The Rate Constant of the Reaction of Superoxide with Nitrogen Monoxide: Approaching the Diffusion Limit

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The reaction of superoxide with nitrogen monoxide has been reinvestigated. Photolysis of alkaline peroxynitrite solutions results in the formation of superoxide and nitrogen monoxide, which subsequently react at a rate of $(1.5 \pm 0.1) \times 10^{10}$ M^{-1} s^{-1}. When hydrogen peroxide is photolyzed in the presence of nitrogen monoxide, hydroxyl radicals are formed; these react with hydrogen peroxide to form superoxide, which then reacts with the nitrogen monoxide present. For the reaction of nitrogen monoxide with superoxide, a rate constant of $(2.0 \pm 0.4) \times 10^{10}$ M^{-1} s^{-1} is derived. Photolysis of nitrite gives rise to hydroxyl radicals and nitrogen monoxide. In the presence of formate and oxygen, the hydroxyl radicals subsequently form superoxide radicals, which, in turn, react with nitrogen monoxide. From these experiments, a rate constant of $(1.3 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1} is derived. We thus report a rate constant of $(1.6 \pm 0.3) \times 10^{10}$ M^{-1} s^{-1}, the weighted average of these three photolysis experiments.

Introduction

Formation of peroxynitrite [systematic name: oxoperoxonitrate(1−)] in vivo has been ascribed to the reaction of superoxide with nitrogen monoxide, reaction 1 (Table 1).[1] Four rate constants are found in the literature for this reaction. Two were determined by pulse radiolysis, 3.8×10^9 M^{-1} s^{-1} [2] and 4.3×10^9 M^{-1} s^{-1},[3] and two by flash photolysis, 6.7×10^9 M^{-1} s^{-1} [4] and 1.9×10^{10} M^{-1} s^{-1}.[5] The pulse radiolysis experiments are based on reactions 3−9 and 15 to generate the reactants superoxide and nitrogen monoxide. Similarly, flash photolysis requires reactions 10, 11, 6, and 7,[4] reaction 2,[5] or reactions 12−15 (this paper). The published rate constants for what is arguably the fastest reaction in biology differ by a factor of 5, an unacceptable range. In experiments previously described,[5] an anaerobic alkaline solution of oxoperoxonitrate(1−) was flashed (reaction 2), which resulted in an immediate increase in absorption at 250 nm and a decrease at 300 nm, near the absorbance maxima of superoxide and oxoperoxonitrate(1−), respectively. Over several microseconds, these absorptions returned to their original values. Even after hundreds of flashes, the kinetics remained unchanged. From these experiments, we concluded that, upon irradiation, oxoperoxonitrate(1−) homolyzes to form superoxide and nitrogen monoxide, which subsequently recombine with a rate constant of $(1.9 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1}. Although our method "should provide the cleanest route for the determination of $k({}^{\bullet}NO + O_2{}^{\bullet-})$",[6] the rate constant was challenged by Merényi et al.,[6] because we reported small gas bubbles after 800 flashes.[5] These bubbles were ascribed[6] to side reactions that we had rigorously ruled out.[5] We show here that these small gas bubbles do not form when the oxoperoxonitrate(1−) is kept at a constant temperature, that a rate constant comparable to 1.9×10^{10} M^{-1} s^{-1} is found when an alkaline solution of hydrogen peroxide is flashed in the presence of nitrogen monoxide, and that the flash photolysis experiment of Huie and Padmaja[4] results in a rate constant higher than the one they reported and more comparable to that found when oxoperoxonitrate(1−) is photolyzed.

Experimental Section

All chemicals used were of the highest purity available. Nitrogen monoxide was further purified by passing it three times through a concentrated potassium hydroxide solution to remove higher oxides. Oxoperoxonitrate(1−) was synthesized according to the method of Kissner et al.[7]

Milli-Q water (Millipore) was used for all solutions. To remove oxygen, solutions were evacuated to boiling at room temperature for 5 min and then saturated with argon; this process was repeated three times. Solutions containing nitrogen monoxide were treated in the same way, except that, after the last evacuation, the desired amount of nitrogen monoxide was added, followed by argon to ambient pressure. The nitrogen monoxide concentration was determined from its partial pressure.

Laser flash photolysis experiments were carried out with an Applied Photophysics LKS 50 instrument. The third (355 nm, 200 mJ/pulse) or fourth (266 nm, 10−100 mJ/pulse) harmonic of a Nd:YAG laser (Quantel Brilliant B) was used for excitation. For the photolysis of hydrogen peroxide, solutions were pumped with a syringe pump (kdScientific kds220) from gastight Hamilton syringes through PVC tubing into a mixer and from there through a glass capillary into a quartz sample cell with a 1 cm optical path length. The photolysis of oxoperoxonitrate(1−) was carried out in a fluorescence quartz cell that could be closed with a Youngs type valve. A water thermostat was used for temperature stabilization.

