Does Peroxynitrite Partition between Aqueous and Gas Phases? Implication for Lipid Peroxidation

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Relative reactivity of alkane and alkylbenzene oxidation by peroxynitrite has been determined in acidic aqueous solutions. The observed data are explained assuming that the reaction simultaneously proceeds in both the gas and the liquid phases. The selectivity in the gas phase was found to be very similar to that of discrete hydroxyl radicals. The ability of peroxynitrite to react with organic substrates in the gas phase suggests that similar processes may occur in lipophilic media. The reaction mechanism and implication for lipid peroxidation are discussed.

Introduction

The recent interest in the chemistry of peroxinitrite[1] is mainly based on its importance in physiological processes (*1−6*). Peroxynitrite can be formed in vivo through a diffusion-controlled recombination reaction [$k = (4.3 \times 10^9)-(1.9 \times 10^{10})$ M^{-1} s^{-1}] (*7−9*) of nitrogen monooxide ($NO^\bullet$) and the superoxide anion ($O_2^{\bullet-}$). Peroxynitrous acid, HOONO, is also known as an intermediate in the oxidation of nitrites to nitrates by hydrogen peroxide in acidic media (*10*) and in the oxidation of hydroxylamine by dioxygen (*11*). The peroxynitrite anion is stable, while peroxynitrous acid ($pK_a = 6.8$) quickly isomerizes ($\tau_{1/2} \approx 1$ s) in a monomolecular reaction and may produce free radicals (*1−6, 12−16*). The reaction is catalyzed by CO_2 (*6, 17−19,* and references cited therein). Despite numerous studies, there is no consensus as to the nature and the yield of these radicals (*14, 16,* and references cited therein). Their high reactivity and involvement in many side reactions make the interpretation of the systems very complex. The hydroxyl radical formation may proceed through the homolysis of peroxynitrous acid (*12, 15, 16, 20*) with a yield of 28% in the absence of CO_2 (*15*). The well-known cytotoxic properties of peroxynitrite are attributed to its ability to react with cell constituents (*1−6, 12, 18, 19*). Polyunsaturated acids in lipid membranes are key targets for peroxynitrite, resulting in lipid peroxidation in the presence of dioxygen (*12, 21*). Recent computational studies (*22*) showed that a direct reaction of peroxynitrite with C−H bonds in hydrocarbons is unlikely to compete with the homolysis of peroxynitrous acid to the discrete hydroxyl radical. The extremely low solubility of hydrocarbons in water does not allow use of routine experimental techniques and approaches to study this reaction. So far, there is only our early experimental work on this subject (*23*). It is worth mentioning that peroxynitrite easily crosses lipid membranes (*24, 25*) by two mechanisms: as an anion through anion channels and in the protonated form by passive diffusion. It has been also pointed out that peroxynitrite-dependent nitration of tyrosine in the aqueous phase is different from nitration in the membrane (*26*). Thus, one could suggest two different pathways of peroxynitrite reactions with lipophilic substrates: (i) radicals are formed in aqueous media and then react with a substrate bound to the membrane surface (heterogeneous reaction); (ii) when crossing a lipid membrane, peroxynitrous acid may undergo homolysis in the lipophilic medium (homogeneous reaction). While the first pathway is obvious, the possibility that the second takes place has never been explored due to numerous experimental complications. In our previous work, we have used alkanes and alkylarenes as probes for peroxynitrite reactivity in water. Alkanes and alkylarenes are the least reactive compounds and can be oxidized only by highly reactive species. The reactivity of alkanes and alkylarenes toward different oxidants is well documented in the literature (*27−30*), which makes them suitable substrates for such studies. We have reported (*23*) that cyclohexane is oxidized by peroxynitrite at pH 7.0 to cyclohexanone and cyclohexanol (2:1), and that nitrocyclohexane is a minor product. Alkylarenes give a mixture of phenols, nitrophenols, and nitroarenes (*31*). The mixture of H_2O_2 and HNO_2, which is known to produce peroxynitrite as an intermediate (*3, 10, 32, 33*), also oxidizes alkanes and alkylbenzenes at pH 4.3 to the same products as authentic peroxynitrite (*23*). The kinetics of the reaction of peroxynitrite with alkanes cannot be explained unless one assumes that the reaction may also proceed simultaneously in the liquid and in the gas phases. The gas-phase reaction can be considered as a model for peroxynitrite reactions in a lipophilic nonpolar environment. In this work, we studied the gas-phase reaction in detail and found that peroxynitrite was as reactive in the gas phase as the hydroxyl radical. Thus, peroxynitrous acid may undergo facile homolysis into discrete hydroxyl radical and nitrogen dioxide in lipophilic medium.

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[1] The term peroxynitrite will be used for both peroxynitrite anion, $ONOO^-$, and peroxynitrous acid, HOONO, if otherwise not specified.

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Experimental Procedures

All chemicals were reagent grade and used without additional purification. The reaction was carried out in acetate buffer (pH 4.3, [NaOAc] = 0.1 M, [HOAc] = 0.25 M) in a closed reaction vessel under vigorous agitation at 25 °C. The mixture of two hydrocarbons, A and B, and methane (stable under reaction conditions and used as an internal standard) was introduced through a rubber septum into the reaction vessel containing $NaNO_2$ solution in acetate buffer. After equilibration, the reaction was initiated by injection of H_2O_2 (as 30% aqueous solution, 0.04−0.06 M final concentration). The $[NaNO_2]$:$[H_2O_2]$ ratio was always 1:1, and at pH 4.3, peroxynitrite formation through the reaction in eq 1 proceeds rather slowly, $\tau_{1/2} = 20-30$ min [$k_{obs} \approx 2$ M^{-1} s^{-1} at 25 °C, estimated from (*12*)].

