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Conversion of nitrite to nitric oxide at zinc *via* *S*-nitrosothiols†

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Nitrite is an important reservoir of nitric oxide activity in the plasma and cells. Using a biomimetic model, we demonstrate the conversion of zinc-bound nitrite in the tris(pyrazolyl)borate complex ^{iPr2}TpZn(NO$_2$) to the corresponding *S*-nitrosothiol RSNO and zinc thiolate ^{iPr2}TpZn–SR *via* reaction with thiols H–SR. Decomposition of the *S*-nitrosothiol formed releases nitric oxide gas.

Nitric oxide (NO) is an endogenous molecule connected to many important biological processes such as vasodilation, neurotransmission and cytoprotection.[1] Although NO is ubiquitous, its lifetime in biological media is very short due to its ready oxidative metabolism to nitrite and nitrate.[2] Thus, reservoirs of NO activity such as nitrite[2–5] and *S*-nitrosothiols[6–10] are of significant importance in physiological processes connected to nitric oxide.

Nitrite serves as an important, air-stable reservoir for nitric oxide activity.[2–5,11,12] Its concentration in human plasma and red blood cells is tightly regulated in the ranges of 0.121 ± 0.009 μM and 0.288 ± 0.047 μM, respectively.[13] Conversion of nitrite to NO requires 1-electron reduction that can be accomplished by several metalloenzymes such as globins, xanthine oxidoreductase and cytochrome *c* oxidase under hypoxic conditions.[2,11,14–16] On the other hand, nitrite can be converted to *S*-nitrosothiols RSNO with thiols H–SR under conditions of high acidity in a redox neutral reaction.[17] Such interconversion is thought to be particularly important in the gut.[16] Moreover, formation of RSNOs from nitrite represents a pathway for ultimate NO generation since RSNOs are prone to NO loss due to their modest RS–NO bond strengths ranging from 20–32 kcal mol^{-1}.[18–20] While this process may take place thermally, enzymes such as CuZn superoxide dismutase catalyze the release of NO from RSNOs with concomitant formation of disulfides.[21] In general, copper ions are particularly effective in promoting release of NO from RSNOs (Scheme 1).[22,23]

$$NO_2^- \xrightarrow{2\,H^+ / e^-} \cdot NO + H_2O$$
$$RSNO \xrightarrow{\Delta \text{ or } Cu^+} 1/2\ RS\text{-}SR + \cdot NO$$

Scheme 1

In 2009, Fago showed that that carbonic anhydrase (CA) can generate nitric oxide from nitrite.[24] Carbonic anhydrase is one of the fastest enzymes known,[25] responsible for maintaining the blood pH by converting carbon dioxide to bicarbonate at a His$_3$Zn^{2+} active site.[25] Due to the rough structural similarity between bicarbonate and nitrite and the ability of CA to bind nitrite which inhibits CO_2 hydration,[24] Fago suggested that CA could also play an important role in NO production from nitrite *via* dehydration of acidified nitrite (HONO) to N_2O_3[24] which readily homolyzes to NO and NO_2.[26] In the absence of such a dehydration–disproportionation sequence, however, reduction of nitrite to nitric oxide at redox inactive zinc requires use of an external reductant.

Herein we employ zinc tris(pyrazolyl)borate complexes that possess a similar coordination environment as found at His$_3$Zn^{2+} centers in many zinc enzymes[27] to examine the reaction of thiols at zinc-bound nitrite. We have previously studied the reactivity of RSNOs, NO, and NO$^+$ at the zinc thiolate linkage employing the thiolate derivatives ^{iPr2}TpZn–SR.[28] We found that RSNOs undergo rapid transnitrosation reactions, formally transferring NO$^+$ between zinc and NO$^+$-bound thiolate residues. Moreover, reaction with NO$^+$ resulted in Zn–SR cleavage to form RSNO while NO_{gas} showed no reactivity with the Zn–SR unit.[28]

Reaction of ^{iPr2}TpZn(NO$_2$)[28] (**1**) with the aromatic thiol H–SAr (Ar = *p*-MeC$_6$H$_4$) at RT in CH_2Cl_2 results in rapid conversion of **1** to the corresponding zinc thiolate ^{iPr2}TpZn–SAr (**2**) (Scheme 2). Compound **2** can be isolated in 70% yield from pentane as colorless crystals. The X-ray structure of **2** (Fig. 1) is similar to that of several related TpZn-thiolates[28–30] with a Zn–S distance of 2.2310(6) Å and Zn–N distances that span 2.0332(19)–2.0487(19) Å. These distances are very similar to those reported by Lippard for a family of tris-(3-phenyl-5-methylpyrazolyl)borate zinc arylthiolates ^{Ph,Me}TpZn–SAr.[30]

