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THE REACTION OF NO WITH SUPEROXIDE

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The rate constant for the reaction of NO with $\cdot O_2^-$ was determined to be $(6.7 \pm 0.9) \times 10^9\,l\,mol^{-1}\,s^{-1}$, considerably higher than previously reported. Rate measurements were made from pH 5.6 to 12.5 both by monitoring the loss of $\cdot O_2^-$ and the formation of the product ^-OONO. The decay rate of ^-OONO, in the presence of $0.1\,mol\,l^{-1}$ formate, ranges from $1.2\,s^{-1}$ at pH 5 to about $0.2\,s^{-1}$ in strong base, the latter value probably reflecting catalysis by formate.

KEY WORDS: Endothelium-derived relaxing factor, nitric oxide, peroxynitrite, superoxide anion.

INTRODUCTION

The identification of the simple inorganic gas nitric oxide (NO) as the endothelium-derived relaxing factor (EDRF)[1], a vasodilator, has engendered a considerable amount of interest in its reactions. Of particular interest is the reaction of NO with the superoxide anion ($\cdot O_2^-$), since this radical had been found to be involved in the deactivation of ERDF[2]. Although NO is known[3] to react with $\cdot O_2^-$ to produce the unstable peroxynitrite (^-OONO) the rate constant for this reaction was recently reported[4] to be relatively slow, $2.7 \times 10^7\ l\ mol^{-1}\ s^{-1}$. This is in contrast with the rate constant for the comparable gas phase reaction[5] of $HO_2\cdot$ with NO of $5.2 \times 10^9\,l\,mol^{-1}\,s^{-1}$. The gas-phase reaction produces the highly reactive radical $\cdot OH$ along with NO_2, and it appears that these two species are also produced to some extent in the aqueous-phase decomposition of ^-OONO[6]. Since the production of these highly reactive free radicals from the relatively unreactive $\cdot O_2^-$ would be of considerable physiological importance, we have remeasured the rate constant for reaction of $\cdot O_2^-$ with NO by laser-flash photolysis.

EXPERIMENTAL

A Questek model 2320 excimer laser[7], operating at 248 nm (KrF), was used to flash photolyze nitrite solutions, typically at $5\,mmol\,l^{-1}$ concentration, in a $0.1\,mol\,l^{-1}$ formate solution saturated with oxygen. The flash photolysis of the nitrite results in the immediate production of NO and $\cdot OH$[8]

$$NO_2^- + h\nu \rightarrow NO + \cdot O^- \qquad (1)$$

$$\cdot O^- + H^+ \rightarrow \cdot OH \qquad (2)$$

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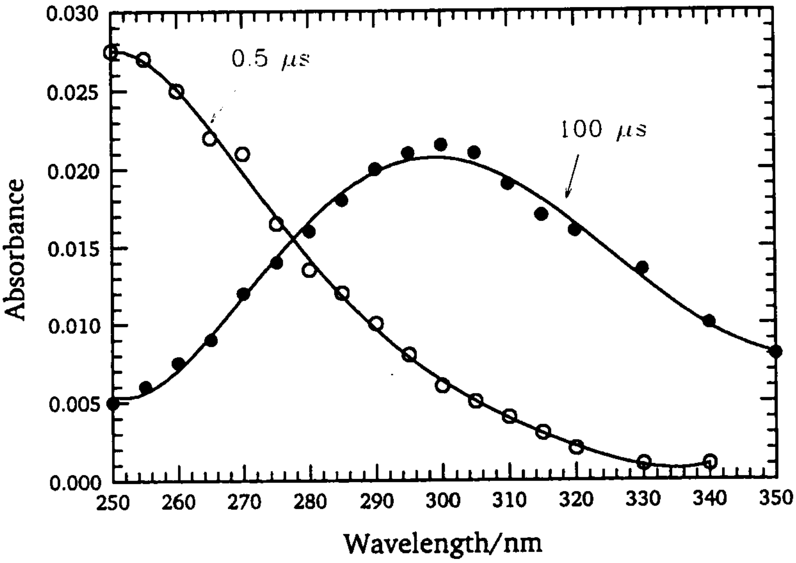

FIGURE 1 Absorption spectra resulting from the 248 nm flash photolysis of an O_2-saturated solution containing 5 mmol l^{-1} $NaNO_2$ and 0.1 mol l^{-1} $NaHCO_2$ at pH 7.5. The open circles are at 0.5 μs and the filled circles at 100 μs after the flash.

