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Nitroxyl (HNO/NO^-) not Nitric Oxide ($NO^\bullet$)

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Abbreviations

CGRP	Calcitonin Gene-Related Peptide
EPR	Electron Paramagnetic Resonance Spectroscopy
Fe-MGD	N-methyl-D-Glucamine Dithiocarbamate Iron
HNO	Nitroxyl Hydride, Hydro nitrogen monoxide
HO-1	Heme Oxygenase 1
NO^-	Nitroxyl Anion, Nitrogen monoxide anion
$NO^\bullet$	Nitric Oxide Radical, Nitrogen monoxide radical
$OONO^-$	Peroxynitrite
RNS	Reactive Nitrogen Species
SOD	Superoxide Dismutase

Table of Contents

1. Abstract

Nitroxyl (NO^-) is an intermediate molecule formed from different reactive nitrogen species (RNS) such as nitric oxide ($NO^\bullet$) and peroxynitrite ($OONO^-$). Nitroxyl (NO^-) is a highly reactive molecule that is thought to be involved in many physiological, biological, and pathological systems associated with the production of RNS. Although the chemical biology of NO^- is largely unknown, some studies suggest that the biological activity of $NO^\bullet$ are actually contributed to NO^-. This short review will explore the nature, formation and some of the chemical and biological properties of the nitroxyl (NO^-).

2. Introduction

Nitroxyl has two main forms; the nitroxyl (HNO) or so called nitrosyl hydride and it anion (NO^-). The NO^- anion is isoelectronic with O_2 and, like O_2, should have a triplet ($^3NO^-$) ground state, whereas the ground state of ^1HNO should be a singlet [1]. Figure 1 shows the energy levels and p*K*a's of different forms of HNO/NO^-.

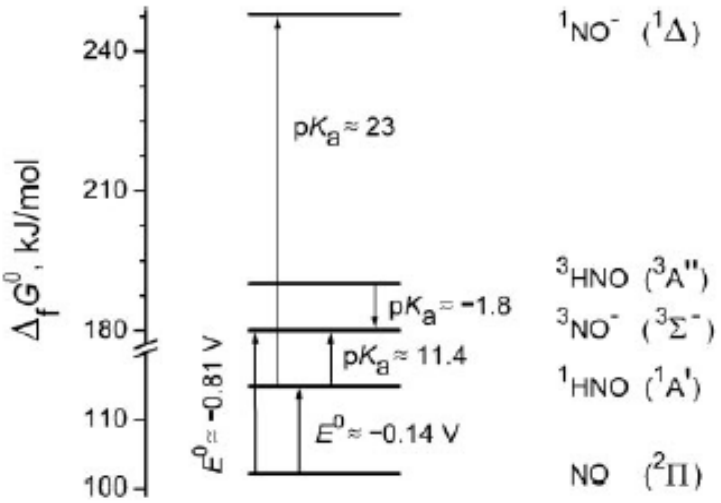


Figure 1. Energy diagram for $NO/HNO/NO^-$ species in aqueous solution at 298 K and 1 mol/kg standard states. Note the energy axis break at 120 kJ/mol. The spectroscopic designations for the electronic states are given in parenthesis. Only the lowest excited states are shown for HNO and NO^-. Adapted from [2]

3. Formation of Nitroxyl

Nitroxyl (HCO/NO^-) could be generated *in vivo* and *in vitro* by different mechanisms that involve two main RNS; peroxynitrite ($OONO^-$) and nitric oxide ($NO^\bullet$). Chemically in aqueous

solution HNO/NO^- can be generated from Angeli's salt (AS, $Na_2N_2O_3$). Its conjugate acid ($H_2N_2O_3$) has consecutive p*K*a values of 2.5 and 9.7 that slow decomposition of the monoprotonated anion occurs through heterolytic N-N bond cleavage. The most used method to generate HNO/NO^- in chemical systems *in vitro* is through photochemical cleavage of Angeli's anion using UV laser flash photolysis to produce HNO^-/NO^- according to reaction 1 [2]:

$$N_2O_3^{2-} \xrightarrow{hv} NO^- + NO_2^- \qquad \text{(Reaction 1)}$$

$^1NO^-$ is an extremely strong base ($^1HNO/NO^-$, p*K*a = 23, figure 1) and, therefore, should be protonated by water nearly instantaneously (reaction 2):

$$^1NO^- + H_2O \rightarrow {}^1HNO + OH^- \qquad \text{(Reaction 2)}$$

Nitroxyl (NO^-) may be produced in biological systems by the reduction of nitric oxide ($NO^\bullet$) by CuZn superoxide dismutase (CuZnSOD), ferrocytochrome c, and ubiquinol and by nitric oxide synthase acting in the absence of adequate tetrahydrobiopterin [3].

4. Detection of Nitroxyl

As mentioned above nitroxyl is highly reactive and converted rapidly to other RNS, which makes the process of detection and differentiation of NO^- form other RNS very difficult. Recently, Xia Y. and coworker described a method to detect NO^- using electron paramagnetic resonance spectroscopy (EPR) spin trapping with N-methyl-D-glucamine dithiocarbamate iron (Fe-MGD) complexes to distinguish nitric oxide and nitroxyl anion. In this method Fe-MGD discriminate $NO^\bullet$ from NO^- dependent on the redox state of the iron complex used. Fe^{2+}-MGD

selectively gives rise to the paramagnetic NO-Fe^{2+} mononitrosyl iron complex (MNIC) when react with $NO^{\bullet}$, while NO^- does not. In contrast, NO^- only reacts with Fe^{3+}-MGD to form a paramagnetic MNIC, figure 2 shows how the redox state of iron is important to distinguish between $NO^{\bullet}$ and NO^- [4].

