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Synthesis and characterization of lithium oxonitrate (LiNO)

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ABSTRACT

The oxonitrate(1−) anion (NO^-), the one-electron reduction product of nitric oxide and conjugate base of HNO, has not been synthesized and isolated due to the inherent reactivity of this anion. The large scale synthesis and characterization of a stable NO^- salt is described here. The lithium salt of oxonitrate (LiNO) was formed by the deprotonation of N-hydroxybenzenesulfonamide with phenyllithium in aprotic, deoxygenated conditions. LiNO exhibited antiferromagnetic paramagnetism as determined by SQUID magnetometry, consistent with a triplet ground state of NO^-. LiNO reacted with HCl to yield nitrous oxide consistent with HNO formation and dimerization. LiNO consumed O_2 in a pH-dependent manner to initially produce peroxynitrite and eventually nitrite. Consistent with the reduction potential of NO, LiNO exhibited an oxidation potential of approximately +0.80 V as determined by reactions with a series of viologen electron acceptors. LiNO also reacted with ferric tetraphenylporphyrin chloride (Fe(TPP)Cl), potassium tetracyanonickelate ($K_2Ni(CN)_4$) and nitrosobenzene in a manner that is identical to other HNO/NO^- donors. We conclude that the physical and chemical characteristics of LiNO are indistinguishable from the experimentally and theoretically derived data on oxonitrate (1−) anion. The bulk synthesis and isolation of a stable $^3NO^-$ salt described here allow the chemical and physical properties of this elusive nitrogen oxide to be thoroughly studied as this once elusive nitrogen oxide is now attainable.

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1. Introduction

The inability to synthesize and isolate the oxonitrate(1−) anion (commonly referred to as nitroxyl anion; NO^-) on a large scale has rendered this enigmatic species experimentally difficult to study. The lack of knowledge concerning this species is rather surprising due to the importance of two species that are intimately related to it, nitric oxide (NO) and nitrosyl hydride (HNO) (Scheme 1). NO, the one electron oxidation product of NO^-, is a critical molecule in many biological processes [1]. HNO, the conjugate acid of NO^-, has also been the subject of recent investigation as it is potentially generated in biological environments and has numerous biochemical targets and pharmacological effects [2]. Once referred to as the "forgotten nitrogen oxide", HNO biochemistry and pharmacology are becoming an emerging field of research [3,4]. For example, HNO has been shown to inhibit thiol-dependent enzymes such as aldehyde dehydrogenase [5], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [6,7] and the DNA repair enzyme, poly(ADP-ribose) polymerase (PARP) [8]. HNO has also been shown to attenuate the N-Methyl-D-aspartate (NMDA) receptor and is proposed to be protective against neuroexcitotoxicity [9]. Administration of HNO prior to an ischemic event has been shown to protect against reperfusion toxicity [10]. One of the more provocative biochemical properties of HNO is its positive inotropic and lusitropic effects in failing hearts [11]. Due to these unique biochemical roles, HNO has been suggested to have potential for pharmacological application, especially for the treatment of heart failure [12].

HNO must be generated in situ from donor compounds, such as sodium trioxodinitrate (Angeli's salt), as HNO dimerizes to form hyponitrous acid which decomposes to water and N_2O (Reaction (1)).

$$2\ HNO \rightarrow [H_2N_2O_2] \rightarrow N_2O + H_2O \quad (1)$$

The direct chemical reduction of NO has been unsuccessfully attempted. The reaction of nitrogen monoxide (NO) with sodium was first reported in 1906 to form a white crystalline material which was reported to be sodium oxonitrate (NaNO) in 1933 [13,14]. This identification was accepted as such until 1963, when the product of the reaction of NO with Na was determined by infrared spectra to be primarily sodium *trans*-hyponitrite (NaN_2O_2), not sodium oxonitrate [15,16].

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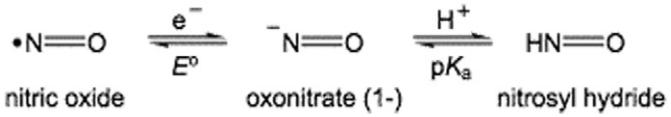

Scheme 1. Oxonitrate (1-) is closely related to nitric oxide and nitrosyl hydride.