$$H_2O_2 + H^+ + NO_2^- \rightarrow HOONO + H_2O \qquad (1)$$

Peroxynitrite thus formed undergoes a fast monomolecular decomposition ($\tau_{1/2} < 1$ s) to either radicals or an activated intermediate, which in turn react with hydrocarbons. At appropriate time intervals, small volumes of the gas phase were withdrawn from the reaction with a gastight syringe and analyzed by GC. Typical total conversion of hydrocarbons was 50−80%. In accordance with eq 5 (see below), lg [A] was plotted versus lg [B], and the slope of the straight line gave the ratio of observed rate constants $k_{obs}{}^{A}/k_{obs}{}^{B}$. For each couple of substrates, the ratio $k_{obs}{}^{A}/k_{obs}{}^{B}$ was measured at least 3 times under the same conditions, and the reproducibility was better than 3%. The values did not depend on the rate of agitation, showing that the reaction was not controlled by mass transfer.

Microsoft Excel was used to treat and to fit the kinetic data. The sums of the squares of the difference between experimental and theoretical values were minimized in the fitting procedure using the Solver subprogram.

Analysis of cyclohexane oxidation products was performed on an “LKHM-80” gas chromatograph equipped with a FID detector and a 1.5% Tween-80 on Inerton-AW packed column. Products were identified and quantified by comparing with authentic samples. The products were extracted with CCl_4 from aqueous solution saturated with NaCl. Nitrobenzene was used as an internal standard.

Results and Discussion

Kinetics of Hydrocarbon Oxidation. Peroxynitrite generated in situ in the reaction of hydrogen peroxide with nitrite resulted in a consumption of hydrocarbons. Neither hydrogen peroxide nor nitrite oxidized these substrates. Methane was found to be completely inert and not consumed in the reaction, and it was used as an internal standard. Typical kinetic traces are given in Figure 1. The rate-limiting step was peroxynitrite formation. Since both H_2O_2 and NO_2^- were consumed in the course of the reaction, the kinetics were not first-order with respect to the hydrocarbon concentration, and the plot lg [RH] versus time was not linear (Figure 1). The concentrations of H_2O_2 and NO_2^- and the pH were optimized to have a reaction time in the range of 50−100 min and hydrocarbon conversions of 50−80%. When two different hydrocarbons, RH^A and RH^B, were competitively oxidized, the plot lg [RH^A] versus lg [RH^B] gave a straight line (Figure 1).

Oxidation Products. Cyclohexane was oxidized to a mixture of cyclohexanol, cyclohexanone, and dicyclohexyl, with nitrocyclohexane a minor product. The alcohol and ketone were not stable under our experimental conditions and underwent slow oxidation to unidentified products. Cyclohexanol was not oxidized to cyclohexanone. The yield of dicyclohexyl was sensitive to dioxygen concentration and decreased when the argon in a headspace was substituted by air. The total product yield (based on peroxynitrite) and the product ratio were dependent on the ratio of the volumes of the gas and liquid phases, $\lambda = V_g/V_l$. The total product yield based on starting nitrite and typical experimental conditions (pH 4.2, 25 °C, $[H_2O_2] = [NaNO_2] = 84$ mM, reaction time 90 min) increased from 0.27% to 0.44% when λ increased from 0.6 to 12. The total yield of identified products did not exceed 60% based on consumed cyclohexane. At high λ, the cyclohexanone, cyclohexanol, dicyclohexyl (under argon), and nitrocycloxane were formed in the ratio 1:0.6:1.2:0.05.

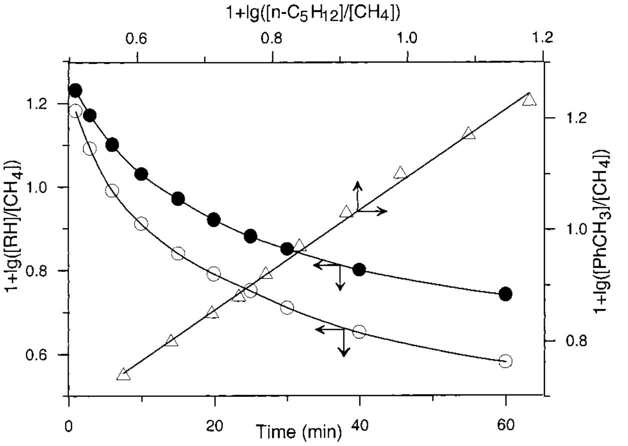


Figure 1. Kinetics of competitive *n*-pentane (○) and toluene (●) oxidation by the $H_2O_2-NaNO_2$ system and the plot lg ([*n*-C_5H_{12}]/[CH_4]) versus lg ([$PhCH_3$]/[CH_4]) (△) in accordance with eq 5. Conditions: $[H_2O_2] = [NaNO_2] = 82$ mM, acetate buffer (pH 4.3, [NaOAc] = 0.1 M, [HOAc] = 0.25 M), 25 °C.

Kinetic Model. Since the oxidation products were not stable under the reaction conditions and their yields were low, it was not possible to monitor the kinetics of product accumulation. Nevertheless, the accurate values of the relative reactivity of hydrocarbons could be obtained by monitoring their relative consumption in the course of the reaction. However, if competitive reactions proceed through a radical mechanism, primary radicals formed from one of the substrates may react with a second substrate, thus contributing to the measured selectivity. Two different reactive species, $HO^•$ and $NO_2{}^•$, can be formed in peroxynitrite decomposition; therefore, the interpretation of the experimental data may be very complicated. Neither $NO_2{}^•$ nor $RO_2{}^•$ reacts with alkanes in these extremely low concentrations at room temperature. Alkanes are also very inert to alcohols and ketones. Alkylarenes are likely to be nonreactive toward these species, but some reactions with NO_2 or $RO_2{}^•$ radicals may take place. Thus, alkanes and alkylarenes seem to be the ideal substrates to study peroxynitrite reactivity.

