Mechanism of Reduction of the Nitrite Ion by Cu^I Complexes

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The reaction of Cu_{aq}^+ with NO_2^- was studied. The results are consistent with the mechanism: $\text{Cu}_{aq}^+ + \text{NO}_2^- \hookrightarrow (\text{Cu}^+\text{NO}_2^-)$, $K = 140 \pm 30 \text{ M}^{-1}$; $(\text{Cu}^+\text{NO}_2^-) + \text{H}^+ \rightarrow \text{Cu}^{2+} + \text{NO} + \text{OH}^-$, $k = 4.6 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$. It is proposed that the copper is bound to the nitrogen in the transient complex $(\text{Cu}^+\text{NO}_2^-)$. In addition, the reaction was studied with $(\text{Cu}^{\text{I}}\text{L}^i)^+$ complexes. The results

Introduction

The one-electron reduction of nitrite to NO is of major importance in a variety of biological processes^[1-3] and in waste water treatment.^[4–6] The reducing agent, or the electro-catalytic active agent,^[7-9] is usually a low valent transition metal complex, e.g. Fe^{II},^[9-13] Co^I,^[14,15] Ru^{II},^[8,16] Cu^I.^[17-20] For example, one of the steps in the important nitrogen cycle is the reduction of nitrite to nitric oxide.^[21] Dinitrogen is released into the atmosphere by the activity of denitrifying bacteria found in soils and sediments.^[22-25] The enzyme nitrite reductase (NIR) is essential for these bacteria.^[1,2] Several mechanistic studies on the reduction of NO₂⁻ by Cu^I complexes in organic solvents were performed in order to elucidate the reduction mechanism, and as plausible models for the enzymatic process.^[26-35] In the present work, we decided to study the mechanism and kinetics of the reaction between $Cu_{aq}^+/(Cu^IL)^+$ complexes and NO_2^{-} . The results indicate that a transient complex, $(Cu^+NO_2^-)$, is formed initially, and that this complex reacts with H_3O^+ to yield the final products.

It is of interest to note that the oxidation of NO by Fe^{III}L complexes proceeds via the reverse mechanism^[35] [see reaction (1)].

$$[Fe^{II}L] + NO \iff [Fe^{II}L \cdot NO]$$

$$\xrightarrow{OH^{-}} [Fe^{II}L] + HNO_{2}$$
(1)

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(NIR = Nitric reductase) enzymes.

Results and Discussion

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When Cu_{aq}^+ is introduced into a solution containing excess NaNO₂ (2.5–100) × 10⁻³ M in the pH range 4.15–5.45 (buffered by 0.10 M acetate, the results are independent of the acetate concentration in the range 0.10–0.50 M), a decrease in the absorption at 355 nm due to nitrite is observed. The kinetics of this process, followed using the stopped-flow technique, obey a first order rate law on Cu^I. The observed rate increases with increasing [NO₂⁻]. However, the results indicate that a limiting rate is observed at high [NO₂⁻] (see Figure 1).

show that the ligands reduce the value of K and that their

effect on k depends on the redox potential of the $(Cu^{II/I}L^{i})$

couple. This reaction is the simplest model for the CuNIR

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Analysis of the results assuming a mechanism involving the fast formation of an intermediate complex fits well with the experimental data [Figure 2, reaction (2)]. The value of k_2 (Table 1) derived from the intercepts of plots analogous to Figure 2 is pH-dependent. A plot of k_2 vs. [H₃O⁺] (Figure 3) yields a straight line ($k_2 = k_{*2}$ [H₃O⁺]). From the slope of the line in this figure one obtains $k_{*2} = (4.6 \pm 0.5) \times 10^5 \text{ m}^{-1} \text{ s}^{-1}$.