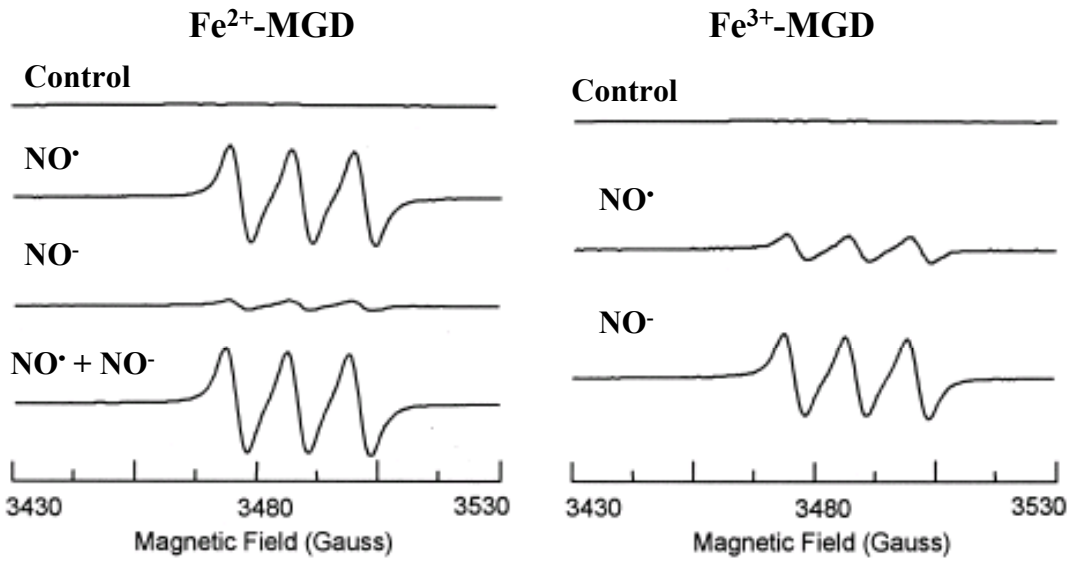


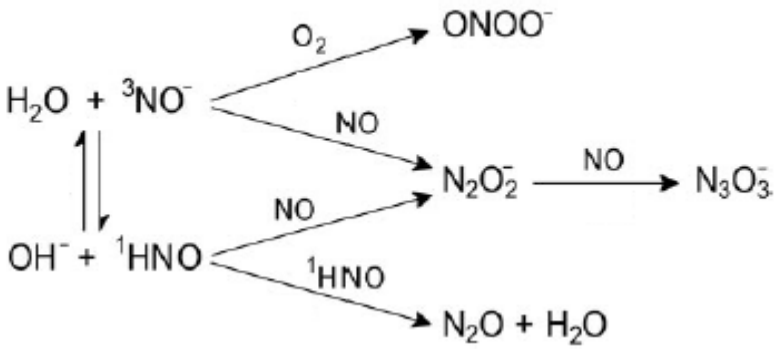
Figure 2 EPR measurements of $NO^{\bullet}$ and NO^- using Fe-MGD showing how the redox state of iron can distinguish between $NO^{\bullet}$ that react with Fe^{2+}-MGD and NO^- that react with Fe^{3+}-MGD. Adapted from [4].

5. Reactions of Nitroxyl

The most important reaction of nitroxyl is the formation of peroxynitrite, where NO^- reacts with molecular oxygen to form preoxynitrite [5] according to reaction 3:

$$NO^- + O_2 \rightarrow ONOO^- \qquad \text{(Reaction 3)}$$

Scheme 1 shows the different pathways of reactions that nitroxyl may take in aqueous solutions that involve Nitric oxide and peroxynitrite.



Scheme 1. Addition of $^3O_2$ to $^3NO^-$ produce peroxynitrite with nearly diffusion-controlled rate (k=2.7 x $10^9 M^{-1}s^{-1}$). In contrast, addition of $^3O_2$ to ^1HNO is spin-forbidden. Both ^1HNO and $^3NO^-$ react sequentially with two NO to generate $N_3O_3^-$ as a long-lived intermediate; the rate laws of $N_3O^-_3$ formation are linear in concentrations of NO and ^1HNO (k = 5.8 x 10^6 $M^{-1}s^{-1}$) or NO and $^3NO^-$ (k = 2.3 x 10^9 $M^{-1}s^{-1}$). Hydroxide catalysis the reactions of ^1HNO with both O_2 and NO through a spin-forbidden deprotonation by OH^- (k = 4.9 x 10^4 $M^{-1}s^{-1}$) of the relatively unreactive ^1HNO into the extremely reactive $^3NO^-$. Dimerization of ^1HNO to produce N_2O happen at rate constant of (k = 8 x 10^6 $M^{-1}s^{-1}$). Adapted from [2]

Nitroxyl can also act as a substrate for CuZnSOD in the presence and absence of O_2 (reactions 4 and 5), also it react with cytochrome c in the absence of O_2 (reaction 6) [3].

$$NO^- + O_2^- + 2H^+ \xrightarrow{SOD} NO + H_2O_2 \qquad k_{SOD}= 2 \times 10^6\ M^{-1}\ s^{-1} \qquad \text{(Reaction 4)}$$

$$NO^- + NO^- + 2H^+ \xrightarrow{SOD} H_2N_2O_2 \qquad k_{SOD}= 2 \times 10^6\ M^{-1}\ s^{-1} \qquad \text{(Reaction 5)}$$

$$\text{cyt c (III)} + NO^- \longrightarrow \text{cyt c (II)} + NO \qquad k_{cyt}= 0.36 \times 10^6\ M^{-1}\ s^{-1} \qquad \text{(Reaction 6)}$$

6. Nitroxyl in Biology

The biology of nitroxyl as its chemistry is not will defined, but different studies showed that it has biological and physiological actions related to other RNS. In one study by Paloocci and others, NO^- derived from Angeli's salt has a unique cardiovascular profile (cardiac contractility assessment, end-systolic elastance, end-diastolic dimension and Isovolumic relaxation), which differs strikingly from $NO^\bullet$. They show that NO^- is a potent, positive inotropic agent endowed with preferential venous dilatative properties in the intact circulation. The inotropic effect of NO^- is redox-sensitive, not related to cGMP-synthesis, and fully antagonized by a (calcitonin gene-related peptide) CGRP-receptor blocker, and demonstrates that a NO^- related species can modulate cardiac contractility by this pathway. By their results the authors suggest that differences between the pharmacological profile of NO^- and traditional $NO^\bullet$ donors may provide an attractive alternative in conditions with increased cardiac loading and depressed function [6].

