

# Viscosity Effects on the Reaction of Peroxynitrite with CO<sub>2</sub>: Evidence for Radical Formation in a Solvent Cage

Sara Goldstein\* and Gidon Czapski

Contribution from the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received June 25, 1998

**Abstract:** Peroxynitrite (ONOO<sup>-</sup>) reacts rapidly with excess of CO<sub>2</sub> to yield in water 30–35% CO<sub>3</sub><sup>•-</sup> and •NO<sub>2</sub> radicals, which oxidize 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferrocyanide. The yield of ABTS<sup>+</sup> decreased upon increasing the glycerol concentration, independent of [ABTS]<sub>0</sub>, and was, within experimental error, identical to that of ferricyanide at the same concentration of glycerol. These results demonstrate that the decrease in the oxidation yields upon increasing the glycerol concentration does not result from a competition between glycerol and ABTS/ferrocyanide for the oxidizing radicals but is due to an increase in viscosity. The oxidation yields were less affected by poly(ethylene glycol) than by glycerol, though the macroscopic viscosities of the polymer solutions were much higher. We conclude that the mechanism of the decomposition of peroxynitrite in the presence of CO<sub>2</sub> takes place through the formation of CO<sub>3</sub><sup>•-</sup> and •NO<sub>2</sub> in a solvent cage. In addition, in the glycerol–water system, the microscopic viscosity is essentially equal to the macroscopic viscosity, whereas in the polymer systems the microscopic viscosity does not approximate the macroscopic viscosity.

## Introduction

Peroxynitrite (ONOOH/ONOO<sup>-</sup>) is unstable in the presence of carbonate,<sup>1,2</sup> and low concentrations of bicarbonate were shown to protect *Escherichia coli* from the toxic effect of peroxynitrite.<sup>3</sup> Lymar and Hurst<sup>4</sup> have shown that ONOO<sup>-</sup> reacts rapidly with CO<sub>2</sub> ( $k_1 = 3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ), apparently forming an adduct whose composition is ONOOCO<sub>2</sub><sup>-</sup>:<sup>4–13</sup>



In the absence of other reactants, nitrate ion was found to be the major product of the reaction of CO<sub>2</sub> with peroxynitrite.<sup>5,12</sup> It has been demonstrated that ONOOCO<sub>2</sub><sup>-</sup> participates in oxidation and nitration processes,<sup>5–13</sup> and that at most 30–35% of peroxynitrite is available for these processes in the presence of excess CO<sub>2</sub>.<sup>11–13</sup> It has been argued that the toxicity of peroxynitrite in biological systems is mainly due to reaction 1

\* To whom correspondence should be addressed. Tel.: 972-2-6586478. Fax: 972-2-6586925. E-mail: SARAG@VMS.HUJI.AC.IL.

(1) Keith, W. G.; Powell, R. E. *J. Chem. Soc. A* **1969**, 1969, 90.  
(2) Radi, R.; Cosgrove, T. P.; Beckman, J. S.; Freeman, B. A. *Biochem. J.* **1993**, 290, 51.

(3) Zhu, L.; Gunn, C.; Beckman, J. S. *Arch. Biochem. Biophys.* **1992**, 298, 452.

(4) Lymar, S. V.; Hurst, J. K. *J. Am. Chem. Soc.* **1995**, 117, 8867.

(5) Uppu, R. M.; Squadrito, G. L.; Pryor, W. A. *Arch. Biochem. Biophys.* **1996**, 327, 335.

(6) Lymar, S. V.; Jiang, Q.; Hurst, J. K. *Biochemistry* **1996**, 35, 7855.

(7) Gow, A.; Duran, D.; Thom, S. R.; Ischiropoulos, H. *Arch. Biochem. Biophys.* **1996**, 333, 42.

(8) Denicola, A.; Freeman, B. A.; Trujillo, M.; Radi, R. *Arch. Biochem. Biophys.* **1996**, 333, 49.

(9) Yermilov, V.; Yoshie, Y.; Rubio, J.; Ohshima, H. *FEBS Lett.* **1996**, 399, 67.

(10) Lemerrier, J.-N.; Padmaja, S.; Cueto, R.; Squadrito, G. L.; Uppu, R. M.; Pryor, W. A. *Arch. Biochem. Biophys.* **1997**, 345, 160.

