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NITROGEN DIOXIDE RADICAL

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Abbreviations.

$Asc^{\bullet -}$: Ascorbate radical
Asc H^- : Ascorbate monanion
CO_2 : Carbon dioxide
E^0 : Standard reduction potential.
H_4B : Tetrahydrobiopterin
H_2O_2 : Hydrogen peroxide
$HO_2^{\bullet}$: Hydrodioxyl
H^+ : Proton
$NO^{\bullet}$: Nitric oxide:
N_2O_3 : Dinitrogen trioxide
N_2O_4 : Dinitrogen tetroxide
O_3 : Ozone
$OONO^-$. : Peroxynitrite
ONOOH : Peroxynitrous acid
ppm : parts per million

Abstract

Nitrogen dioxide is a reddish-brown,corrosive, highly oxidizing nitrogen-centered free radical gas with a characteristic pungent odor.Either directly or indirectly,it plays a key role in the cellular oxidative and nitrosative stress .The unpaired electron in nitrogen dioxide is delocalized on both nitrogen and oxygen atoms ,so that nitrogen and/or oxygen atoms of the nitrogen dioxide radical ($NO_2^{\bullet}$) can participate in the formation of chemical bonds. The redox properties of $NO_2^{\bullet}$ will help us to understand its reactivity towards the most likely biological targets .The reaction studies presented in this report are aimed to gain an insight into the basic chemistry of this

radical. The chemical origin and fate of $NO_2^{\bullet}$ *invivo* are paramount in understanding their potential contributions to pathophysiological and toxicological mechanisms. The goal of this review is to emphasize the chemistry of nitrogen dioxide radical and to examine the variety of outcomes in its interactions with biological materials under either physiological or pathophysiological conditions.

Introduction.

Nitrogen dioxide is an oxidizing and nitrating agent. It has one unpaired electron and therefore is an oxidizing radical. This toxic radical is formed in living cells during physiological processes or it can enter the living system through inhaled air. Studies show evidence of $NO_2^{\bullet}$ involved in a variety of destructive pathways in living systems like lipid peroxidatioin, generation of carcinogenic nitrosamines, nitration of tyrosines and antitrypsin inactivation. It has also come to notice that an arsenal of antioxidants is involved in eliminating this harmful radical from biological systems. With this biological relevance in background, the various aspects of $NO_2^{\bullet}$ chemistry relevant to understand the physiological consequences of $NO_2^{\bullet}$ are reviewed in this paper.

Sources of $NO_2^{\bullet}$

Nitrogen dioxide is a constituent of air pollution. This atmospheric pollutant is discharged into air from automobile exhausts, power plant emissions and general combustion processes. During combustion of organic materials $NO^{\bullet}$ and $NO_2^{\bullet}$ radicals are formed. The $NO^{\bullet}$ formed reacts further with O_2 to form. more $NO_2^{\bullet}$.

$$2NO^{\bullet} + O_2 \rightarrow 2NO_2^{\bullet} \qquad (\text{gas phase}, k = 2 \times 10^6\ M^{-2}\ s^{-1}) \qquad (1)$$

The primary source of $NO_2^{\bullet}$ in the troposphere is from the rapid reaction of $NO^{\bullet}$ with ozone [Atkinson et al.,1992].

$NO^{\bullet} + O_3 \rightarrow NO_2^{\bullet} + O_2$ (gas phase, k =1.1 x 10^7 L mol^{-1} s^{-1}) (2)

$NO_2^{\bullet}$ is also formed in the atmosphere by the reaction of nitric oxide with hydroperoxyl radical.. [De more et al ,1992; Wayne et al, 1990]

$NO^{\bullet} + HO_2^{\bullet} \rightarrow NO_2^{\bullet} + HO^{\bullet}$ (gas phase, k = 5.2 x 10^9 L mol^{-1} $s^{-1)}$ (3)

The above mentioned reactions of nitrogen dioxide suggests its central role in troposphere chemistry.

Endogenous formation of nitrogen dioxide:

Redox biology of $NO_2^{\bullet}$ plays an important role in various aspects of oxidative and nitrosative stress.The identification of $NO_2^{\bullet}$ as a key intermediate in many physiological processes reinforces the possibility that nitrogen dioxide is formed frequently in biological systems. [Moncada et al , 1991] .

In biological systems , $NO_2^{\bullet}$ can be derived from peroxynitrites ,nitrites or from autoxidation of nitric oxide.The reaction between nitric oxide and oxygen is termed nitric oxide autoxidation. In the gas phase and in hydrophobic layers of the cellular membranes, nitric oxide autoxidation reaction initially produces $NO_2^{\bullet}$, which then further reacts with an additional NO molecule to form the nitrosating species , N_2O_3. [2]. The autoxidationof NO is of particular significance in the lung lining epithelial fluid where the oxygen concentration is high.

