44 °C, any volatile material was removed under dynamic vacuum to leave the nonvolatile liquid IX. The ^{1}H NMR spectrum in $(CD_3)_2SO$ showed a peak at δ 10.8 (OH). The ^{19}F NMR spectrum (n-$C^1F_3C^2F_2C^3F_2C^4F_2)_2P(O)OH$) had a triplet at ϕ −80.38 (C^1F_3) ($J_{C^1F_3-C^2F_2}$ = 9.33 Hz), a broad peak at ϕ −120.4 (C^2F_2), a doublet of multiplets centered at ϕ −121.4 (C^4F_2) ($J_{C^4F_2-P}$ = 68.35 Hz), and a triplet at ϕ −125.4 (C^3F_2) ($J_{C^3F-C^4F_2}$ = 11.5 Hz). The ^{31}P NMR spectrum gave a pentet centered at δ −2.4. The positive CI mass spectrum has a molecular ion at m/e 503 (M^+ + H) 2.4%, as well as peaks at m/e 466 ($C_8F_{17}PO^+$) 0.7%, 447 ($C_8F_{16}PO^+$) 32.2%, 283 ($C_4F_9O_2PH^+$) 0.7%, 219 ($C_4F_9^+$) 0.9%, 215 ($C_3F_6O_2PH_2^+$) 1.6%, 169 ($C_3F_7^+$) 5.6%, 119 ($C_2F_5^+$) 9.9%, 100 ($C_2F_4^+$) 13.8%, 81 (CF_2P^+) 100%, 69 (CF_3^+) 69%, 65 ($PO_2H_2^+$) 2.1%, and 51 (CF_2H^+) 5.6%.

Anal. Calcd for $C_4F_{19}PO_2H$: C, 19.12; H, 0.2; P, 6.17. Found: C, 19.38; H, 0.25; P, 5.99.

Preparation of $(n$-$C_4F_9)_2P(O)OH\cdot H_2O$ (XI). A 30:70 mixture of $(n$-$C_4F_9)_3PO$ and $(n$-$C_4F_9)_3PF_2$ (15 g) and 100 mmol of water were transferred into a flask. After the flask had warmed to 25 °C, an exothermic reaction occurred. The mixture was allowed to remain at 25 °C for 10 h. After removal of the volatile materials, HF, C_4F_9H, and H_2O, a sublimable (70 °C, 0.1 torr) white solid, XI, remained in 90% yield. In $(CD_3)_2SO$ the proton NMR spectrum had a resonance peak at δ 10.1 (OH). The ^{19}F NMR spectrum (n-$C^1F_3C^2F_2C^3F_2C^4F_2)_2P(O)OH\cdot H_2O$ showed a triplet of triplets at ϕ −81.78 (C^1F_3) with $J_{C^1F_3-C^2F_2}$ = 9.4 Hz and $J_{C^1F_3-C^3F_2}$ = 2.57 Hz, a broad peak at ϕ −121.45 (C^2F_2), a doublet of multiplets at ϕ −122.4 (C^4F_2), and a multiplet of triplets at ϕ −126.5 (C^3F_2) ($J_{C^3F_2-C^4F_2}$ = 12.5 Hz). The ^{31}P NMR spectrum showed a pentet centered at δ −0.9 with J_{P-CF_2} = 66.41 Hz.

Anal. Calcd for $C_8F_{18}PO_3H_3$: C, 18.46; H, 0.57; P, 5.76. Found: C, 18.89; H, 0.51; P, 6.46.

Preparation of n-$C_4F_9P(O)(OH)_2\cdot H_2O$ (XII). XI (5 mmol, 2.65 g) was transferred into a thick-walled Pyrex tube. To this was added dis-

tilled water (15 mmol, 0.27 g). The tube was evacuated at −196 °C and sealed off. It was heated at 150 °C for 36 h. Left behind after evacuation of the volatile materials was a sublimable (110 °C, 0.1 torr) white solid, XII. The ^{1}H NMR spectrum in $(CD_3)_2SO$ gave δ 12.5 (OH). The ^{19}F NMR spectrum ($(n$-$C^1F_3C^2F_2C^3F_2C^4F_2)P(O)(OH)_2\cdot H_2O$) had a triplet of triplets at ϕ −80.25 (CF_3) ($J_{C^1F_3-C^2F_2}$ = 9.62 Hz, $J_{C^1F_3-C^3F_2}$ = 3.18 Hz), a quartet centered at ϕ −121.9 (C^2F_2), a triplet of triplets of doublets centered at ϕ −123.7 (C^4F_2) ($J_{C^4F_2-P}$ = 78.12 Hz, $J_{C^4F_2-C^3F_2}$ = 13.04 Hz, $J_{C^4F_2-C^2F_2}$ = 2.63 Hz), and a triplet at ϕ −126.26 (C^3F_2). The ^{31}P NMR had a triplet centered at δ −3.98. The CI$^+$ mass spectrum has a molecular ion peak at m/e (M^+ + H) 32.6%, as well as peaks at m/e 281 ($C_4F_8PO_3H_2^+$) 2.6%, 231 ($C_3F_6PO_3H_2^+$) 0.9%, 181 ($C_2F_4PO_3H_2^+$) 6.4%, 131 ($CF_2PO_3H_2^+$) 56.1%, 100 ($C_2F_4^+$) 32.8%, 82 ($PO_3H_3^+$) 48.2%, 81 ($PO_3H_2^+$) 100%, 69 (CF_3^+) 69.3%, and 51 (CF_2H^+) 17.5%.