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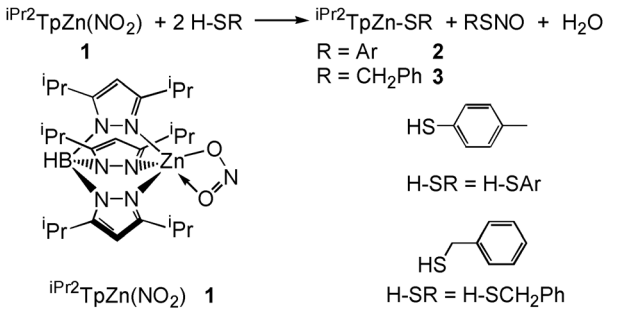


Scheme 2

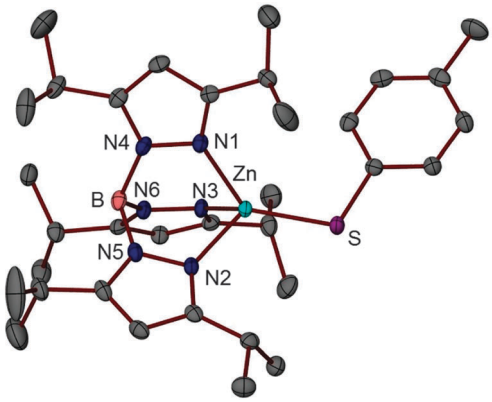
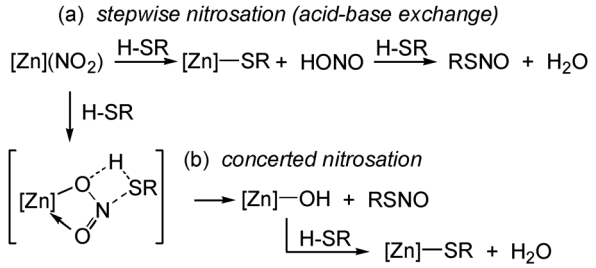


Fig. 1 X-ray structure of ^{iPr2}TpZn–SAr (**2**).

A green color also develops upon reaction of **1** with the aromatic thiol H–SAr which indicates formation of the corresponding *S*-nitrosothiol ArSNO.[31] The intensity of this green color decreases over time due to the decomposition of the thermally unstable aromatic *S*-nitrosothiol[18] to the corresponding disulfide ArS–SAr. After 10 min at RT, addition of 2.3 eq. H–SAr to ^{iPr2}TpZn(NO$_2$) (**1**) in $CDCl_3$ resulted in a 45% yield of ArSNO (δ 2.47 ppm for *p*-Me signal) and a 79% yield of the zinc thiolate ^{iPr2}TpZn–SAr (**2**) (δ 2.23 ppm for *p*-Me signal) as monitored by ^{1}H NMR spectroscopy. The disulfide ArS–SAr was also observed (δ 2.31 ppm for *p*-Me signal), representing the thermal decomposition product of the corresponding *S*-nitrosothiol ArSNO. After 24 h, no ArSNO was present as it was completely converted to the disulfide ArS–SAr observed in 86% overall yield (Scheme 5).

The qualitative rate of reaction of **1** with thiols appears highly dependent on the nature of the thiol. Addition of the primary alkyl thiol H–SCH$_2$Ph (2.3 eq.) to **1** under similar conditions in $CDCl_3$ results in a slow conversion of **1** to the corresponding zinc-thiolate ^{iPr2}TpZn–SCH$_2$Ph (**3**)[28] (Scheme 2). After 1 h, the reaction afforded a 32% yield of **3** as judged by observation of the ^{iPr2}TpZn–SC*H*$_2$Ph benzylic ^{1}H NMR resonance at δ 4.03 ppm. Stirring for 24 h gave an 86% yield of **3** after which time the *S*-nitrosothiol PhC*H*$_2$SNO (δ 4.68 ppm) and disulfide PhC*H*$_2$S–SC*H*$_2$Ph (δ 3.60 ppm) were present in 50% and 12% yields, respectively. Water was also observed in this transformation (64% yield). The especially bulky thiol H–SCPh$_3$ did not react with ^{iPr2}TpZn(NO$_2$) (**1**) under these conditions.

We considered two possible mechanisms in the conversion of zinc-bound nitrite to *S*-nitrosothiol (Scheme 3). In the first,

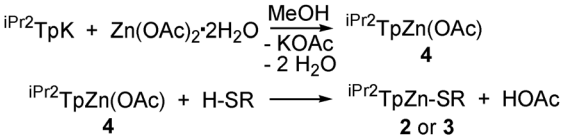


Scheme 3

acid–base reaction between ^{iPr2}TpZn(NO$_2$) (**1**) and H–SR could give ^{iPr2}TpZn–SR and HONO (Scheme 3a). The HONO thus generated could react with a second equivalent of H–SR to give RSNO and H$_2$O.[17] Alternatively, H–SR could directly react with the zinc-bound nitrite anion in ^{iPr2}TpZn(NO$_2$) to give ^{iPr2}TpZn–OH and RSNO. The highly reactive zinc-hydroxide[32] ^{iPr2}TpZn–OH would be expected to rapidly give ^{iPr2}TpZn–SR and H$_2$O under these conditions (Scheme 3b).[29,30]