(11) Goldstein, S.; Czapski, G. *Inorg. Chem.* **1997**, 36, 5113.

(12) Lymar, S. V.; Hurst, J. K. *Inorg. Chem.* **1998**, 37, 294.

(13) Goldstein, S.; Czapski, G. *J. Am. Chem. Soc.* **1998**, 120, 3458.

as the concentration of bicarbonate in vivo varies between 12 and 30 mM, resulting in a CO<sub>2</sub> concentration of about 1 mM.<sup>4,6,7,11–13</sup> The mechanism of this reaction has been extensively investigated,<sup>6–12</sup> and possible intermediates have been suggested, such as the couple CO<sub>3</sub><sup>•-</sup> and •NO<sub>2</sub>, NO<sub>2</sub><sup>+</sup>, or O<sub>2</sub>NOCO<sub>2</sub><sup>-</sup>. Recently, we have established the identity of the intermediate as the couple CO<sub>3</sub><sup>•-</sup> and •NO<sub>2</sub>.<sup>13</sup>

It has been well established that the rate of diffusion of radicals away from a solvent cage decreases upon increasing the viscosity of the solvent.<sup>14</sup> Therefore, if a caged radical pair is formed, changes in the viscosity of the solvent should affect the product yields and the rate constant for the decomposition of the free radicals initiators. Pryor et al.<sup>15</sup> found that increasing the viscosity of the solvent using poly(ethylene glycol) has little or no effect on the rate of peroxynitrite decomposition, and they concluded that the viscosity test does not support homolysis of ONOOH into free •OH and •NO<sub>2</sub> radicals. The present study shows a decrease in the oxidation yields upon increasing the viscosity of the solvent when peroxynitrite reacts with an excess of CO<sub>2</sub> in the presence of various reactants. It is therefore concluded that the decomposition of peroxynitrite in the presence of CO<sub>2</sub> takes place via the formation of CO<sub>3</sub><sup>•-</sup> and •NO<sub>2</sub> in a solvent cage.

## Experimental Section

**Materials.** Glycerol, anhydrous (Baker A. R., 99.9%, density 1.257 g/mL at 25 °C) was used without further purification. Glycerol–water solutions were made up by weight and are reported in weight percents of glycerol. Poly(ethylene glycol) (PEG) 4000 and 20000 were purchased from Fluka. The polymer solutions were prepared by

(14) Koenig, T.; Fischer, H. In *Free Radicals*; Kochi, J. K., Ed.; John Wiley & Sons: New York, 1976; Vol. 1, Chapter 4, pp 157–189 and references therein.

(15) Pryor, W. A.; Jin, X.; Squadrito, G. L. *J. Am. Chem. Soc.* **1996**, 118, 3125.

dissolving weighed samples in water. 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferrocyanide were dissolved in water and then mixed with glycerol or PEG solutions. The macroscopic viscosity was measured in a single-bulb Ubbelohde viscometer and compared to that of water ( $\eta = 0.8885$  cP at 25 °C<sup>16</sup>).

**Preparation of Peroxynitrite.** Peroxynitrite was synthesized in a quenched-flow system by the reaction of nitrite with acidified hydrogen peroxide. This system was optimized as recently described,<sup>17</sup> to produce relatively high yields of ONOO<sup>-</sup> with minor known residuals of NO<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>. A syringe pump (WPI, model SP 230IW) with a flow rate of 45 mL/min was used to inject 0.63 M NaNO<sub>2</sub> and 0.60 M H<sub>2</sub>O<sub>2</sub> in 0.7 M HClO<sub>4</sub> into a first mixing chamber through four tangential inlets. The combined solution was allowed to react after passing through a short delay line (170 ms) connected to a second mixing chamber, where 3 M NaOH was injected with the same flow rate to quench the reaction. The yield of ONOO<sup>-</sup> was determined from its absorption at 302 nm using  $\epsilon = 1670$  M<sup>-1</sup> cm<sup>-1</sup>.<sup>18</sup> The stock solution of ca. 0.12 M ONOO<sup>-</sup> contained about 80 mM nitrate, 10 mM nitrite, and practically no residual H<sub>2</sub>O<sub>2</sub>.