$$Cu^{+}_{aq} + NO_{2}^{-} \xleftarrow{K_{2app}} [Cu^{+}NO_{2}^{-}]$$

$$\frac{k_{2}}{H^{+}} Cu^{2+}_{aq} + NO + OH^{-}$$
(2)

$$-d[NO_2^{-}]/dt = (k_2 K_{2app}[NO_2^{-}][Cu_{aq}^{+}])/(1 + K_{2app}[NO_2^{-}])$$

$$1/k_{obsd.} = 1/(k_2 K_{2app}[NO_2^{-}]) + 1/k_2$$

The results indicate that a mechanism in which an unreactive $Cu^{I}-NO_{2}^{-}$ complex is formed [reactions (3) and (4)] could yield the same rate law. However, this would require that the reaction according to reaction (4) is a third order process. Thus, this mechanism seems unlikely.

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Figure 1. Dependence of $k_{\rm obsd.}$ on the [NO₂⁻]. The experiments were performed in solutions containing 5.0×10^{-4} M Cu⁺_{aq}, 0.10 M NaClO₄, 0.10 M acetic acid, 0.023 M NH₄⁺, helium saturated at a final pH of: a. 4.3, b. 4.65, c. 5.25.



Figure 2. Dependence of $1/k_{obsd.}$ on $1/[NO_2^{-}]$. The experiments were performed in solutions containing 5.0×10^{-4} M Cu_a⁺, 0.10 M NaClO₄, 0.10 M acetic acid, 0.023 M NH₄⁺, helium saturated, the final pH: 4.3 (-----), 4.65 (—)

 $Cu_{aq}^{+} + NO_{2}^{-} \rightarrow (Cu^{+}NO_{2}^{-})$ (3)

 $Cu_{ag}^{+} + NO_{2}^{-} \rightarrow P \tag{4}$

$$Cu_{aq}^{+} + NO_{2}^{-} + H_{3}O^{+} \rightarrow Cu_{aq}^{2+} + NO + OH^{-}$$
 (5)

K_{2app} (M ⁻¹)	$k_2 (M^{-1}S^{-1})$	[H ₃ O ⁺] (M)	рН
77	2.9	$3.5 imes 10^{-6}$	5.45
62	3.4	4.0×10^{-6}	5.40
62	5.0	1.3×10^{-5}	4.90
152	8.2	2.0×10^{-5}	4.70
120	12	2.2×10^{-5}	4.60
132	12.7	4.0×10^{-5}	4.40
110	23	5.0×10^{-5}	4.30
154	33	7.1×10^{-5}	4.15

The value of k_2 (Table 1), derived from the intercepts of plots analogous to Figure 2, is pH dependent.



Figure 3. Dependence of k_5 on [H₃O⁺]. The experiments were performed in solutions containing 5.0×10^{-4} M Cu⁺_{aq}, 0.10 M NaClO₄, 0.10 M acetic acid, 0.023 M NH₄⁺, helium saturated.

The apparent values of K_{2app} , derived from the slopes of the straight lines, e.g. from Figure 2 (at several pH's in this pH range) are presented in Table 1. The average of K_{2app} in the pH range 4.15–4.70 is 130 ±25 m⁻¹ (Table 1). When the pH is increased, pH \geq 4.7, the formation of CuOH $[K_{sp}(CuOH) = 1 \times 10^{-14} \text{ m}^{-2} \text{ [}^{36]}]$ causes an apparent decrease in K_{2app} , (Table 1). The contribution of reaction (6)^[37] to K_{2app} was examined, $K_2 = K_{2app}(1 + K_a[H^+])$.

$$HNO_2 \stackrel{\leftarrow}{\rightarrow} NO_2^- + H^+; pK_a = 3.25$$
(6)

This analysis indicates that reaction (6) has a minor effect on K_2 , $K_2 = 140\pm30 \text{ m}^{-1}$. From K_2 , it can be calculated that when $[\text{NO}_2^-] = 7 \text{ mM}$, the observed rate of reaction is ca. 50% of the maximum rate. Therefore, the binding of NO_2^- to Cu_{aq}^+ is ca. 100 times weaker than that to CuNIR, in which $K_{\text{M}} = 0.074 \text{ mM}.^{[38]}$

