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The reaction of $ONOO^-$ with carbonyls: Estimation of the half-lives of $ONOOC(O)O^-$ and $O_2NOOC(O)O^-$ †

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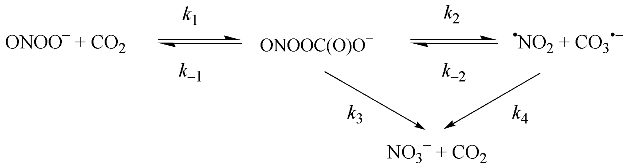
The equilibrium constant for the formation of an adduct between a carbonyl and a hydroperoxide ion varies linearly with the acid dissociation constant of the hydroperoxide. Based on this relationship, the half-life of the adduct formed between $ONOO^-$ and CO_2, $ONOOC(O)O^-$, is estimated to be shorter than 100 ns. Consequently, this adduct should not play any role whatsoever in chemical or biological systems. O_2NOO^- and CO_2 are believed to be involved in a fast equilibrium reaction forming an adduct, $O_2NOOC(O)O^-$. This adduct does not appear to homolyse either along the O–O or the N–O bond. Furthermore, at realistic CO_2 concentrations, the equilibrium should be shifted far to the $O_2NOO^- + CO_2$ side. Therefore, the rate of self-decomposition of O_2NOO^- into NO_2^- and O_2 is unaffected by the presence of bicarbonate.

Introduction

The importance of the coupling of $^{\bullet}NO$ with $O_2^{\bullet -}$ to yield $ONOO^-$ in biological systems was first suggested by Beckman *et al.*,[1] and since then a growing interest has centered on the chemical and biological properties of peroxynitrite ($ONOOH$/$ONOO^-$). The decomposition of peroxynitrite is often accompanied by the formation of peroxynitrate (O_2NOOH/O_2NOO^-),[2,3] and therefore the chemical properties of this peroxo species are also of great importance. Peroxynitrite ion reacts relatively fast with CO_2, *i.e.* $k = (2.9 \pm 0.3) \times 10^4\ M^{-1}\ s^{-1}$ at 24 °C,[4] whereas peroxynitrate was found to be stable in the presence of bicarbonate.[2,3] It was found that CO_2 reacts with $ONOO^-$ to yield NO_3^- as a final product,[5,6] and that this process proceeds *via* the formation of about 33% $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ in the bulk of the solution,[2,3,5–10] which are capable of oxidizing and nitrating a large variety of substrates.[2–8] The reaction of $ONOO^-$ with CO_2 most probably forms an adduct to one of the two C=O bonds of CO_2, *i.e.* $ONOOC(O)O^-$, which homolyses to form about 33% $^{\bullet}NO_2$ and $CO_3^{\bullet -}$ free radicals, and the remainder forms NO_3^- and CO_2 (Scheme 1).



Scheme 1

The decay of $ONOO^-$ in the presence of excess CO_2 and the formation of several oxidized or nitrated substrates were shown to occur in the same kinetic step using the stopped-flow technique, which indicates that $ONOOC(O)O^-$ does not accumulate within 2–3 ms, *i.e.* the lifetime of $ONOOC(O)O^-$ must be significantly shorter than 2–3 ms.[8,10] Merényi and Lind[11] estimated a lifetime for $ONOOC(O)O^-$ on the order of submicroseconds. Surprisingly, it was found that peroxynitrate is stable in the presence of bicarbonate,[2,3] though there is no reason to assume that it reacts differently from peroxynitrite with CO_2. It is also expected that the half-life of $O_2NOOC(O)O^-$ would be significantly longer than that of $ONOOC(O)O^-$ because similar species such as O_2NOOH[12] and O_2NOOCH_3[13] are relatively stable, as they show no tendency to homolyse along the O–O bond. Under physiological conditions, where the most abundant species are $ONOO^-$ and O_2NOO^-, *i.e.* $pK_a(ONOOH) = 6.6$,[14] $pK_a(O_2NOOH) = 5.9$,[15] and the concentration of CO_2 exceeds 1 mM, the toxicity of these peroxo species are most probably governed by the products of their reaction with CO_2. Therefore, the determination of the half-lives of $ONOOC(O)O^-$ and $O_2NOOC(O)O^-$ is important due to their potential role as reactive species in biochemical systems. In the present study we estimate the half-life of $ONOOC(O)O^-$ by relating the experimental data to the rate constants in Scheme 1, and explain why peroxynitrate is stable in the presence of bicarbonate.