**Kinetic Measurements.** Kinetic measurements were carried out using the Bio SX-17MV sequential Stopped-flow apparatus from Applied Photophysics with a 1-cm-long mixing cell. The oxidation of ABTS and ferrocyanide by peroxynitrite/CO<sub>2</sub> was followed at 660 ( $\epsilon_{660}(\text{ABTS}^+) = 12\,000$  M<sup>-1</sup> cm<sup>-1</sup>) and 420 nm ( $\epsilon_{420}(\text{Fe}(\text{CN})_6^{3-}) = 1000$  M<sup>-1</sup> cm<sup>-1</sup>), respectively. Peroxynitrite in 0.01 M NaOH was mixed in a 1:5 ratio with ABTS or ferrocyanide in 60 mM phosphate buffers containing bicarbonate, glycerol, or PEG. The concentration of CO<sub>2</sub> in the buffer solutions was determined by measuring the pH of these solutions and using  $\text{p}K = 6.2$  for the hydration of CO<sub>2</sub>.<sup>19</sup> Under all experimental conditions, the reaction of peroxynitrite with CO<sub>2</sub> was faster than CO<sub>2</sub> equilibration with HCO<sub>3</sub><sup>-</sup>.<sup>4,19,20</sup> The concentration of CO<sub>2</sub> immediately after mixing was five-sixths that at equilibrium prior to mixing. Peroxynitrite concentration was measured before mixing. The pH of the mixture was measured at the outlet of the flow system. The kinetic measurements were carried out at 25 °C.

## Results

When two solutions with different viscosities are mixed, the formation of shadows on the image of the cell occurs by the interception of convergent rays that, while passing through the cell, have been deflected by any inhomogeneous layers of liquid within the cell. The latter is called the Schlieren effect, and it diminishes when the solution becomes homogeneous.<sup>21</sup> The time needed for the mixture to become homogeneous depends on the viscosity of the solution that is mixed with water and on the efficiency of the mixer. We found that, when water was mixed with 83%, 76%, and 69% glycerol at a 1:5 ratio using the Bio SX-17MV stopped-flow apparatus, the mixture became homogeneous within 50 s, 10 s, and 50 ms, respectively. Therefore, the highest concentration of glycerol used in this study was 69% (59% after mixing,  $\eta = 6.88$  cP). Higher viscosity could be achieved with PEG 20000 as the mixtures become homogeneous faster than in the case of glycerol.

The concentration of CO<sub>2</sub> for kinetics measurements was chosen so as to obtain a half-life longer than 150 ms but still considerably shorter than that of the self-decay of peroxynitrite, which decomposes to yield ca. 40% highly oxidizing intermediate.<sup>22</sup> The rate constant of the self-decay of peroxynitrite at pH

< 9 and 25 °C is given by eq 2 ( $\text{p}K_a(\text{ONOOH}) = 6.6 \pm 0.1$ ),<sup>23-25</sup>

$$k_d = 1.2[\text{H}^+]/(K_a + [\text{H}^+]) \text{ s}^{-1} \quad (2)$$

and that of the oxidation by peroxynitrite/CO<sub>2</sub> is given by eq 3.<sup>4</sup>

$$k_{\text{oxd}} = 3 \times 10^4 [\text{CO}_2] K_a / (K_a + [\text{H}^+]) \text{ s}^{-1} \quad (3)$$

For  $k_{\text{oxd}} > 10k_d$  and  $\ln 2/k_{\text{oxd}} > 0.15$  s, the oxidation process should be carried out at  $\text{pH} > 7$  and in the presence of  $[\text{CO}_2] < 160$   $\mu\text{M}$ . Under these conditions and limiting concentrations of peroxynitrite, ABTS is a better scavenger than ferrocyanide due to its high absorption ( $\epsilon_{660} = 12\,000$  M<sup>-1</sup> cm<sup>-1</sup>), and relatively low concentrations of peroxynitrite can be used. The rate constants for the reactions of \*NO<sub>2</sub> with ferrocyanide and ABTS are  $3 \times 10^6$  and  $2.2 \times 10^7$  M<sup>-1</sup> s<sup>-1</sup>, respectively,<sup>26</sup> and those for CO<sub>3</sub><sup>-</sup> with ferrocyanide and ABTS are  $5 \times 10^8$  (ref 26) and  $2.9 \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> (this work),<sup>27</sup> respectively. The rate constant for the reactions of CO<sub>3</sub><sup>-</sup> with glycerol has been determined to be  $1.4 \times 10^4$  M<sup>-1</sup> s<sup>-1</sup> (this work),<sup>28</sup> and that for PEG is expected to be of the same order of magnitude. The rate constants for the reactions of \*NO<sub>2</sub> with glycerol and PEG are expected to be considerably lower than those for CO<sub>3</sub><sup>-</sup> with these substrates.<sup>26</sup> Therefore, both ABTS and ferrocyanide compete efficiently with glycerol and PEG for CO<sub>3</sub><sup>-</sup> and \*NO<sub>2</sub> radicals.