Solutions of NaNO₂ were mixed with Cu_{aq}^+ at a final pH of 2.20 (at this pH the nitrite is present mostly as HNO₂). Figure 4 describes $k_{obsd.}$ as a function of [NaNO₂] at this acidic pH. It is apparent that there is no limiting rate (at higher pH's and the same NO₂⁻ concentrations a deviation from linearity is observed, as shown in Figure 1). Though

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 HNO_2 is a stronger oxidant than $NO_2^{-,[37]}$ there is no reason to suggest that reaction (7) occurs.



Figure 4. Dependence of $k_{\rm obsd.}$ on [NaNO₂]. The experiments were performed in solutions containing 5.0×10^{-4} M Cu⁺_{aq}, 0.10 M Na-ClO₄, 0.023 M NH₄⁺, the final pH was 2.20, helium saturated

 $Cu_{aq}^{+} + HNO_{2} \stackrel{\leftarrow}{\rightarrow} (Cu^{+}HNO_{2})^{+}$ (7)

Moreover, if $(Cu^{+}HNO_{2})^{+}$ were formed its pK_{a} is expected to be lower than the pK_a of HNO₂. Therefore, when the pH \ge 4.15, (Cu^INO₂⁻) is clearly the complex formed. At pH values lower than the pK_a of HNO₂, the NO₂⁻ concentration is low so that the apparent value for $k_{*5}K_5$ is expected to be lower at pH 2.20 than that observed at pH 4.15-5.45. The slope of the line in Figure 4 equals the apparent value of $k_{*2}K_2[H_3O^+] = 4.0 \times 10^4$, i.e. $k_{*2}K_2$ (at pH 2.20) = 5.5 × 10⁶ M⁻²sec⁻¹ as compared to $k_{*2}K_2 = 4.6 \times$ $10^5 \times 140 = 6.4 \times 10^7 \text{ m}^{-2} \text{sec}^{-1}$ at pH = 4.15-4.70. Thus, at pH 2.20 the apparent value of $k_{*2}K_2$ equals ca. 10% of that observed at pH 4.15-4.80. The nitrite concentration at pH 2.20 is ca. 10% of its concentration at pH \ge 4.15. This result is in agreement with the suggested mechanism: It should be pointed out that if HNO₂ were the oxidant, a lower apparent rate is expected also at pH 2.20, as the concentration of HNO_2 is not proportional to $[H_3O^+]$ below the pK_a of HNO₂. However, the nonlinear dependence of the observed rate at $pH \ge 4.1$ and the linear dependence at pH 2.20 on $[NO_2^-]$ + [HNO₂], proves that the active oxidant is NO_2^- .

The yield of NO from the reaction was measured by two techniques: 1. EPR, by measuring the effect of the addition of NO to 4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline-1-oxyl 3-oxide (PN) on the EPR spectrum. 2. Mass spectrometry. Both techniques revealed that [NO] $\geq 75\%$ [Cu_{aq}]. This result demonstrates that the yield of NO is quantitative as some dioxygen always enters the samples during the handling of the solutions.

The mechanism observed here is in agreement with that observed for several other Cu^I complexes in methanol/ acetonitrile.^[26,28,29] However, the rate constant is considerably higher for Cu⁺_{aq} than for those complexes. This observation is attributed to the stabilization of Cu^I by the ligands used in those studies, caused by an anodic shift of the redox potential of the Cu^{II/I} couple. Therefore, it seemed of interest to study the mechanism and kinetics of reduction of NO₂⁻ by (Cu^IL)⁺ complexes in aqueous solutions. Two complexes were studied:

1. A copper(I) complex which is a stronger reducing agent than Cu_{aq}^+ . For this purpose, $(Cu^IL^1)^+$ ($L^1 = 2,5,8,11$ -tetramethyl-2,5,8,11-tetraazadodecane, Figure 5) was chosen, $E^0(Cu^IL/Cu^IL^1) = 0.116 \text{ V}$ (vs. NHE),^[39] at pH 5.0. At this pH $k[NO_2^- + (Cu^IL^1)^+] = 35\pm10 \text{ m}^{-1}\text{s}^{-1}$ is obtained. The observed rate is nearly independent of [H₃O⁺] in the pH range 5.0–6.0. This rate is considerably higher than that obtained for Cu_{aq}^+ over this pH range. The lack of dependence on [H₃O⁺] suggests that, in this case, H₂O acts as the proton donor, i.e. the general mechanism for the decomposition of the complex [$(Cu^IL^i)^+NO_2^-$] is according to reaction (8).



Figure 5. $L^1 = 2,5,8,11$ -tetramethyl-2,5,8,11-tetraazadodecane

$$[(Cu^{I}L^{i})^{+}NO_{2}^{-}] + H_{3}O^{+}/H_{2}O \rightarrow (Cu^{II}L^{i})^{2+} + NO + (OH^{-} + H_{2}O)/2OH^{-}$$
(8)

From the results, which indicate that the observed rate increases linearly with $[NO_2^{-}]$, it can be estimated that $K = [(Cu^IL^1)^+NO_2^{-})]/([(Cu^IL^1)^+][NO_2^{-}]) \le 7 \text{ m}^{-1}$, i.e. the ligation of NO₂⁻ to the complex is considerably weaker than to Cu_{aq}^+ . This is probably due to steric hindrance caused by the ligand L¹, which is a very bulky ligand, or to the competition for a ligation site between a terminal tertiary amine group of L¹ and NO₂⁻.

2. Copper(I) complexes which are weaker reducing agents^[40,41] than Cu_{aq}^+ . For this purpose, the complexes $[Cu^{I}(CH_3CN)_n]^+$ were chosen. These solutions contain a mixture of several species: $[Cu_{aq}^+, Cu^{I}(CH_3CN)]^+$, $[Cu^{I}(CH_3CN)_2]^+$, $[Cu^{I}(CH_3CN)_3]^+$, and the detailed composition of each solution can be calculated from the known stability constants of these complexes.^[40,41] Solutions of $[Cu^{I}(CH_3CN)_n]^+$ 5.0 × 10⁻⁴ M were mixed with NO₂⁻ [(2.5–100) × 10⁻³ M) at different pH's (4.0–4.8) and different CH₃CN concentrations (0.048–0.96) M. The results demonstrate that:



Figure 6. Dependence of $k_{\rm obsd.}$ on [NO₂⁻]. The experiments were performed in solutions containing 5.0×10^{-4} M Cu⁺_{aq}, 0.10 M Na-ClO₄, 0.10 M acetic acid, 0.048 M CH₃CN, the final pH was 4.40, helium saturated.

FULL PAPER

$$[Cu^{I}(CH_{3}CN)_{n}]^{+} + NO_{2}^{-} \xleftarrow{K_{9}} [Cu^{I}(CH_{3}CN)_{n}NO_{2}^{-}]$$

$$\xrightarrow{k_{9}} \text{ products} \qquad (9)$$

The observation that $K_9 < K_2$ is probably due to one or more of the following facts:

I. Competition between one of the CH_3CN ligands and NO_2^- for the same ligation site. II. A change of hybridization from sp to sp³ is required for the binding of NO_2^- to $[Cu^I(CH_3CN)_2]^+$, which is the major complex present in the solution.

b) The observed rate increases linearly with $[H_3O^+]$ at all the CH₃CN concentrations studied, e.g. Figure 7. The slope of the graph equals $k_9K_9 = 4.0 \times 10^5 \text{ m}^{-2}\text{sec}^{-1}$. $k^*_2K_2$ for the Cu⁺_{aq} system equals 140 × 4.6 × 10⁵ = 6.4 × 10⁷ M⁻²\text{sec}^{-1}, i.e. at this CH₃CN concentration, acetonitrile reduces the rate of reaction by a factor of 150.



