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Review article

A comparison of the chemistry associated with the biological signaling and actions of nitroxyl (HNO) and nitric oxide (NO)

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ABSTRACT

Nitric oxide (NO) and nitroxyl (HNO) are reported to have numerous biological activities with significant therapeutic potential. Many of these activities are overlapping. The chemistry by which these two species act is likely to be distinct in spite of their apparent close structural similarities. Discussed in this review is the chemistry of NO and HNO with their likely biological targets — thiolproteins, metalloproteins (more specifically iron heme proteins) and free radical processes. Based on the chemistry discussed, it can be concluded that the biological actions of NO are likely due primarily to its interactions with metal centers and reaction with radical species. The likely biological targets for HNO are, similarly, metal centers and radical species (albeit with different chemistry compared to NO). HNO is also particularly good at directly modifying thiols while NO-mediated thiol modification requires other reactants to be present and is not as facile. Thus, a fundamental difference between NO and HNO that likely distinguishes them with regards to their biological activity is the greater propensity for HNO to react with thiols compared to NO.

1. Introduction

The biological chemistry and activity of nitrogen oxides in general have been of significant interest since the discovery of endogenous generation of nitric oxide (NO) in mammalian systems [1,2]. For example, NO has been reported to be involved in numerous biological/physiological functions including the maintenance of vascular tone, as an immune response effector and as a regulator of the central nervous system [3]. More recently the one-electron reduced and protonated congener of NO, nitroxyl (HNO) has been found to elicit numerous biological effects, some of which have significant therapeutic potential [4]. Interestingly, NO and HNO possess significant overlap with regards to their biological activity. For example, both species can have profound effects on the cardiovascular system, can be involved in redox biochemical processes and have been proposed as pharmacological agents involving these two distinct activities (vide infra). Many early reports on the biological actions of HNO proposed that it can be converted to NO in a biological milieu and that the activity could be due to NO (as opposed to HNO itself) [5]. Thus, a fundamental and important question associated with discerning the mechanisms of HNO and NO biology is with regard to the possible interconversion of these closely related chemical species. That is, can the biological effects of HNO be due to conversion to NO (and vice versa)? HNO and NO are related simply by a single electron and proton. The reduction potential for the $NO,H^+/HNO$ couple is −0.55 V (vs. NHE, pH 7) [6,7]. This value makes reduction of NO to HNO very difficult in a biological system. The $NO/^3NO^-$ couple is, of course, even less favorable ($E^{\circ\prime} = -0.8$ V, vs. NHE). Also, the H–NO bond strength is only 46–47 kcal/mol [8,9], indicating the potential for HNO to be easily oxidized by hydrogen atom abstraction. These values indicate that the conversion of HNO to NO can occur in the presence of even weak oxidants. Thus, HNO can conceivably be converted to NO but it would be difficult to convert NO to HNO via simple one-electron reduction processes in most biological systems. Based on this, it is reasonable to speculate that some of the biological activity of HNO can be due to conversion to NO. As stated above, some early studies considered that the biological actions of HNO were indeed due to conversion to NO [10]. In many cases, this possibility cannot be entirely ruled out (or at least it needs to be considered that maybe some NO is generated in a solution of HNO). One way of distinguishing the biological actions of HNO versus NO is to take advantage of the greater reactivity of HNO over NO with thiols [11]. That is, as discussed in more detail below, HNO readily reacts with thiols under conditions where little reaction with NO (or NO-derived species) occurs. Therefore, if the addition of thiols (e.g. cysteine, under the proper conditions) blocks the activity of an HNO-donor, then it can be concluded that HNO and not NO was responsible for this activity. Regardless, rigorous studies indicate that the chemistry of HNO and NO are distinct and there is currently little doubt that distinct biology from both species is observed based on their chemical differences.

The three most prevalent biological targets for both NO and HNO have been reported to be 1) metals/metalloproteins (especially iron heme proteins), 2) thiols/thiol proteins, and 3) free radical processes. Herein the chemistry of NO and HNO with these three target classes are compared and contrasted and the mechanisms by which they

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alter or react with common biological targets to affect physiological function is discussed.

