Peroxynitrite chemistry

This century old area of research has been experiencing a renaissance during the last decade, with the annual number of publications on the subject increasing from only one in 1990 to nearly 200 in the late-1990s. This renewed interest is stimulated by the discovery of biological roles of nitric oxide, distinguished by the 1998 Nobel prize, and the recognition that the conversion of nitric oxide into peroxynitrite may play major roles in human diseases associated with oxidative stress and in cellular defense against invading pathogens.

Occurrence. Peroxynitrite ($ONOO^-$) is a structural isomer of nitrate (NO_3^-) that contains a peroxo bond (Fig. 1). The physiological route to $ONOO^-$ is provided by the combination of nitric oxide ($^{\bullet}NO$) with superoxide ($^{\bullet}O_2^-$), an extremely rapid reaction occurring upon every encounter of these radicals (Fig. 1; the upper dot denotes radical species). Both $^{\bullet}NO$ and $^{\bullet}O_2^-$ are the oxygen metabolic products simultaneously generated in a number of cell types within a human body. Compared to its precursors, peroxynitrite is a much stronger oxidant capable of oxidizing proteins, nucleic acids, and lipids.

In the environment, peroxynitrite can be produced by the action of ultraviolet or ionizing radiation upon the nitrate ion, which induces its isomerization (Fig. 1). Because NO_3^- is among the three most abundant anions present in the cloud water of the Earth's atmosphere, photochemical $ONOO^-$ generation can be significant, particularly in stratospheric aerosols, where the flux of solar ultraviolet light is high. A man-made environment, where the extensive radiation-induced generation of $ONOO^-$ occurs, is found within the nuclear waste storage tanks at Hanford, Washington, which contain about 16,000 cubic meters (60 million gallons) of highly radioactive, nitrate-saturated liquids and solids generated during the Cold War nuclear weapons

production.

Reactivity. Although almost 180 kJ/mol higher in energy than NO_3^- and, therefore, inherently unstable, peroxynitrite anion does not isomerize through a concerted bond rearrangement. In the absence of acids, peroxynitrite solutions decompose with the half-life of approximately 20 hours to nitrite (NO_2^-), oxygen, and NO_3^- in a complex set of radical reactions initiated by the slow dissociations of the ON-OO$^-$ and ONO-O$^-$ bonds (Fig. 1).

The ONOO$^-$ anion is a moderately strong Lewis base (electron pair donor), which is the most prominent characteristic determining its reactivity. Accordingly, both major pathways through which peroxynitrite performs its oxidative chemistry begin with the neutralization by an acid (Fig. 2.) The combination of ONOO$^-$ with a Lewis acid (electron pair acceptor, L) creates the ONOOL$^-$ adduct, which then decomposes either through homolytic (radical) or heterolytic (polar) O-O bond cleavage. In the latter case, an O atom is transferred, i.e., the two-electron oxidation of L, e.g., the oxidation of trivalent chromium in alkali,

$$ONOO^- + Cr^{III} \rightarrow NO_2^- + Cr^{V}{=}O$$

Similar reactions are presumed to occur for some trivalent arsenic and antimony compounds and for several ketones (R_1R_2CO).

A homolytic decomposition of ONOOL$^-$ transfers $^{\bullet}O^-$, i.e., the one-electron oxidation of L, creating a geminate radical pair within a solvent cage (Fig. 2). The caged radicals then either recombine to give NO_3^- and regenerate L or diffuse apart to become the free radicals. Carbon dioxide (CO_2) is the most important, unexpected, and well-studied Lewis acid reacting via these pathways. It rapidly binds to ONOO$^-$ forming an unstable adduct, which almost instantly breaks up to afford the carbonate ($^{\bullet}CO_3^-$) and nitrogen dioxide ($^{\bullet}NO_2$) radicals (Fig. 3a). In the absence