Pulse radiolysis experiments were carried out by irradiation of the samples with a Febetron 705 accelerator as described earlier.[8]

Results

Flash Photolysis of ONOO$^-$. We repeated the photolysis of oxoperoxonitrate(1−)[5] under argon, reaction 2 (Table 1).

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TABLE 1: Rate Constants Relevant to the Reaction of Superoxide with Nitrogen Monoxide

reaction no.	reactants	products	rate constant
1	$O_2^{\bullet -} + NO^{\bullet}$	$ONOO^-$	$1.6 \times 10^{10}\ M^{-1}\ s^{-1}$ (this paper)
2	$ONOO^- + h\nu$	$O_2^{\bullet -} + NO^{\bullet}$	
3	H_2O (pulse radiolysis)	$e^{\bullet -}_{aq}$, H^+, $^{\bullet}OH$, $H^{\bullet}$, H_2O_2, H_2	
4	$NO_2^- + e^{\bullet -}_{aq}$	$NO_2^{\bullet 2-}$	$4 \times 10^{9}\ M^{-1}\ s^{-1}$ [9]
5	$NO_2^{\bullet 2-} + H_2O$	$NO^{\bullet} + 2\ OH^-$	$5 \times 10^{4}\ s^{-1}$ [10]
6	$HCOO^- + {}^{\bullet}OH$	$CO_2^{\bullet -} + H_2O$	$3.2 \times 10^{9}\ M^{-1}\ s^{-1}$ [9]
7	$CO_2^{\bullet -} + O_2$	$CO_2 + O_2^{\bullet -}$	$2.0 \times 10^{9}\ M^{-1}\ s^{-1}$ [11]
8	$O_2 + e^{\bullet -}_{aq}$	$O_2^{\bullet -}$	$1.9 \times 10^{10}\ M^{-1}\ s^{-1}$ [11]
9	$O_2 + H^{\bullet}$	$HO_2^{\bullet}$	$1.2 \times 10^{10}\ M^{-1}\ s^{-1}$ [11]
10	$NO_2^- + h\nu$	$NO^{\bullet} + O^{\bullet -}$	
11	$O^{\bullet -} + H^+$	$^{\bullet}OH$	$pK = 11.9$ [9]
12	$H_2O_2 + h\nu$	$2\ {}^{\bullet}OH$	
13	$HO_2^- + {}^{\bullet}OH$	$HO_2^{\bullet} + OH^-$	$7.5 \times 10^{9}\ M^{-1}\ s^{-1}$ [9]
14	$H_2O_2 + {}^{\bullet}OH$	$HO_2^{\bullet} + H_2O$	$2.7 \times 10^{7}\ M^{-1}\ s^{-1}$ [9]
15	$HO_2^{\bullet}$	$O_2^{\bullet -} + H^+$	$pK = 4.7$ [12]
16	$H_2O_2 + e^{\bullet -}_{aq}$	$HO^- + HO^{\bullet}$	$1.1 \times 10^{10}\ M^{-1}\ s^{-1}$ [9]

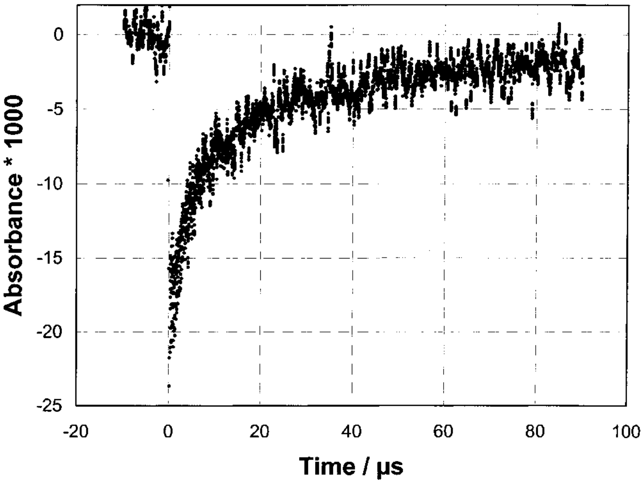

Figure 1. Photolysis of oxoperoxonitrate(1−) with 355 nm laser light to superoxide and nitrogen monoxide and subsequent recombination monitored at 310 nm. One thousand flashes were given to the *same* argon saturated solution of 200 μM oxoperoxonitrate(1−) at pH 12, which contained 60 μM nitrite as a contaminant. For measurement and display, the first 10 and then 10 times 99 experiments were averaged. The main source of experimental scatter is the stability of the Xe-arc lamp over ca. 30 min, the duration of the experiment. The observed kinetics follows a second-order law over 4.5 half-lives with a rate constant k_1 of $(1.5 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$.

