

Direct Observation of Intermediates in the Reaction of Peroxynitrite with Carbon Dioxide

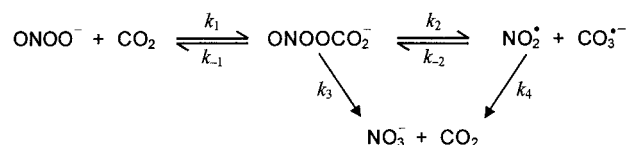
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Peroxynitrite (ONOO^- , oxoperoxonitrate(1-)) reacts with carbon dioxide to form an adduct that absorbs with a maximum at 640 nm and an extinction coefficient of $ca. 2 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$. Within 0.1 s at 4°, this absorption decreases while the maximum is shifted to lower wavelengths, which indicates that trioxocarbonate(1-) radicals ($\text{CO}_3^{\cdot-}$) are formed. This interpretation is supported by the observation of a weak ESR signal at $g = 2.013$.

Introduction. – *Radi et al.* [1] proposed in 1993 that peroxynitrite (oxoperoxonitrate(1-)), an inorganic toxin formed *in vivo*, reacts with hydrogencarbonate(1-). Subsequently, *Lymar* and *Hurst* showed that the peroxynitrite anion reacts with carbon dioxide (CO_2), but not with hydrogencarbonate(1-) or carbonic acid [2]. Rate constants of $3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at 25° [2] and $5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at 37° [3] have been reported. Based on a cellular concentration of CO_2 of 1.3 mM [3] and the stated rate constants, peroxynitrite should disappear with a rate of $ca. 50 \text{ s}^{-1}$, which is higher than that due to the reaction with glutathione peroxidase (15 s^{-1}), glutathione (6 s^{-1}), or other cellular components [4][5]. Thus, the reaction with carbon dioxide is the main pathway for the disappearance of peroxynitrite *in vivo*. It has been suggested that an adduct with the formula ONOOCO_2^- (1-carboxylato-2-nitrosodioxidane) is formed [1], which was shown to be strongly oxidizing [6]. It has also been reported that this adduct is responsible – and even essential – for nitration reactions of peroxynitrite [7][8]. Two estimates for the lifetime of the adduct have been given: 1) shorter than 3 ms [9] and 2) submicroseconds [10]. The stable products of the reaction of peroxynitrite with CO_2 are nitrate and carbon dioxide, which can be formed directly by the adduct (*Scheme, Reactions 1 and 3*) [5][11][12], or *via* the reaction of the homolysis products nitrogen dioxide and the trioxocarbonate(1-) radical (*Scheme, Reactions 1, 2, and 4*) [11][13–15].

Scheme



Given the estimates for the lifetime of ONOOCO_2^- , it may be too short-lived to be observed by stopped-flow spectrophotometry, but if the trioxocarbonate(1-) radical is formed, one should be able to detect it at 600 nm [16][17].

Results and Discussion. – Rapid mixing of excess CO_2 with peroxyxynitrite resulted within 2 ms unexpectedly in a transient absorption with a broad maximum at 640 nm. Over a period of *ca.* 100 ms, this absorption decreased, and the maximum was shifted to lower wavelengths. The spectra shown in *Fig. 1* were obtained with an *OLIS RSM 1000* multi-wavelength stopped-flow spectrophotometer, and identical spectra (not shown) were obtained with an *Applied Photophysics SX17MV* single-wavelength instrument. The initial absorption at 640 nm increases with the peroxyxynitrite concentration in the range 0.03–0.5 mM. The extinction coefficient is *ca.* $2 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$. The lifetimes of $4 \pm 1 \text{ ms}$ at $10^\circ - 12^\circ$, and of *ca.* 15 ms at 4° , exceed that of a solvent cage by 8 orders of magnitude [18], and, therefore, the absorption at 640 nm is unlikely to be due to a $[\text{NO}_2/\text{CO}_3^{\cdot-}]$ radical pair. Trioxocarbonate(1–) and nitrogen dioxide radicals absorb at 600 and 400 nm with extinction coefficients of $1860 \text{ M}^{-1} \text{ cm}^{-1}$ and $200 \text{ M}^{-1} \text{ cm}^{-1}$, respectively [16]. The weak extinction coefficient of nitrogen dioxide and its fast dimerization rate [19] of $4.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ prevent observation of this radical. Nitrogen monoxide scavenges both trioxocarbonate(1–) [17] and nitrogen dioxide radicals [20]. To obtain information about the species absorbing at 640 nm, we mixed CO_2 and peroxyxynitrite in the presence of 0.6 mM nitrogen monoxide. Under these conditions the lifetimes of nitrogen dioxide and trioxocarbonate(1–) radicals are less than $1 \mu\text{s}$. The absorption at 640 nm was observed to the same extent and with a similar rate of decay, but a shift of the maximum was not observed in this case (not shown). Control experiments with peroxyxynitrite alone, or CO_2 alone, or mixing CO_2 with a 10 mM hydroxide solution, or with an alkaline-decayed peroxyxynitrite solution did not produce any transient absorptions above 450 nm. We conclude that the adduct ONOOCO_2^- is responsible for the absorption at 640 nm, and that formation of the trioxocarbonate(1–) radical causes the shift toward 600 nm.