Discussion

The reaction of $ONOO^-$ with CO_2

In a recent publication the equilibrium constant K_5 was determined to be $0.32\ M^{-1}$ using NMR spectroscopy.[16]

$$HCO_3^- + H_2O_2 \rightleftharpoons HOOCO_2^- + H_2O \quad K_5 = 0.32\ M^{-1} \quad (5)$$

With $pK_a(H_2O_2) = 11.7$ and $pK(CO_2) = 6.36$ for the hydration of CO_2,[17] we obtain $K_6 = 7 \times 10^4\ M^{-1}$.

$$CO_2 + HOO^- \rightleftharpoons HOOC(O)O^- \quad K_6 = 7 \times 10^4\ M^{-1} \quad (6)$$

The equilibrium constant K_1 was calculated by assuming that the equilibrium constant for the formation of an adduct between the C=O double bond of a certain compound and a hydroperoxide ion varies linearly with the acid dissociation constant of the hydroperoxide. Specifically, if the two hydroperoxide anions HOO^- and $ONOO^-$ add to the C=O bond of CO_2, this assumption implies that $\log K_6 - \log K_1 \approx pK_a(H_2O_2)$

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$-$ pK_a(ONOOH) = 11.7 − 6.6 = 5.1, whence $K_1 \approx 1$ M^{-1}. The validity of the above relationship will be demonstrated below for the addition of these two hydroperoxides to acetaldehyde and acetone, *i.e.* $\log K_7 - \log K_8 \approx 5$.

$$R^1R^2C{=}O + HOO^- \rightleftharpoons R^1R^2C(OOH)O^- \quad (7)$$

$$R^1R^2C{=}O + ONOO^- \rightleftharpoons R^1R^2C(OONO)O^- \quad (8)$$

Our recent study[13] on the reactions of CH_3CHO and $(CH_3)_2CO$ with $ONOO^-$ has disclosed the equilibrium constant of reaction 8 to be ≈2 × 10^{-5} and ≈6 × 10^{-8} M^{-1}, respectively. The equilibrium constant of reaction 7 was calculated using eqns. 9–11.

$$R^1R^2C{=}O + H_2O_2 \rightleftharpoons R^1R^2C(OOH)OH \quad (9)$$

$$R^1R^2C(OOH)OH \rightleftharpoons R^1R^2C(OOH)O^- + H^+ \quad (10)$$

$$R^1R^2C(OONO)OH \rightleftharpoons R^1R^2C(OONO)O^- + H^+ \quad (11)$$

The equilibrium constants of reaction 9 for acetaldehyde and acetone are known (see Table 1). Utilizing the well-known linear relationship between the pK_a of a nucleophile and that of the nucleophile–carbonyl adduct,[20] we estimated pK_{10} ≈ 13.0 and ≈ 13.3 for acetaldehyde and acetone, respectively.[21] Thus, the K_7-values are calculated from Hess' law, using the values of K_9, K_{10} and p$K_a(H_2O_2)$, *i.e.* $K_7 = K_9K_{10}/K_a(H_2O_2)$. All the relevant equilibrium constants are compiled in Table 1. Comparing K_7 with K_8 for acetaldehyde and acetone, we find that $\log K_7 - \log K_8 \approx 4.5$ and ≈ 5, respectively, which is almost the same as the difference between the pK_a values of the conjugate acids of the nucleophilic hydroperoxides, *i.e.* p$K_a(H_2O_2)$ − pK_a(ONOOH) = 11.7 − 6.6 = 5.1. This good fit confirms the validity of our assumptions, which formed the basis for our estimated $K_1 \approx 1$ M^{-1}.

The Gibbs energy of formation of $ONOO^-$ has been determined to be 16.4 kcal mol^{-1},[23] and the literature values of the Gibbs energies for CO_2, $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ are −92.2, −89.5 and 15.1 kcal mol^{-1}, respectively.[24,25] Hence, $(\Delta G_1^{\circ} + \Delta G_2^{\circ}) = -RT\ln(K_1K_2) = 1.4$ kcal mol^{-1}, $K_1K_2 = 0.1$, $K_1 \approx 1$ M^{-1} and $K_2 \approx 0.1$ M.