The effect of 59% glycerol and 130 g/L PEG 20000 on the absorption of ABTS<sup>+</sup> and ferricyanide was less than 3%, and therefore was ignored. Under all experimental conditions, the formation of ABTS<sup>+</sup> and ferricyanide was first order, and the yields were determined via extrapolation to half the time required for the mixture to become homogeneous.

The reaction of peroxynitrite with an excess of CO<sub>2</sub> in the presence of ABTS was studied at pH 7.6 (50 mM phosphate buffer) for  $[\text{peroxynitrite}] < 75$   $\mu\text{M}$  and  $[\text{CO}_2] < 160$   $\mu\text{M}$ . Some kinetic traces at various viscosities are given in Figure 1, and these show the increase in the Schlieren effect and the decrease in the oxidation yield upon increasing the glycerol concentration. The rates and yields measured at various concentrations of ABTS and glycerol are given in Table 1.

When glycerol was replaced by PEG 4000 and PEG 20000, the yields of ABTS<sup>+</sup> were considerably higher than those measured in the presence of glycerol, though the viscosities of the polymer solutions were higher (Table 2).

The yield of ferricyanide was measured under the conditions where 0.22 mM peroxynitrite reacted with 0.35 mM CO<sub>2</sub> in the presence of 50 mM ferrocyanide at pH 7.5 (50 mM phosphate buffer). The oxidation yields were measured to be  $65 \pm 2\%$ ,  $23 \pm 2\%$ , and  $17 \pm 2\%$  in the presence of 0%, 52%, and 59% glycerol, respectively. These yields are, within

(23) Logager, T.; Sehested, K. *J. Phys. Chem.* **1993**, *97*, 6664.

(24) Goldstein, S.; Czapski, G. *Free Radical Biol. Med.* **1995**, *19*, 195.

(25) Kissner, R.; Nauser, T.; Bugnon, P.; Lye, P. G.; Koppenol, W. H. *Chem. Res. Toxicol.* **1997**, *10*, 87.

(26) Ross, A. B.; Mallard, W. G.; Helman, W. P.; Buxton, J. V.; Huie, R. E.; Neta, P. *NIST Standard Reference Database 40*, Version 2.0, 1994.

(27) The rate constant of the reaction of CO<sub>3</sub><sup>-</sup> with ABTS has been determined using pulse radiolysis of N<sub>2</sub>O-saturated solutions containing 0.3 M carbonate and 22–91  $\mu\text{M}$  ABTS at pH 11 to be  $(2.9 \pm 0.2) \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> by following the formation of ABTS<sup>+</sup> at 420 nm.

(28) The rate constant of the reaction of CO<sub>3</sub><sup>-</sup> with glycerol has been determined using pulse radiolysis of N<sub>2</sub>O-saturated solutions containing 0.3 M carbonate and 5 mM glycerol at pH 11 to be  $(1.4 \pm 0.4) \times 10^4$  M<sup>-1</sup> s<sup>-1</sup> by following the decay of CO<sub>3</sub><sup>-</sup> at 600 nm.

(16) *Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1985; pp 10–96.

(17) Saha, A.; Goldstein, S.; Cabelli, D.; Czapski, G. *Free Radical Biol. Med.* **1998**, *24*, 653.