Figure 7. Dependence of k on $[H_3O^+]$. The experiments were performed in solutions containing 5.0×10^{-4} M $[Cu^I(CH_3CN)_n]^+$, 0.10 M NaClO₄, 0.10 M acetic acid, 0.96 M CH₃CN, helium saturated.

c) The observed rate at constant pH and $[NO_2^-]$ decreases with [CH₃CN], (e.g. Figure 8).



Figure 8. Dependence of k on [CH₃CN]. The experiments were performed in solutions containing 5.0×10^{-4} M [Cu^I(CH₃CN)_n]⁺, 0.10 M NaClO₄, 0.10 M acetic acid, 0.10 M NO₂⁻, helium saturated. solid triangle: result in the absence of CH₃CN, solid circles: results in presence of CH₃CN.

If Cu_{aq}⁺ were the only reacting species in solutions containing CH₃CN, i.e. if the rate of reaction of $[Cu^{I}(CH_{3}CN)_{n}]^{+}$ were negligible, then the expected observed rate in solutions containing 0.96 mol·dm⁻³ CH₃CN at pH 4.4 would be $k_{*2}K_2 \times [H_3O^+] \times ([Cu_{aq}^+]/[Cu^I]_T) =$ $140 \times 4.6 \times 10^{5} \times 4 \times 10^{-5} \times (2.1 \times 10^{-9}/5.0 \times 10^{-4}) =$ $0.011 \text{ M}^{-1}\text{s}^{-1}$. (Cu_{aq} concentration in this equation was calculated using the stability constants^[40,41] of the $[Cu^{I}(CH_{3}CN)_{n}]^{+}$ complexes). However, $k_{obsd.} = 24 \text{ m}^{-1}\text{s}^{-1}$ is obtained under these conditions. These results clearly demonstrate that the $[Cu^{I}(CH_{3}CN)_{n}]^{+}$ complexes react with NO₂⁻ and contribute to the observed rates of reaction. The results are not accurate enough to evaluate the rate constant of the reaction of each of the $[Cu^{I}(CH_{3}CN)_{n}]^{+}$ species accurately. However, the results (Figure 8) clearly demonstrate that the rate constants decrease with an increase in n.

Conclusions

The value of $K_2 = [(Cu^+NO_2)]/([Cu_{aq}^+][NO_2^-]) = 140\pm25$ M⁻¹ is similar to values reported in the literature for Cu^I complexes with other ligands in organic solvents,^[28,29] but higher than the values measured in this work for $[(Cu^IL^1)^+NO_2^-]$ $K \leq 7$ M⁻¹ and for $[(Cu^I(CH_3CN)_n)^+NO_2^-]$, $K \leq 30$ M⁻¹. The stability constant of the (Cu⁺NO_2^-) complex suggests that nitrite binds to the copper via the nitrogen atom, as the stability constants of Cu^I complexes with oxo-anions are expected to be considerably lower.^[42,43]

Experimental Section

Materials and Methods: NaNO₂, CuSO₄, Cu⁰, NH₃, L¹ (L¹ = 2,5,8,11-tetramethyl-2,5,8,11-tetraazadodecane), acetonitrile, acetic acid, NaClO₄, and HClO₄ were purchased from Aldrich. PN [4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline-1-oxyl 3-oxide] was prepared according to modified procedures, along with small amounts of 4,4,5,5-tetramethyl-2-(1-pyryl)-2-imidazoline.^[44] All solutions were prepared using A.R. grade chemicals and from distilled water further purified by passing through a Milli Q Millipore setup, final resistivity >10 mΩ/cm. The experiments were carried out at [NaClO₄] = 0.1 mol·dm⁻³, at room temperature, 22±2 °C.

