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Peroxynitrite

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Abbreviations
$HO^{\bullet}$: hydroxyl radical
$^{\bullet}NO$: Nitric oxide
$^{\bullet}NO_2$: Nitrogen dioxide
NO_2^+ : Nitronium
$O_2^{\bullet -}$: Superoxide
$ONOO^-$: Peroxynitrite
ONOOH : Peroxynitrous acid

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I. Abstract

Peroxynitrite is an important biological oxidant that is produced from the reaction of nitric oxide and superoxide radicals. Peroxynitrite reactions and decomposition products are involved in multiple biological reactions that play a significant role in its biological activity. Peroxynitrite can react with a number of biomolecules, including thiols, amines, lipids and proteins. Several assays have been developed to detect peroxynitrite.

II. Introduction

Peroxynitrite ($ONOO^-$) is an oxidant produced *in vivo* by activated macrophages, neutrophils and endothelial cells (Figure 1).

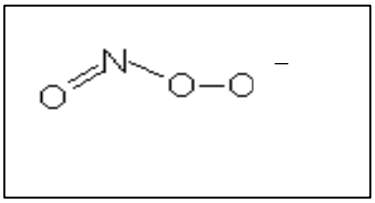


Figure 1. The structure of Peroxynitrite.

When protonated to peroxynitrous acid (ONOOH), it is highly reactive and yields oxidizing and nitrating species [1]. Peroxynitrite is stable enough to diffuse over at least one cell diameter under physiological conditions, and more stable at alkaline pH [2].

III. Formation of peroxynitrite

Peroxynitrite formation by endothelial cells, neutrophils, and macrophages occurs by the diffusion-controlled reaction between cell-derived nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet -}$) [3].

$$^{\bullet}NO + O_2^{\bullet -} \xrightarrow{5 \times 10^9\ M^{-1} s^{-1}} ONOO^-$$

In the laboratory, peroxynitrite is produced by rapid mixing and quenching of nitrite and acidified hydrogen peroxide. Acidified nitrite attacks hydrogen peroxide to produce peroxynitrous acid [2].

$$HONO + HOOH \longrightarrow HOONO + H_2O$$

The later can be stabilized by rapidly quenching the reaction with an excess of NaOH to form peroxynitrite anion.

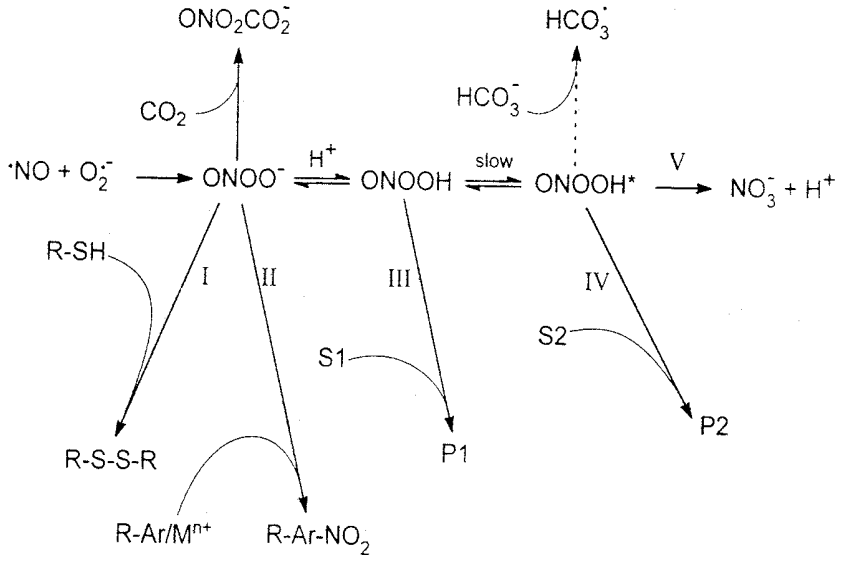
Peroxynitrite can also be produced by bubbling ozone through alkaline azide [3]. In another procedure, Lies et al (1993) showed that peroxynitrite can be produced by the reaction between alkaline hydrogen peroxide and organic nitrites [4] as follows:

$$HOO^- + R{-}O{-}N{=}O \longrightarrow ONOO^- + ROH$$

IV. Reactions of peroxynitrite

At physiological pH, about 20% of peroxynitrite is protonated to peroxynitrous acid (HOONO), with a pk_a of 6.8. This fraction of peroxynitrous acid plays a significant role in the biological activity of the compound. One- and two-electron oxidations can be performed by peroxynitrite. It can also react with a number of biomolecules, including thiols, amines, lipids and proteins [2].

