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LETTERS

Evaluation of Activation Volumes for the Conversion of Peroxynitrous to Nitric Acid

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Peroxynitrous acid, an inorganic toxin of biological importance, acts both as an oxidizing and a nitrating agent during its conversion to nitric acid. In discussions of the mechanism of this conversion, activation volumes have been invoked to distinguish between possible mechanisms, viz., homolysis of the O−O bond versus rotation via the N−O bond of peroxynitrous acid. A reinvestigation of the activation volume for the conversion of peroxynitrous acid to nitric acid by high-pressure stopped-flow spectrophotometry yielded an average value of 6.9 ± 0.9 cm^3 mol^{-1} at 25 °C. Activation volumes currently cited in the literature for this process range from 6 to 10 cm^3 mol^{-1} in the temperature range 18−25 °C. Such moderately positive values do not support a definite conclusion regarding the mechanism of the conversion.

Peroxynitrite[1] is formed in vivo from the diffusion-controlled reaction of superoxide with nitrogen monoxide, $k = (1.6 \pm 0.3) \times 10^{10}$ M^{-1} s^{-1}.[2] This reaction is biologically relevant especially near activated macrophages, which produce micromolar concentrations of nitrogen monoxide. In contrast, inside cells where nitrogen monoxide is found at lower signal-level concentrations (nM), the presence of ca. 10 μM superoxide dismutase prevents the reaction with superoxide, and peroxynitrite formation is unlikely. While at physiological pH a significant part of the peroxynitrite combines with carbon dioxide[3] to form an adduct that may be oxidizing itself and decays mostly to carbon dioxide and nitrate,[4] at lower pH and inside membranes, the decay of peroxynitrous acid may become physiologically relevant. During this decay, a strong oxidant is formed, the nature of which is a matter of debate. The current mechanistic viewpoints are that the conversion of peroxynitrous to nitric acid can proceed by way of homolysis of the O−O bond to produce free nitrogen dioxide and hydroxyl radicals to an extent of 10−40%,[5−8] or via a rotation along the N−O bond followed by an intramolecular HO transfer.[9−11] Activation volumes have been invoked by disputing research groups in attempts to differentiate between these two possible mechanisms. Initially, small positive values that favor the intramolecular HO-transfer mechanism were found, but in later studies, authors reported significantly larger values that were interpreted in terms of a homolysis mechanism. In the meantime, the research groups involved have repeated their measurements; herein we report our evaluation of the existing data.

Tetramethylammonium peroxynitrite was synthesized and purified according to Bohle et al.[12] Starting materials were synthetic-grade potassium superoxide from Aldrich, tetramethylammonium hydroxide from Fluka, and 99.9% nitrogen monoxide from Linde. A solution of peroxynitrite at ca. 1 mM was prepared in freshly prepared 10 mM analytical-grade sodium hydroxide (Siegfrid) and frozen at −80 °C in small quantities. Immediately prior to an experiment, the frozen peroxynitrite pellets were dissolved in freshly prepared 10 mM sodium hydroxide cooled in an ice bath in quantities suitable to obtain

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TABLE 1: Activation Volumes for the Reaction ONOOH → NO$_3^-$ + H$^+$