Anal. Calcd for $C_4F_9PO_4H_4$: C, 15.09; H, 1.25; P, 9.74. Found: C, 13.90; H, 1.27; P, 9.18.

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Kinetics of the Reaction of Nitric Oxide with Sulfite and Bisulfite Ions in Aqueous Solution

David Littlejohn, K. Y. Hu, and S. G. Chang*

The kinetics of the reaction of dissolved nitric oxide with sulfite and bisulfite ions was studied over a pH range of 4–10 by monitoring the reaction product, *N*-nitrosohydroxylamine-*N*-sulfonate (NHAS), with rapid-mixing, continuous-flow and stopped-flow systems. The kinetics of the reaction can be described as d[NHAS]/dt = k_a[NO][HSO$_3^-$] + k_a[NO][SO$_3^{2-}$] where k_a = 32 ± 10 M^{-1} s^{-1} and k_b = 620 ± 100 M^{-1} s^{-1} at T = 298 K. The reaction was also studied at 284 and 323 K. The hydrolysis rate of NHAS was determined over a pH range of 4–6.

There have been several studies[1–4] of the reaction of nitric oxide with forms of dissolved sulfur dioxide. Most of the studies were done under dissimilar conditions, and are not directly comparable. An early study was done by Terres and Lichti,[1] who observed the decrease in pressure of nitric oxide in the presence of K_2SO_3, $KHSO_3$, and $SO_2\cdot H_2O$. They were unable to obtain rate constants, but decided that a $(ONSO_3)^{2-}$ intermediate was involved and that the reaction between nitric oxide and bisulfite ion was first order in both species. Nunes and Powell[2] investigated the reaction of NO with SO_3^{2-} at pH 13–14 by observing the decrease in pressure of NO over the sulfite solution in a well-stirred reactor. The rate expression they obtained consisted of a sulfite-dependent term and a sulfite-independent term. They found that the dissolution process that NO undergoes influenced the kinetics. Takeuchi et al.[3] observed the loss of nitric oxide diluted with nitrogen after it either flowed over a sulfite solution surface or was bubbled through a sulfite solution. They found the reaction had second-order de-

pendence on nitric oxide and zero-order dependence on sulfite concentration. Their results, although not quantitative, indicated that the reaction was very fast. Martin et al.[4] used a stopped-flow system to observe the reaction between dissolved nitric oxide and dissolved sulfur dioxide at pH ≤3 by monitoring $SO_2\cdot H_2O$ at 280 mm. They found that the reaction was very slow and could only obtain an upper limit for the rate. Since none of these studies measured the rates of the reactions of predissolved NO with SO_3^{2-} and with HSO_3^-, we decided to investigate the kinetics of these systems.

Experimental Section

For the experiments, a continuous-flow rapid-mixing system was assembled. The system could also be used for stopped-flow experiments. The system was constructed entirely of glass and plastic to minimize contamination by metal ions. House-deionized water was run through two nonmetallic deionizing columns and stored in a plastic container prior to use in the experiments. Both tanks for the reactant solutions were filled with the processed water and degassed by passing argon through them for 2 h. Nitric oxide (Matheson, CP) was purified by passing it through a concentrated sodium hydroxide solution and then through a cold trap at dry ice temperature. The treated nitric oxide was then passed through one of the tanks for at least 1 h. Checks done on the dissolved NO concentration indicated this was sufficient to saturate the solution. A lower flow of nitric oxide was passed through the solution during the