2. Reactions of nitric oxide with iron heme proteins

Although NO has been reported to react with numerous biological targets, there is little doubt that the primary and most established biological receptor for NO is the ferrous-heme containing enzyme soluble guanylate cyclase (sGC) [12]. This enzyme converts guanosine triphosphate (GTP) to the second messenger cyclic guanosine monophosphate (cGMP). Thus, much of NO physiology is due to the actions of elevated cellular cGMP levels. The interaction of NO with sGC involves its coordination to a regulatory iron heme group associated with the enzyme [13]. Binding of NO to the ferrous heme of sGC results in a weakening of the trans axial bond (referred to as trans-activation) leading to dissociation of the histidine ligand [14]. This ability to trans-activate is fairly unique to NO as a ligand and has been proposed to be responsible for the specificity of NO as a regulator of sGC. It has also been noted that sGC appears to be specifically designed to react solely with NO (as opposed to biological concentrations of CO or O_2, for example), contributing to its specificity of action [15,16]. The ability for NO to labilize the trans ligand is partially due to electron donation from a partially filled π^* orbital on NO to the d_z^2 orbital on the metal (for example, [17,18]). This interaction weakens the trans-ligand bond to the metal resulting in loss of the ligand and generation of a 5-coordinate complex. Importantly, NO is fairly unique in this regard as ligands such as O_2 and CO do not transactivate and generally form stable 6-coordinate complexes.

Unlike the two other biologically relevant diatomics CO and O_2, NO will also bind oxidized metalloproteins such as ferric heme proteins. NO is considered an amphoteric ligand in that it can bind to metals as either "NO^+" (which is isoelectronic with CO and therefore binds in a linear fashion) or "NO^-" (which is isoelectronic with O_2 and typically binds in a bent fashion). Coordination of NO to, for example, ferric hemes normally results in a linear complex that can be described as an Fe^{2+}–NO^+ complex. As such, a nitrosyl ligand bound to a ferric heme is often times subject to attack by nucleophilic species such as water (generating the ferrous heme and nitrite) or a thiol (generating the ferrous heme and an S-nitrosothiol) (Reactions 1 and 2) [19].

$$\text{heme–Fe}^{\text{III}} + \text{NO} \rightarrow [\text{heme–Fe}^{\text{III}}\text{–NO} \leftrightarrow \text{heme–Fe}^{\text{II}}\text{–NO}^+]. \quad (1)$$

$$\text{Nuc–H} + \left[\text{heme–Fe}^{\text{III}}\text{–NO} \leftrightarrow \text{heme–Fe}^{\text{II}}\text{–NO}^+\right] \rightarrow \text{heme–Fe}^{\text{II}} + \text{Nuc–NO} + \text{H}^+ \quad (2)$$
$$(\text{Nuc–H} = \text{RS} - \text{H}, \text{HO–H}, \text{etc.}).$$

$$\text{heme–Fe}^{\text{II}} + \text{NO} \rightarrow \text{heme–Fe}^{\text{II}}\text{–NO}. \quad (3)$$

The ferric nitrosyl complexes of hemoglobin and myoglobin, for example, undergo reductive nitrosylation in the presence of excess NO whereby Reactions 1 and 2 are followed by rapid coordination of NO to the ferrous heme (Reaction 3).

Generally speaking, binding of NO to ferrous hemes of many metalloproteins is more favorable than binding to the corresponding ferric species. For example, the k_{on} and k_{off} for NO binding to ferrous myoglobin are $1.7\times10^7\ M^{-1}s^{-1}$ and $1.2\times10^{-4}\ s^{-1}$, respectively, whereas the k_{on} and k_{off} for NO binding to ferric myoglobin are $1.9\times10^5\ M^{-1}s^{-1}$ and $13.6\ s^{-1}$, respectively [20]. Thus, using these values the NO association constant for the ferric and ferrous forms of myoglobin can be calculated to be approximately $1.4\times10^{11}\ M^{-1}$ for ferrous Mb and only $1.4\times10^4\ M^{-1}$ for ferric Mb. It needs to be mentioned that all iron heme proteins do not behave identically and the differences in the kinetics between the ferrous and ferric species may not be this extreme. For example, the binding of NO to the metal center in many cases is reported to occur via a dissociative mechanism and therefore the k_{on} values will be a function of the nature of the dissociating ligand as well as other protein factors [21].

