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NITRIC OXIDE: THE MAGIC GAS OF JOHN DALTON AND JOHN PRIESTLEY

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Abbreviations:

BH₄ - tetrahydrobiopterin, CaM – calmodulin, Cat – catalase, cGMP – cyclic guanosine monophosphate, EDRF – endothelium-derived relaxing factor , EPR – electron paramagnetic resonance, Hb – hemoglobin, Mb – myoglobin, NADPH – nicotinamide-adenine dinucleotide phosphate, NOS – nitric oxide synthase, sGC – soluble guanylate cyclase, SOD – superoxide dismutase, TTPS – mesotetrakis (4-sulfonatophenyl) porphyrin

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1. Abstract

Nitric oxide is a small, gaseous, paramagnetic radical with a high affinity for interaction with ferrous hemoproteins such as sGC and Hb. Nitric oxide is produced in macrophages, neutrophils, endothelial cells and in the cerebellum. Nitric oxide was identified as EDRF and acts not only as a potent endogenous vasodilator, but also has a role in inflammation, thrombosis, platelet adhesion, neutrophil aggregation, regulation of blood pressure, neurotransmission and synaptic plasticity in the brain. This review paper will discuss nitric oxide chemistry, biosynthesis and measurement.

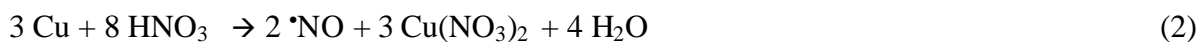
2. Introduction

Nitrogen (N) forms oxides in which nitrogen exhibits each of the positive oxidation numbers from +1 to +5. Nitric oxide ($\bullet\text{NO}$) is a simple molecule, consisting of a single oxygen bonded to one nitrogen atom. It is in fact one of the 10 smallest stable molecules in nature and also a remarkably stable free radical. Nitric oxide was discovered by John Priestley more than 200 years ago. In a paper dating from 1805, John Dalton used the reaction of nitric oxide with oxygen to develop his Law of Simple Multiple Proportions, which was the first great success of the Atomic Theory of Matter.

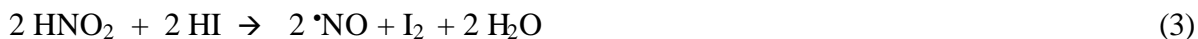
Nitric oxide can be prepared in several ways. The lightning that occurs during a thunderstorm brings about the direct union of atomic nitrogen and atomic oxygen in the air to produce small amounts of nitric oxide, as does heating the two elements together. On an industrial scale, $\bullet\text{NO}$ is produced by the oxidation of ammonia with a platinum catalyst [1]:



In the laboratory, $\bullet\text{NO}$ was prepared by John Priestley by the action of nitric acid on metals (*e.g.* copper) [1]:

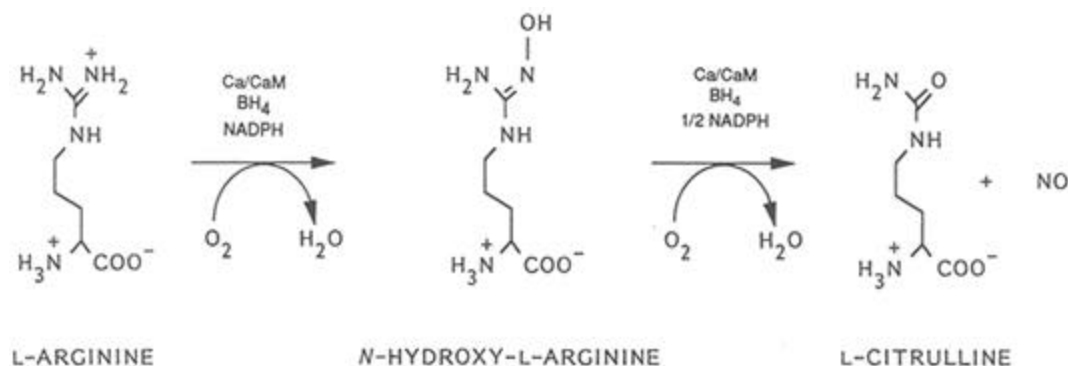


On the laboratory scale, there are additional methods for the production of $\bullet\text{NO}$, *e.g.* the reduction of acidified nitrite(nitrous acid) by a suitable reducing agent [1]:



3. Biosynthesis of nitric oxide

Nitric oxide is synthesized from L-arginine and molecular oxygen [2] as shown in Figure 1 (from [3]) by NOS, a family of four isoenzymes that represent distinct gene products :



4. Physical and chemical properties of nitric oxide

At room temperature and pressure at 1 atm, $\bullet\text{NO}$ is a colorless gas, consisting of diatomic molecules. The molecular weight of $\bullet\text{NO}$ is 30.01 and its composition is 46.68 % N and 53.32 % O. Its boiling point is 151.7 °C. Gaseous $\bullet\text{NO}$ is the most thermally stable oxide of nitrogen and is also the simplest known thermally paramagnetic molecule, *i.e.* molecule with an unpaired electron. Gaseous $\bullet\text{NO}$ is one of the environmental pollutants generated by internal-combustion engines, resulting from the reaction of nitrogen and oxygen in the air during the combustion process [4].