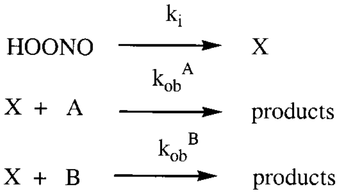
In the simplest case, when a competitive oxidation of A and B by a single reactive species X formed from peroxynitrite proceeds exclusively in a liquid phase (Scheme 1), then the consumption of each substrate can be described by eqs 2 and 3:

$$d[A]/dt = k_{obs}{}^{A}[A][X] \qquad (2)$$

$$d[B]/dt = k_{obs}{}^{B}[B][X] \qquad (3)$$

where $k_{obs}{}^{A}$ and $k_{obs}{}^{B}$ are the reaction rate constants of X

Scheme 1



Scheme 2

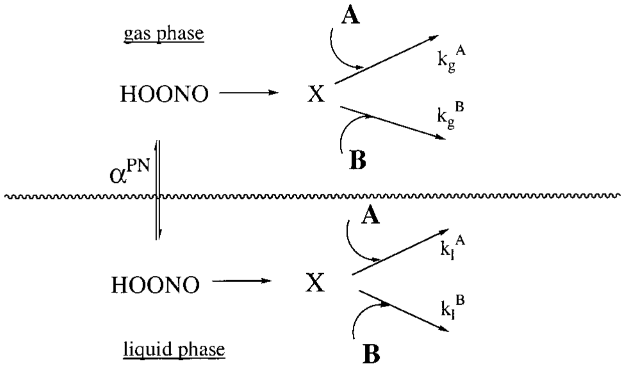


Table 1. Relative Rate Constants for Oxidation of Toluene and *n*-Pentane in H_2O_2−HNO_2/Acetate Buffer System (pH 4.3, [NaOAc] = 0.1 M, [HOAc] = 0.25 M) at 25 °C

λ	k^t_{obs}/k^5_{obs} (exp)	$(k^t/k^5)^a$	k^t_{obs}/k^5_{obs} (theor)^b
0.65	0.61	0.022	0.61
1.20	0.70	0.015	0.70
2.31	0.83	0.012	0.84
5.61	1.04	0.0093	1.04
21.0	1.28	0.0081	1.28

[a] Calculated using eq 6. [b] Calculated using eq 8 with $k_l^t/k_g^t\alpha^X = 0.34$, $k_l^5/k_g^5\alpha^X = 1.20$, $k_g^t/k_g^5 = 1.43$, and $k_l^t/k_l^5 = 0.40$.

with A and B, respectively. Dividing eq 2 by eq 3 gives eq 4, which after integration gives eq 5:

$$d[A]/d[B] = (k_{obs}{}^{A}/k_{obs}{}^{B})[A]/[B] \qquad (4)$$

$$\lg [A] = (k_{obs}{}^{A}/k_{obs}{}^{B}) \lg [B] + \text{const} \qquad (5)$$

Thus, the relative consumption of A and B in competitive oxidation by X is described by eq 5. It is important that eq 5 does not depend on time and concentration of X. By plotting lg [A] versus lg [B], one can determine the ratio of rate constants $k_{obs}{}^{A}/k_{obs}{}^{B}$ from the slope of the straight line. It is worth mentioning that the [A]/[B] ratio may differ substantially in the beginning and in the end of the reaction. The higher the difference between the reactivity of A and B, the more substantial change is in the [A]/[B] ratio in the course of the reaction. A better accuracy is obtained at higher conversions of each substrate. Therefore, the reactivities of both substrates should be close to each other. If the substrates are volatile, this approach requires the use of a reactor without a headspace. However, it is known (*34*) that a mixture of nitrite and hydrogen peroxide at pH 4.8−5.2, which is used to generate pernitrous acid in situ, produces dioxygen as a product. Dioxygen thus formed results in the inevitable formation of small gas bubbles in the reaction solution. Since low molecular weight hydrocarbons are volatile and very poorly soluble in water, they easily escape into these bubbles and substantially increase the experimental error. Therefore, eq 5 cannot be used, and the distribution of hydrocarbons between gas and liquid phases should be taken into account.

If we assume that the reaction proceeds in the liquid phase with the rate constants k^A and k^B and the reactor has a headspace (gas-phase) volume V_g and a liquid-phase volume V_l, then their ratio can be calculated as (*23, 27*):

$$k^A/k^B = (k_{obs}{}^{A}/k_{obs}{}^{B})\{(1 + \alpha^A\lambda)/(1 + \alpha^B\lambda)\} \qquad (6)$$

where $k_{obs}{}^{A}/k_{obs}{}^{B}$ is the ratio of observed rate constant obtained from eq 5; α^A and α^B are the ratios of the concentrations of A and B in the gas and liquid phases [the Henry constant K_A of A is $K_A = [A]_l/P_A$, where $[A]_l$ is the concentration in the liquid phase (expressed in molar) and P_A is the partial pressure (in atm units) of A; then $\alpha^A = 0.041/K_A$ at 25 °C, respectively, and $\lambda = V_g/V_l$]. The values α^A and α^B can be measured independently (*27*).