(1) Terres, E.; Lichti, H. *Angew. Chem.* **1934**, *47*, 511.
(2) Nunes, T. L.; Powell, R. E. *Inorg. Chem.* **1970**, *9*, 1916.
(3) Takeuchi, H.; Ando, M.; Kizawa, N. *Ind. Eng. Chem. Process Des. Dev.* **1977**, *16*, 303.
(4) Martin, L. R.; Damschen, D. E.; Judeikis, H. S. *Atmos. Environ.* **1981**, *15*, 191.
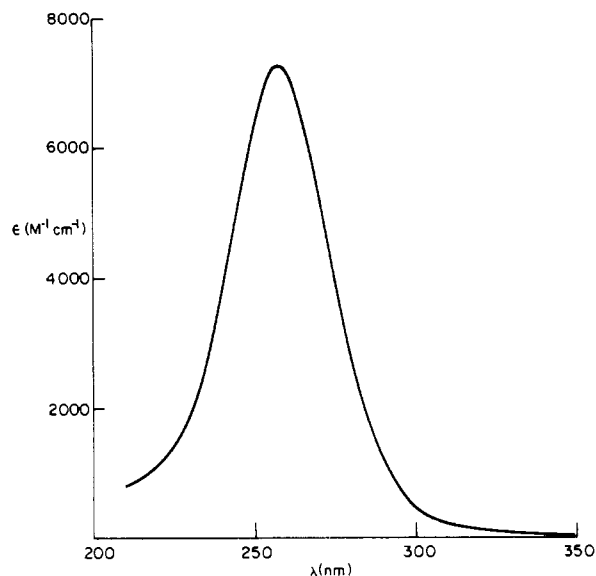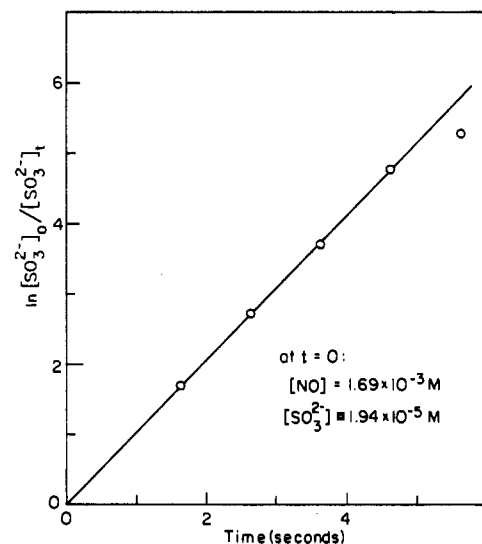
Figure 1. UV absorption spectrum of NHAS (*N*-nitrosohydroxyl-amine-*N*-sulfonate).

experiments. The sulfite and/or bisulfite solution was prepared by weighing out reagent grade sodium sulfite and/or sodium metabisulfite. Argon was passed through the tank containing the sulfite or bisulfite ion solution during the experiments. The exhaust gases from the tanks were passed through a water column to provide about 3 kPa of pressurization to the system. The pHs of the solutions were adjusted by the addition of sodium hydroxide or hydrochloric acid, of necessary. After preparation, the two solutions were passed through flowmeters and a mixer and then into either a 1 cm- or a 5-cm path length UV cell. The cell was located in a temperature-controlled compartment in a Cary 219 spectrophotometer. For experiments done at temperatures other than room temperature, the tanks containing the reactants were surrounded by a water bath to bring the solutions to the appropriate temperature. The pH of the mixed solution was monitored with an in-line pH probe downstream of the cell.
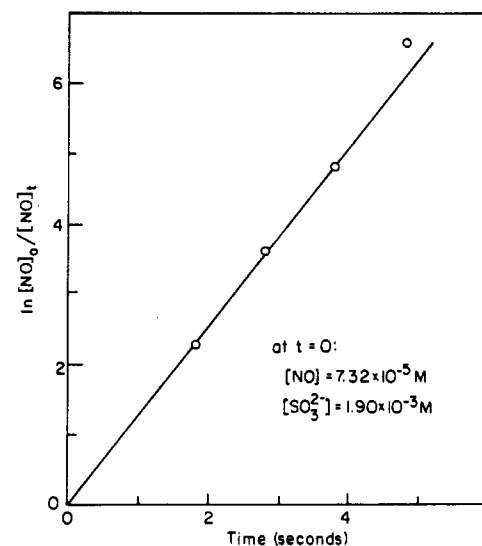
Ackerman and Powell[5] found that the only product of the reaction of NO with SO_3^{2-} was *N*-nitrosohydroxylamine-*N*-sulfonate (NHAS), which has a strong UV absorption. We prepared NHAS by the method given in the literature[5] and measured the absorption spectrum, shown in Figure 1. The extinction coefficient determined at 258 nm was found to be in good agreement with Ackerman and Powell's value and was found to vary negligibly over the temperature range 284–323 K. NHAS was monitored in the wavelength range between 258 and 300 nm, where absorptions by other species would not interfere. The 5-cm cell was used except in all cases where the NHAS absorption was too strong, in which case the 1-cm cell was used.