Although sodium is thermodynamically able to reduce NO to NO^- (Reaction (2)), a secondary reaction of NO^- with excess NO occurs with a near diffusion controlled rate constant to form hyponitrite radical ($N_2O_2^-$) (Reaction (3)) [17].

$$Na + NO \rightarrow NaNO \quad (2)$$

$$NaNO + NO \rightarrow NaN_2O_2 \quad (3)$$

Therefore the large-scale synthesis of NO^- salts cannot be accomplished by the direct reduction of NO and thus NO^- salts have not yet been synthesized, except by the condensation of NO and alkali metal molecular beams at 20 K [18,19]. The inability to synthesize NO^- salts has resulted in very limited empirical data concerning the physical and chemical properties of this nitrogen oxide.

Recent investigation on the pKa of HNO and the revision of the reduction potential of NO has concluded that the chemical properties of HNO are quite different from that of its conjugate base, NO^- [20,21]. Most striking is the electronic angular momentum (spin multiplicity) of the respective ground state species, as HNO is a singlet molecule while NO^- is a triplet. This implies that the deprotonation of HNO is more complicated than for most Brönsted–Lowry acid–base systems, as it is a "spin-forbidden" process (Reaction (4)), and the rate constant for the deprotonation of HNO, and consequently the protonation of NO^-, is much slower than "spin-allowed" proton transfer reactions [21].

$$^1HNO \rightleftharpoons H^+ + {}^3NO^- \quad (4)$$

The two species are also predicted to behave very differently in regards to their respective chemical reactivity, as HNO reactivity is thought to be dominated by its electrophilic character, while NO^- is thought to be a potent one-electron reducing agent as the (NO/NO^-) couple has been determined to be −0.80 V (vs. NHE) by theoretical and experimental methods [17,20].

In order to study the chemical, biochemical and pharmacological properties of NO^-, the isolation and chemical characterization of this nitrogen oxide were undertaken. We report here the synthesis and isolation of an oxonitrate(1−) (NO^-) salt derived not from the reduction of nitric oxide, but from the deprotonation of N-hydroxybenzenesulfonamide. The salt mixture containing LiNO formed from this synthesis exhibits physical and chemical characteristics consistent with the several reported theoretical and experimental properties of NO^-. From these results, we conclude that the NO^- anion has been for the first time synthesized and isolated on a large scale and allows for the direct chemical study of this unique molecule.

2. Experimental

2.1. General considerations and reagents

All manipulations were performed under an atmosphere of purified argon using standard Schlenk and glovebox techniques unless otherwise stated and all glassware was cleaned in a KOH/EtOH bath and rinsed in 2N HCl followed by double distilled water and subsequently flame dried on a high vacuum line. Magnetic stir bars were cleaned in *aqua regia* for at least one week prior to use. Diethyl ether and THF were distilled from sodium/benzophenone ketyl onto excess sodium. N-hydroxybenzenesulfonamide was purchased from TCI (Tokyo, Japan); ^{15}N-hydroxybenzenesulfonamide was synthesized according to the literature using $^{15}NH_3OH^+$ Cl^- (99%) (Cambridge Isotope, Cambridge, MA); potassium tetracyanonickelate was synthesized according to the literature [21] and twice recrystalized from water; an oxoperoxonitrite (1−) solution was prepared according to the literature [22]; all other chemicals were purchased from Aldrich (St. Louis, MO) and used without further purification. All UV–visible spectra were recorded on a Shimadzu 2501-UVPC instrument at 298 K.

2.2. Synthesis of lithium oxonitrite (LiNO)

Approximately 10 mL of diethyl ether was vacuum transferred into a 25 mL Schlenk flask charged with N-hydroxybenzenesulfonamide (75 mg, 0.43 mmol) at 77 K. The solution was warmed to room temperature and an atmosphere of argon introduced. Another Schlenk flask containing a magnetic stir bar, 0.5 mL of 1.8 M phenyllithium (0.9 mmol) was degassed by multiple freeze–pump–thaw cycles and filled with argon. The N-hydroxybenzenesulfonamide solution was slowly transferred via cannula to the rapidly stirring phenyllithium solution under a positive pressure of argon. A white precipitate was rapidly formed and collected by Schlenk filtration, dried under high vacuum and stored under argon.