While we do not have unambiguous data that unequivocally suggest one pathway over the other, at this stage we believe that RSNO and zinc thiolate formation may occur *via* an acid–base reaction (Scheme 3a). Since the pK_a's of HONO and HOC(O)Me are similar (3.3 and 4.7 in water)[33,34] we examined the thiol reactivity of the zinc acetate complex ^{iPr2}TpZn(OAc) (**4**). This zinc acetate complex **4** may be isolated in 73% yield by addition of ^{iPr2}TpK to Zn(OAc)$_2$·2H$_2$O in methanol (Scheme 4). The X-ray structure of **4** shows highly unsymmetrical binding of the acetate ligand with Zn–O distances of 1.9385(17) and 2.578(2) Å (Fig. 2), similar to other TpZn(OAc) complexes.[35] Interestingly, this species bears semblance to ^{iPr2}TpZn(NO$_2$) (**1**) which also exhibits highly unsymmetrical binding of the nitrite ligand with Zn–O distances of 1.981(2) and 2.430(2) Å.[28]

Reaction of ^{iPr2}TpZn(OAc) (**4**) with H–SAr (pK_a = 6.8)[36] and H–SCH$_2$Ph (pK_a = 9.4)[37] in $CDCl_3$ (10.8 and 15.4 in DMSO,



Scheme 4

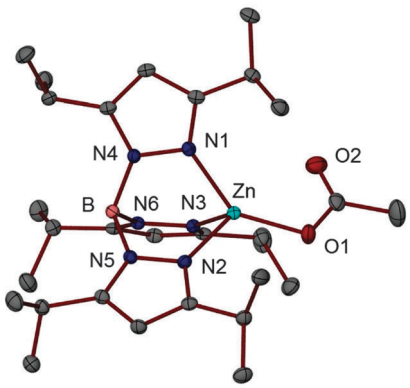
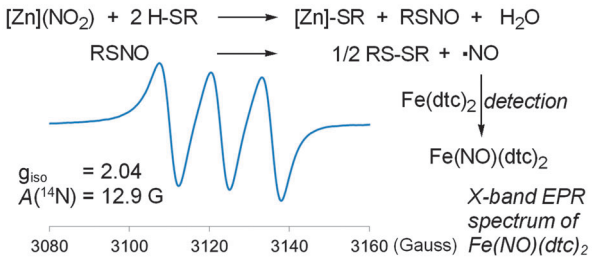


Fig. 2 X-ray structure of ^{iPr2}TpZn(OAc) (**4**).



Scheme 5

respectively)[36,38] resulted in conversion to the zinc thiolates **2** and **3** in 63% and 41% yields, respectively, along with acetic acid (Scheme 4). Some degradation of the ^{iPr2}Tp ligand to the corresponding free pyrazole was also observed, likely due to the presence of free acetic acid. We note ^{iPr2}TpZn(NO_3)[28] that bears the much less basic nitrate anion did not react with either H–SAr or H–SCH_2Ph.

RSNOs are thermally unstable towards loss of NO to give the corresponding disulfide RS–SR, especially *S*-nitrosothiols with aromatic substituents that have weak ArS–NO bonds (18–21 kcal mol^{-1}).[20] Thus, we anticipated the eventual formation of NO_{gas} in the reactions of **1** with H–SR. The headspace gas of the reaction between ^{iPr2}TpZn(NO_2) (**1**) and H–SAr in CH_2Cl_2 was collected and bubbled into a solution of the NO-trap Fe(dtc)$_2$ (dtc = bis(*N*,*N*-diethylthiocarbamate)). EPR characterization of the NO-trap solution clearly indicated the presence of Fe(NO)(dtc)$_2$,[39–41] demonstrating ultimate formation of NO_{gas} from the zinc-bound nitrite in **1** (Scheme 5).

S-Nitrosothiols result from the reaction of thiols H–SR with nitrite bound to the Lewis acidic zinc center in ^{iPr2}TpZn(NO_2). The qualitative rate for *S*-nitrosothiol and zinc thiolate formation is greatly dependent on the pK_a and size of the thiol H–SR. Observation of the zinc thiolate ^{iPr2}TpZn–SR upon addition of thiol H–SR to ^{iPr2}TpZn(OAc) (with release of HOAc) suggests that RSNO generation may occur *via* an acid–base reaction between ^{iPr2}TpZn(NO_2) (**1**) and H–SR, perhaps assisted by the thiophilicity of the zinc center. Generation of NO_{gas} occurs *via* decomposition of the resulting *S*-nitrosothiol RSNO, indicating that thiols ultimately serve as a reducing agents that enable the conversion of zinc-bound nitrite to NO. Thus, these studies suggest that zinc-based enzymes could promote NO formation from nitrite in the presence of thiols such as glutathione (0.5 mM in red blood cells)[42] *via* the intermediacy of the corresponding *S*-nitrosothiols RSNOs. In addition to serving as ready sources of NO in the biological milieu, such *S*-nitrosothiols are also involved in post-translational protein modification important in health and disease.[6]

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