A molecular model was established for the $\bullet\text{NO}$ molecule. The nitrogen and the oxygen atom are bound by a double bond. There are two pairs of non-bonding electrons on the oxygen atom and one pair of non-bonding electrons on the nitrogen atom. Nitric oxide has a linear molecular shape, and the electron pair geometry around both the nitrogen and oxygen atoms is trigonal planar. Nitric oxide has molecular polarity: the partial specific negative charge is on the oxygen

atom and the partial specific positive charge is on the nitrogen atom [4]. Thus, $\bullet\text{NO}$ has an unpaired electron in the highest occupied molecular orbital (HOMO) (Figure 2 , from [1]):

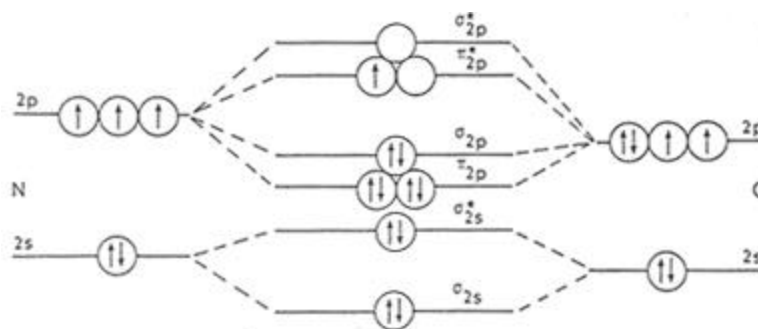


Figure 1. Molecular orbital diagram for NO in its ground state

According to the molecular orbital theory, $\bullet\text{NO}$ has three fully occupied bonding orbitals with the unpaired electron residing in a fourth antibonding orbital. This extra electron weakens the overall bonding of nitrogen by one-half a bond. Thus, the nitrogen and the oxygen in $\bullet\text{NO}$ are effectively held together by 2.5 bonds (3 bonding minus 0.5 antibonding orbitals). The bond distance and bond energy for $\bullet\text{NO}$ are 1.2 Å and 149.9 kcal/mol, respectively [4].

The electrostatic field of $\bullet\text{NO}$ repels hydrogen ions, accounting for the hydrophobic properties of this molecule that allow it to freely diffuse through biological membranes [4]. Biological membranes and other hydrophobic tissue compartments are important sites for disappearance of $\bullet\text{NO}$ and for formation of $\bullet\text{NO}$ -derived reactive species [5]. Nitric oxide exhibits a low level of solubility in water ($\sim 1.7 \times 10^{-3}$ mol/l at 25 °C and $P_{\text{NO}} = 1$ atm), comparable in magnitude to the solubilities of N_2 , O_2 and CO . However, $\bullet\text{NO}$ is approximately nine times more soluble in a hydrophobic solvent such as hexane than in water [5]. Therefore, nitric oxide is more soluble in lipid structures than in aqueous environment.

Nitric oxide does not exhibit a strong tendency to dimerize. The presence of dimers N_2O_2 in the gas phase is barely detectable by infrared spectroscopy, although the participation of this species as an intermediate is probable in the cases of several of the reactions of $\bullet\text{NO}$ [1].

Like other nitrogen oxides, $\bullet\text{NO}$ is thermodynamically unstable, as indicated by its large positive Gibbs energy of formation ($\Delta_f G^\circ_{298} = 86.32 \text{ kJ/mol}$). In consequence the synthesis of $\bullet\text{NO}$ from its constituent elements N_2 and O_2 occurs to an appreciable extent only at elevated temperatures, and does so in electric arc processes (including natural lightning), fossil-fired power plants, internal combustion engines and other high temperature processes carried out in the atmosphere. Despite the thermodynamic instability of $\bullet\text{NO}$, its decomposition is kinetically hindered; the gas can be stored indefinitely at 1 atm pressure and ordinary temperatures without detectable decomposition. Under elevated pressure, however, disproportionation of $\bullet\text{NO}$ to form N_2O and $\bullet\text{NO}_2$ is known to occur [4]:



Nitric oxide can rapidly and directly react with the unpaired electron on both organic and oxygen-centered radicals to yield a variety of highly reactive intermediates [4].