This kinetic model and eq 6 were used to determine the relative activities of alkanes and alkylarenes toward various oxidants (*27, 35*). As seen from Figure 1, eq 5 was found to also be valid for the reactions with peroxynitrite, and thus the ratios k^A/k^B were determined for different hydrocarbons. However, in the case of peroxynitrite, these values of k^A/k^B appeared to depend on λ. For example, for toluene (k^t) and *n*-pentane (k^5), the ratio k^t/k^5 was 0.022, 0.012, and 0.0081 at $\lambda = 0.65$, 2.3, and 21, respectively. To rationalize such dependence, we had to assume (*23*) that the reaction occurred simultaneously in both the gas and the liquid phase (Scheme 2). In this case, the ratio of the observed rate constants $k_{obs}{}^{A}/k_{obs}{}^{B}$ can be expressed with the rate constants that apply to the liquid ($k_l{}^{A}$, $k_l{}^{B}$) and the gas phase ($k_g{}^{A}$, $k_g{}^{B}$) reactions as follows:

$$\frac{k^A_{obs}}{k^B_{obs}} = \frac{(k^A_g\alpha^A\alpha^X\lambda + k^A_l)(1 + \alpha^B\lambda)}{(k^B_g\alpha^B\alpha^X\lambda + k^B_l)(1 + \alpha^A\lambda)} \qquad (7)$$

where α^X is the ratio of the concentrations of the reactive species X in the gas and liquid phases. This equation can easily be replaced by eq 8:

$$\frac{k^A_{obs}}{k^B_{obs}}\cdot\frac{(1 + \alpha^A\lambda)}{(1 + \alpha^B\lambda)} = \frac{(k^A_g/k^B_g)(\alpha^A/\alpha^B) + (k^A_l/k^B_g\alpha^B\alpha^X\lambda)}{1 + (k^B_l/k^B_g\alpha^B\alpha^X\lambda)} \qquad (8)$$

The values $k_g{}^{A}/k_g{}^{B}$, $k_l{}^{A}/k_g{}^{B}\alpha^X$, and $k_l{}^{B}/k_g{}^{B}\alpha^X$ were determined from the best least-squares fitting of $k_{obs}{}^{A}/k_{obs}{}^{B}$ calculated from eq 8 to experimental data in a wide range of $\lambda = 0.5$−30 at constant and optimal nitrite and acetate concentrations. Some results obtained are given in Table 1. The relative reaction rate constants, $k_l{}^{A}/k_l{}^{B}$, were calculated as the ratio $(k_l{}^{A}/k_g{}^{B}\alpha^X)/(k_l{}^{B}/k_g{}^{B}\alpha^X)$. We have chosen *n*-pentane as a standard; the relative reactivities of other hydrocarbons are expressed as $k_g{}^{A}/k_g{}^{5}$ and $k_l{}^{A}/k_l{}^{5}$.

The described kinetic model suggests that a reactive species X is in equilibrium partition between aqueous and gas phases. However, the reactive species formed from peroxynitrite (i.e., the hydroxyl radical) should be short-lived and unlikely to diffuse from the aqueous into the gas phase. However, ONOOH itself could also partition between the two phases. Therefore, a more complex Scheme 2 has been considered. It gives the same eq 8,

Table 2. Distribution Coefficients, α, and Relative Gas-Phase Rate Constants, $(k/k^5)_g$, for Oxidation of Hydrocarbons by $H_2O_2-HNO_2$/Acetate Buffer System (pH 4.3, [NaOAc] = 0.1 M, [HOAc] = 0.25 M) and by Hydroxyl Radical at 25 °C

hydrocarbon	α	$H_2O_2-HNO_2$	$HO^{\bullet}$ [a]	hydrocarbon	α	$H_2O_2-HNO_2$	$HO^{\bullet}$ [a]
1. propane	29	0.32	0.33	10. cyclohexane-d_{12}	8.0	0.62	0.78
2. *n*-butane	37	0.65	0.66	11. benzene	0.23	0.33	0.32
3. *n*-pentane	49	1.0	1.0	12. toluene	0.26	1.4	1.7
4. *n*-hexane	65	1.3	1.6	13. toluene-d_8	0.26	1.4	
5. *n*-heptane	86	1.7	2.0	14. *o*-xylene	0.18	3.2	3.8
6. *n*-octane	110	2.2	2.3	15. *m*-xylene	0.29	5.4	6.4
7. isobutane	48	0.58	0.64	16. *p*-xylene	0.26	3.4	4.1
8. cyclopentane	7.7	1.2	1.3	17. pseudocumene	0.21	8.4	11
9. cyclohexane	8.0	1.6	1.8	18. mesitylene	0.36	13	17

[a] The data are taken from (*27, 28, 30*).

but α_{obs} should be used instead of α^X, where $\alpha_{obs} = K\alpha^{PN}$, and α^{PN} is the distribution coefficient for ONOOH and K is a function of substrates, nitrite and acetate concentrations. The complexity of the reaction does not allow a simple estimation of α^{PN} in our system. Our rough estimation gives its upper level to be <0.01−0.001. However, regardless of the kinetic model used, the obtained ratios, k_g^A/k_g^B and k_l^A/k_l^B, do not depend on α^X, λ, or on concentrations of any of the possible substrates (including nitrite, acetate, and hydrogen peroxide).

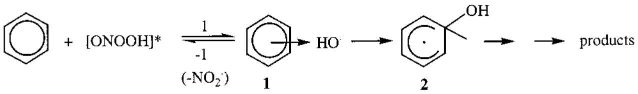
Peroxynitrite Reactivity in the Liquid Phase. Since the solubility of alkanes in water is very low ($\alpha \approx 10^1-10^2$) and the possible reactive species, the hydroxyl radical, quickly reacts with nitrite [5.3×10^9 M^{-1} s^{-1} (*20*), 6×10^9 M^{-1} s^{-1} (*39*)], the contribution of the liquid-phase pathway to their overall oxidation rate appeared to be very low (less than 5−10%), even at the lowest ratios of gas- to liquid-phase volumes ($\lambda = 0.32$). Therefore, an accurate determination of the relative reaction rate constants for alkanes k_l^A/k_l^5 was not possible in the liquid phase.

