II) can negatively affect the reaction rate.[22] Our data do not support such an involvement by Fe(II), as the reaction remains pseudo first order through completion (Figure 4). The lack of an apparent effect may be the result of only minute quantities of Fe(II) being produced in our experiments.

Nevertheless, to meet the requirements of first-order dependence on Fe(III) and first-order, or less than first-order, dependence on hydroxylamine, the reaction must involve, as an initial step, the formation of an activated complex comprising a single Fe(III) ion and a single NH_2OH molecule. For example, the role of H^+ can be explained if we consider that the initial reactants are $Fe(OH)_2^+$ and free NH_2OH (Table II). A reaction sequence similar to the one following, then, would explain the dependencies on iron and hydroxylamine:

$$Fe(OH)_2^+ + NH_2OH = [Fe(OH)_2NH_2OH]^+ \quad (7)$$

$$[Fe(OH)_2NH_2OH]^+ = [FeOHNHOH]^+ + H_2O \quad (8)$$

$$[FeOHNHOH]^+ = [FeOHNHO] + H^+ \quad (9)$$

$$Fe(III) + FeOHNHO = Fe(II) + Fe(OH)^+ + HNO + H_2O \quad (10)$$

$$2HNO = N_2O + H_2O \quad (11)$$

Electron transfer and reorganization of the transition complex (eq 8) would need to be rate-limiting and independent of ferric ion and hydroxylamine to maintain first-order dependencies; however, hydroxylamine (or hydroxylammonium) may play an inhibitory role at this point, thus accounting for the less than first-order relationship. The involvement of a second ferric ion (eq 10) should follow decomposition of the activated complex to assure both the reaction order and the stoichiometry of iron according to eq 1. In this reaction, the form of Fe(III) is not specified. The steps remaining after eq 10 also must be fast and must favor dimerization of oxidized N. Because the probability of encounters among reactive intermediates is low, the sequence must involve a number of highly specific reactions leading to the production of N_2O.[11]

Equation 1 does not adequately describe the reaction of Fe(III) with hydroxylamine. One or more reactions, at least one of which is catalyzed by light, yield nitrogenous products other than N_2O. However, it also is apparent from other studies[7] that the amount of Fe(II) produced is consistent with eq 1. If this is true, then the competing reactions either must occur after the Fe(III) has oxidized the hydroxylamine to a +1 intermediate, as postulated in the above reaction sequence, or if they involve the formation of different ferric hydroxylamine complexes, then they must adhere to the same stoichiometry; later steps could then lead to any of a number of products.

Further understanding of the oxidation of hydroxylamine by Fe(III) requires additional study of the competing reactions and their products, particularly with respect to the role of light. Whether the reaction catalyzed by light is the same as the reaction

(22) Mitchell, A. D. *J. Chem. Soc.* **1926**, *129*, 336.

that remains operative in the dark and whether these reactions proceed by formation of separate complexes with iron or, alternatively, involve the reorganization of an oxidized nitrogenous intermediate are questions that need to be resolved before the reaction mechanism can be fully elucidated.

Acknowledgment. This study was funded by the Marine Chemistry Program of the National Science Foundation under Grant No. OCE-8409069, for which we are deeply grateful. We also acknowledge support from the Air Resources Laboratory (GMCC) of the National Oceanic and Atmospheric Administration. We thank Dave Carlson and James Krueger for valuable discussions and for reviewing the manuscript.

Registry No. NH_2OH, 7803-49-8; Fe^{3+}, 20074-52-6; N_2O, 10024-97-2.

Contribution from the Section de Biologie, U. 219 INSERM, Bât. 112, Institut Curie, Centre Universitaire, 91405 Orsay Cedex, France

Electron Paramagnetic Resonance Studies of the Intramolecular Coordination in Superstructured Iron(III) Porphyrins

Corinne Schaeffer,* Michel Momenteau, Joël Mispelter, Bernard Loock, Christiane Huel, and Jean-Marc Lhoste

Received February 28, 1986

The electron paramagnetic resonance spectra of iron(III) both face hindered porphyrins (basket-handle porphyrins) in which oxygen or nitrogen ligand donors are incorporated into one of the two handles are reported in the absence or in the presence of exogenous ligands. The ESR spectra of the ferric chloride complexes of compounds α-5,15-[2,2′-(6-hydroxyundecanediamido)diphenyl]-β-10,20-[2,2′-(dodecanediamido)diphenyl]porphyrin ((**11**)Cl), α-5,15-β-10,20-bis[2,2′-(6-hydroxyundecanediamido)diphenyl]-porphyrin ((**13**)Cl), and α-5,15-[2,2′-(6-hydroxyundecanediamido)diphenyl]-β-10,20-[2,2′-(pyridine-3,5-diyldipropionamido)diphenyl]porphyrin ((**14**)Cl) in the presence of tributylamine are characteristic of high-spin five-coordinated complexes with a strong rhombic distortion attributed to the intramolecular coordination of the alkoxo group. A similar spectrum is obtained directly from the compound {α-5,15-[2,2′-(6-oxyundecanediamido)diphenyl]-β-10,20-[2,2′-(3,3′-(*p*-phenylene)dipropionamido)diphenyl]-porphyrin}iron(III) (**12**), which elutes from thin-layer chromatography without exogenous counterion. Addition of sodium alkoxide or 1-methylimidazole to a chloroform solution of these compounds produces low-spin six-coordinated complexes. Their ESR spectra depend not only on the nature of the appended ligand but also on the nature of the starting counterion of iron(III) and on the exogenous ligand type. From the analysis of ESR *g* values, which describes the tetragonal and rhombic distortion of the octahedral ligand field, it was possible to identify the axial ligands in mixed-ligand or bis(ligand) complexes. The formation of the different complexes are discussed with respect to the electronic properties and constraint of the axial ligands.