of reductants, these radicals react with each other regenerating CO_2, hence the catalysis of $ONOO^-$ isomerization to NO_3^-. Because carbon dioxide is ubiquitous in biological fluids, being present at a concentration level of one millimole per liter or greater, it is projected that nearly all of the $ONOO^-$ that might be generated in these environments will, within 0.02-0.03 seconds, react preferentially with CO_2. Since both $^{\bullet}CO_3^-$ and $^{\bullet}NO_2$ are potent oxidants, a bystander compound, otherwise unreactive toward $ONOO^-$, can become oxidized in the presence of CO_2. For example, CO_2 promotes the nitration of guanine in deoxyribonucleic acid (DNA) and the oxidation of phenols, most notably, tyrosine residues of proteins (Fig. 3b). The *in vitro* experiments have shown that the nitration of only one or, in some cases, a few tyrosine residues of an enzyme can seriously compromise its biological function. Because the yields of major oxidation products, nitrotyrosine and dityrosine (Fig. 3b), depend upon the medium pH and its tyrosine, CO_2, and $ONOO^-$ contents, both the extent and the type of CO_2-mediated protein damage by $ONOO^-$ may vary appreciably in different tissues. Reportedly, the dietary polyphenols consumed with vegetables, tea, chocolate, or wine can act as protectors by scavenging the peroxynitrite-derived radicals. Aldehydes (RHCO), which can be viewed as partially reduced CO_2, are weaker Lewis acids; accordingly, they react with $ONOO^-$ in the same manner, but much more slowely.

Several porphyrins (P) of trivalent manganese (PMn^{III}) have been reported to scavenge $ONOO^-$ through the Lewis acid homolytic pathway in Fig. 2, with the specific rates up to a thousand times greater than that for CO_2. In this case, the tetravalent manganese porphyrin ($PMn^{IV}=O$) is the reaction product, along with $^{\bullet}NO_2$. Being weaker oxidants than $^{\bullet}CO_3^-$, the $Mn^{IV}P=O$ species react more selectively. Although also capable of oxidizing tyrosine, they

preferentially react with natural antioxidants, such as ascorbic and uric acids. Analogous porphyrins of trivalent iron are also extremely efficient $ONOO^-$ scavengers. Further progress in this direction may lead to the development of drugs protecting cellular proteins and DNA against peroxynitrite by redirecting its reactivity toward less critical targets.

A somewhat different reactivity arises when $ONOO^-$ accepts a hydrogen ion (H^+) to become peroxynitrous acid (ONOOH, Fig. 2). Unlike most hydroperoxides, ONOOH is a relatively strong acid; its pK value is 6.6. As a result, very rapidly interconverting $ONOO^-$ and ONOOH coexist at comparable amounts in neutral (pH 7) solutions, a situation that has occasionally resulted in ambiguity in the assignment of observed reactivity to one of these species. Peroxynitrous acid is the only known hydroperoxide capable of spontaneous O-O bond scission at ambient temperature; within a few seconds, it decomposes via the two competing pathways producing both NO_3^- and a pair of hydroxyl ($^{\bullet}OH$) and $^{\bullet}NO_2$ radicals (Fig. 2). The highly reactive $^{\bullet}OH$ radical can almost indiscriminately oxidize a wide variety of organic and inorganic compounds; accordingly, the mutagenic DNA lesions and strand breaks, oxidation of amino acids, inactivation of enzymes, and initiation of lipid peroxidation have all been observed upon incubation with peroxynitrite *in vitro*. However, the $^{\bullet}OH$-mediated reactions of ONOOH will be of little or no significance *in vivo*, because of the competition from the more rapid CO_2-catalyzed pathway and from the direct oxidations by ONOOH.

As with all hydroperoxides, ONOOH can engage in an oxygen atom transfer to electron donors (D, Fig. 2), e.g., the oxidation of dimethyl sulfide to dimethyl sulfoxide

$$ONOOH + (CH_3)_2S \rightarrow HNO_2 + (CH_3)_2SO$$

The two sulfur-containing amino acids, methionine ($RSCH_3$) and cysteine (RSH), and their

naturally-occurring selenium analogs react in a similar fashion. Their reactivity is sufficiently high to compete with the spontaneous decomposition of ONOOH in most, and even with CO_2-catalyzed reactions in some, cellular environments. Unlike tyrosine oxidation, the oxidation of these amino acids can be reversed, sometimes rapidly, by the cellular enzymatic reduction systems. For example, the remarkably efficient catalytic destructions of peroxynitrite by a bacterial enzyme, peroxiredoxin, and by a mammalian enzyme, glutathione peroxidase, are thought to be carried on by the oxidation-reduction cycling of their respective cysteine and selenocysteine residues.