The experimental second order rate constant for the reaction of $CO_3^{\bullet -}$ with $^{\bullet}NO_2$ has been determined to be $k_R = (4.3 \pm 1) \times 10^8$ M^{-1} s^{-1} using the pulse radiolysis technique.[26] The authors started with approximately equimolar amounts of $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ (a few times 10^{-5} M each), and their findings were consistent with a single kinetic step to yield CO_2 and NO_3^-, *i.e.* peroxynitrite was not formed under these conditions. According to the model given in Scheme 1, $k_R = \{k_4 + k_{-2}(k_{-1} + k_3)/(k_{-1} + k_2 + k_3)\}$, and since $k_3/(k_2 + k_3) = 0.67$,[2,3,5–10] it follows that k_R equals $(k_4 + 0.67k_{-2})$ if $k_{-1} \ll k_2$ or $(k_4 + k_{-2})$ if $k_{-1} \gg k_2$.

As the adduct can never accumulate, k_{-2} is not measurable and has to be estimated. Merényi *et al.*[27] have demonstrated that the reaction of $^{\bullet}OH/O^{\bullet -}$ with $^{\bullet}NO_2$ generates almost equal amounts of $ONOOH/ONOO^-$ and $NO_3^- + H^+$. This suggests that oxygen-centered radicals react with $^{\bullet}NO_2$ in such a way that initially *ca.* half of the reaction results in N–O coupling while the other half gives rise to O–O coupling. We thus have reason to believe that the oxygen-centered $CO_3^{\bullet -}$ radical should react with $^{\bullet}NO_2$ in a similar way to $^{\bullet}OH/O^{\bullet -}$.

Assuming a generous range of uncertainty, we set therefore $k_4/k_{-2} = 0.1$–10. This implies that the lowest possible value of k_{-2} is ≈ 3 × 10^7 M^{-1} s^{-1}. However, if we consider the global equilibrium, characterized by $K_1K_2 = 0.1$, we can define the rate constant for radical formation as $k_f = 0.33 \times 2.9 \times 10^4 \approx 10^4$ M^{-1} s^{-1}, and therefore the rate constant of $ONOO^-$ formation from the radicals, $k_b = 0.1/k_f \approx 10^5$ M^{-1} s^{-1}. Since $k_b = k_{-1}k_{-2}/(k_{-1} + 3k_2)$, it follows that k_2 is at least two orders of magnitude higher than k_{-1}, whence $4.3 \times 10^8 = k_4 + 0.67k_{-2}$ applies. Thus, $k_{-2} \approx (0.31 - 6.9) \times 10^8$ M^{-1} s^{-1} and with $K_2 \approx 0.1$ M, one calculates $k_2 \approx (0.31 - 6.9) \times 10^7$ s^{-1}.

Table 1 Summary of the equilibrium constants of reactions 7–11 for acetaldehyde and acetone[a]

	Acetaldehyde	Acetone
K_9/M^{-1}	48[b]	0.086[c]
K_{10}/M^{-1}	≈1 × 10^{-13}[d]	≈5 × 10^{-14}[d]
K_{11}/M^{-1}	≈5 × 10^{-13}[e]	≈1.6 × 10^{-12}[e]
K_7/M^{-1}	≈2	≈2 × 10^{-3}
K_8/M^{-1}	≈2 × 10^{-5}[e]	≈6 × 10^{-8}[e]
$\log K_7 - \log K_8$	≈5	≈4.5

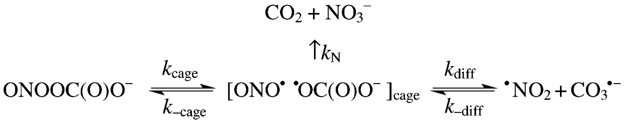
[a] $K_7 = K_9K_{10}/K_a(H_2O_2)$, p$K_a(H_2O_2)$ = 11.7. [b] Ref. 18. [c] Ref. 19. [d] Ref. 21. [e] Ref. 13.

The overall rate constant of the adduct decomposition is $(k_{-1} + k_2 + k_3)$, where $k_3/k_2 \approx 2$.[2,3,5–10] Assuming the steady-state approximation for $ONOOC(O)O^-$ and the radicals, rate law 12 is obtained, where $\beta = k_4/(k_4 + k_{-2})$.

$$-\frac{d[ONOO^-]}{dt} = \frac{k_1(\beta k_2 + k_3)}{k_{-1} + \beta k_2 + k_3}[ONOO^-][CO_2] = 2.9 \times 10^4[ONOO^-][CO_2] \quad (12)$$

As $K_1 \approx 1$ and since k_{-1} is much smaller than $(\beta k_2 + k_3)$, *i.e.* $k_1 \approx k_{-1} \approx 2.9 \times 10^4$ M^{-1} s^{-1}, the overall rate constant of the adduct decomposition turns out to be larger than 10^7 s^{-1}. This estimation confirms and refines our previous conclusion that the half-life of $ONOOC(O)O^-$ is very short, *i.e.* $t_{1/2} < 0.1$ µs. Consequently, it should not play any role whatsoever in chemical or biological systems, except in the trivial sense of being an ephemeral intermediate on route from peroxynitrite to the oxidative radicals $CO_3^{\bullet -}$ and $^{\bullet}NO_2$.