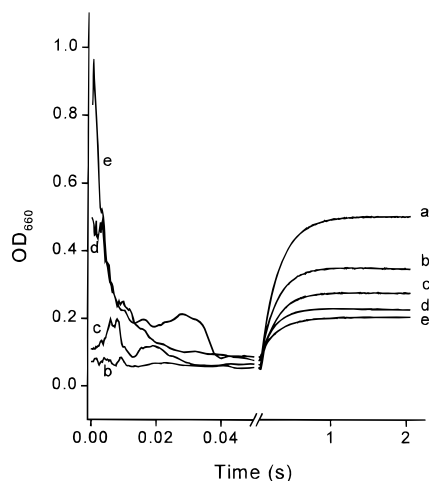
(18) Halfpenny, E.; Robinson, P. L. *J. Chem. Soc. (A)* **1952**, 928.

(19) Alberty, R. A. *J. Phys. Chem.* **1995**, *99*, 11028.

(20) Kern, D. M. *J. Chem. Educ.* **1960**, *37*, 14.

(21) *McGraw-Hill Dictionary of Scientific and Technical Terms*, 3rd ed.; Parker, S. P., Ed.; McGraw-Hill Book Co.: New York, 1983.

(22) Goldstein, S.; Squadrito, G. L.; Pryor, W. A.; Czapski, G. *Free Radical Biol. Med.* **1996**, *21*, 965 and references therein.



**Figure 1.** Kinetic traces obtained for the reaction of 64–68  $\mu\text{M}$  peroxyntirite with 160  $\mu\text{M}$   $\text{CO}_2$  in the presence of 2 mM ABTS at pH 7.6 (50 mM phosphate buffer) in the absence (a) and in the presence of 21.9% (b), 30.7% (c), 52% (d), and 59% (e) glycerol.

**Table 1:** Rates and Yields of the Oxidation of ABTS by Peroxyntirite (PN)/ $\text{CO}_2$  in the Presence and Absence of Glycerol at pH 7.6

[ABTS], mM	[PN], $\mu\text{M}$	[ $\text{CO}_2$ ], $\mu\text{M}$	[glycerol], wt % (g/L)	$\eta$ , cP	$k_{\text{oxd}}$ , $\text{s}^{-1}$	[ABTS <sup>+</sup> ]/[PN], %	1/cage
2.0	69	137		0.89	$4.5 \pm 0.3$	$59.2 \pm 1.2$	1.420
2.0	59	146	7.5 (76)	1.06	$4.2 \pm 0.1$	$51.3 \pm 0.2$	1.345
2.0	64	146	14.8 (153)	1.32	$4.4 \pm 0.1$	$45.4 \pm 0.2$	1.294
2.0	64	146	21.9 (229)	1.70	$4.7 \pm 0.2$	$40.0 \pm 1.0$	1.250
2.0	62	146	30.7 (328)	2.31	$5.0 \pm 0.2$	$34.5 \pm 0.5$	1.208
2.0	64	146	43 (469)	3.72	$5.3 \pm 0.3$	$24.6 \pm 0.4$	1.140
2.0	68	146	52 (586)	5.10	$5.4 \pm 0.4$	$20.0 \pm 1.0$	1.111
2.0	70	146	59 (670)	6.88	$4.7 \pm 0.5$	$16.2 \pm 0.9$	1.088
0.89	73	146	59 (670)	6.88	$5.1 \pm 0.3$	$14.8 \pm 0.3$	
3.6	73	146	59 (670)	6.88	$5.1 \pm 0.3$	$16.4 \pm 0.2$	
6.7	75	160	59 (670)	6.88	$6.2 \pm 0.8$	$16.1 \pm 0.9$	

**Table 2:** Yields of ABTS<sup>+</sup> Measured in the Peroxyntirite (PN)/ $\text{CO}_2$  System in the Absence and Presence of PEG and Glycerol at pH 7.6  $\pm 0.1$

[ABTS], mM	[PN], $\mu\text{M}$	[ $\text{CO}_2$ ], $\mu\text{M}$	[solute], g/L	$\eta$ , cP	[ABTS <sup>+</sup> ]/[PN], %
2.0	69	137		0.89	$59.2 \pm 1.2$
2.2	60	160	100 (PEG 20000)	10.24	$41.7 \pm 0.9$
2.2	68	160	128 (PEG 20000)	13.95	$33.1 \pm 1.2$
2.0	80	149	213 (PEG 4000)	7.57	$37.5 \pm 1.0$
2.0	70	146	670 (glycerol)	6.88	$16.2 \pm 0.9$

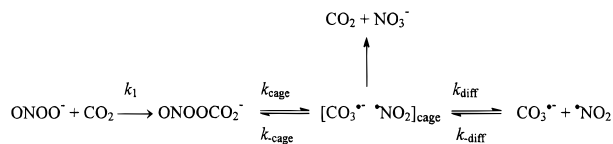
experimental error, identical to those obtained in the ABTS system under the same viscosities.