Solutions of $[Cu^{I}(NH_{3})_{2}]^{+}$ and $(Cu^{I}L^{i})^{+}$ were prepared via the conproportionation process, reaction (10). Solid copper metal was added to deaerated solutions containing CuSO₄ and an excess of NH₃, or L^{*i*} at the appropriate pH values.

$$\operatorname{Cu}_{\mathrm{s}}^{0} + (\operatorname{Cu}^{\mathrm{II}}\operatorname{L}_{n})_{\mathrm{aq}}^{2+} + (2m-n)\operatorname{L} \stackrel{\leftarrow}{\to} 2(\operatorname{Cu}^{\mathrm{I}}\operatorname{L}_{m})_{\mathrm{aq}}^{+}$$
(10)

 Cu_{aq}^+ was obtained by injecting $[Cu^I(NH_3)_2]^+$ into an acetate buffer solution (acidic solution), or into an HClO₄ solution containing the nitrite. Under these conditions the following reactions (11) and (12) occur.

$$[Cu1(NH_3)_2]^* \quad \xrightarrow{fast} Cu^*_{aq} + 2NH_3 \tag{11}$$

$$NH_3 + H^+ \xrightarrow{fast} NH_4^+$$

Thus, at pHs considerably lower than the pK_a of NH_4^+/NH_3 all the $[Cu^I(NH_3)_2]^+$ is transformed into Cu^+_{aq} (The ligand exchange of Cu^I is fast^[45]).

Kinetic Measurements: Stopped-flow experiments were carried out using an SX.18 Applied PhotoPhysics stopped-flow instrument that enables kinetic measurements of reactions with lifetimes ≥ 2 msec. A 05–109 Spectra Kinetics Monochromator, which enables measurements in the range of 200–700 nm, was used. The optical path was 10 mm. All the kinetic runs are the average of at least a triplicate. The error limit for the rate constants and for the equilibria constants is $\pm 10\%$.

The time-dependence of the absorbances at 355 nm was observed . This wavelength is the maximum of the absorption band of $\rm NO_2^-$ for the reactions of $\rm Cu_{aq}^{-}$ and $\rm Cu^I(\rm CH_3\rm CN)_{\it n}^+$. Alternatively, the absorbance of the band at 635 nm, the maximum of the absorption band of $\rm (Cu^{II}L^1)^{2+}$ for the reaction of $\rm (Cu^{I}L^1)^+$, was observed.

Electron Paramagnetic Resonance (EPR) spectra were recorded using a Bruker EMX-220 digital X-band (v = 9.4 GHz) spectrometer equipped with a Bruker EP 4241VT temperature control system. All spectra were recorded at room temperature (T = 297 K) with the following parameters: 20.12 mW nonsaturating microwave power, 100 kHz field modulation of 0.1 mT amplitude. EPR spectra were processed using the Bruker WIN-EPR and MicrocalcTM OriginTM software.

Solutions of $(Cu^{I}L)^{+}$, nitrite and PN were mixed producing 4,4,5,5tetramethyl-2-(1-pyryl)-2-imidazoline (PPN), which has a completely different EPR spectrum (Figure 9) from PN (PN reacts only with NO to produce PPN).

Mass Spectra: The mass spectrometer used in this study was a Balzers Quadrupole model QMG-421 instrument, with an SEM (secondary electron multiplier) detector.



Figure 9. The EPR spectrum of PPN (bold line), formed by mixing 1:1 1.0×10^{-3} M [Cu^I(NH₃)₂]⁺ with 5.0×10^{-2} M NaNO₂; this solution was mixed 4:1 with 1.55×10^{-3} M PN. The experiment was carried out in solutions containing also 0.08 M NaClO₄ and 0.08 M acetic acid. The EPR spectrum of PN (thin line) which does not change after mixing 1:1 2.0×10^{-4} M PN with 0.03 M NaNO₂, 0.10 M NaClO₄, 0.20 M acetic acid. The final pH was 4.7, the solutions were helium-saturated, v = 9.424 GHz.

Acknowledgments

(12)

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FULL PAPER

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