Alkylarenes are more soluble ($\alpha \approx 0.2-0.4$), allowing their relative reactivity in aqueous solutions to be determined. All alkylarenes studied appeared to react with peroxynitrite at the same rate as benzene, $k_l^{ArH}/k_l^{PhH} = 1.0 \pm 0.1$. The hydroxyl radical has the same reactivity pattern toward alkylarenes in aqueous solutions (*27, 29*). Thus, our data do not contradict the assumption that the HO−ONO bond undergoes homolysis to form the discrete hydroxyl radical and $NO_2^{\bullet}$. The latter one is unlikely to react with the hydrocarbons studied, but in water undergoes fast dimerization and then hydrolysis to nitrate and nitrite.

Peroxynitrite Reactivity in the Gas Phase. The data obtained are listed in Table 2 alongside known values for the relative reactivity of the hydroxyl radical. An excellent correlation between the reactivity of the $H_2O_2 + HNO_2$ system and the hydroxyl radical in the gas phase is observed. Kinetic isotope effects $(KIE)_g$ for c-C_6H_{12}/c-C_6D_{12} oxidation were also similar, 2.65 and 2.50, respectively.

The similarity of substrate selectivity in the gas-phase reaction of alkanes with HOONO and $HO^{\bullet}$ suggests that the same reactive species are involved. Since the hydroxyl radical itself is unlikely to diffuse from water into the gas phase, peroxynitrous acid is probably distributed between both phases. Its homolysis may proceed easily in the gas phase, since for this reaction $\Delta G° = 7.2$ kcal/mol (*5*) and $\Delta H° = 23$ kcal/mol (*36*). As an alternative to the homolysis of HOONO to the discrete hydroxyl radical, an activated intermediate, [ONOO]*, could be formed (*4*) in the gas phase. Such an intermediate can abstract the hydrogen atom from alkanes in a rate-limiting step and show very similar relative reactivity to that of $HO^{\bullet}$:

Scheme 3



$$RH + [ONOOH]^* \rightarrow \{R^{\bullet}...H^{\bullet}...HO^{\bullet}...NO_2^{\bullet}\} \rightarrow R^{\bullet} + H_2O + NO_2^{\bullet} \quad (9)$$

The identified products of cyclohexane oxidation by the $H_2O_2-HNO_2$ mixture are in good agreement with intermediate formation of alkyl radicals. Their fast reaction with dioxygen gives alkylperoxy radicals, and their recombination should form the major products, cyclohexanone and cyclohexanol, with a 1:1 ratio. The experimentally observed ratio, 1:0.5, is lower; since the alcohol was less stable than ketone, the latter underwent further oxidation 2−3 times slower. The recombination of alkyl radicals, or their reaction with nitrogen dioxide, forms dicyclohexyl and nitrocyclohexane, respectively. Thus, the observed products are typical for the reaction with an intermediate formation of alkyl radicals.

Similar to hydroxyl radicals (*29, 30*), the activated intermediate ONOOH* is likely to react with the aromatic nuclei of alkylarenes as shown in Scheme 3. The fast preequilibrium 1 is followed by the slow transformation of the intermediate charge-transfer complex **1** into a σ-complex, the cyclohexadienyl radical, **2**. In this case, the reactivity (k_{ArH}) is controlled by the basicity (lg K_{ArH}) and the ionization potential (I_{ArH}) of a given alkylarene (*35*):

$$\lg (k^{ArH}) = a (\lg K^{ArH}) + b(I^{ArH}) \quad (10)$$

If benzene is chosen as a standard hydrocarbon, then the reactivity of other alkylarenes, ArH, can be expressed as

$$\lg (k^{ArH}/k^{PhH}) = a \{\lg (K^{ArH}/K^{PhH})\} + b(I^{ArH} - I^{ArH}) \quad (11)$$

The above eq can be reduced to eq 12:

$$\lg (k^{ArH}/k^{PhH})/\lg (K^{ArH}/K^{PhH}) = b(I^{ArH} - I^{ArH})/\lg (K^{ArH}/K^{PhH}) + \text{const} \quad (12)$$

Indeed, in accordance with eq 12, plots of lg (k^{ArH}/k^{PhH})/lg (K^{ArH}/K^{PhH}) versus $(I^{ArH} - I^{PhH})$/lg (K^{ArH}/K^{PhH}) yield straight lines ($R = 0.985$) for alkylrene oxidation in the gas phase by $H_2O_2-HNO_2$ and by hydroxyl radical (Figure 2) with the same slopes. However, unlike in the gas phase, the formation of the charge-transfer complex,

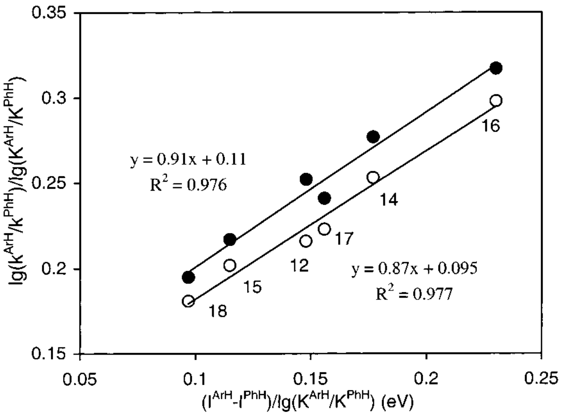


Figure 2. Plot of lg (k^{ArH}/k^{PhH})/lg (K^{ArH}/K^{PhH}) versus ($I^{ArH} - I^{PhH}$)/lg (K^{ArH}/K^{PhH}) for the gas-phase oxidation of alkylarenes by the H_2O_2−$NaNO_2$ system (○) and by the hydroxyl radical (●). The numbers on the plot and in Table 2 designate the same alkylarenes. The data for the hydroxyl radical were taken from (*30*).