## Discussion

Table 1 shows that the yield of ABTS<sup>+</sup> decreases upon increasing [glycerol] and is independent of [ABTS]<sub>0</sub> at a constant [glycerol]. These results demonstrate that the decrease in the oxidation yield upon increasing [glycerol] does not result from a competition between ABTS and glycerol for the oxidizing radicals. This is further supported by the observation that the yields of ferricyanide are, within experimental error, identical to those of ABTS<sup>+</sup> at the same viscosities. We conclude that the decrease in the oxidation yields upon increasing the glycerol concentration is due to increased viscosity. Therefore, the mechanism of the decomposition of peroxyntirite in the presence of  $\text{CO}_2$  takes place through the formation of  $\text{CO}_3^{\bullet-}$  and  $\bullet\text{NO}_2$  in a solvent cage.

Two mechanisms can account for these observations, which are kinetically indistinguishable. According to mechanism I,<sup>5,10,12,29</sup>  $\text{CO}_3^{\bullet-}$  and  $\bullet\text{NO}_2$  are produced in a solvent cage via

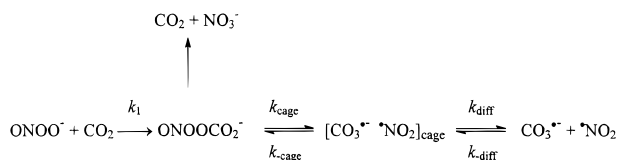
### Mechanism I



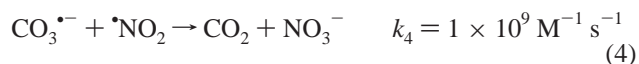
homolytic cleavage of the  $-\text{O}-\text{O}-$  bond of  $\text{ONOOCO}_2^-$ . The limiting oxidation yield of 30–35% represents the yield of radicals escaping the cage, whereas 65–70% combine in the cage to form  $\text{NO}_3^-$  and  $\text{CO}_2$ .

According to mechanism II, the adduct decomposes into  $\text{CO}_2$  and  $\text{NO}_3^-$  and also undergoes homolysis in parallel reactions

### Mechanism II



via two different transition states.<sup>6,8,10,11,13</sup> The free radicals, which are inert to the solvent molecules, have been previously suggested to react via reaction 4.<sup>30</sup>



However, according to both mechanisms the final product of the decomposition of peroxyntirite in the presence of  $\text{CO}_2$  will be nitrate. Adding low concentrations of reactive compounds should divert the radicals away from combination in the bulk of the solution, and hence up to 35% oxidation yield can be obtained. This is the case with both mechanisms, as scavenging radicals from the cage requires very high concentrations of scavengers,<sup>14</sup> which exceed by orders of magnitude those which were used.<sup>11–13</sup>

Noyes has provided a quantitative model for the efficiency of geminate reactions.<sup>14,31</sup> For this model, the probability of recombination of two radicals separated with normal thermal energies to distance  $R_0$  ( $\beta_0'$ ) is given as the reciprocal of this probability for  $\eta > 1$  cP by eq 5,<sup>14</sup>

$$\frac{1}{\beta_0'} = \frac{R_0}{2b} \left( 1 + \frac{A_T}{\alpha\eta} \right) \quad (5)$$

where  $b$  is the radius of the diffusing radical,  $R_0$  is the initial separation attained by the centers of the two radicals,  $\alpha$  is the probability per collision that the pair will react, and  $A_T = (mkT/24)^{1/2}/\pi b^2$ .<sup>14,31</sup> In Figure 2,  $1/\beta_0'$  is plotted as a function of  $1/\eta$ . A linear plot was obtained with intercept =  $R_0/2b = 1.05$  and slope/intercept =  $A_T/\alpha = 3.25 \times 10^{-3}$  poise. Such a linearity was also found in the case of simple identical radical pairs such as methyl<sup>32</sup> or trifluoromethyl<sup>33</sup> but also for geminate *tert*-butoxy radicals<sup>33</sup> as well as for the pair of *tert*-butoxy and cumyl radicals formed in the thermal decomposition of *tert*-butylper-

(29) Merényi, G.; Lind, J. *Chem. Res. Toxicol.* **1997**, *10*, 1216.