Another class of biological molecules that are capable of exceptionally rapid peroxynitrite scavenging is represented by heme proteins, which contain iron porphyrin active sites. These include a number of peroxidases, cytochromes, and hemoglobin. Depending, apparently, upon the oxidation state of heme iron and the protein structure, some of them react with $ONOO^-$ through the Lewis acid route, while the others take the donor pathway and scavenge ONOOH (Fig. 2). In the cellular and subcellular compartments, e.g., red and white blood cells or mitochondria, with high content of heme proteins, they have the capacity to rival CO_2 -directed peroxynitrite reactivity. This, however, does not always constitute protection, because the nascent products of peroxynitrite reactions with hemes are, in many cases, strong oxidants in their own right.

Environment. The oxidation of natural and anthropogenic sulfur dioxide (SO_2) by hydrogen peroxide (H_2O_2) in atmospheric fog and clouds is a major contributor to the infamous acid rain. Under the typical cloud water conditions, the similar reaction of SO_2 with ONOOH is about a hundred times more rapid than with H_2O_2. Accordingly, the presence of even relatively

small amounts of $ONOO^-$ has a capacity to adversely affect the environment. In the stratospheric clouds, the $^{\bullet}OH$ radical created during ONOOH decomposition is capable of initiating a chain reaction of ozone destruction in the presence of halogen-containing compounds. Although the contributions of peroxynitrite to atmospheric chemistry have not yet been completely accounted for, a correlation between the conditions favoring $ONOO^-$ formation and the depletion of ozone over the Earth's polar regions has been observed.

Biology. Peroxynitrite has been implicated as a causative agent in a number of human diseases, including neurodegenerative disorders, atherosclerosis, ischemic reperfusion injury, inflammation, and sepsis. The connection is made primarily based on (1) the likelihood of simultaneous (in place and time) generation of large fluxes of $^{\bullet}NO$ and $^{\bullet}O_2^-$; (2) the *in vitro* observation of the exceptionally high peroxynitrite cytotoxicity and its adverse effects upon critical cellular components, and (3) immunohistological assays using nitrotyrosine-specific antibodies that reveal copious tyrosine nitration in the affected tissues (Fig. 4).

While deleterious in normal cells, the massive oxidative damage becomes beneficial when inflicted upon invading bacteria and parasites. The preponderance of circumstantial evidence has led to a suggestion that peroxynitrite may be one of the key bactericidal agents generated by several types of phagocytes, the specialized cells dedicated to combating microbial infection. Upon activation, these cells have been shown to induce nitration of added phenols and nitrotyrosine lesions in bacteria, consistent with the involvement of peroxynitrite.

Finally, peroxynitrite formation may play a regulatory role by controlling the biological activity of its nitric oxide and superoxide precursors through both their consumption and the oxidative inactivation of the enzymes involved in their metabolism. Furthermore, there appears

to be evidence of peroxynitrite involvement in cellular signaling events and in modulation of the immune response.

All the physiological roles of peroxynitrite are predicated on the idea that it is produced in biological systems in significant quantities, which still remains a contentious point, mainly because the short lifetime of peroxynitrite under the physiological conditions has prevented its accumulation and detection by the direct means. Although nitrotyrosine found in cells (e.g., Fig. 4) is widely accepted as a biomarker of peroxynitrite chemistry, it is also recognized that, at least in certain cells and tissues, there exist other pathways of the •NO metabolism that may yield nitrotyrosine without the intermediacy of peroxynitrite. Clearly, the unraveling of the extremely complex biological roles of peroxynitrite is still in its infancy. As with any rapidly evolving field, controversies abound and much more research will have to be done before a comprehensive understanding emerges.

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Figure legends.

Fig. 1. Peroxynitrite formation and decomposition reactions.

Fig. 2. Major peroxynitrite reactivity pathways. L = Lewis acid; D = electron donor; parentheses denote a geminate (contact) radical pair.