1, is the rate-limiting step for the hydroxyl radical in the liquid phase. This rate is diffusion-controlled (*29*); therefore, in aqueous solutions, the reaction proceeds with about the same rate for all alkylarenes studied.

So far, there is no direct experimental proof that peroxynitrite may partition between the two phases and react with hydrocarbons in the gas phase. However, there are several indirect lines of evidence. First, the model of the lower level (when the reactions in the gas phase are not taken into account) has been successfully used in our previous studies of alkane oxidation by various oxidants *(27, 35)*. The data obtained using this method were the same as those obtained by other techniques and methods. This lower level model applied to alkane oxidation by peroxynitrite gives the observed "relative reaction rate constants", dependent on the ratio of volumes of gas and liquid phases, λ. Second, the higher the λ, the higher are the deviations in alkane selectivity from the selectivity of the hydroxyl radical. Third, the extended model (assuming a reaction in the gas phase) gives these relative reaction rate constants independent of λ. Fourth, assuming that peroxynitrite produces the hydroxyl radical in the gas phase, the model quantitatively describes the observed selectivity of alkane oxidation. Or, vice versa, the extended model gives the selectivity for peroxynitrite reactions with alkane in the gas phase similar to that of the hydroxyl radical. Fifth, if the hydroxyl radical is formed from peroxynitrite in the liquid phase, it should react mostly with an excess of nitrite; therefore, only a small proportion of the alkanes (which are very poorly soluble in water) is oxidized in water. Sixth, the higher the ratio of gas-phase to liquid-phase volumes, the higher is the alkane oxidation product yield. Thus, all these above observations support the assumption that alkanes are oxidized by peroxynitrite in the gas phase.

Is Peroxynitrate an Alternative Reactive Species? Under our experimental conditions, peroxynitrite is generated in situ from hydrogen peroxide and nitrite at pH 4.3. However, under these conditions, peroxynitric acid, O_2NOOH, can also be formed. Indeed, one of the known methods to synthesize peroxynitric acid includes a reaction of HNO_2 with an excess of H_2O_2 at low pH (*37*). Peroxynitrate is likely to be formed through intermediate formation of peroxynitrite. In fact, its formation from peroxynitrite has been shown in a wide variety of experimental conditions (*38*). The most likely mechanism of O_2NOOH formation includes a reversible recombination of $HO_2^•$ and $NO_2^•$:

$$HO_2^{\bullet} + NO_2^{\bullet} \rightleftharpoons O_2NOOH \qquad (13)$$

where $k_{13} = 1.8 \times 10^9\ M^{-1}\ s^{-1}$ (*39*) and $k_{-13} = 0.05\ s^{-1}$ (*40*), 0.026 s^{-1} (*41*), and 0.0046 s^{-1} (*42*) at 25 °C.

Peroxynitric acid [pK_a = 5.85 (*39*) and 5.9 (*40*)] is a relatively stable compound, and its lifetime varies in the range (0.6−8) × 10^3 s (*41*, and references cited therein). Moreover, it partitions between the gas and aqueous phase with Henry's Law constant $K_A = 1.26 \times 10^4$ M atm^{-1} ($\alpha = 0.041/K_A \approx 3 \times 10^{-6}$) (*41*). Thus, the possible formation of peroxynitric acid and its reaction with alkanes in the gas phase should also be considered in our system. The major pathway of O_2NOOH decay is its homolysis to $HO_2^•$ and $NO_2^•$ radicals (eq 13). The bond strengths of H−OOH and H−NO_2 are 369 and 328 kJ mol^{-1}, respectively, and are lower than in alkanes and toluene, 400 kJ mol^{-1} in H−cyclohexyl and 376 kJ mol^{-1} in H−$CH_2C_6H_5$ (*43*); the hydrogen atom abstraction from alkanes by these radicals is thermodynamically unfavorable. The homolysis of O_2NO−OH to form very reactive $NO_3^•$ and $HO^•$ radicals has never been observed and is estimated to be 94 kJ mol^{-1}, less favorable than reaction 13 [ab initio calculations at the MP4/6-31+G* level (*44*)]. Direct two-electron oxidation of hydrocarbons by O_2NOOH is also very unlikely in our system, since the products formed from cyclohexane oxidation are typical for intermediate alkyl radical formation. Thus, hydrocarbon oxidation by peroxynitrate is very unlikely to occur in our system.

Implications for Lipid Peroxidation. Our finding is relevant to the understanding of lipid peroxidation induced by peroxynitrite. Peroxynitrite is known to permeate rapidly through lipid membranes (*24, 25*) via anion channels and, in the protonated form, by passive diffusion. Meanwhile, peroxynitrite, being a powerful oxidant, initiates lipid peroxidation (*18* and references cited therein). Such a peroxidation may be caused by free radicals formed from peroxynitrous acid that have undergone homolysis in aqueous medium. This study shows that peroxynitrite, that diffuses into a hydrophobic milieu, can produce these species with the same reactivity as that of the hydroxyl radical. The physiological and toxicological relevance of this reaction depends on several factors: peroxynitrite solubility in a hydrophobic environment, its lifetime, and the radical yield. So far none of these factors have been studied. From our kinetic data, we roughly estimated the upper level of α^{PN} to be <0.01−0.001. The Henry's Law K_A constant for peroxynitric acid is 12 600 ± 4700 M atm^{-1} (*41*); thus, $\alpha = 0.041/K_A \approx 3 \times 10^{-6}$. A similar value of $\alpha = 5 \times 10^{-7}$ has been reported for H_2O_2 (*45*). Thus, the value of α^{PN} could also be in the range 10^{-6}−10^{-7}. We note, however, that a distribution coefficient for H_2O_2 in the two-phase system benzene/H_2O is $\alpha = 0.013$ (*46*), which is several orders of magnitude higher than for the distribution between gas and aqueous phases. Therefore, peroxynitrous acid solubility could also be much higher in lipophilic media than α^{PN} for the case of water/gas.