$NO_2^{\bullet} + NO^{\bullet} \rightarrow N_2O_3$ (4)

$N_2O_3 + H_2O \rightarrow 2\ HNO_2$ (5)

Another important chemical reaction which results in nitrogen dioxide formation *in vivo* is the homolytic fission of conjugate acid of $OONO^-$. Decomposition of $OONO^-$ is suggested to proceed through peroxynitrous acid (ONOOH, pKa 6.8), which results in the formation of intermediates *trans*-$ONOOH^\bullet$, $NO_2^\bullet$ and $OH^\bullet$.[2]

$$OONO^- + H^+ \leftrightarrow \textit{cis}\text{- ONOOH.} \quad (6)$$

$$\textit{cis}\text{- ONOOH} \rightarrow \textit{trans}\text{-}ONOOH^\bullet \quad (7)$$

$$\textit{trans}\text{-}ONOOH^\bullet \rightarrow [NO_2^\bullet \mid OH^\bullet] \rightarrow NO_3^- \quad (8)$$

In another reaction peroxynitrate reacts with carbon dioxide *in vivo* to form nitrogen dioxide along with carbonate radicals, via homolysis of O-O bond in $ONOOCO_2^-$ [3].

$$OONO^- + CO_2 \leftrightarrow ONOOCO_2^- \rightarrow NO_2^\bullet + CO_3^{\bullet -} \quad (9)$$

$NO_2^\bullet$ radical can be generated in cellular environment by the oxidation of NO_2^-, a process that can be mediated by myeloperoxidase.[4]. Increased levels of NO_2^- has been noticed at sites of inflammation, which in turn can lead to higher concentration of $NO_2^\bullet$ at inflammatory sites.

$$H_2O_2 + NO_2^- + H^+ \rightarrow NO_2^\bullet + HO + H_2O \quad (10)$$

The reaction of oxyhemoglobin (HbO_2)with nitrite has been proposed to yield $NO_2^\bullet$ and H_2O_2. [Kosaka et al.,1981].

$$HbO_2 + NO_2^- \rightarrow Hb^{+3} + NO_2^\bullet + H_2O_2 . \quad (11)$$

$NO_2^\bullet$ formed will further react with a second oxyhemoglobin

$$HbO_2 + NO_2^\bullet \rightarrow Hb^{3+} + NO_2^- + O_2 \quad (12)$$

Reactions

Nitrogen dioxide reacts rapidly with other free radicals, reasonably fast with one electron reductants ,but reacts much slowly by addition or hydrogen abstraction reaction.[5].

In gas phase and in non aqueous solvents, $NO_2^{\bullet}$ dimerises to form N_2O_4 [5]

$$2NO_2 \leftrightarrow N_2O_4 \quad (13)$$

The rate constant for the formation of the dimer is $\sim 5 \times 10^8$ L mol^{-1} s^{-1} in the gas phase [Borrell et al.,1988] and is 4.5×10^8 L mol^{-1} s^{-1} in aqueous solution [Gratzel et al.,1969;Broszkiewicz,1976].The dimer reacts rapidly in aqueous solution to form nitrite (NO_2^-) and nitrate (NO_3^-).

$$N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + H^+ \quad (14)$$

$NO_2^{\bullet}$ reaction with radicals:

Nitrogen and/or oxygen atoms of the $NO_2^{\bullet}$ radical can participate in the formation of chemical bonds with the target radical, because the unpaired electron is delocalized on both nitrogen and oxygen atoms. The combination of $NO_2^{\bullet}$ radicals with other radicals is a very rapid reaction ,and in many cases, the rates of this reactions are close to the diffusion -controlled limit.[6].

Nitrogen dioxide also reacts with organic radicals,leading to the formation of nitro compounds and nitrosoxy derivatives. For instance Prutz *et al* have reported that $NO_2^{\bullet}$ reacts with tyrosine radicals to form 3-nitrotyrosine,which is toxic.[10]

$$NO_2^{\bullet} + TyrO^{\bullet} + H^+ \rightarrow Tyr(NO_2)OH. \ (k_{32} \sim 3 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}). \quad (15)$$