To ensure that the reactants would be properly mixed in the mixer, it was checked by observing the reaction of $Fe^{II}(NTA)$ with NO. The $Fe^{II}(NTA)$ + NO reaction has a rate constant on the order of 10^8 M^{-1} s^{-1},[6] and the mixed solution should react completely before entering the cell if the reactants are well mixed. This was observed to be the case. Total flow rates of 0.5–11.5 mL/s were used and the observation of the reacting solution varied from 0.25 to 9 s after mixing. The reaction was usually studied with one or the other of the reactants being in excess of the other. The NO concentration after mixing ranged from 4.5 × 10^{-5} to 1.7 × 10^{-3} M and the SO_3^{2-}/HSO_3^- concentration after mixing ranged from 1.2 × 10^{-5} to 5.0 × 10^{-3} M.

The reaction was studied in two ways. In continuous-flow measurements, the flow rates of the reactants were stabilized and a spectrum of the reaction mixture was taken from 350 to 230 nm. In stopped-flow measurements, the flow rates of the reactants were stabilized and one wavelength where NHAS alone absorbed was monitored. The flow of the reactants was stopped abruptly and the change in NHAS concentration vs. time was monitored. The results of the experiments at high pH conditions were analyzed to obtain a rate constant for NO + SO_3^{2-}. This rate constant was then used to correct low and intermediate pH experiments to obtain the rate constant for NO + HSO_3^-. The hydrolysis rate for NHAS was obtained from the stopped-flow experiments at low

(5) Ackermann, M. N.; Powell, R. E. *Inorg. Chem.* **1967,** *6,* 1718.
(6) Lin, N.; Littlejohn, D.; Chang, S. G. *Ind. Eng. Chem. Process Des. Dev.* **1982,** *21,* 725.

Figure 2. Plot of ln $([SO_3^{2-}]_0/[SO_3^{2-}]_t)$ vs. time.

Figure 3. Plot of ln $([NO]_0/[NO]_t)$ vs. time.

pH, and this was used to correct the low pH experiments for loss of the reaction product.

Results and Discussion

The order dependence of the reactants on the rate of NHAS production was determined by observing the reactions under pseudo-first-order conditions. It was also separately determined by observing the effect on the rate of varying the concentration of one reactant while keeping the other reactant at a fixed concentration. The results indicate that the reaction is first order in both reactants. In stopped-flow experiments done under pseudo-first-order conditions, the absorbance curve for NHAS could be used to calculate the limiting reactant concentration profile with time. When the concentration of NO is much larger than that of SO_3^{2-}, the integrated form of the rate expression is

$$\ln\left[\frac{[SO_3^{2-}]_0}{[SO_3^{2-}]_t}\right] = k[NO]_0 t$$

ln $([SO_3^{2-}]_0/[SO_3^{2-}]_t)$ is plotted vs. time in Figure 2. The straight line indicates first-order dependence on SO_3^{2-}. The initial concentrations after mixing were [NO] = 1.69 × 10^{-3} M and $[SO_3^{2-}]$ = 1.94 × 10^{-5} M, and the solution pH was 9.2.

When the concentration of SO_3^{2-} is much larger than that of NO, the rate expression is

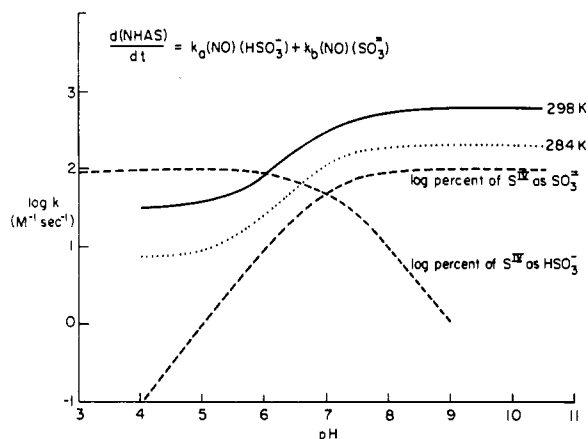$$\ln\left[\frac{[NO]_0}{[NO]_t}\right] = k[SO_3^{2-}]t$$

Figure 4. pH dependence of the observed rate constant.

Figure 3 illustrates first-order dependence on NO with a straight line plot of ln $([NO]_0/[NO]_t)$ vs. time. The initial reactant concentrations after mixing were $[NO] = 7.32 \times 10^{-5}$ M and $[SO_3^{2-}] = 1.90 \times 10^{-3}$ M at pH 9.4.