Introduction

The state and the nature of the heme iron–ligand bonds are closely related to the function of hemoproteins. Thus, imidazole is known to coordinate axially to iron in hemoglobin, myoglobin, cytochromes, and some peroxidases.[1-3] Anionic ligands such as imidazolate,[4] mercaptide,[5] and phenoxide[6] have also been proven as proximal iron ligands in other type of hemoproteins. In particular, the latter ligand may play a determinant role in controlling the catalytic activity of catalase.[7] Such a ligand is also found in several hemoglobin mutants that permanently remain oxidized (Fe(III)) in vivo where the proximal or distal histidines are replaced by tyrosine, in either the α or β chain.[6,8,9] Simulations of the coordination sphere of iron in these hemoproteins have been previously performed by reactions of simple iron porphyrins with free alkoxides and phenoxides.[10-14] Another approach is to use compounds in which the axial ligand is covalently attached to the porphyrin ring. These compounds permit the strict control of the coordination number on the iron and eliminate the need for excess free ligand in solution.[15-18] We have applied with success this concept in the ether- and amide-"basket-handle" porphyrins, both involving a pyridine or imidazole ligand on one side,[19] in order to study the main factors for O_2 and CO binding in hemoglobin and myoglobin.[20]

The present work reports on the ESR study of the interaction of iron(III) amide-basket-handle porphyrins in which alcohol, alkoxide, pyridine, or imidazole are inserted into one or both handles with several neutral or anionic ligands. The results provide new insights into the magnetic properties of alkoxide complexes, which are compared to those of well-established ferric porphyrin derivatives.

Results

Formulas of the functionalized amide-basket-handle porphyrins (a-BHP) we have investigated are shown in Figure 1. They

(1) Antonioni, E.; Brunori, M. *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North-Holland: Amsterdam, 1971.
(2) Margoliash, E. *Heme and Hemoproteins*; Chance, B., Estabrook, R. W., Yonetani, T. Eds.; Academic: London, 1966.
(3) Mathews, F. S.; Levine, M.; Argos, P. *J. Mol. Biol.* **1972**, *64*, 449–464.
(4) Morrison, M.; Schonbaun, G. R. *Annu. Rev. Biochem.* **1976**, *45*, 861–888.
(5) (a) Stern, J. O.; Peisach, J. *J. Biol. Chem.* **1974**, *249*, 7495–7498. (b) Cramer, S. P.; Dawson, J. H.; Hodgson, K. O.; Hager, L. P. *J. Am. Chem. Soc.* **1978**, *100*, 7282–7290.
(6) Greer, J. *J. Mol. Biol.* **1971**, *59*, 107–126.
(7) Reid, T. J., III; Murthy, M. R. N.; Sigignono, A.; Tanaka, N.; Musick, W. D. L.; Rossman, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4767–4771.
(8) Pulsinelli, P. D.; Perutz, M. F.; Nagel, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3870–3874.
(9) Gerald, P. S.; Ejron, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 1758–1764.
(10) Peisach, J.; Gersonde, K. *Biochemistry* **1977**, *16*, 2539–2545.
(11) Ainscough, E. W.; Addison, A. W.; Dolphin, D.; James, B. R. *J. Am. Chem. Soc.* **1978**, *100*, 7585–7591.
(12) Tang, S. C.; Koch, S.; Papaefthymiou, G. C.; Foner, S.; Fraukel, R. B.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 2414–2433.
(13) (a) Otsuka, T.; Ohya, T.; Satō, M. *Inorg. Chem.* **1984**, *23*, 1777–1779. (b) Otsuka, T.; Ohya, T.; Sato, M. *Inorg. Chem.* **1985**, *24*, 776–782.
(14) Quinn, R.; Nappa, M.; Valentine, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 2588–2595.
(15) Chang, C. K.; Traylor, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2647–2650.
(16) Momenteau, M.; Rougée, M.; Loock, B. *Eur. J. Biochem.* **1976**, *71*, 63–76.
(17) Momenteau, M.; Loock, B.; Bisagni, E.; Rougée, M. *Can. J. Chem.* **1979**, *57*, 1804–1813.
(18) Collman, J. P.; Braumau, J. L.; Doxsee, K. M.; Halbert, T. R.; Bunneberg, E.; Linder, R. E.; La Mar, G. N.; Del Gaudio, J.; Lang, G.; Spartalian, K. *J. Am. Chem. Soc.* **1980**, *102*, 4182–4192.
(19) (a) Part 2: Momenteau, M.; Mispelter, J.; Loock, B.; Lhoste, J. M. *J. Chem. Soc., Perkin Trans. 1* **1985**, *61*–70. (b) Part 3: Momenteau, M.; Mispelter, J.; Loock, B.; Lhoste, J. M. *J. Chem. Soc., Perkin Trans. 1* **1985**, 221–231.
(20) Lavalette, D.; Tetreau, C.; Mispelter, J.; Momenteau, M.; Lhoste, J. M. *Eur. J. Biochem.* **1985**, *145*, 555–565.

0020-1669/86/1325-4577$01.50/0