In view of the above, a detailed mechanism is given in Scheme 2, where $ONOOC(O)O^-$ is assumed to decompose *via* homolysis of the O–O bond forming $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ in a solvent cage.

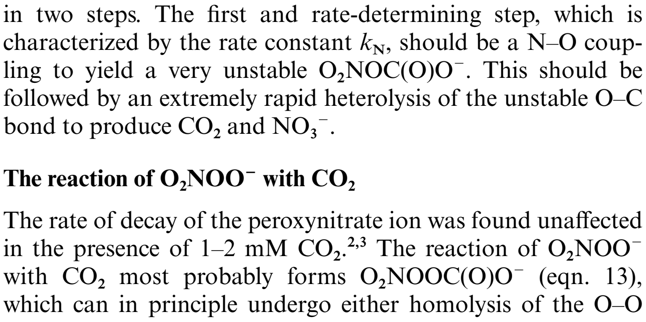


Scheme 2

The formation of CO_2 and NO_3^- most probably occurs in two steps. The first and rate-determining step, which is characterized by the rate constant k_N, should be a N–O coupling to yield a very unstable $O_2NOC(O)O^-$. This should be followed by an extremely rapid heterolysis of the unstable O–C bond to produce CO_2 and NO_3^-.

The reaction of O_2NOO^- with CO_2

The rate of decay of the peroxynitrate ion was found unaffected in the presence of 1–2 mM CO_2.[2,3] The reaction of O_2NOO^- with CO_2 most probably forms $O_2NOOC(O)O^-$ (eqn. 13), which can in principle undergo either homolysis of the O–O bond forming $^{\bullet}NO_3$ and $CO_3^{\bullet -}$ (eqn. 14) or homolysis of the N–O bonds forming $^{\bullet}NO_2$ and $CO_4^{\bullet -}$ (eqn. 15, Scheme 3).



Scheme 3

The equilibrium constant of reaction 13 is calculated, similarly to the calculation of equilibrium 1, by assuming that the equilibrium constant for the formation of an adduct between a carbonyl and a hydroperoxide ion varies linearly with the acid dissociation constant of the hydroperoxide, *i.e.* $\log K_6 - \log K_{13} \approx pK_a(H_2O_2) - pK_a(O_2NOOH) = 11.7 - 5.9 = 5.8$, whence $K_{13} \approx 0.1\ M^{-1}$.

The Gibbs energy of formation of O_2NOO^- has been determined to be 9.4 kcal mol^{-1}.[28] Using the literature values of $\Delta_f G^\circ(CO_3^{\bullet -}) = -89.5$ kcal mol^{-1} [24] and $\Delta_f G^\circ({}^{\bullet}NO_3) = 31.3$ kcal mol^{-1},[24] one calculates $(\Delta G^\circ_{13} + \Delta G^\circ_{14}) = 24.6$ kcal mol^{-1}, *i.e.* $K_{13}K_{14} \approx 1 \times 10^{-18}$ and $K_{14} \approx 1 \times 10^{-17}$ M. The latter low value reveals the impossibility of the homolysis along the O–O bond, *i.e.*, k_{14} is extremely small.

The value of $K_{13}K_{15}$ was estimated as follows. We assume that $HO_2^{\bullet}$ ($pK_a(HO_2^{\bullet}) = 4.8$)[25] can be treated as a hydroperoxide in its reaction with carbonyl compounds, *i.e.* K_{16} is described by a similar linear free energy relationship as those with *bona fide* hydroperoxide anions, and hence $\log K_6 - \log K_{16} \approx 11.7 - 4.8$ (Fig. 1) and $K_{16} \approx 10^{-2}\ M^{-1}$.

