This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

All material contained in this paper is copyright of the author, or the owner of the source that the material was taken from. This work is not intended as a threat to the ownership of said copyrights.

Nitrosoperoxocarboxylate

By

Sarita Menon

B-180 Med Labs

Free Radical and Radiation Biology

The University of Iowa

IA-52242.

077:222 Spring 2003

February 12, 2003

Abbreviations

$ONOOCOO^-$: Nitrosoperoxocarboxylate

$ONOO^-$: Peroxynitrite

$^{\bullet}NO$: Nitric Oxide

$O_2^{\bullet-}$: Superoxide

CO_2 : Carbon dioxide

Abstract:

Nitrosoperoxocarboxylate ($ONOOCOO^-$) is a reactive nitrating and oxidizing species that is a first reaction intermediate formed from peroxynitrite and carbon dioxide. This is an extremely fast reaction with rate constant of $k = 5.8 \times 10^4\ M^{-1}s^{-1}$. In biological systems this reaction seems to be a predominant one, due to large amounts of CO_2 present in the tissues. This intermediate performs important biological reactions like nitration of tyrosine residues, which is also important in signaling and affects enzyme expression, which is modulated by phosphorylation.

Hence this paper reviews the nitrosoperoxocarboxylate species, its chemistry and kinetics along with detection method and the biological significance.

The Species :

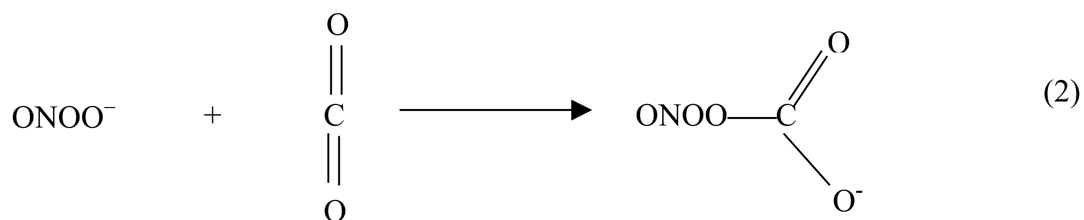
Nitrosoperoxocarboxylate ($ONOOCOO^-$) or 1 carboxylato-2-nitrosodioxidane is the first intermediate formed in the reaction with peroxynitrite (ONO_2^-) and CO_2. Radi et al in 1993 first proposed that biological oxidations by nitroperoxides were actually mediated by the secondary oxidations by intermediates formed from nitroperoxide/bicarbonate reaction. This was based on the observation, that peroxynitrite-induced luminol chemiluminescence, was greatly enhanced by bicarbonate. Carbon dioxide is found ubiquitously within the cell and bicarbonate is the main buffering system in tissues. The nitroperoxide and bicarbonate reaction therefore is of prime biological relevance.

The Chemistry and Kinetics:

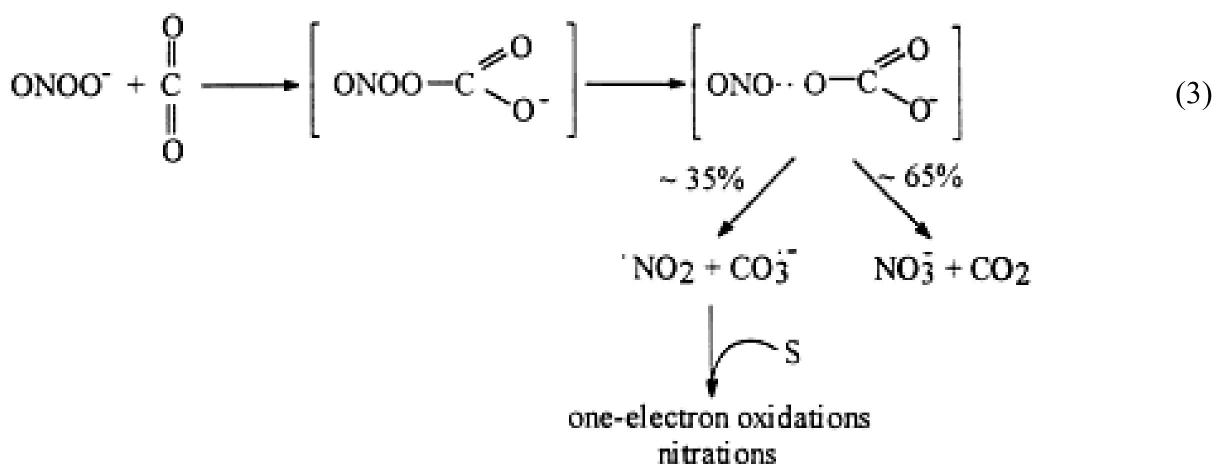
Peroxynitrite is formed from a very fast reaction between $^{\bullet}NO$ and $O_2^{\bullet -}$ (1), with a rate constant of k = (6.7 – 19) x 10^9 M^{-1} s^{-1}.