Different mechanisms for the reactivity of peroxynitrite are shown in scheme I [5]. In scheme I, we can see that peroxynitrite anion ($ONOO^-$) directly reacts with sulfhydryls (RSH) to yield the corresponding disulfide (RSSR) (reaction I). Peroxynitrite anion will also nitrate aromatics (R-Ar) to give nitroderivative in a reaction catalyzed by transition metal M^{n+} (reaction II).



Scheme I. Reactions of peroxy nitrite. See text for explanation (Adapted from 5).

When protonated to peroxynitrous acid (ONOOH), it can react with biomolecules (S1) such as cytochrome c^{2+}. It can also undergo a rate-limiting transition to vibrationally activated intermediate (ONOOH*). The activated intermediate can isomerize to nitric acid (reaction V) or oxidize a target molecule (S2) such as benzoate or DMSO with a reactivity similar to that of hydroxyl radical (reaction IV). Bicarbonate anion can react with ONOOH* (dashed arrow), whereas carbon dioxide reacts with peroxynitrite anion ($k=5.8\times10^4\ M^{-1}s^{-1}$ at 37°C) to form an adduct, $ONO_2CO_2^-$. Since the concentration of CO_2 is high both intra- and extracellularly (around 1-2 mM), the later reaction represents one of the major routes of peroxynitrite activity in vivo [19].

The reaction of $ONOO^-$ with CO_2 results in the formation of nitrocarbonate anion, which has a biological importance in being able to oxidize substances via one- and two-electron pathways, as well as nitrosylate a wide variety of compounds [9, 10].

$$ONOO^- + CO_2 \longrightarrow ONOOCO_2^- \longrightarrow O_2NOCO_2^-$$

Radi R. (1998), reported rate constants for the reactions of peroxynitrite with biomolecules and three other relevant synthetic compounds in the physiological pH range (7.2-7.6) as shown in Table I [19].

Table 1. Rate Constants of Peroxynitrite Reactions with Biomolecules and Some Other Relevant Compounds at Physiological pH[a] (Adapted from 19).

Reaction	k_s (M^{-1} s^{-1})	Reaction	k_s (M^{-1} s^{-1})
Fe(III)TMPyP	2.2×10^6 [b]	Cu-Zn SOD	10^3-10^5 [b]
Mn(II)TMPyP	1.8×10^6 [c]	CO_2	4×10^4 [b]
Ebselen	1.6×10^6 [c]	Bovine serum albumin	6×10^3 [b]
Myeloperoxidase	$>10^6$ [d]	Cysteine	5×10^3 [b]
Horseradish peroxidase	7×10^5 [c]	Glutathione	1.35×10^3 [b]
Alcohol dehydrogenase	3×10^5 [e]	Methionine	1.8×10^2 [c]
Aconitase	1.4×10^5 [c]	Tryptophan	1×10^2 [b]
Cycochrome *c*	1.3×10^4 [c]	Ascorbate	1×10^2 [c]
Oxyhemoglobin	1×10^4 [c]		

Reported rate constants were obtained from the literature and represent the apparent values (pH-dependent) in the physiological pH range (7.2-7.6) for the reactions of peroxynitrite with biomolecules and three other relevant synthetic compounds. The synthetic compounds reported at the top of the table represent molecules that have been proposed as compounds that may interact and attenuate the toxic effects promoted by peroxynitrite.[b] T = 37°C.[c] T = 25°C.[d] T = 12°C.[e] T = 23°C.

Moreover, different decomposition products may play a role in the biological activity of peroxynitrite. In homolytic radical generation, both hydroxyl radical ($HO^\bullet$) and nitrogen dioxide ($^\bullet NO_2$) are produced [6].

$$HOONO \longrightarrow HO^\bullet + NO_2^\bullet$$

In another decomposition reaction, it might undergo heterolytic decomposition to yield nitronium and hydroxyl ion [7].

$HOONO \longrightarrow OH^- + NO_2^+$

Or a dismutation to give either nitrogen dioxide and nitrosodioxyl radical, or nitrite and oxygen [8].