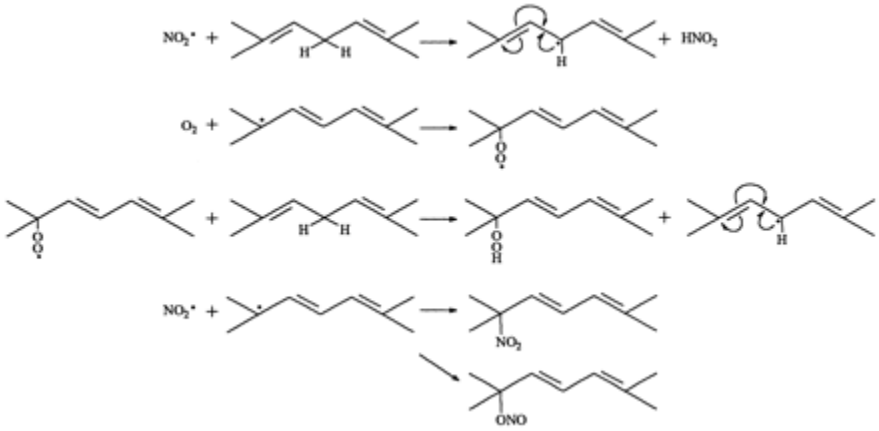
Nitrotyrosine could interfere with signal tranduction or get incorporated into the microtubule protein *tubulin*, and by distorting the cytoskelton ,leads to cell death .

Abstraction reactions :

Abstaction reactions of NO_2• has more significance because of its role in lipid peroxidation. $NO_2^{\bullet}$ can initiate lipid peroxidation by abstracting the allylic hydrogen atom from PUFA .[7]

$$LH + NO_2^{\bullet} \rightarrow L^{\bullet} + HNO_2 \quad (16)$$

Scheme.1 illustrates how $NO_2^{\bullet}$ reacts with a linoleate- type fatty acid (k~ $10^6 M^{-1} s^{-1}$) to produce the corresponding conjugated diene.



Scheme.1. $NO_2^{\bullet}$ mediated oxidation of unsaturated fatty acids. [10].

According to this mechanism, $NO_2^{\bullet}$ abstracts an allylic hydrogen atom to form a resonance-stabilized, carbon-centered radical. This radical can combine with oxygen to form a peroxyl radical, which can propagate lipid oxidation. In the absence of oxygen, the allylic radical can combine with a molecule of $NO_2^{\bullet}$ to form an allylic nitro or nitrite compound. Alternatively, $NO_2^{\bullet}$ also can add to the double bond and form a carbon-centered radical, which can react with oxygen to form a nitro-peroxyl radical or, in the absence of oxygen, react with another molecule of $NO_2^{\bullet}$ to form a dinitro compound. [10].

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Addition reactions:

The addition reaction of $NO_2^{\bullet}$ to unsaturated bonds of membrane lipids is of biological importance.The addition reaction of $NO_2^{\bullet}$ with alkenes generate carbon-centered radicals that can react with O_2 to form peroxyl radicals. These peroxyl radicals formed will participate in the chain reaction that propagates lipid peroxidation. [7]

Electron transfer reactions

$NO_2^{\bullet}$ is a moderately strong one-electron oxidant. The reduction potential of $NO_2^{\bullet}$ / NO_2^- couple determines the oxidizing strength. Estimates of the potential in the range of E^0 ($NO_2^{\bullet}$ / NO_2^-) in the range of ~ + 0.89 V to + 1.05 V (vs NHE) have been discussed by Stranbury *et al* ,with possibly the most values near + 1.04V .[10].

$NO_2^{\bullet}$ reactswith polyunsaturated linker in β-carotene (CAR), primarily through electron transfer to produce the radical-cation. ($k = 1.1 \times 10^8$ L mol^{-1} s^{-1}) [10]

$$NO_2^{\bullet} + CAR \rightarrow NO_2^- + CAR^{\bullet+} \quad (17)$$

Scavengers of $NO_2^{\bullet}$

Thiols and ascorbate are important antioxidants that minimizes the deleterious effects of $NO_2^{\bullet}$ in cytoplasm. But in lipid compartments (cell membranes) ,vitamin - E plays a major role in eliminating the nitrosative stress involving $NO_2^{\bullet}$. The oxidation of thiols by $NO_2^{\bullet}$ occurs with $K_{16} \sim 2 \times 10^3$ L mol^{-1} s^{-1}. [8].

$$NO_2^{\bullet} + RSH \rightarrow NO_2^{\bullet} + RS^{\bullet} + H^+ \quad (18)$$

Ascorbate is a much more effective antioxidant in thermodynamic terms than thiols,since at pH 7, the reduction potential of ascorbyl radicals, E^0 ($Asc^{\bullet -}$, H^+/ Asc H^-) is ~ +0.3 V. i.e, about 0.4 V lower than that of thiyl radical/thiolate couple. [10].

$NO_2^{\bullet} + Asc\,H^- \rightarrow NO_2^- + Asc^{\bullet -} + H^+$ (19)

It was reported that urates acts as a major scavenger of $NO_2^{\bullet}$ in plasma[7]. The hydrophilic scavenger tetrahydrobiopterin (H_4B) also plays a key role in eliminating $NO_2^{\bullet}$ .Nitrogen dioxide reacts with H_4B at a rate constant of 9.4×10^8 L mol^{-1} s^{-1} .[7] . This scavenger is considered less important since the cytosolic concentration of H_4B is only a few micromolars,.