The results obtained by Nunes and Powell[2] indicated that two forms of nitric oxide exist in solution. They referred to them as dissolved NO and hydrolyzed NO. They obtained a value for the rate constant for the reaction of the former compound with sulfite ion and indicated that the latter reacted more quickly, but they were unable to obtain rate data. In our work, the nitric oxide is dissolved in solution prior to mixing with the sulfite solution and the reaction we observe should be that of the hydrolyzed form of NO with SO_3^{2-}.

The possibility that the observed reaction was catalyzed by trace metal ions was tested by doing three experimental runs at 25 °C and pH 9–10: the first with standard solutions, the second with 5.0×10^{-4} M EDTA added, and the third with 1.0×10^{-5} M Cu^{2+} added. Nine rate constant measurements were obtained under similar conditions for each run. The averaged rate constants for the runs are as follows: standard solutions, $k = 635 \pm 132$ M^{-1} s^{-1}; solutions with added EDTA, $k = 643 \pm 134$ M^{-1} s^{-1}; solutions with added Cu^{2+}, $k = 712 \pm 165$ M^{-1} s^{-1}. The addition of EDTA had no effect on the value of the rate constant obtained. The addition of Cu^{2+} to the reactant solutions yielded a slightly higher value for the rate constant. However, these values are within the experimental uncertainties of the others.

The variation in the measured values of the rate constant appear to be associated with instability in the flows of the reactant solutions, small bubbles in the mixer, and flow noise in the observation cell. We attempted to minimize the influence of these factors.

Experiments were done in which the pH of the mixed solution was varied from 4 to 10.5 to observe what influence pH has on the reaction rate. The rate slowed as the pH was decreased, suggesting that NO reacts more slowly with bisulfite ion than sulfite ion. A plot of log k vs. pH is shown in Figure 4, where k is defined as

$$k = \frac{d[NHAS]/dt}{[NO]([HSO_3^-] + [SO_3^{2-}])}$$

Also plotted as dashed lines are the log percent of the total S(IV) oxide as HSO_3^- and SO_3^{2-}. The curves indicate that both sulfite and bisulfite ions react with NO. If the reaction occurred only between SO_3^{2-} and NO, the experimentally obtained curve would be expected to follow the dashed line for SO_3^{2-}, rather than leveling out at lower pHs. Runs were not done below pH 4 because the NHAS hydrolysis became quite rapid, and also there was the possibility of interference from $SO_2 \cdot H_2O$, which has an absorption maximum near 280 nm.

We also studied the temperature dependence of the reaction by putting the reactant tanks in a thermostated bath, passing the reactant solutions through a second thermostated bath just prior to mixing, and regulating the temperature of the sample com-

Table I

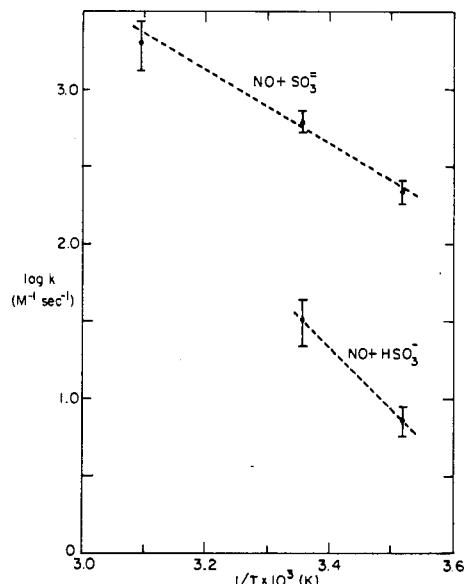| T, K	k_a, M^{-1} s^{-1}	k_b, M^{-1} s^{-1}
284	7.5 ± 2	220 ± 35
298	32 ± 10	620 ± 100
323		2000 ± 500

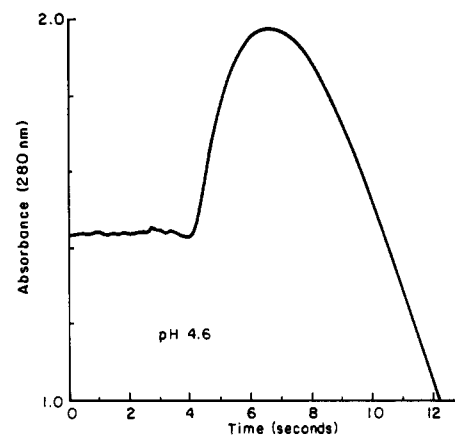**Figure 5.** Arrhenius plot for NO + SO_3^{2-} and NO + HSO_3^-.