Fig. 3. CO_2-catalyzed peroxynitrite decomposition (a) and tyrosine oxidation (b). R = CH_2-CH(CO_2^-)(NH_3^+)

Fig. 4. Immunostaining for nitrotyrosine (dark) in a degenerating spinal motor neuron (large central triangular cell) of a patient suffering from amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease). Magnification 130x. (Courtesy of Liliana Viera and Joseph Beckman, University of Alabama at Birmingham.)

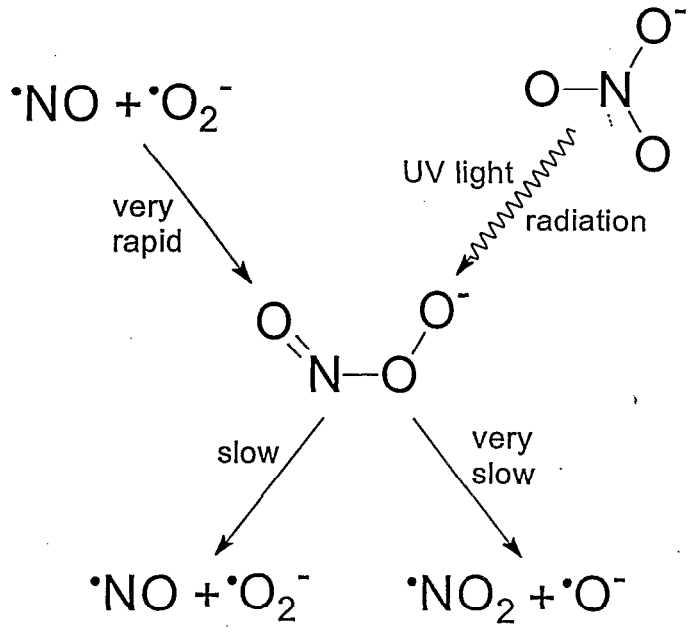