Nitric oxide is also capable of reacting with dioxygen complexes of iron hemeproteins. Indeed, one of the first convenient assays for NO relied on the reaction with oxyhemoglobin to give methemoglobin (Reaction 4) [22].

$$\text{HbFe}^{\text{II}}\text{O}_2 + \text{NO} \rightarrow \text{HbFe}^{\text{III}} + \text{NO}_3^-. \quad (4)$$

Due to the fact that many ferrous heme dioxygen complexes can be viewed as ferric superoxide complexes (Fe^{III}–O_2^-), Reaction 4 is analogous to the well known and studied reaction of NO with superoxide to give, initially, peroxynitrite ($ONOO^-$) which then degrades to NO_3^- (Reaction 5) (although it is reported that peroxynitrite may not be an intermediate in this reaction [23]).

$$\text{NO} + \text{O}_2^- \rightarrow \text{ONOO}^- \rightarrow \text{NO}_3^-. \quad (5)$$

2.1. Reactions of HNO with iron heme proteins

One study has reported that HNO is also capable of activating sGC, possibly via coordination to the ferrous heme [24]. However, this has been disputed and it remains to be unequivocally determined whether HNO itself can activate this enzyme [25] or under what conditions this may occur. Regardless, HNO is able to coordinate metalloproteins in ways distinct from NO. For example, work from Farmer's lab has established that HNO coordination complexes with myoglobin can be made either by reducing ferrous–NO complexes (Reaction 6) or by directly trapping HNO by the ferrous heme protein (Reaction 7) [26–28]. In both cases, HNO binding to the metal is via coordination through the nitrogen atom of HNO. Unlike NO, HNO ligation to ferrous hemes is not expected to labilize the trans ligand due to the fact that HNO has unoccupied π^* orbitals.

$$\text{MbFe}^{\text{II}}\text{–NO} + \text{e}^- + \text{H}^+ \rightarrow \text{MbFe}^{\text{II}}\text{–HNO}. \quad (6)$$

$$\text{Mb–Fe}^{\text{II}} + \text{HNO} \rightarrow \text{MbFe}^{\text{II}}\text{–HNO}. \quad (7)$$

Much like the analogous O_2 complexes, the ferrous myoglobin HNO complexes are low-spin (diamagnetic d^6). Probably the most distinguishing and diagnostic characteristic of the ferrous myoglobin HNO complexes is the ^1H NMR resonance for the bound HNO at about 15 ppm. The HNO adducts with ferrous myoglobin are inherently stable under strict anaerobic conditions and can be stored for extended periods of time without degradation [29]. However, exposure of the ferrous HNO myoglobin complex to O_2 results in rapid ferric myoglobin formation and exposure to NO or NO_2^- gives a ferrous–NO complex [28]. Interestingly, it appears that the ferrous HNO myoglobin adduct can react with excess HNO to also give the NO adduct (Reaction 8) [29]. Of particular note is the possibility that a ferrous–HNO complex in sGC could be converted to the ferrous–NO complex by a variety of species (i.e. NO, HNO, NO_2^-) leading to enzyme activation (possibly explaining the results of Miller and coworkers [24]). Currently, the mechanisms of these degradation pathways are not established.

$$\text{MbFe}^{\text{II}}\text{–HNO} + \text{HNO} \rightarrow \text{MbFe}^{\text{II}}\text{–NO}(\text{mechanism unknown}). \quad (8)$$

HNO also reacts rapidly with ferric heme proteins. For example, reaction of HNO with ferric myoglobin (metmyoglobin) results in the formation of the ferrous nitrosyl myoglobin (Reaction 9) [30].

$$\text{HNO} + \text{Fe}^{\text{III}}\text{Mb} \rightarrow \text{ON–Fe}^{\text{II}}\text{Mb} + \text{H}^+. \quad (9)$$

As mentioned above, the generation of the ferrous–NO complex of sGC results in enzyme activation. Under normal biological conditions,