$2HOONO \longrightarrow H_2O + NO_2 + {}^{\bullet}ONO_2$

$2ONOO^- \longrightarrow NO_2^- + O_2$

V. Biological importance of peroxynitrite

Several studies demonstrated that peroxynitrite toxicity is due to a) its ability to oxidize thiols and thiol containing proteins and membrane lipids [11,12], and b) its ability to nitrate phenols, including tyrosines of SOD and other proteins. Bovine Cu,Zn superoxide dismutase reacted with peroxynitrite to form a stable yellow protein-bound adduct identified as nitrotyrosine [13]

$ONOO^- + Cu^{2+}, ZnSOD \longrightarrow ONOO\text{-}Cu^{1+}, ZnSOD$

$ONOO\text{-}Cu^{1+}, ZnSOD + H^+ \longrightarrow NO_2^+ + HO\text{-}Cu^{1+}, ZnSOD$

Peroxynitrite has been shown to be involved in tissue damage in a number of pathological conditions in humans and experimental animals, *e.g*., atherosclerosis [14], ischemia-reperfusion injury [15], and renal allograft rejection, where activated cellular infiltrate produces high levels of both superoxide and nitric oxide. These reactive oxygen species interact to form peroxynitrite, a potent oxidant that can modify proteins to form 3-nitrotyrosine [16].

VI. Assays of peroxynitrite

Different methods are used to detect and assay peroxynitrite. In one method, the stock solution of peroxynitrite is diluted in NaOH and increase in absorbance at 302 nm is measured as shown in Figure 2 [20].

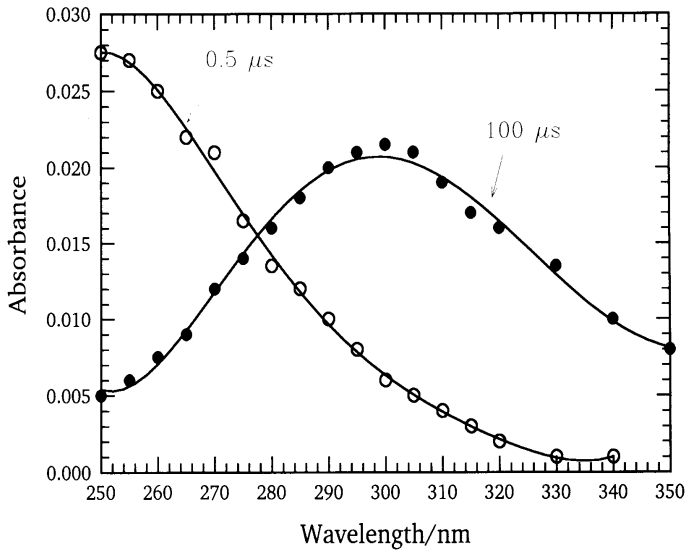


Figure 2 Absorption spectra resulting from 248 nm flash photolysis of an O_2-saturated solution containing 5 $\mu moll^{-1}$ $NaNO_2$ and 0.1 $\mu moll^{-1}$ $NaHCO_2$ at pH 7.5. The open circles are at 0.5 μs and the filled circles at 100 μs after the flash (Adapted from 20).

Radi R. *et al* showed that peroxynitrite reacts with luminol to yield chemiluminescence, which was greatly enhanced by bicarbonate [17]. Monoclonal antibodies can also be used to detect 3-nitrotyrosine residues in proteins and tissue samples (Figure 3), where the involvement of peroxynitrite ($ONOO^-$) in inflammatory diseases has been implicated by detection of 3-nitrotyrosine, a characteristic protein oxidation product, in various inflamed tissues [18].

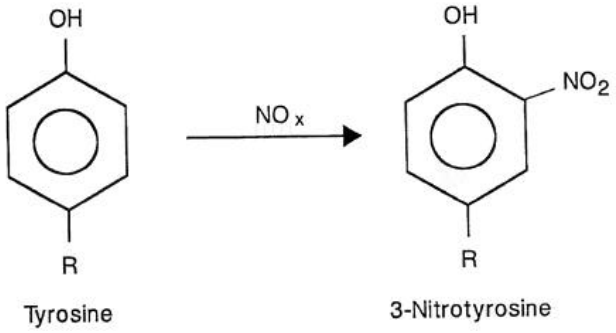


Figure 3
Nitration of tyrosine by reactive nitrogen species NOx can be detected using monoclonal antibodies (Adapted from 21).

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