activation vol/ cm^3 mol^{-1}	conditions	technique	ref
9.6 ± 1.0	pH 4.1, 0.15 M HCO$_2^-$, 3 mM NO$_2^-$, P = 0.1 and 150 MPa, ambient T	pulse radiolysis	15
10.7 ± 1.9	pH 4.1, 0.15 M HCO$_2^-$, 3 mM NO$_2^-$, P → 150 MPa, 18 °C	pulse radiolysis	17
10.0 ± 0.3	pH 4.1, 3 mM NO$_3^-$, 4 mM H$_2$PO$_4^-$, P → 150 MPa, 19 °C	pulse radiolysis	17
10.5 ± 1.1	pH 5.6, 3 mM NO$_3^-$, 4 mM H$_2$PO$_4^-$, P → 150 MPa, 18−19 °C	pulse radiolysis	17
6.0 ± 0.7	pH 4.5, 0.15 M H$_2$PO$_4^-$, 53 μM NO$_2^-$, P → 100 MPa, 19.5 °C	stopped-flow	18
9.7 ± 1.4	pH 4.5, 0.15 M H$_2$PO$_4^-$, 0.5 → 15 mM NO$_2^-$, P → ? MPa, 20 °C	stopped-flow	19
6.2 ± 1.1	pH 3.6, 5 mM H$_2$PO$_4^-$, 50 → 150 MPa, 25 °C^a	stopped-flow	c
7.6 ± 1.3	pH 3.6, 0.1 M H$_2$PO$_4^-$, 10 → 130 MPa, 25 °C^b	stopped-flow	c
7.8 ± 1.1	pH 3.6, 5 mM M H$_2$PO$_4^-$, 10 → 130 MPa, 25 °C^b	stopped-flow	c
6.0 ± 0.5	pH 4.2, 0.1 M H$_2$PO$_4^-$, 0.1 → 140 MPa, 25 °C^a	stopped-flow	c
6.6 ± 1.0	pH 3.6, 5 mM H$_2$PO$_4^-$, 0.1 → 140 MPa, 25 °C^a	stopped-flow	c
4.8 ± 0.2	pH 3.3, 10 mM H$_2$PO$_4^-$, 5 → 180 MPa, 3 °C^a	stopped-flow	c

^{a} Experiments carried out in Zürich. ^{b} Experiments carried out in Erlangen. Slow decomposition of the alkaline peroxynitrite solution occurred during the experiments; see Discussion. ^{c} This work.

the concentrations desired; these solutions were protected from light and atmospheric carbon dioxide. The acidification to effect the peroxynitrous acid decomposition was carried out with phosphoric acid/phosphate buffers of various composition prepared from analytical grade reagents (Fluka). Ultrapure water obtained from a Milli-Q unit was used to prepare all aqueous solutions.

For low ionic strength experiments, the ca. 10 mM sodium hydroxide solution containing peroxynitrite was mixed with ca. 20 mM phosphoric acid to obtain a pH between 3 and 5. The actual pH value was determined by mixing equal volumes of 5 mL of the buffer and the peroxynitrite solutions in a small beaker and measuring the pH with a glass electrode. For high-ionic-strength experiments, either 0.2 M sodium dihydrogen phosphate, or a mixture of 0.2 M sodium dihydrogen phosphate with ca. 20 mM phosphoric acid, was mixed with peroxynitrite. The former system resulted in a final pH of 5−6, the latter in a range of 3−5, as in the low-ionic-strength experiments.

Both high-pressure stopped-flow systems employed are of well-established designs: one has a sealed internal electrical drive (Erlangen, Germany),[13] whereas the other (Zürich, Switzerland) has an external pneumatic drive (Hi-Tech HPSF-56, Salisbury, U.K.) according to a design by the group of Prof. A. Merbach at EPFL (Lausanne, Switzerland).[14] Both instruments are fully thermostated. Both systems allow a delay of 30 min prior to measurement, after the pressure increase, to dissipate the heat generated during adiabatic compression. The peroxynitrite concentration after mixing was kept in the range −100−600 μM to ensure a reasonable absorbance signal for peroxynitrous acid at the observation wavelength of 260 nm. During one series of experiments (in Erlangen), substantial decay of the alkaline peroxynitrite solutions was observed inside the stopped-flow apparatus. This decay may have been caused by contact with the drive-syringe piston seals, which are composed of a Teflon and bronze material. The initial concentration dropped from 600 to 300 μM during a set of experiments. In the HI−Tech apparatus, the seals of which are made of Teflon only, the peroxynitrite was stable for hours, and the initial peroxynitrite concentration could be kept at 100−150 μM. Pressure ranges for the various experiments carried out at 25 °C described here were 50−150 MPa (Zürich, May 2001, C. Thomas), 10−130 MPa (Erlangen, July 2001, R. Kissner and M. S. A. Hamsa), and 0.1−140 MPa (Zürich, December 2001, R. Kissner). The pressure range in an experiment at 3 °C (Zürich, December 2002, R. Kissner) was 5−180 MPa.