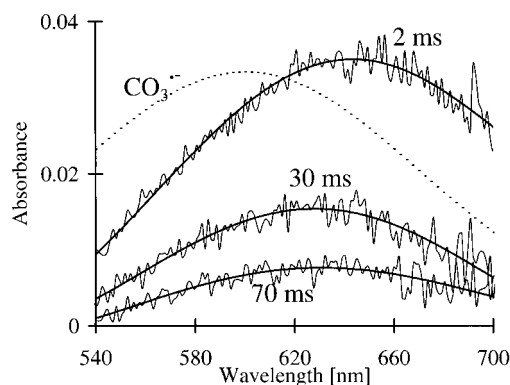


Fig. 1. Absorption spectrum of the adduct of CO_2 with ONOO^- (38 mM CO_2 (pH 3.6) and 0.3 mM peroxyxynitrite (pH 12.5), prior to mixing). This absorption is observed during *ca.* 100 ms at 4° in which time the maximum was shifted from 640 nm to lower wavelengths. The spectrum of $\text{CO}_3^{\cdot-}$ [16] (dashed line) is shown for comparison.

Further support for the formation of the trioxocarbonate(1–) radical was obtained by mixing a solution of peroxyxynitrite (0.22 mM, pH 12.5) with a solution saturated with CO_2 (19 mM, pH 3.6) at room temperature in the cavity of an ESR spectrometer. A weak signal at $g = 2.013$ was observed (*Fig. 2*). As the lifetime of nitrogen dioxide in

alkaline water is ten times shorter than that of the trioxocarbonate(1-) radical, the ESR signal is not derived from nitrogen dioxide. The simple structure of the ESR spectrum implies a *doublet*, and, therefore, it is not due to a $[\text{NO}_2/\text{CO}_3^-]$ radical pair. We conclude that the radical observed is the trioxocarbonate(1-), also because, within the error, this value is identical to that reported for the CO_3^- radical in irradiated calcite, $g = 2.0115$ [21]. No signal was observed when either peroxyxynitrite or CO_2 was omitted.

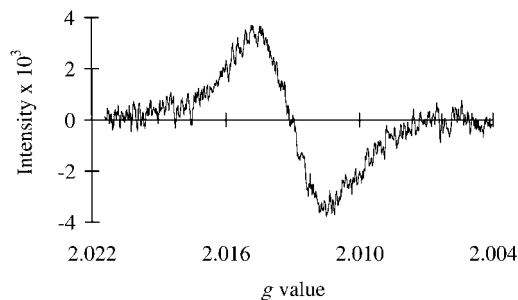


Fig. 2. ESR Spectrum of the CO_3^- radical. A solution of peroxyxynitrite (0.22 mM, pH 12.5) was mixed 1:1 with sat. aq. CO_2 (19 mM, pH 3.6) at room temperature in the cavity of a *Bruker EMX 080* ESR spectrometer equipped with a flow reactor at maximal flow rate. The sum of 40 scans is shown.

Based on the presumed short lifetime of ONOOCO_2^- , oxidations and nitrations have been ascribed to the radical products NO_2 and CO_3^- . The observation that this adduct has a lifetime in the ms range makes it necessary to study its reactivity. Our findings show that indeed trioxocarbonate(1-) radicals are formed during the decay of ONOOCO_2^- , but do not indicate that this is the only mode of decay of the adduct. Qualitatively, the yield of formation of trioxocarbonate(1-) radicals is small, and most of the adduct is likely to decay directly to nitrate and CO_2 (*Scheme 2, Reaction 3*).

Methods and Materials. – ONOOK was produced from the reaction of solid KO_2 with gas NO as described in [22]. Nitrogen monoxide was obtained from *Linde*, CO_2 and Ar from *Pangas*. All other chemicals were obtained at the highest purity from *Fluka* and *Merck*. Stopped-flow studies were carried out with *OLIS RSM 1000* and *Applied Photophysics SX17-MV* stopped-flow spectrophotometers. CO_2 (38 mM, pH 3.6) was mixed with peroxyxynitrite (0.03–0.50 mM, pH 12.5). Sat. CO_2 solns. were prepared from deionized water (*Millipore Milli-Q*) deoxygenated with Ar and then saturated with CO_2 gas at 0° to maximize the CO_2 concentration. Directly after mixing the pH was estimated to be 12, due to dilution. During 100 ms, which equals or exceeds the duration of the experiments, the pH is not expected to have dropped below 9, given a $k(\text{OH}^- + \text{CO}_2)$ of $8.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. Loss of dissolved CO_2 was prevented by the use of a gas-tight syringe equipped with a water jacket. The CO_2 and the peroxyxynitrite solns. were kept at 0° . Stopped-flow experiments were carried out between 5° and 10° . ESR Experiments were carried out by continuously mixing peroxyxynitrite (0.22 mM, pH 12.5) at a ratio of 1:1 with a CO_2 -sat. soln. (19 mM, pH 3.6) at r.t. in the cavity of a *Bruker EMX 080* instrument.

Note added in proof. – Independently, *Bonini et al.* also obtained evidence for the trioxocarbonate(1-) radical by ESR spectroscopy. They report a signal at $g = 2.0113$ [23].

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