Figure 6. UV absorption vs. time at pH 4.6.

partment. The curve of log k vs. pH at 284 K is also shown in Figure 4. Only the reaction of NO with SO_3^{2-} was studied at 323 K because of problems with the development of bubbles in the mixed solution, which created unpredictable changes in the liquid volume between the mixer and the cell. This introduced uncertainty in the reaction time between mixing and viewing. The problem was greater at the slow flow rates that would have been needed to study the NO + HSO_3^- reaction. The formation of the product can be expressed as

$$\frac{d[NHAS]}{dt} = k_a[NO][HSO_3^-] + k_b[NO][SO_3^{2-}]$$

The values for k_a and k_b are listed in Table I. These values are plotted as log k vs. $1/T$ in Figure 5. For NO + SO_3^{2-}, a preexponential term of $A = 3.2 \times 10^{10}$ M^{-1} s^{-1} and an activation energy of $E_a = 10.6$ kcal/mol are obtained where $k = Ae^{-E_a/RT}$. For NO + HSO_3^-, a preexponential term of 2.6×10^{14} M^{-1} s^{-1} and an activation energy of 17.6 kcal/mol are obtained.

It was necessary to correct runs done at low pH for the hydrolysis of the NHAS product. Figure 6 shows the curve of absorption vs. time for a typical stopped-flow experiment at pH 4.6. The flow was stopped at 4 s on the scale shown in the figure. The absorbance initially rises due to the continued reaction of NO
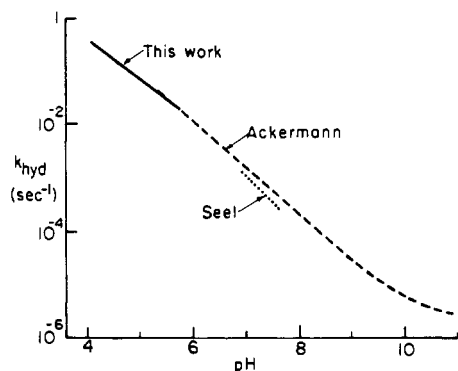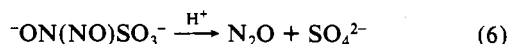
Figure 7. pH dependence of the NHAS hydrolysis rate.

with HSO_3^- and SO_3^{2-}. As the reactants are depleted, the hydrolysis of NHAS causes the absorbance to decrease. The decaying absorbance curves obtained from these experiments were used to determine the hydrolysis rate constant for NHAS as a function of pH. Short intervals of the decay curve were used to calculate values of the rate constant. Intervals of the curve taken shortly after the flow was stopped yielded values that were low, due to the continuing formation of NHAS by $NO + HSO_3^-$ and SO_3^-. At longer times, the values became consistent when the NHAS formation reaction no longer occurred at a significant rate. These values were averaged to obtain the hydrolysis rate constant. This is shown in Figure 7, along with results obtained at higher pH conditions by Ackermann[7] and Seel and Winkler.[8] Seel used EDTA to bind any trace amounts of metal ions present in solution and obtained a lower hydrolysis rate constant than Ackermann, suggesting the hydrolysis may be catalyzed by metal ions. The NHAS product is not completely stable even at high pH conditions. Ackermann[7] indicates that some decomposition exists even at pH 14. When absorption cross-section measurements were performed at 323 K with a pure NHAS solution at pH 11-12, it was noted that the concentration decreased at a rate of about 1%/min. Ackermann and Powell[5] found that NHAS would slowly oxidize in alkaline solutions when exposed to air, forming nitrite and sulfate ions.

The first-order dependence of the rate on both NO and SO_3^{2-}/HSO_3^- indicates the formation of an intermediate that rapidly reacts with a second NO to form the NHAS product. This is in agreement with what Terres and Lichti[1] and Nunes and Powell[2] have observed. The reaction scheme is developed by Nunes and Powell is consistent with our observations. Here NO_{diss} denotes the dissolved form of NO and NO_{hyd} indicated the hydrolyzed form.

$$NO(g) = NO_{diss} \tag{1}$$

$$NO_{diss} \rightarrow NO_{hyd} \tag{2}$$

$$NO_{diss} + SO_3^{2-} \rightarrow (ONSO_3)^{2-} \tag{3}$$

$$NO_{hyd} + SO_3^{2-} \rightarrow (ONSO_3)^{2-} \tag{4}$$

$$NO + (ONSO_3)^{2-} \rightarrow {}^-ON(NO)SO_3^- \tag{5}$$

$${}^-ON(NO)SO_3^- \xrightarrow{H^+} N_2O + SO_4^{2-} \tag{6}$$

This reaction sequence should also apply to HSO_3^-, although Nunes and Powell did not study the reaction at low pH. It was their work with the reaction of NO with SO_3^{2-} and with $SnCl_2$ in the presence of Cu(I) that prompted Nunes and Powell to propose that NO exists in two forms in aqueous solution.