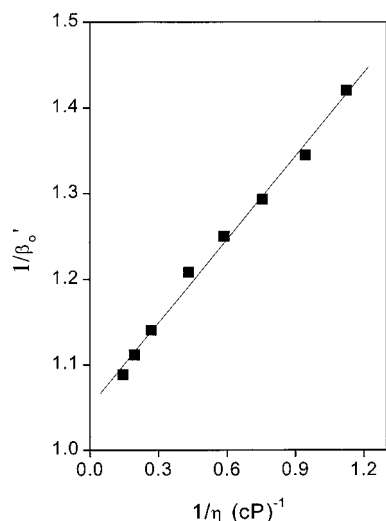
(30) Lilie, J.; Hanrahan, R. J.; Henglein, A. *Radiat. Phys. Chem.* **1978**, *11*, 225.

(31) Noyes, R. M. *Prog. React. Kinet.* **1961**, *1*, 1129.

(32) Dobis, O.; Pearson, J. M.; Szwarc, M. *J. Am. Chem. Soc.* **1968**, *90*, 278.

(33) Chakravorty, K.; Pearson, J. M. *J. Am. Chem. Soc.* **1968**, *90*, 283.





**Figure 2.** Dependence of 1/cage efficiency on 1/η. The data were taken from Table 1.

oxy- $\alpha$ -phenyl isobutyrate.<sup>35</sup> Equation 5 predicts that heavier radicals should give lower combination yields, but an increase in the radius of the diffusing radicals leads to an increase in combination yields, so the two effects tend to cancel each other.<sup>14</sup> Therefore, variations in the slopes in different systems, not accounted for by size changes, are related to different  $\alpha$ . The value of  $A$  was estimated to be  $\sim 1 \times 10^{-5}$  poise  $\text{deg}^{-1/2}$ ,<sup>32,33,35</sup> and hence we calculate that, in our system,  $\alpha = 0.05$ , which is within the range found for trifluoromethyl radicals ( $\alpha = 0.03\text{--}0.1$ )<sup>33</sup> and somewhat higher than that found for the pair of *tert*-butoxy and cumyl radicals ( $\alpha = 0.02$ ).<sup>35</sup>

The decrease in the oxidation yield upon increasing the viscosity suggests the formation of  $\text{CO}_3^{\bullet-}$  and  $\bullet\text{NO}_2$  in a solvent cage. However, this observation cannot distinguish between mechanisms I and II, as the formation of  $\text{CO}_3^{\bullet-}$  and  $\bullet\text{NO}_2$  in any mechanism must take place via the formation of a caged radical pair. The question is whether the stable products (65–70%) are formed by  $[\text{CO}_3^{\bullet-} \bullet\text{NO}_2]_{\text{cage}} \rightarrow \text{CO}_2 + \text{NO}_3^-$  (mechanism I) or by  $\text{ONOOCO}_2^- \rightarrow \text{CO}_2 + \text{NO}_3$  (separate transition states, mechanism II). In either mechanism, the yield of  $\text{ABTS}^+$  will decrease with viscosity, as the radical formation reaction must compete with product formation. The two mechanisms might have different viscosity dependencies, but that is a quantitative matter, which we were unable to predict. Mechanism I is the simpler of the two mechanisms. Mechanism II requires both an  $\text{--O--O--}$  bond scission and an O transfer from  $\text{O=C--}$  to  $\text{=N}$ . It is very unlikely that the two processes occur simultaneously. If the oxygen is transferred first, then it is certainly to be expected that a similar process would be efficient in the cage, i.e., mechanism I. If a five-member ring is postulated