The $\cdot OH$ then reacts rapidly with formate to produce $\cdot CO_2^-$ ($k = 3.2 \times 10^9\,l\,mol^{-1}\,s^{-1}$)[9] which then reacts with O_2 to produce $\cdot O_2^-$ ($k \sim 3 \times 10^9\,l\,mol^{-1}\,s^{-1}$)[10]

$$\cdot OH + HCO_2^- \rightarrow H_2O + CO_2^- \tag{3}$$

$$\cdot CO_2^- + O_2 \rightarrow CO_2 + \cdot O_2^- \tag{4}$$

Solutions were flowed through a 4 cm long quartz reaction cell, masked so that only the central 2.5 cm is irradiated by the laser. Light from an ILC Technology 300 W xenon arc lamp, a Hammamatsu 75 W xenon arc lamp, or a Hammamatsu water-cooled deuterium lamp was focused through the reaction cell onto the entrance slit of a Kratos monochromator. The light was detected by a RCA 4840 photomultiplier. The signal from the photomultiplier was measured relative to the signal sampled just before the pulse by a differential amplifier, and monitored by a Tektronix 7612D transient recorder. Results from 5–10 flashes typically were averaged before kinetic analysis. All experiments were carried out at room temperature.

RESULTS

The flash photolysis of an O_2-saturated, 0.1 mol l^{-1} formate solution containing 5 mmol l^{-1} nitrite led to the appearance of an optical absorption with a peak below 250 nm within 0.5 μs, identified as $\cdot O_2^-$ (Figure 1). This absorption decayed by a second-order process to a residual, long-lived, absorption while a second absorption grew simultaneously with a maximum near at 300 nm (Figure 1). This latter absorption was identified as ^-OONO.

$$\cdot O_2^- + NO \rightarrow {}^-OONO \tag{5}$$

Taking the absorptivity of $\cdot O_2^-$ at 260 nm to be[11] 1940 l mol^{-1} cm^{-1}, and assuming complete conversion of the $\cdot O_2^-$ to $^-$OONO, we calculate an absorptivity of 1730 l mol^{-1} cm^{-1} for the peroxynitrite, in good agreement with the reported value of 1670.[12] From a comparison of the amount of $\cdot O_2^-$ generated upon flash photolysis of this solution with the yield of $\cdot SO_4^-$ obtained by the flash photolysis of $S_2O_8^{2-}$ at the same laser energy, assuming that $\cdot SO_4^-$ is produced with a quantum yield of 2, we estimate that the quantum yield for O^- production from NO_2^- is approximately 0.2 at 248 nm.

Rate measurements were carried out both by monitoring the decay of the absorbance at 260 nm and the growth in absorbance at 300 nm. The experimental data were fit to a second-order rate equation by a weighted linear least-squares routine to give a value of $k/\varepsilon l$. Since the reactant $\cdot O_2^-$ and the product $^-$OONO have overlapping absorption spectra, an effective absorptivity must be used to calculate the rate constant.[13] This is simply the difference between the absorptivities of the two species at each wavelength. At 260 nm, the resulting value is 1495 l mol^{-1} cm^{-1} while at 300 nm, it is 1380 l mol^{-1} cm^{-1}.