For the reaction of dissolved NO with SO_3^{2-} (reaction 3), Nunes and Powell obtained $-d[NO]/dt = 0.45[NO][SO_3^{2-}]$ s^{-1}, which is about 3 orders of magnitude slower than the rate we have

obtained for the reaction of hydrolyzed NO with SO_3^{2-}. From their temperature dependence, we calculate a preexponential term of 4.9×10^5 M^{-1} s^{-1} and an activation energy of 8.2 kcal/mol for reaction 6. The activation energy is fairly close to the value we have obtained for reaction 2, 10.6 kcal/mol, as would be expected. They estimated that the rate constant for conversion of dissolved NO to hydrolyzed NO was about 0.14 s^{-1} at 298 K.[9]

Using the above rate constants, we modeled the stopped-flow experiments done with $NO + SO_3^{2-}$ at 25 °C in an attempt to determine if our results showed evidence for the presence of the dissolved form of NO in addition to the hydrolyzed form. Since the hydrolyzed NO would react more quickly than the dissolved NO, the rate of NHAS formation should slow down as the hydrolyzed NO is consumed in a stopped-flow experiment. From the comparison of the calculations and the observed NHAS curves, we estimate that $K_{eq} = [NO_{hyd}]/[NO_{diss}]$ is at least 10. There is considerable uncertainty in this value.

Since the NO is dissolved in solution well in advance of our experiments, there would be little evidence for two forms of NO in our experiments if the equilibrium constant is large. In an attempt to independently confirm the presence of two forms of NO in solution, Raman spectra were recorded of solutions of NO under approximately 34 bar pressure. The only NO peak observed was at 1873 cm^{-1}. This is slightly shifted from the gas-phase peak of 1876 cm^{-1}, but the shift is similar to that observed for O_2 and N_2. It is less than the shift due to the formation of $(NO)_2$ in the liquid ($\nu_{NO} = 1862$ cm^{-1})[10] and much less than the shifts due to the formation of ferrous chelate nitrosyls ($\nu_{NO} = 1750-1800$ cm^{-1}; observed in this laboratory). It may be that there is only a small quantity of dissolved NO present, or NO may be completely converted into the hydrolyzed form.

The results obtained by Nunes and Powell do not appear to be in error. The mixing system they used apparently was not diffusion-limited, since the reaction rates were reported to be independent of stirrer amplitude and the rate they obtained for dissolution of CO_2 in a pH 9 buffer was in good agreement with literature values. They also obtained the same value for k_2- (forward) in both the $NO + SO_3^{2-}$ system and the $NO + Sn(II)$ system.

The results obtained by Takeuchi and co-workers appear to be inconsistent with the results obtained by us and by Nunes and Powell. In the system used by Takeuchi et al., the NO absorption rate was dependent on the stirring speed in the reaction vessel. They attempted to correct for this by calculating the NO absorption rate per unit surface area. They then plotted the log of the NO absorption rate per unit surface area against log of the partial pressure of NO to obtain the rate dependence on NO. They found that their NO absorption rate was not dependent on SO_3^{2-} concentration in work done with 350 ppm NO and SO_3^{2-} concentrations of 0.02–0.8 M. Most of their work was done in the presence of NO_2.

Takeuchi et al. appear to be unaware of the work by Nunes and Powell and do not mention the reaction product. It is possible that their results are due to the conversion of dissolved NO to hydrolyzed NO. If this was the limiting step in their system, the rate dependence on sulfite concentration would appear to be zero. Also, it is not clear whether their correction for the influence of the stirring speed in their system was adequate.

It is uncertain whether NO reacts with dissolved sulfur dioxide. The possibility of interference from the UV absorption of $SO_2 \cdot H_2O$ and the rapid hydrolysis of NHAS prevented the investigation of this reaction by the method we used. At the highest pH used by Martin et al.,[4] the S(IV) should have been in the form of both $SO_2 \cdot H_2O$ and HSO_3^-. The HSO_3^- should have reacted with the NO, but the absorption decrease due to the loss of S(IV) may have been offset by the absorption increase due to NHAS. A better method to monitor the reaction may be to observe the decrease in pressure of NO over a solution of $SO_2 \cdot H_2O$. The reaction appears to be slow enough that diffusion should not be

(7) Ackermann, M. N. Ph.D. Thesis, University of California, 1966.
(8) Seel, F.; Winkler, R. Z. *Naturforsch., A: Astrophys., Phys., Phys. Chem.* **1963**, *18A*, 155.

(9) Nunes, T. L.; Powell, R. E. *Inorg. Chem.* **1970**, *9*, 1912.
(10) Herzog, T.; Schwab, G. M. Z. *Phys. Chem. (Munich)* **1969**, *66*, 190.

the limiting process if the solution is well stirred.