$$^{\bullet}NO + O_2^{\bullet -} \longrightarrow ONOO^- \quad (1)$$

The $ONOO^-$ form, with a pKa = 6.8, is a predominant species at biological pH (7.4). Carbon dioxide is found in high concentrations in tissues (~ 1 mM) along with its bicarbonate form. Peroxynitrite thus reacts very fast with carbon dioxide (k = 5.8 x 10^4 M^{-1} s^{-1}), forming the adduct nitrosoperoxocarboxylate ($ONOOCOO^-$) (2). This species has an absorbance at 650 nm and disappears at a rate of ca. 2505 s^{-1}.



Due to the predominance of formation of the nitrosoperoxocarboxylate, most of the biological reactivity of peroxynitrite is now being ascribed to this adduct. The nitrosoperoxocarboxylate adduct formation is the rate limiting step and this species further decomposes to form secondary reactive species. As seen from reaction (3), it is believed that the nitrosoperoxocarboxylate adduct decomposes to nitrate and carbon dixode. However in the presence of substrates, it undergoes homolytic cleavage of the weak O-O bond and results in the formation of the carbonate radical and nitrogen dioxide radical species. In biological systems these species are associated with reactions including aromatic nitrations on protein tyrosine residues.



<u>The Detection</u>:

Homolysis of the nitrosoperoxocarboxylate adduct generates the carbonate radical which can be detected by EPR. Since the peroxynitrite and carbon dioxide reaction is extremely fast, the carbonate radical can be detected only by continuous fast flow EPR. Fig 1 shows an EPR spectrum of the carbonate radical detected by fast flow mixing of concentrated solutions of peroxynitrite and bicarbonate at pH 6.9.

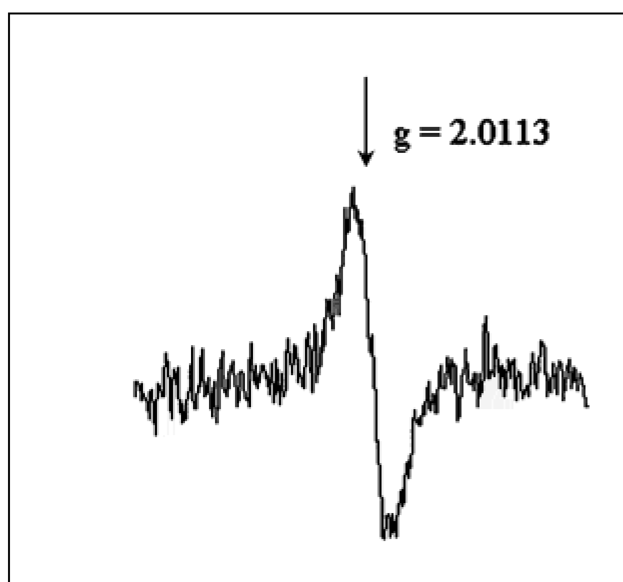


Fig 1 EPR continuous low spectra of the carbonate radical produced from mixing peroxynitrite and bicarbonate solutions at room temperature and under nonequilibrium conditions. Peroxynitrite is present at 40 mM , pH 6.4 and bicarbonate 100 mM in 0.1 M phosphate buffer , pH 6.4. (1).

Other methods of detection include stopped flow spectroscopy at 302 nm, to study peroxynitrite decomposition in the presence of carbon dioxide.

<u>The Biology</u>

The intermediates of peroxynitrite/CO_2 reaction are nitrating, and oxidizing species including nitrosoperoxocarboxylate. Biologically important reaction of this species is nitration of tyrosine residues. Although these nitrations can be pathological, they are also known to play a role in signal transduction. Due to the fast reaction kinetics of nitrosoperoxocarboxylate, scavengers of this reaction are limited. In order to scavenge this reaction, the commonly used scavengers would have to be at toxic levels in order to compete and quench the reaction of peroxynitrite with CO_2.

Some of the biotargets that directly interact with peroxynitrite are heme containing proteins like hemoglobin, seleno-proteins like glutathione peroxidase, peroxidases like myeloperoxidase, proteins with zinc thiolate centers such as DNA – binding transcription factors, and synthetic antioxidant ebselen.

Table-1 shows the comparision in reactivities of peroxynitrite with various biotargets in blood. While most of the peroxynitrite reacts with CO_2, only hemeproteins compete with CO_2. Myeloperoxidase, glutathione peroxidase, serum albumin, and other small antioxidants like glutathione react too slowly and/or are present at very low concentrations, for effective competition

Table 1

Biomolecule	kapp (M^{-1} s^{-1}	biomolecule (M)	k x [biomolecule] (s-1)
Hemoglobin	2.5×10^4	2.3×10^{-3}	58
CO_2	4.6×10^4	1×10^{-3}	46
Myeloperoxidase	4.8×10^6	2×10^{-7}	1
Glutathione peroxidase	2×10^4	$< 1.5 \times 10^{-6}$	<0.03

.