Biological importance of NO_2

Acute effects of higher dose nitrogen dioxide are hypoxaemia, acidosis, pulmonary edema and pneumonitis. Chronic exposure to nitrogen dioxide in the inhaled air can damage the lung tissues. Increased rates of respiratory disease has been detected at exposure levels above 0.053 p p m. Higher levels of exposure > 3-4 p.p.m can cause more severe lung damage by inactivating the alpha -1 antitrypsin , leading to emphysema.

The $NO_2^{\bullet}$ can dissolve in lung lining epithelial fluid to form nitric and nitrous acid.

$2\,NO_2^{\bullet} + H_2O \rightarrow HNO_3 + HNO_2$ (20)

$HNO_3 \rightarrow H^+ + NO_3^{\bullet}$ (21)

$HNO_2 \leftrightarrow H^+ + NO_2^{\bullet}$ (22)

This nitrous acid (HNO_2) formed can produce mutations by deaminating DNA bases,e.g. by converting cytosine to uracil, adenine to hypoxanthine, and guanine to xanthine.[9] . Lipid peroxidation is also noted with high levels $NO_2^{\bullet}$. Nitration of surfactant proteins by $NO_2^{\bullet}$ impairs the alveolar compliance and can lead to emphysema..[9]. Even though less evidence favours the carcinogenic nature of $NO_2^{\bullet}$, reactions of $NO_2^{\bullet}$ with secondary or tertiary amines can generate carcinogenic amines .

Detection of $NO_2^{\bullet}$

For environmental monitoring of $NO_2^{\bullet}$, it is measured by electrochemical, chemiluminescence and colour indicator techniques. Other feasible methods for measurement of $NO_2^{\bullet}$ are laser diode, gas chromatography-mass spectrometry or differential optical absorption spectroscopy. Methods for detection and analysis of endogenous $NO_2^{\bullet}$ has not been satisfactorily developed yet .

Conclusion

Most of the studies about $NO_2^{\bullet}$ is directed in studying nitrogen dioxide as an atmospheric pollutant. As the studies on this radical suggests its key role in cellular oxidative and nitrosative stress, it becomes necessary to conduct considerable amount of work to establish the reaction of this radical in physiological systems.

References

Journal:

1.O'Donnell VB, Eiserich JP, Chumley PH, Jablonsky MJ, Krishna NR, Kirk M, Barnes S, Darley – Usmar VM,Freeman BA. (1999). Nitration of unsaturated fatty acids by nitric oxide –derived reactive nitrogen species peroxynitrite,nitrous acid, nitrogen dioxide and nitronium ion . *Chem. Res Toxicol.**12**, 83 – 92.*

2.Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP,Wink DA. (2002). A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. *Ann.N.Y. Acad,Sci*. **962**: 195 –206.

3.Meli R, Nauser T,Latal P, Koppenol WH. (2002). Reaction of peroxynitrite with carbon dioxide:intermediates and determination of the yield of CO_3^{-} and $NO_2^{\bullet}$. *J Biol Inorg Chem* 7 :31 – 36.

4.Byun J, Mueller DM, Fabjan JS, Heinecke JW.(1999) .Nitrogen dioxide radical generated by the myeloperoxidase-hydrogen peroxide-nitrite system promotes lipid peroxidation of low density lipoprotein. *FEBS Lett.* Jul 23;**455:** 243-6.

5. Huie RE, (1994). The reaction kinetics of $NO_2^{\bullet}$. *Toxicology **89:** 193 – 216*.

6.Neta P, Huie RE., Ross AB.(1988). Rate constants for reactions of inorganic radicals in aqueous solutions.*J.Phys.Chem. Ref.Data* .**17**, 1027 –1284.

7.Eleonora F., Martin .N. H, Peter W. (2002). Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH. *Free Radical Biology & Medicine*, vol **32**. 1314 –1323.

8.Singh RJ, Goss SP, Joseph J, Kalyanaraman B. (1998). Nitration of gamma-tocopherol and oxidation of alpha-tocopherol by copper-zinc superoxide dismutase/H2O2/NO2-: role of nitrogen dioxide free radical. *Proc Natl Acad Sci U S A* .**95** pp(22):12912-7.

Book:

9. Halliwell B,Gutteridge JMC.(1999). *Free Radicals in Biology and Medicine* pp 577-579.

Chapter in Edited Book:

10.Wardman P.(1998). Nitrogen dioxide in biology:correlating chemical kinetics with biological effects.In: AlfassiZB, *N-Centered Radicals*. pp156-179.

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