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Reactions of O2 with Hemerythrin, Myoglobin, and Hemocyanin: Effects of D2O on Equilibration Rate Constants and Evidence for H-Bonding

Graeme D. Armstrong and A. Geoffrey Sykes*

On and off rate constants associated with the binding of O_2 to hemerythrin (octamer from *Phascolopsis gouldii*), myoglobin (sperm whale), and hemocyanin (monomer from *Panulirus interruptus*) have been determined by using the temperature-jump and stopped-flow methods. When H_2O is replaced by D_2O, the following effects are observed: hemerythrin, k_{on} unchanged, k_{off} 19% decrease; myoglobin, k_{on} 17% decrease, k_{off} 16% decrease; hemocyanin, k_{on} and k_{off} unchanged. These effects are consistent with H-bonding effects in the case of the oxy forms of hemerythrin and myoglobin but not in the case of hemocyanin. The results obtained support the recently proposed structure for binding of O_2 at the hemerythrin active site.

Introduction

Dioxygen is known to bind to a metal (or metals) in different ways as illustrated in A–D.[1] The O_2 in A is assigned a peroxo
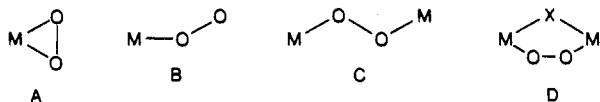
O_2^{2-} formal oxidation state. In B a superoxo O_2^- state is found, while for C and D both the superoxo and peroxo states are known. It has been established that B is relevant to O_2 binding in myoglobin (and hemoglobin), with the implication that the Fe(III)–superoxo description applies.[2,3] From vibrational spectroscopy[4,5] and EXAFS studies[6] the μ-peroxo structure (D) is relevant in the case of hemocyanin. Recently, from EXAFS,[7] X-ray crystallography,[8] and resonance Raman spectroscopy,[9] it has been proposed that hemerythrin binds O_2 in a novel and at present quite unique manner as illustrated in E. Although

E
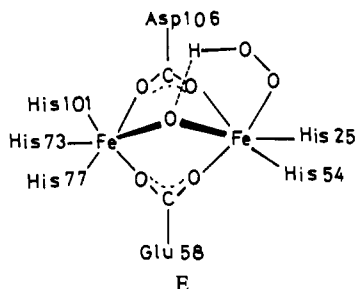
somewhat different, a μ-hydroperoxo ligand is known to be present in the dicobalt(III) complex $[(en)_2Co(\mu$-$O_2H,NH_2)Co(en)_2]^{4+}$, which at present remains an isolated example.[10] Since the peroxo description applies to E,[11] it appears that protonation and H-bonding are responsible for the O_2 taking up its unusual orientation, when sideways-on binding (to one Fe) or bridging (to two Fe's) might otherwise have been expected. In order to confirm (or disprove) this feature of the oxyhemerythrin active site, we set out to study the effect of replacing solvent H_2O by D_2O on the kinetics of O_2 binding. To help assess the data, it was also decided to carry out similar studies with myoglobin and hemocyanin. It is known in the case of oxymyoglobin that there is H-bonding of the bound O_2 to the distal histidine,[2,12] whereas no H-bonding has been reported for the symmetrical binding of O_2 to hemocyanin.[5] Monomer forms were used except in the case of hemerythrin, where it is known that there is no cooperativity effect between the different subunits. Also the resonance Raman work referred to was with the octamer from *Phascolopsis gouldii*. A comparison of rate constants for the octamer and monomer forms of hemerythrin from *Themiste zostericola* has been made in a previous temperature-jump study from this group.[13] The protein sources were those for which X-ray crystallographic or EXAFS information is available. Thus *P. gouldii* hemerythrin (Hr), sperm whale myoglobin (Mb),[2] and *Panulirus interruptus* hemocyanin (Hc)[14] were selected for investigation.

Experimental Section

Protein. Methemerythrin from the marine worm *P. gouldii*, obtained from the Marine Biological Laboratory, Woods Hole, MA, was supplied by Professor D. M. Kurtz. Oxyhemerythrin (2 mL, 1 mM) was prepared by dialysis against 0.05 M $Tris/H_2SO_4$ at pH 8.55 containing sodium dithionite (BDH) (1 g/L) and purified by Sephadex G-100 gel filtration chromatography (column 1000 × 1.5 cm) to remove excess reductant and denatured protein. Sperm whale myoglobin (Sigma Chemicals) (50 mg), mostly in the met form, was dissolved in 10 mL of Tris/HCl (5 mM, pH 8.4), reduced with sodium dithionite, and then dialysed against the same

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