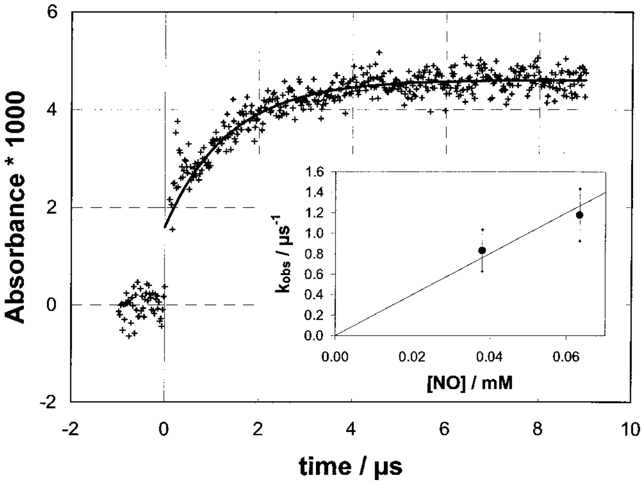

Figure 2. Oxygen-free production of superoxide in the presence of excess nitrogen monoxide (38 μM) monitored at 310 nm. Hydrogen peroxide (83 mM) in alkaline solution (1.7 mM KOH) is photolyzed by 266 nm laser light to hydroxyl radicals which oxidize more hydrogen peroxide to superoxide. The absorption increase shown here is attributed to the formation of oxoperoxonitrate(1−). A rate constant k_1 of $2 \times 10^{10}\ M^{-1}\ s^{-1}$ is derived from this particular experiment (fit). Inset: dependence of the observed rate constant (95% confidence) on the $NO^{\bullet}$ concentration.

Solutions of approximately 200 μM oxoperoxonitrate(1−) and 60 μM nitrite at pH 12 were flashed with laser pulses of 6 ns duration and approximately 200 mJ energy at a wavelength of 355 nm, and 1000 laser pulses were delivered to the same sample. The first 10 and then every 99 traces were averaged, and the 11 resulting traces are shown in Figure 1. With extinction coefficients at 310 nm of 100 and 1620 $M^{-1}cm^{-1}$ for superoxide and oxoperoxonitrate(1−), respectively, a recombination rate of $(1.5 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$ was determined. Results obtained at a single wavelength, 310 nm, were analyzed. The noise in Figure 1 is mainly due to digitization. In the thermostated cell, no bubbles were detected after this experiment, in contrast to the results presented before.[5] In the presence of oxygen, degradation of approximately 50% of oxoperoxonitrate(1−) could be observed during the 1000 laser pulses. However, the values measured for k/ϵ were the same for all 1000 flashes, and again, no bubbles were visible. Although a thermostat was used, the temperature of the solution during this experiment increased by 1 °C.

Flash Photolysis and Pulse Radiolysis of H_2O_2 in the Presence of $NO^{\bullet}$. We also determined the rate constant for the reaction of superoxide with nitrogen monoxide by anaerobically generating superoxide from hydrogen peroxide in the presence of nitrogen monoxide (see reactions 12−15). An argon-saturated solution of 100 mM hydrogen peroxide in a 50 mL syringe was mixed with a solution of either 120 or 200 mbar of nitrogen monoxide in 10 mM potassium hydroxide in a 10 mL syringe. The mixture with a partial pressure of 20 mbar of nitrogen monoxide (38 μM) was flashed with 5 ns laser pulses at 266 nm. Absorbance vs time traces were recorded between 265 and 340 nm. This experiment was carried out with 36 separate samples. The laser energy was tuned such that the resulting absorption did not exceed 6×10^{-3} units at 310 nm and no more than 4 μM oxoperoxonitrate(1−) was formed to maintain pseudo-first-order conditions for the experiment where 38 μM nitrogen monoxide was present. A total of 44 solutions with a nitrogen monoxide concentration of 63 μM (33 mbar) were treated accordingly. These results are shown in the inset of Figure 2. On the assumption that reaction 1 is bimolecular, a line was drawn through the origin, and a rate constant of $(2.0 \pm 0.4) \times 10^{10}\ M^{-1}\ s^{-1}$ was calculated. For the best line, but one that is not forced through the origin, a rate constant k_1 of $(1.4 \pm 0.2) \times 10^{10}\ M^{-1}\ s^{-1}$ is found. This experiment was also carried out by pulse radiolysis and is based on reactions 16, 14