Conclusions. A competitive oxidation of alkanes and alkylarenes by an aqueous H_2O_2 + HNO_2 mixture shows that reaction is likely to proceed simultaneously in the liquid and in the gas phase. The reactive species formed in the gas phase has the same reactivity as that of the hydroxyl radical. This requires that peroxynitrous acid, after its formation, might diffuse into the gas phase. In water, the homolytic cleavage of the HO−ONO bond may produce hydroxyl radicals, or, alternatively, an activated intermediate [ONOOH]* is formed. The similar diffusion from water to a lipophilic environment may take place in cells. Subsequent peroxynitrite decomposition into radicals may cause lipid peroxidation and other oxidative damage of membrane constituents.

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References

(1) Beckman, J. S. (1996) The physiological and pathophysiological chemistry of nitric oxide. In *The Biological Chemistry of Nitric Oxide* (Lancaster, J., Ed.) Academic Press, New York.

(2) Pfeiffer, S., Mayer, B., and Hemmens, B. (1999) Nitric Oxide: Chemical puzzles posed by a biological messanger. *Angew. Chem., Int. Ed. Engl.* **38**, 1715−1731.

(3) Edwards J. O., and Plumb, R. C. (1994) The chemistry of peroxonitrites. In *Progress in Inorganic Chemistry* (Karlin, K. D., Ed.) pp 599−635, Wiley, New York.

(4) Pryor, W. A., and Squadrito, G. L. (1995) The chemistry of peroxynitrite: A product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* **268**, L699−L722.

(5) Koppenol, W. H. (1999) Chemistry of peroxynitrite and its relevance to biological systems injury. *Met. Ions Biol. Syst.* **36**, 597−619.

(6) Hurst, J. K., and Lymar, S. V. (1999) Cellular generated inorganic oxidants as natural microbicidal agents. *Acc. Chem. Res.* **32**, 520−528.

(7) Huie, R. E., and Padmaja, S. (1993) The reaction rate of nitric oxide with superoxide. *Free Radical Res. Commun.* **18**, 195−199.

(8) Goldstein, S., and Czapski, G. (1995) The reaction of $NO^•$ with $O_2^{•-}$ and $HO_2^•$: A pulse radiolysis study. *Free Radical Biol. Med.* **19**, 505−510.

(9) Kissner, R., Nauser, Th., Bugnon, P., Lye, P. G., and Koppenol, W. H. (1997) Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. *Chem. Res. Toxicol.* **10**, 1285−1292.

(10) Halfpenny, E., and Robinson, P. L. (1952) Pernitrous acid. The reaction between hydrogen peroxide and nitrous acid, and the properties of an intermediate product. *J. Chem. Soc.*, 928−938.

(11) Hughes, M. N., Nicklin, H. G., and Shrimanker, K. (1971) Autoxidation of hydroxylamine in alkaline solutions. II. Kinetics. Acid dissociation constant of hydroxylamine. *J. Chem. Soc. A*, 3485−3487.

(12) Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., and Freeman, B. A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1620−1624.

(13) Crow, J. P., Spruell, C., Chen, J., Gunn, C., Ischiropoulos, H., Tsai, M., Smith, C. D., Radi, R., Koppenol, W. H., and Beckman, J. S. (1994) On the pH-dependent yield of hydroxyl radical products from peroxynitrite. *Free Radical Biol. Med.* **16**, 331−338.

(14) Richeson, C. E., Mulder, P., Bowry, V. W., and Ingold, K. U. (1998) The complex chemistry of peroxynitrite decomposition: New insights. *J. Am. Chem. Soc.* **120**, 7211−7219.

(15) Gerasimov, O. V., and Lymar, S. V. (1999) The Yield of Hydroxyl Radical from the Decomposition of Peroxynitrous Acid. *Inorg. Chem.* **38**, 4317−4321.

(16) Coddington, J. W., Hurst, J. K., and Lymar, S. V. (1999) Hydroxyl Radical Formation during Peroxynitrous Acid Decomposition. *J. Am. Chem. Soc.* **121**, 2438−2443.

(17) Lymar, S. V., and Hurst, J. K. (1998) CO_2-Catalyzed One-Electron Oxidations by Peroxynitrite: Properties of the Reactive Intermediate. *Inorg. Chem.* **37**, 294−301.

(18) Goldstein, S., and Czapski, G. (1997) The Effect of Bicarbonate on Oxidation by Peroxynitrite: Implication for Its Biological Activity. *Inorg. Chem.* **36**, 5113−5117.

(19) Squadrito, G. L., and Pryor, W. A. (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radical Biol. Med.* **25**, 392−403.

(20) Merenyi, G., Lind, J., Goldstein, S., and Czapski, G. (1999) Mechanism and thermochemistry of peroxynitrite decomposition in water. *J. Phys. Chem. A* **103**, 5685−5691.

(21) Radi, R., Beckman, J. S., Bush, K. M., and Freemen, B. A (1991) Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* **288**, 481−487.