Nitric oxide may be oxidized by one electron to give nitrosonium ion (NO^+) or reduced by one electron to form nitroxyl anion (NO^-) which are important intermediates in the chemistry of $\bullet\text{NO}$ [4]:



5. Reactions of nitric oxide

A. Reaction of $\bullet\text{NO}$ with O_2

In the gas phase, the reaction of $\bullet\text{NO}$ with O_2 results in the formation of nitrogen dioxide ($\bullet\text{NO}_2$) as the terminal product. The kinetics of this reaction is second order in $\bullet\text{NO}$ (Rxn 6). In

aqueous solution, however, the reaction of $\cdot\text{NO}$ with dioxygen occurs with the overall stoichiometry of Rxn 8, and the rate of disappearance of $\cdot\text{NO}$ is given by (9) [5,6] :



$$-d [\cdot\text{NO}] / dt = k[\cdot\text{NO}]^2[\text{O}_2], \quad k = 2 \times 10^6 \text{ M}^{-2} \text{ s}^{-1} \quad (7)$$



$$-d [\cdot\text{NO}] / dt = 4k [\cdot\text{NO}]^2 [\text{O}_2], \quad k = 2 \times 10^{-6} \text{ M}^{-2} \text{ s}^{-1} \quad (9)$$

B. Reaction of $\cdot\text{NO}$ with $\text{O}_2^{\cdot-}$

The reaction of $\cdot\text{NO}$ with superoxide ($\text{O}_2^{\cdot-}$) results in peroxynitrite which is stable. Its conjugate acid, $\text{O}=\text{NOOH}$, has a pK_a of 6.8 [8] :



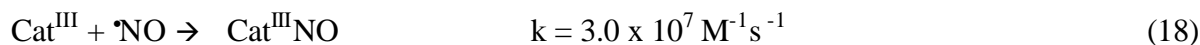
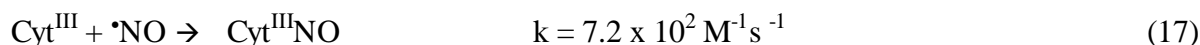
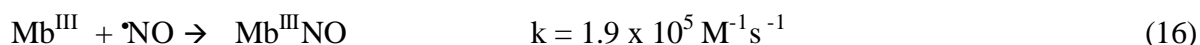
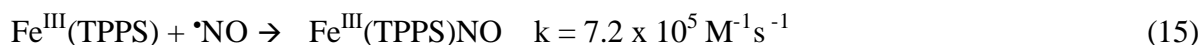
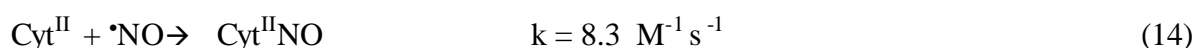
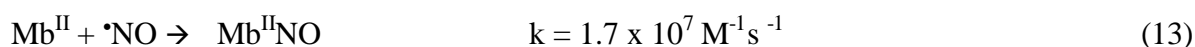
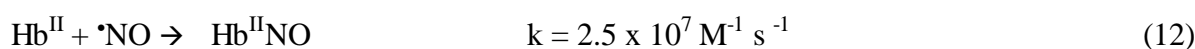
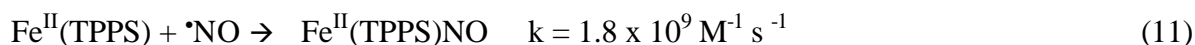
The formation of peroxynitrite *in vivo* transforms two relatively unreactive free radicals, $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$, into a much more reactive species. This reaction occurs with one of the largest rate constants known for reactions of $\cdot\text{NO}$. The rate of formation of peroxynitrite is first order in the concentrations of both $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$ and therefore on the activities of both NOS and SOD. The biological effects of peroxynitrite can be prevented by limiting the formation of peroxynitrite from $\cdot\text{NO}$ by lowering the concentration of $\text{O}_2^{\cdot-}$ using SOD or SOD mimics [8].

C. Reactions with transition metals and their complexes

Nitric oxide rapidly reacts with transition metals, which have stable oxidation states differing by one electron. Nitric oxide is unusual in that it reacts with both the ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms of iron. Complexes of ferric iron with $\cdot\text{NO}$ are called nitrosyl compounds and will nitrosate (add an NO^+ group) many compounds, while reducing the iron to the ferrous state. The binding of $\cdot\text{NO}$ with ferrous iron is reversible and occurs with a remarkably high affinity. Nitric

oxide binds more tightly to ferrous iron, but has a greater ability than CO to bind the iron in the ferric state, producing a diamagnetic complex [4].