At 260 nm and 25 °C we observed the decay of peroxynitrous acid near pH 4 at pressures up to 150 Mpa. Both at low and high ionic strength, activation volumes of 6 to 8 cm^3 mol^{-1}
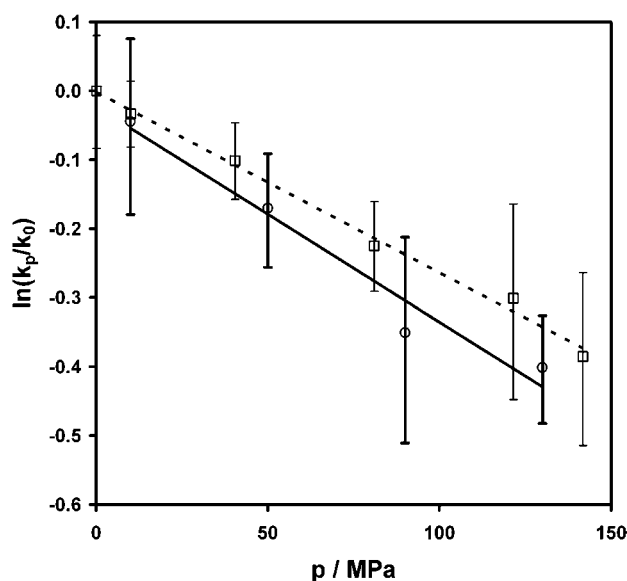

Figure 1. Pressure dependence of the rate constant for the decay of peroxynitrous acid at low ionic strength (5 mM). Squares: 25 °C, peroxynitrite 120 μM, final pH = 3.6, $\Delta V^{\ddagger}$ = +6.6 ± 1.0 cm^3 mol^{-1}. Circles: 25 °C, peroxynitrite 300−600 μM, final pH = 3.6, $\Delta V^{\ddagger}$ = +7.8 ± 1.1 cm^3 mol^{-1}. k_0 = 1.15 s^{-1} at 25 °C. Error bars represent the 95% confidence interval for an additional determination.

were found (Table 1, Figure 1). As there is agreement that, at lower temperature, there is a quantitative conversion of peroxynitrous acid to nitrate, we also carried out experiments at 3 °C and found an activation volume of 4.8 cm^3 mol^{-1} (Figure 2). These results are summarized along with available literature data in Table 1. It should be noted that the literature data[15−19] were selected so as to reflect the currently preferred data as quoted by the individual authors. For example, an earlier value[16] of 2 cm^3 mol^{-1} is not included because the measurements were carried out at a pH too close to the pK_a of peroxynitrous acid. For the determination of the activation volumes, two different instrumental techniques were employed. In one method, the peroxynitrous acid is generated through pulse radiolysis of a nitrate solution, or of a solution that contains a mixture of formate and nitrite. In the other method, a stable alkaline peroxynitrite solution is acidified in situ in a stopped-flow instrument.