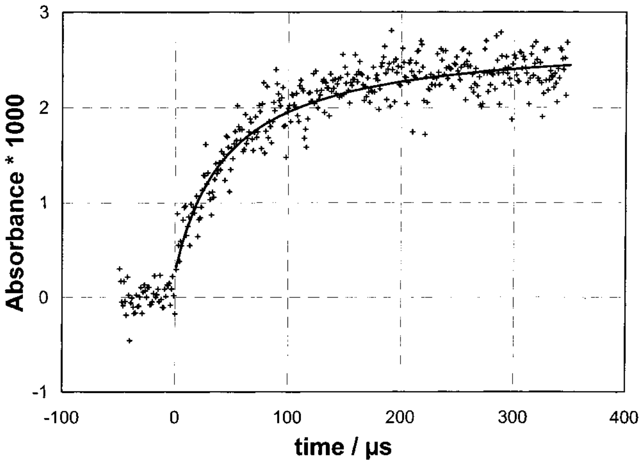

Figure 3. Oxoperoxonitrate(1−) is produced by photolysis with 355 nm light of an aereated aqueous solution containing 4.5 mM nitrite, 90 mM formate and 8 mM phosphate buffer (pH 8.2). Superoxide and nitrogen monoxide are produced in aequimolar amounts through reactions 10, 11, 6, and 7. The trace shown is an average of five experiments observed at 310 nm. The corresponding least-squares second-order fit (solid line) leads to a rate constant k_1 of 1.2 × 10^{10} M^{-1} s^{-1}.

and 15. Because of higher noise, only a lower limit for the rate constant could be derived ($k_1 > 10^{10}$ M^{-1} s^{-1}, results not shown).

Flash Photolysis of NO_2^- in the Presence of O_2 and HCO_2^-. We repeated the flash photolysis experiments of Huie and Padmaja[4] under slightly different conditions. An air or dioxygen saturated solution of 4.5 mM nitrite, 90 mM formate, and 8 mM phosphate buffer at pH 8.2 was flashed either with the third (355 nm) or fourth (266 nm) harmonic of the Nd:YAG laser. The yields of both superoxide and nitrogen monoxide were ca. 8 times lower than those in the experiments of Huie and Padmaja.[4] Although similar spectra were obtained, the rate constant was $(1.3 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1} based on 78 averages of two experiments, which is twice the value reported by Huie and Padmaja.[4]

Pulse Radiolysis of NO_2^- in the Presence of O_2 and HCO_2^-. We also repeated the determination of k_1 by pulse radiolysis (not shown) and found no major deviations from published data.[2,3]

Discussion

The present results show that three different photolysis-based experiments all yield rate constants $k_1 \approx 1.6 \times 10^{10}$ M^{-1} s^{-1} for the reaction of superoxide with nitrogen monoxide. Pulse radiolysis experiments of nitrite-containing formate solutions result in a significantly lower value, a finding for which we have as yet no explanation. As this method relies on a larger number of consecutive reactions (reactions 3−9, Table 1) than the flash photolysis methods to yield superoxide and nitrogen monoxide, it is possible that one of these is rate-limiting.

We show that, when an oxoperoxonitrate(1−) solution is irradiated at constant temperature under argon, no bubbles form even after 1000 pulses, that there is no degradation, and that the results are reproducible (Figure 1). From these results, a rate constant of $(1.5 \pm 0.1) \times 10^{10}$ M^{-1} s^{-1} was calculated for k_1. We also generated oxoperoxonitrate(1−) by photolysis of an oxygen-free, alkaline hydrogen peroxide solution that contained nitrogen monoxide and by pulse radiolysis of such solutions (not shown). A rate constant k_1 of $(2.0 \pm 0.4) \times 10^{10}$ M^{-1} s^{-1} was obtained. Reproduction of the experiments of Huie and Padmaja[4] resulted in a rate constant k_1 of $(1.3 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1}. The error of the results of the flash photolysis experiments with hydrogen peroxide is higher than that of the other two experiments. In addition, uncertainties in the nitrogen monoxide concentration and the mixing may increase the error to 30%. If we weigh the results of these three different methods with their corresponding errors, we obtain a value for k_1 of $(1.6 \pm 0.3) \times 10^{10}$ M^{-1} s^{-1}, in agreement with our earlier value of $(1.9 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1}. We propose the value of $(1.6 \pm 0.3) \times 10^{10}$ M^{-1} s^{-1} for further use.

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