Generally, the reactions of paramagnetic metal complexes with $\bullet\text{NO}$ are facile. The rates are strongly affected by a number of factors: the type of metal, the types of ligands coordinated to the metal, the degree of unsaturation and/or lability of the ligands, and the spin state of the metal. Shown below are the kinetic properties of some reactions involving $\bullet\text{NO}$ and heme proteins [7] :

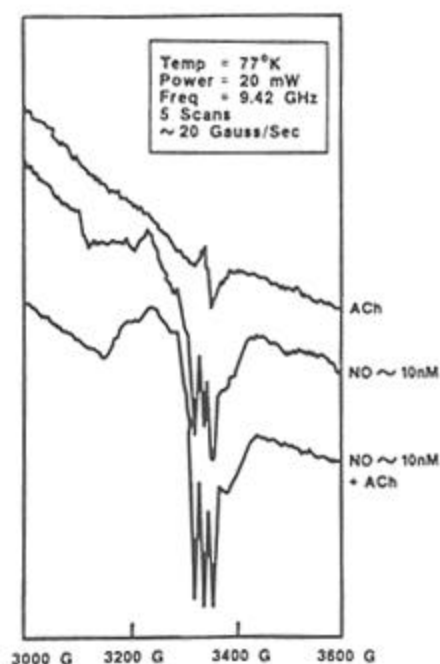


These direct reactions of $\bullet\text{NO}$ with metalloproteins are important to the bioregulatory activities of $\bullet\text{NO}$. Thus, the binding of $\bullet\text{NO}$ to the ferrous heme iron of guanylate cyclase is important for the activation of signal transduction pathways, since it activates guanylate cyclase and thereby increases the synthesis of the intracellular messenger cGMP. The binding of $\bullet\text{NO}$ to the ferrous heme groups being reversible, this allows the guanylate cyclase to turn off immediately after the $\bullet\text{NO}$ gradient has dissipated. It is assumed that guanylate cyclase is activated by a reversible equilibrium of $\bullet\text{NO}$ with the ferrous heme group. In addition to the widely known $\bullet\text{NO}$ complexes of ferrous hemes, $\bullet\text{NO}$ forms complexes with iron-sulfur proteins, other non-heme proteins as well as copper proteins [4].

6. Measurement of nitric oxide

Measurement of $\bullet\text{NO}$ in biological specimens is difficult both because of the small amounts present and the lability of $\bullet\text{NO}$ in the presence of O_2 [2]. The most widely known assays use one of these three strategies to detect nitric oxide: (1) $\bullet\text{NO}$ is "trapped" by reduced Hb, forming a stable adduct that is detected by EPR (detection threshold ~ 1 nmol) (as shown in figure 3, from [10]); (2) $\bullet\text{NO}$ oxidizes reduced Hb to methemoglobin, which is detected by spectrophotometry (detection threshold ~ 1 nmol); and (3) $\bullet\text{NO}$ interacts with ozone producing light, "chemiluminescence" (detection threshold ~ 20 pmol) [10].

Figure 3. Detection of nitric oxide by electron paramagnetic resonance spectroscopy (using a hemoglobin-agarose spin trap). Effluent from isolated canine femoral arteries (with intact endothelium) was measured using a hemoglobin-agarose trap by EPR (see ref 38 for methods). The top trace showed that the femoral arteries did not produce detectable amounts of NO when given acetylcholine (ACH 10^{-6} M), though EDRF activity was confirmed. The middle trace confirms the ability of the assay to detect authentic NO (10 nM) (note the characteristic triplet pattern of hyperfine splitting between 3200 and 3400 G). The third trace shows that when the NO was passed through the femoral artery, in combination with acetylcholine, NO was readily detected. The authors concluded that the EPR assay is sensitive to nanomolar amounts of NO, in vitro and in rings, and that ACH-induced vasodilatation in this model is NO-independent. This figure is reproduced with permission from Greenberg et al. (38).



7. Summary

Nitric oxide is the classical example of a simple molecule endowed with fascinatingly complex biological functions. Long thought upon as being merely an ozone-destroying pollutant, $\bullet\text{NO}$ is an essential physiological signaling molecule in cell-cell communication. It has

emerged as a possible component of therapies for diseases such as neonatal persistent pulmonary hypertension, stroke-induced ischemia, adult respiratory-distress syndrome, septic shock, post-angioplasty restenosis, and impotence. Its role in carcinogenesis is controversial [9]. This, as well as many other aspects of NO biology, remains to be further studied and understood.

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