Most rate measurements were carried out at pH 7.5 and with 5 mmol l^{-1} NO_2^-, but experiments were also done at pH values ranging from 5.6 to 12.5 and with 1 mmol l^{-1} NO_2^-. Laser flash energies ranging from 75 to 245 mJ were employed. The derived second-order rate constant was the same regardless of experimental condition, with an average value of $(6.7 \pm 0.9) \times 10^9$ l mol^{-1} s^{-1}, where the uncertainty is one standard deviation. The yield of product was a linear function of flash energy, indicating no processes of higher order in the photon flux.

At high pH values, a secondary reaction forming $^-$OONO was also observed at very long times. We attribute this to the competetion of O_2 for $\cdot O^-$, leading to O_3^- and the subsequent formation of HO_2^-, which then reacts with NO_2^- to produce $^-$OONO. This reaction did not interfere with the rate measurements on the reaction of O_2^- with NO.

We have also measured rate constants for the loss of the product $^-$OONO. At pH 5, 6, 7, and 7.4, rate constants of 1.2, 0.7, 0.4 and 0.3 s^{-1} were measured. The value at pH 5 is uncertain due to a weak signal. At high pH, the rate constants leveled off at about 0.2 s^{-1}.

DISCUSSION

The rate constant reported here for the reaction of $\cdot O_2^-$ with NO, 6.7×10^9 l mol^{-1} s^{-1}, is quite fast, close to the rate constant for diffusion. It is also close to the rate constant for the gas-phase reaction of $HO_2\cdot$ with NO. It is, however, a factor of 200 times greater than the previously reported value for this rate constant in aqueous solution.[4] This lower rate constant was derived from a study in which a solution of 10 mmol l^{-1} formate, 73 μmol l^{-1} O_2, and 80 μmol l^{-1} NO at pH 7.4 was pulse irradiated and the formation of $^-$OONO at 302 nm monitored. Under the conditions of those experiments, about half of the electrons from the pulse would be expected to react with the NO, forming NO^-.

$$e_{aq}^- + NO \rightarrow NO^- \qquad (6)$$

NO might also compete with reaction (4) for CO_2^-, forming additional NO^-.

$$\cdot CO_2^- + NO \rightarrow CO_2 + NO^- \qquad (7)$$

The NO^- may then react with O_2[14]

$$NO^- + O_2 \rightarrow {}^-OONO \qquad (8)$$

This latter reaction may result in the relatively slow buildup of ^-OONO observed in the pulse radiolysis experiments; the present results suggest that the reaction of NO with $\cdot O_2^-$ would be complete wthin less than 1 μs under the conditions of those experiments.

The rate constants measured in this work for the decomposition of ^-OONO can be compared to measurements made on peroxynitrite prepared from the reaction of H_2O_2 with NO_2^-. Earlier work[15] at 1°C resulted in a rate constant of about $0.1\,s^{-1}$ below pH 6, decreasing to about $7 \times 10^{-4}\,s^{-1}$ at pH 9. More recent work[16] at 37°C indicated a low pH rate constant of $0.65\,s^{-1}$, decreasing to about $0.05\,s^{-1}$ at pH 9. Other work at high pH also found very slow decomposition.[11] The present results are generally in agreement with these measurements, allowing for the temperature difference, except at high pH, where our values are too high. It was pointed out previously[15] that the use of buffers at pH > 9 led to much faster rates of loss of ^-OONO than expected and to somewhat erratic results. The $0.1\,mol\,l^{-1}$ formate employed in the present experiments probably behaved similarly to these buffers and is the likely explanation for our high rate constants at high pH.

The physiological importance of the reaction of NO with $\cdot O_2^-$ has been questioned recently,[17] partly based on the relatively low rate constant which had been reported. The present results show, however, that the reaction is quite fast. The possible importance of the conversion of NO to NO^- by reduced superoxide dimutase was also raised in that paper. Our interpretation of the pulse radiolysis results suggest that any NO^- produced under aerobic conditions will be converted rapidly to ^-OONO.

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