In other studies nitroxyl was found to be involved in many biological systems, it was found to regulate NMDA receptor/channel that plays a crucial role in the function of the CNS [7], also, Nitroxyl was found to enhanced human neutrophil migration [8]. Further more, nitroxyl induces heme oxygenase (HO-1) activity and protein expression in analogy with other reactive nitrogen species [9]. Nitroxyl also affects other anitoxidant enzymes. Niketic, V. and colleges found that, exposure of Mn and FeSODs, but not CuZnSOD, to $NO^\bullet$ leads to nitrosonium and nitroxyl ions generation, which cause enzyme modification and inactivation. This inactivation was accompanied by extensive structural alterations, including the cleavage of enzyme polypeptide chains [10].

7. Summary

Nitroxyl is a highly reactive nitrogen species *in vivo* and *in vitro,* although its chemistry still unclear, studies showed that nitroxyl may contribute to many biological activity that related to RNS production and action specifically $NO^{\bullet}$, in which nitroxyl formation may play critical role in $NO^{\bullet}$ actions *in vivo*. In fact, some studies as mentioned above, suggest that nitroxyl may oppose that action of $NO^{\bullet}$, or even the action of $NO^{\bullet}$ is really comes from the generation of NO^{-}. Finally, more studies are needed to understand the chemical and biological properties of nitroxyl that will help in reveling the important role of this RNS.

7. References

1. Ellis HB, Ellison GB. (1983). Photoelectron spectroscopy of HNO^- and DNO^-. *Journal of Chemical Physics*. **78:** 6541–6558.

2. Shafirovich V, Lymar SV. (2002). Nitroxyl and its anion in aqueous solutions: spin states, protic equilibria, and reactivities toward oxygen and nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America*. **99:**7340-7345.

3. Liochev SI, Fridovich I. (2002). Nitroxyl (NO^-): a substrate for superoxide dismutase. *Archives of Biochemistry & Biophysics*. **402:**166-171.

4. Xia Y, Cardounel AJ, Vanin AF, Zweier JL. (2000). Electron paramagnetic resonance spectroscopy with N-methyl-D-glucamine dithiocarbamate iron complexes distinguishes nitric oxide and nitroxyl anion in a redox-dependent manner: applications in identifying nitrogen monoxide products from nitric oxide synthase. *Free Radical Biology & Medicine*. **29:**793-797.

5. Kirsch M, de Groot H. (2002). Formation of peroxynitrite from reaction of nitroxyl anion with molecular oxygen. *Journal of Biological Chemistry*. **277:**13379-13388.

6. Paolocci N, Saavedra WF, Miranda KM, Martignani C, Isoda T, Hare JM, Espey MG, Fukuto JM, Feelisch M, Wink DA, Kass DA. (2001). Nitroxyl anion exerts redox-sensitive positive cardiac inotropy in vivo by calcitonin gene-related peptide signaling. *Proceedings of the National Academy of Sciences of the United States of America*. **98:**10463-10468.

7. Colton CA, Gbadegesin M, Wink DA, Miranda KM, Espey MG, Vicini S. (2001). Nitroxyl anion regulation of the NMDA receptor. *Journal of Neurochemistry*. **78:**1126-1134.

8. Vanuffelen BE, Van Der Zee J, De Koster BM, Vansteveninck J, Elferink JG. (1998). Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration. *Biochemical Journal*. **330:**719-722.

9. Naughton P, Foresti R, Bains SK, Hoque M, Green CJ, Motterlini R. (2002). Induction of heme oxygenase 1 by nitrosative stress. A role for nitroxyl anion. *Journal of Biological Chemistry*. **277:**40666-40674.

10. Niketic V, Stojanovi S, Nikoli A, Spasi M, Michelson AM. (1999). Exposure of Mn and FeSODs, but not Cu/ZnSOD, to NO leads to nitrosonium and nitroxyl ions generation which cause enzyme modification and inactivation: an in vitro study. *Free Radical Biology & Medicine*. **27:**992-996.