(22) Shustov, G. V., Spinney, R., and Rauk, A. (2000) Mechanism of peroxynitrite oxidation of aliphatic CH bonds in saturated and unsaturated hydrocarbons. A theoretical model for the CH oxidation of lipids. *J. Am. Chem. Soc.* **122**, 1191−1199.

(23) Rudakov, E. S., Lobachev, V. L., Geletii, Yu. V., and Balavoine, G. G. A. (1996) Unusual kinetics of the reactions of alkanes and arenes with peroxynitrous acid: synchronous reactions in aqueous and gas phases under conditions of the kinetic distribution method. *Russ. J. Org. Chem.* **32**, 500−507.

(24) Marla, S. S., Lee, J., and Groves, J. T. (1997) Peroxynitrite rapidly permeates phospholipid membranes. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 14243−14248.

(25) Denicola, A., Souza, J. M., and Radi, R. (1998) Diffusion of peroxynitrite across erythrocyte membranes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3566−3571.

(26) Goss, S. P. A., Singh, R. J., Hogg, N., and Kalyanaraman, B. (1999) Reactions of $NO^•$ and $NO_2^•$ and peroxynitrite in membranes: Physiological implications. *Free Radical Res. Commun.* **31**, 597−606.

(27) Rudakov, E. S. (1985) *Reactions of alkanes with oxidants, metal complexes and radicals in solution*, p 186, Naukova dumka, Kiev (in Russian).

(28) Atkinson, R. (1986) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. *Chem. Rev.* **86**, 69−201.

(29) Sehested, K., Corfitzen, H., Christensen, H. C., and Hart, E. J. (1975) Rates of O^-, OH, and H with methylated benzenes in aqueous solution. Optical spectra of radicals. *J. Phys. Chem.* **79**, 310−315.

(30) Perri, R. A., Atkinson, R., and Pitts, J. N. (1977) Kinetics and mechanism of gas-phase reaction of OH radicals with aromatic hydrocarbons over the temperature range 296−473 K. *J. Phys. Chem.* **81**, 296−304.

(31) Halfpenny, E., and Robinson, P. L. (1952) The nitration and hydroxylation of aromatic compounds by pernitrous acid. *J. Chem. Soc.*, 939−946.

(32) Anbar, M., and Taube, H. (1954) Interaction of nitrous acid with hydrogen peroxide and with water. *J. Am. Chem. Soc.* **76**, 6243−6247.

(33) Goldstein, S., Cabelli, D., and Czapski, G. (1998) Determination of optimal conditions for synthesis of peroxynitrite by mixing acidified hydrogen peroxide with nitrite. *Free Radical Biol. Med.* **24**, 653−659.

(34) Mahoney, L. R (1970) Evidence for formation of hydroxyl radicals in izomerization of pernitrous acid to nitric acid in aqueous solution. *J. Am. Chem. Soc.* **92**, 5262−5263.

(35) Lobachev, V. L., Rudakov, E. S., and Zaytchuk, E. V. (1996) Kinetics, kinetic isotope effects, and substrate selectivity of alkylbenzene oxidation in aqueous permanganate solutions: IV. The activation parameters for reactions with the MnO_4^- anion. *Kinet. Catal.* **37**, 165−168.

(36) Koppenol, W. H., Moreno, J. J., Pryor, W. A., Ischiropoulos, H., and Beckman J. S. (1992) Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem. Res. Toxicol.* **5**, 834−842.

(37) Appelman, E. H., and Gosztola, D. J. (1995) Aqueous peroxynitric acid ($HOONO_2$): a novel synthesis and some chemical and spectroscopic properties. *Inorg. Chem.* **34**, 787−791.

(38) Hodges, G. R., and Ingold, K. U. (1999) Cage-escape of geminate radical pairs can produce peroxynitrate from peroxynitrite under a wide variety of experimental conditions. *J. Am. Chem. Soc.* **121**, 10695−10701.

(39) Loegager, T., and Sehested, K. (1993) Formation and decay of peroxynitric acid: a pulse radiolysis study. *J. Phys. Chem.* **97**, 10047−10052.

(40) Goldstein, S., and Czapski, G. (1997) Reactivity of peroxynitric acid (O_2NOOH): a pulse radiolysis study. *Inorg. Chem.* **36**, 4156−4162.

(41) Régimbal, J.-M., and Mozurkewich, M. (1997) Peroxynitric acid decay mechanisms and kinetics at low pH. *J. Phys. Chem.* **101**, 8822−8829.

(42) Lammel, G., Perner, D., and Warneck, P. (1990) Decomposition of pernitric acid in aqueous solution. *J. Phys. Chem.* **94**, 6141−6144.

(43) *CRC Handbook of Chemistry and Physics* (2000) (Lide, D. R., Ed.) 81st ed., CRC Press, Boca Raton, FL.

(44) Antonovskii, V. L., and Bozhenko, K. V. (1995) Ab initio study of the conformational mobility and thermochemistry of radical dissociation of peroxynitric acid. *Dokl. Akad. Nauk (Transl. Dokl. Chem.)* **345**, 196−198.

(45) O'Sullivan, D. W., Lee, M., Noone, B. C., and Heikes, B. G. (1996) Henry's law constant determinations for hydrogen peroxide, methyl hydroperoxide, hydroxymethyl hydroperoxide, ethyl hydroperoxide, and peroxyacetic acid. *J. Phys. Chem.* **100**, 3241−3247.

(46) Yurzhenko, T. I., Gromova, G. N., and Khaizer, V. B. (1946) Study of various types of peroxides as initiators of emulsion polymerization. *Zh. Obshch. Khim.* **16**, 1505−1520 (in Russian).

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