Fig. 1. Lymar, Peroxynitrite chemistry

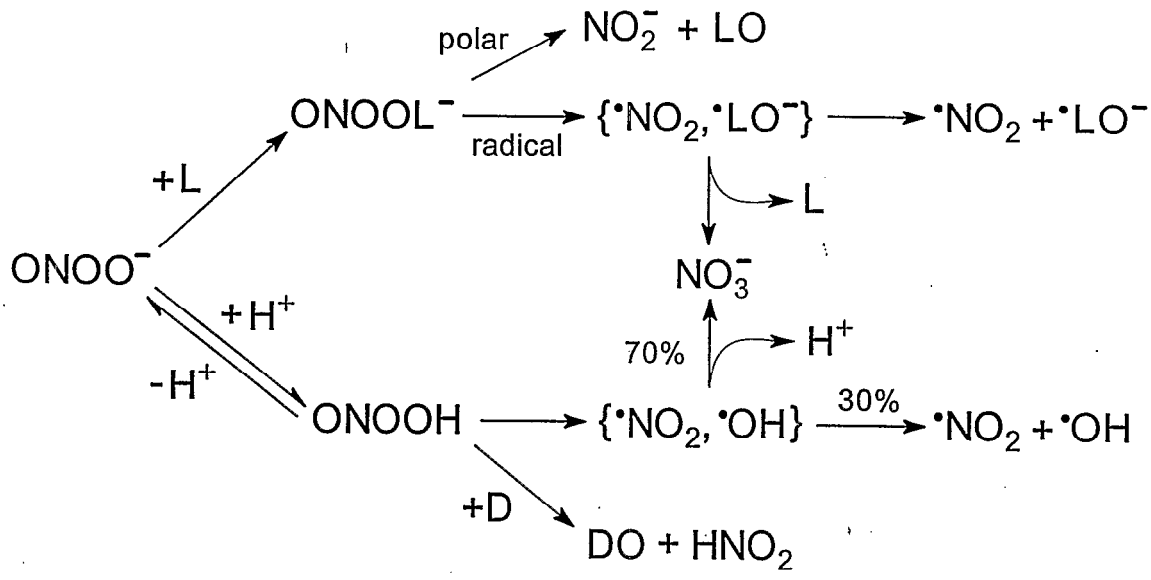

Fig. 2. Lymar, Peroxynitrite chemistry

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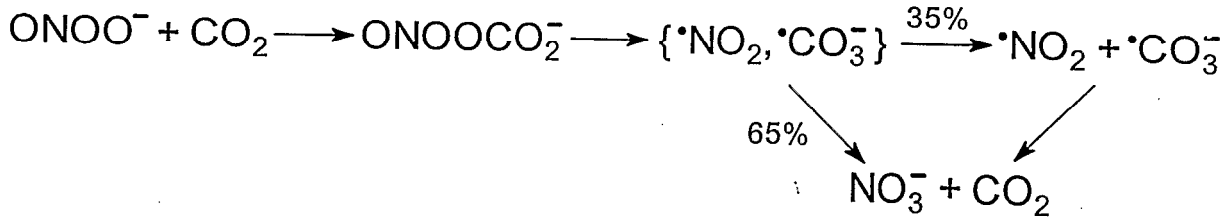

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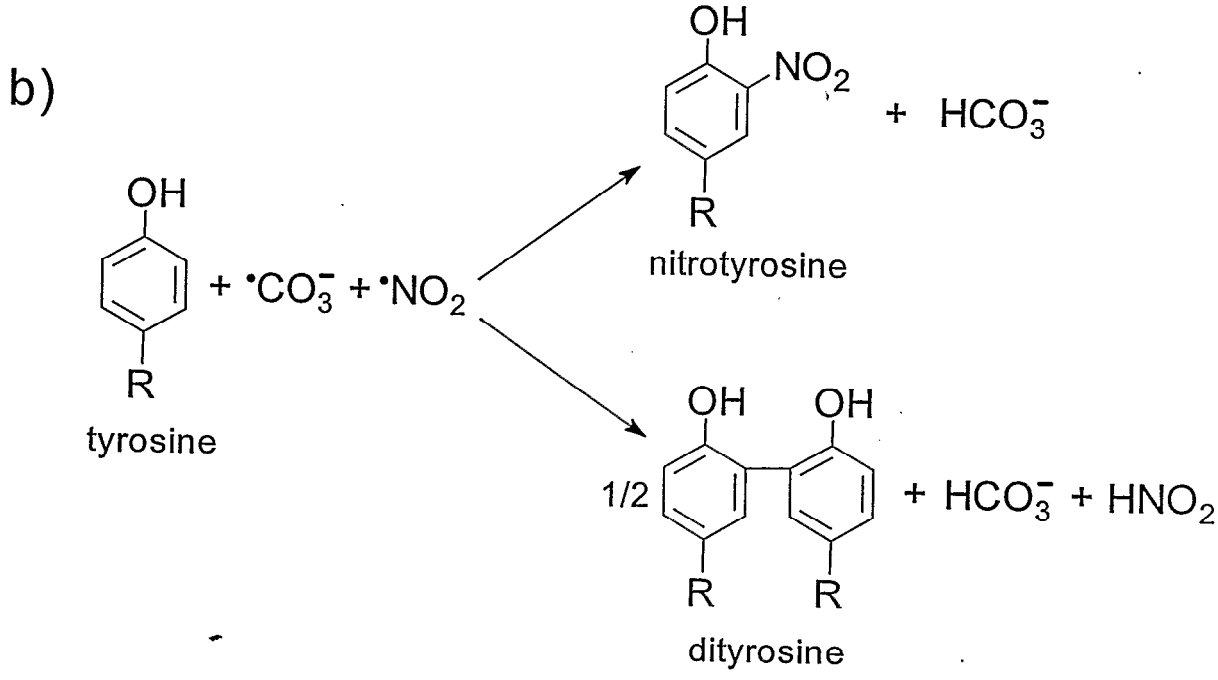

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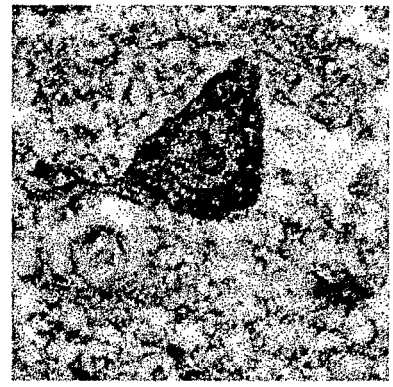

Fig. 4. Lymar, Peroxynitrite chemistry