Carbon dioxide has been shown to decrease ablility of peroxynitrite to oxidize glutathione and thiol groups of bovine serum albumin. Likewise peroxynitrite/CO_2 reaction intermediate- nitrosoperoxocarboxylate is also shown to protect the enzyme glutathione peroxidase.

Table 2: Effect of bicarbonate on peroxynitrite mediated inactivation of Glutathione Peroxidase. (GSH-Px).

Compound	**Concentration (mM)**	**% Protection**
Benzoate	100	9.2 ± 1.1
DMSO	100	10.1 ± 2.0
Bicarbonate	40	57 ± 5.0
Bicarbonate	25	55 ± 6.0

Since rate of peroxide anion reaction with CO_2 is about 5.8 $x10^4$ M^{-1} s^{-1}, under the experimental conditions in the above reaction, GSH-Px should be completely protected from peroxynitrite. However, only a partial inactivation was seen even in the presence of 40 mM bicarbonate, suggesting that the bicarbonate/peroxynitrite adduct, nitrosoperoxocarboxylate or its derivatives may inactivate GSH-Px. While there are other reports that show, carbon dioxide assisted enhanced ability of peroxynitrites to nitrate aromatic compounds (Fig 2), and decrease the amount hyroxylated products.

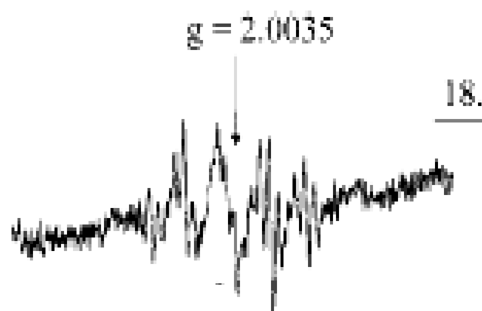


Fig 2. Continous flow EPR spectra of the tyrosyl radical produced from mixing 2 mM peroxynitrite and 3.7 mM tyrosine and 50 mM bicarbonate in 0.3 M phosphate buffer at pH 7.4.

Discussion:

Peroxynitrite production resulted in extensive nitration of tyrosine residues, but it was known that peroxynitrite in itself was a poor nitrating reagent, although it could hydroxylate aromatic compounds. The small nitration yields that were observed, in absence of bicarbonate was attributed to the presence of adventitious

CO_2 dissolved in the solutions. It was earlier thought that it was superoxide dismutase that was interacting with peroxynitrite, transforming it into a nitrating reagent, but the slow rate of superoxide dismutase reaction with peroxynitrite could not be explained when compared to the fast reaction kinetics of peroxynitrite with other biotargets. Thus nitrosoperoxocarboxylate as a reaction intermediate can best explain the biological nitrations. These tyrosine nitration reactions also are important signal transduction molecules, because nitration of tyrosine modulates phosphorylation and controls enzymatic activity.

Peroxynitrite is formed from $^{\bullet}NO$ and $O2^{\bullet -}$, a reaction catalyzed by superoxide dismutase (SOD). In principle increasing SOD concentrations can prevent the biological effects of peroxynitrite, and thereby reducing $O2^{\bullet -}$ levels. Another way of scavenging the radicals would be to trap reaction intermediates like nitrosoperoxocarboxylate and block the deleterious biological effects of peroxynitrite.

References:

1. Bonini MG, Radi R., Sueta GF., Ferreira AM., Augusto O., (1999). Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide. *J. Biol. Chem*. **274**: 10802-10806.
2. Denicola A, Freeman BA, Trujillo M, Radi R. (1996) Peroxynitrite reaction with carbon dioxide/ bicarbonate: Kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys*., **333**: 49-58.
3. Koppenol WH. (1998), Peroxynitrite Uncloaked? *Chem Res. Toxicol*. **11**: 716-717.
4. Meli R, Nauser T, Latal P, Koppenol WH (2002). Reaction of peroxynitrite with carbon dioxide: intermediates and determination of the yield of $CO_3^{\bullet-}$ and $NO_2^{\bullet}$. *J Biol Inorg Chem* **7**:31-36.
5. Padmaja S, Squadrito GL., Pryor WA (1998). Inactivation of Glutathione Peroxidase by peroxynitrite. *Arch Biochem Biophys*, **349**: 1-6.
6. Squadrito GL., Pryor WA. (1998). The Nature of Reactive species in systems that produce peroxynitrite. *Chem Res. Toxicol* **11**: 718-719.
7. Squadrito GL., Pryor WA. (1998). Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite and carbon dioxide. *Free Radical Biology and Medicine* **25**:392-403.