2.3. SQUID measurements

Measurements were recorded using a Quantum Designs Superconducting Quantum Interference Device (SQUID) magnetometer with MPMSR2 software (San Diego, CA, USA). Magnetic susceptibility measurements were performed at 0.150 kOe. A sample of LiNO (~10 mg) was loaded into a plastic straw and centered within the magnetometer using the DC centering scan. Data was acquired from 5 to 300 K.

2.4. Reaction of LiNO with O_2

Oxygen consumption studies were performed in a 25 mL two-neck round bottom flask fitted with a rubber septum and the electrode housed in a gastight PTFE plug. A 5 mg sample of powdered LiNO was dropped into 15 mL of a stirring air saturated aqueous solution of varying pH. The rubber septum was quickly replaced and the dissolved oxygen concentration was measured by a Clark type O_2 electrode (YSI, Yellow Springs, OH). The aqueous solutions used were pH 14 (1.0 N NaOH), pH 13 (0.1 N NaOH), pH 9 (0.1 M carbonate buffer) and pH 7 (0.1 M phosphate buffer). The UV spectra of the reaction between LiNO and O_2 were measured by dissolving LiNO (4.4 mg) in 2.2 mL of O_2-saturated 1.0 N NaOH. The solution was placed into a quartz cell and the UV spectra were immediately recorded at room temperature. The reaction spectra were referenced to a cell prepared by adding 2.0 mL of argon saturated 1.0 N NaOH via gas-tight syringe to a quartz cell fitted with a ground-glass joint and stopcock containing 4.0 mg of LiNO. An authentic oxoperoxonitrite (1−) solution was also reacted with approximately 3 mg LiNO in deoxygenated 1.0 N NaOH and the UV spectra were recorded over 30 min. $Li^{15}NO$ (6 mg) was reacted with O_2 by adding the salt into 2 mL of an O_2 saturated 2.0 N KOD solution and stirred at room temperature for 15 min. The solution was transferred to a 10 mm NMR tube with an external reference of liquid ammonia. The ^{15}N-NMR spectrum was recorded at 298 K on a Bruker 360 MHz instrument equipped with a multinuclear broadband probe.

2.5. Reaction of LiNO with HCl

The reaction of LiNO with HCl was studied in aqueous and organic solvents. Deoxygenated aqueous HCl solution (2 mL, 2.0 N) was added via syringe to 7 mg of LiNO in a 10 mL Schlenk tube and stirred for 10 min. The gaseous products were analyzed by gas chromatography

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as previously described [23]. In an analogous reaction, 2.0 N HCl in diethyl ether was degassed via multiple freeze–pump–thaw cycles and vacuum transferred onto the LiNO sample at 77 K. After warming to room temperature the reaction was stirred for 10 min under an atmosphere of argon and the products were analyzed as above.

2.6. Reaction of LiNO with viologens

The viologens, 1,1′-dimethyl-4,4′-bipyridinium (MV^{2+}), 7,8-dihydro-2,12-dimethyldipyrido[1,2-a:2′,1′-c][1,4]diazepiinedium ($V2^{2+}$) and 1′-methyl-4,4′-bipyridinium (MMV^{+}), were dissolved in 1.0 M NaOH to form a 1.4 mM solution. These solutions were then deoxygenated by purging with argon for 1 h. The reactions were initiated by adding via syringe 3 mL of the viologen solution to 20 mg of LiNO in a deoxygenated flask. The reaction was monitored in a gas-tight quartz cuvette. The one-electron reduced violgen species were confirmed by reduction with Zn(Hg).