to form followed by  $\text{--O--O--}$  bond breakage, the resulting very short-lived complex  $\text{O}_2\text{NOCO}_2^-$  should also be easily formed in a cage. We also note that the quantum calculations of Houk et al.<sup>36</sup> did not reveal any low-energy transition state, except for homolysis of the  $\text{--O--O--}$  bond. Thus, cage formation of nitrate and  $\text{CO}_2$  is expected in any event, and it would be surprising if another process with nearly the same rate were to occur. This study does suggest other interesting work which could distinguish between the two mechanisms: The reaction of  $\text{CO}_3^{\bullet-}$  with  $\bullet\text{NO}_2$  should be studied to see what fraction of the products are  $\text{CO}_2 + \text{NO}_3^-$  and what fraction is  $\text{ONOOCO}_2^-$ . Unfortunately, this is not an easy experiment to do, as the lifetime of  $\text{ONOOCO}_2^-$  was estimated to be less than  $1 \mu\text{s}$ .<sup>29</sup>

Table 2 shows that the effect of added solutes on the oxidation yield of  $\text{ABTS}^+$  is in the following order: glycerol > PEG 4000 > PEG 20000. In glycerol–water systems, the high viscosity is a direct result of the high degree of hydrogen bonding present, which results in a closely bonded molecular framework. The space available for diffusion of the radicals through this framework is quite small, and consequently, the microscopic viscosity is essentially equal to the macroscopic viscosity.<sup>37,38</sup> In the polymer systems, the actual space between the polymer molecules may be very large, and the radicals diffuse at a rapid rate through the network, as though they were diffusing through a low-viscosity solvent. Thus, the microscopic viscosity in polymer systems does not approximate the macroscopic viscosity, as has been demonstrated for polyisobutylene<sup>39</sup> and PEG (present study). Thus, when testing the effect of the change in viscosity on diffusion rates and product yields, the use of polymers should be avoided.

It is worthwhile to note that Merényi and Lind<sup>29,40</sup> have recently suggested a scheme similar to mechanism I for the self-decomposition of  $\text{ONOOH}$ . Utilizing thermokinetic arguments, they have provided strong evidence for the homolysis of  $\text{ONOOH}$  into  $\bullet\text{OH}$  and  $\bullet\text{NO}_2$ , where a caged radical pair is formed, and in water ca. 40% of the radicals escape the cage and ca. 60% combine to form nitrate. However, in this case, the decomposition rate of peroxynitrite is relatively slow, and peroxynitrite may react, to some extent, directly with glycerol and PEG (unpublished results), as relatively high concentrations of these substrates are required in order to increase the solvent viscosity. In addition,  $\bullet\text{OH}$  radicals will be scavenged by glycerol and PEG to yield the corresponding organic radicals, and in aerated solutions the peroxy radicals, which both may react with  $\text{ONOOH}$ . These three processes may enhance the rate of peroxynitrite decomposition, whereas increasing the viscosity via the addition of the organic substrates reduces the rate of peroxynitrite decomposition, and the two effects may cancel each other. Therefore, the lack of an effect of viscosity on the rate of peroxynitrite decomposition does not rule out homolysis of  $\text{ONOOH}$  into free  $\bullet\text{OH}$  and  $\bullet\text{NO}_2$  radicals.

**Acknowledgment.** This research was supported by Grant 4129 from The Council For Tobacco Research and by The Israel Science Foundation. We thank Drs. Harold A. Schwarz, Gabor Merényi, Giuseppe, L. Squadrito, and William A. Pryor for stimulating discussions and valuable suggestions.

JA9822254

(34) Keifer, H.; Traylor, T. *J. Am. Chem. Soc.* **1967**, *89*, 6667.

(35) Herkes, F. E.; Friedman, J.; Bartlett, P. D. *Int. J. Chem. Kinet.* **1969**, *1*, 193.

(36) Houk, K. N.; Condroski, K. R.; Pryor, W. A. *J. Am. Chem. Soc.* **1996**, *118*, 13002.

(37) Williams, J. W.; Cady, L. C. *Chem. Rev.* **1934**, *14*, 171.

(38) Lauffer, M. A. *Biochem. J.* **1961**, *1*, 205.

(39) Heppell, G. E. *Photochem. Photobiol.* **1965**, *4*, 7.

(40) Merényi, G.; Lind, J. *Chem. Res. Toxicol.* **1998**, *11*, 243.