From the data in Table 1, it is clear that the data from the pulse-radiolysis studies yield an average activation volume of 10.2 ± 1.1 cm^3 mol^{-1}. Stopped-flow data from in situ acidificaton of peroxynitrite range from 6 to 8, with an average value of 6.7 ± 0.9 cm^3 mol^{-1}. Hurst and co-workers recently

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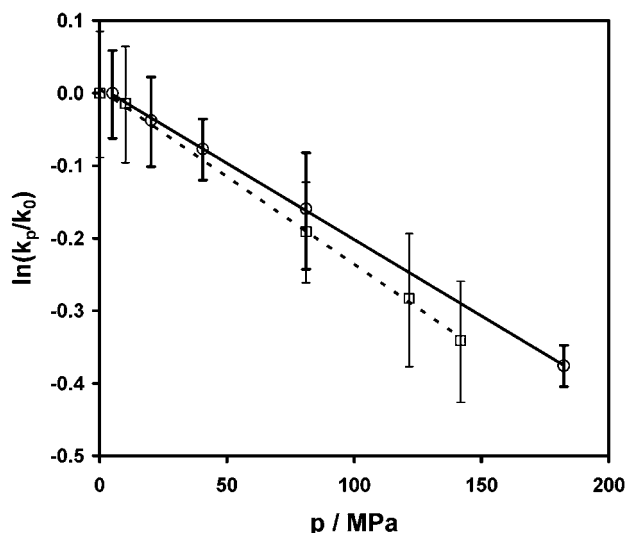*J. Phys. Chem. A, Vol. 107, No. 51, 2003* **11263**

Figure 2. Pressure dependence of the rate constant for the decay of peroxynitrous acid at different temperatures. Squares: 25 °C, ionic strength 0.1 M, peroxynitrite 150 μM, final pH = 4.2, $\Delta V^{\ddagger} = +6.0 \pm 0.5$ cm^3 mol^{-1}. Circles: 3 °C, ionic strength 0.1 M, peroxynitrite 135 μM, final pH = 3.8, $\Delta V^{\ddagger} = +4.8 \pm 0.2$ cm^3 mol^{-1}. $k_0 = 0.10$ s^{-1} at 3 °C. Error bars represent the 95% confidence interval for an additional determination.

reported stopped-flow data giving rise to an average value of 9.7 ± 1.4 cm^3 mol^{-1}.[19]

There is one activation volume in Table 1 that requires comment. At 3 °C a value of 4.8 ± 0.2 cm^3 mol^{-1} is reported. At low temperature, there is nearly quantitative conversion of peroxynitrous acid to nitric acid without formation of nitrite and dioxygen.[10] In this case, homolysis does not seem to be the likely reaction pathway.

All in all, the average values quoted above are, indeed, not very disparate when one considers that different laboratories, using different experimental techniques, different peroxynitrite preparations, and different reaction media were employed. Thus, it is safe to conclude that the conversion of peroxynitrous to nitric acid is characterized by a moderately positive activation volume. What does this mean in terms of the mechanism of the conversion reaction? It is reasonable to expect that the mechanism involving rotation around the N−O bond followed by intramolecular HO transfer requires a small but moderate volume increase in the transition state, i.e., in line with the lower numbers reported in Table 1.[20] On the other hand, the suggested homolysis mechanism is expected to require a substantial volume increase in going to the transition state, i.e., in line with the higher numbers reported in Table 1.[17−20] This means that the range of experimental values in Table 1 does not enable definitive discrimination between the two possible mechanisms

on the basis of the reported activation volumes, and a more detailed mechanistic interpretation based on these values is, at present, pure speculation. Careful ab initio studies may provide predictions of volume changes associated with the two proposed reaction mechanisms and allow more detailed interpretation of the data.

References and Notes

(1) Systematic names: O$_2^{\bullet-}$, dioxide($\bullet$1−); NO$^{\bullet}$, oxidonitrogen($\bullet$); NO$_2^-$, dioxonitrate(1−); NO$_3^-$, trioxonitrate(1−); ONOO$^-$, oxoperoxonitrate(1−). The trivial names superoxide, nitrogen monoxide, nitrite, nitrate, and peroxynitrite, respectively, are allowed. Leigh, G. J., Ed. *Nomenclature of Inorganic Chemistry*; Blackwell Scientific Publications: Oxford, U.K., 1990. Koppenol, W. H. *Pure Appl. Chem.* **2000**, *72*, 437−446.

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