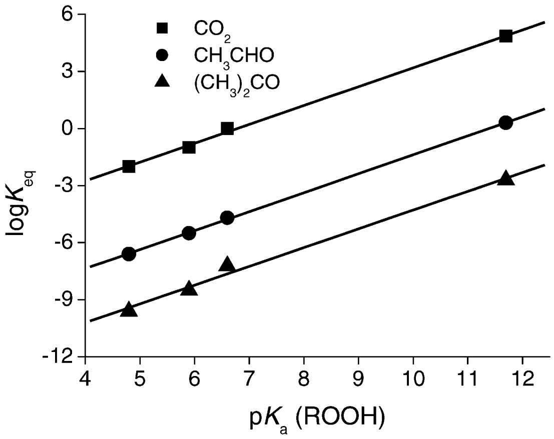


Fig. 1 The relationship between the pK_a's of H_2O_2, ONOOH, O_2NOOH and $HO_2^{\bullet}$ and the equilibrium constants of the formation of the adducts between the hydroperoxide ions and CO_2, CH_3CHO and $(CH_3)_2CO$.

$$O_2^{\bullet -} + CO_2 \rightleftharpoons {}^{\bullet}OOC(O)O^- \qquad (16)$$

Using the literature values $\Delta_f G^\circ(O_2^{\bullet -}) = 7.6$ kcal mol^{-1} [24] and $\Delta_f G^\circ(CO_2) = -92.2$ kcal mol^{-1}, one calculates $\Delta_f G^\circ(CO_4^{\bullet -}) = -81.8$ kcal mol^{-1}, which finally yields $(\Delta G^\circ_{15} + \Delta G^\circ_{13}) \approx 16.1$ kcal mol^{-1}, *i.e.* $K_{13}K_{15} \approx 1 \times 10^{-12}$ and $K_{15} \approx 10^{-11}$ M. The last value, in particular, appears to be a very reasonable estimate, since the corresponding constants for other peroxynitrates, K_{17} and K_{18}, have previously been determined to be 1.4×10^{-11} [12] and $4.9 \times 10^{-11}\ M^{-1}$,[13] respectively.

$$O_2NOOH \rightleftharpoons {}^{\bullet}NO_2 + HO_2^{\bullet} \qquad (17)$$

$$O_2NOOCH_3 \rightleftharpoons {}^{\bullet}NO_2 + CH_3O_2^{\bullet} \qquad (18)$$

We are thus confident that K_{15} is smaller than 10^{-10} M, and since k_{-15} cannot exceed *ca.* $1 \times 10^{10}\ M^{-1}\ s^{-1}$, k_{15} should be smaller than 1 s^{-1}. With $K_{13} \approx 0.1\ M^{-1}$ and given that the highest possible concentration of CO_2 is well below 0.1 M, $K_{13}[CO_2]k_{15}$ can hardly exceed 10^{-2} s^{-1}. The latter value implies that less than 1% of O_2NOO^- can decompose *via* its reaction with CO_2, given that the rate of the self-decomposition of O_2NOO^- is *ca.* 1 s^{-1} at room temperature.[15] Therefore, the rate of the decomposition of O_2NOO^- into NO_2^- and O_2 is unaffected by the presence of realistic concentrations of CO_2.

Conclusions

Peroxynitrite ion reacts relatively fast with CO_2 forming an adduct, $ONOOC(O)O^-$, whose half-life is estimated to be shorter than 100 ns. Consequently, this adduct should not play any role whatsoever in chemical or biological systems. O_2NOO^- and CO_2 are believed to be involved in a fast equilibrium reaction forming an adduct, $O_2NOOC(O)O^-$. This adduct does not appear to homolyse either along the O–O or the N–O bond. Furthermore, at realistic CO_2 concentrations, the equilibrium should be shifted far to the $O_2NOO^- + CO_2$ side. Therefore, the rate of self-decomposition of O_2NOO^- into NO_2^- and O_2 is unaffected by the presence of bicarbonate.

Acknowledgements

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21 $pK(R^1R^2C(OH)_2)$s, *i.e.* $pK(CH_3CH(OH)_2)$ and $pK((CH_3)_2C(OH)_2)$, are 13.6 and 14.5, respectively,[22] and pK_{11}, *i.e.* $pK(CH_3CH(OONO)(OH))$ and $pK((CH_3)_2C(OONO)(OH))$ have been recently derived as ≈12.3 and ≈11.8, respectively.[13] Using the above values, as well as $pK_a(ONOOH) = 6.6$, $pK_a(H_2O_2) = 11.7$, $pK_a(H_2O) = 15.7$, and the linear free energy relationship between the pK_a of the conjugate acid of a nucleophile and the pK of the corresponding adduct to a carbonyl group[19] which in the present case implies $((pK_{10} - pK_{11})/(pK(R_1R_2C(OH)_2) - pK_{11}) \approx (11.7 - 6.6)/(15.7 - 6.6)$, we estimated $pK_{10} \approx 13.0$ and ≈ 13.3 for acetaldehyde and acetone, respectively.
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