2.7. Reaction of LiNO with Fe(TPP)Cl

In a typical experiment, 10 mL Schlenk tube was charged with a magnetic stir bar, ~1 mg meso-tetraphenylporphyrin iron(III) chloride (Fe(TPP)Cl) and excess amount of LiNO (~10 mg). To this solid mixture, approximately 10 mL of diethyl ether was vacuum transferred at 77 K from sodium/benzophenone ketyl. The flask was warmed to room temperature, filled with an atmosphere of argon and stirred for 2 h. The spectral time course of the LiNO reaction was initiated by adding 3 mL of a 5×10^{-5} M Fe(TPP)Cl solution in diethyl ether via syringe to a quartz cell fitted with a stopcock containing 3 mg of LiNO. The visible spectrum was recorded every 15 min at room temperature.

2.8. Reaction of oxonitrate salts with $K_2Ni(CN)_4$

A 122 mM solution of potassium tetracyanonickelate ($K_2Ni(CN)_4$) in 1.0 N NaOH was deoxygenated by multiple vaccum/argon fill cycles. To this solution 13 mg of LiNO or 20 mg N-hydroxymethanesulfonamide was added, and stirred overnight at room temperature and the visible spectra were recorded.

2.9. Reaction of LiNO with nitrosobenzene

Nitrosobenzene (3 mg) and excess LiNO (~10 mg) were deoxygenated by multiple vacuum/argon fill cycles and approximately 3 mL of anhydrous, degassed THF was vacuum transferred into the reaction flask at 77 K. The flask was warmed to room temperature and the reaction was stirred at room temperature for 12 h. The solvent was removed under reduced pressure to yield a yellow solid. Approximately 2N HCl was added to this yellow solid and the aqueous phase was extracted with chloroform. The crude product in $CHCl_3$ was spotted on a TLC plate along with nitrosobenzene and authentic cupferron. The mobile phase was ethyl acetate/hexanes (1:1) with 1% acetic acid and spots were visualized under UV light.

2.9.1. Copper cupferron formation from LiNO reaction with nitrosobenzene

The yellow solid product (~10 mg) was dissolved in distilled water and a concentrated $CuSO_4$ solution (>1 M) was added dropwise to form a precipitate. The precipitate was dissolved in chloroform and the visible spectrum was recorded.

3. Results and discussion

3.1. Synthesis of lithium oxonitrate

Lithium oxonitrate(1−) (LiNO) was synthesized by reacting one equivalent of N-hydroxybenzenesulfonamide with two equivalents of phenyllithium in deoxygenated, anhydrous diethyl ether at room temperature (Scheme 2). A white precipitate was rapidly formed upon the addition of the N-hydroxybenzenesulfonamide ether solution to a vigorous stirring phenyllithium solution, which was then collected by Schlenk filtration and dried in vacuo (Supplemental Fig. 1). The LiNO salts produced are not pure as the decomposition of the N-hydroxysulfonamide yields the corresponding sulfinate salt (RSO_2^-). Due to the reactivity of NO^-, separation of LiNO from the RSO_2^- byproduct was not achieved. However the RSO_2^- anion is relatively inert with respect to the chemistry of NO^- (vide infra) and does not limit the use of these salts as an NO^- source. Thus the precipitated product of the reaction is referred to as LiNO, although it should be understood that this is a mixture of salts as shown in Scheme 2.

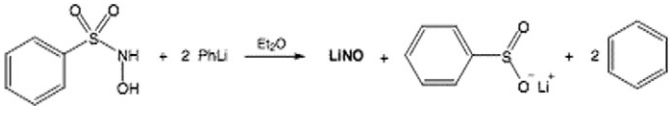
The yield of LiNO from the reaction shown in Scheme 2 is calculated from the yield of the reaction of Fe(TPP)Cl to Fe(TPP)NO (vide infra) (Reaction (5)). This reaction was chosen as the standard reference reaction used to calculate the apparent yield of LiNO, as the reaction conditions do not favor reactant or product decomposition over the time of the reaction. The yields derived from this method for a typical synthesis of LiNO is around 20%. Although this yield is relatively low, it is the total yield of two processes; the conversion of N-hydroxybenzenesulfonamide to NO^- and the reaction of NO^- with the ferric porphyrin to form the ferrous nitrosyl. Along with low yields and limited solubility, the exact amount or concentration of NO^- is a difficult, if not impossible, value to quantify. These factors make kinetic analysis of reactions with LiNO difficult and currently only appropriate for pseudo-first order type reaction conditions. Although the yields of LiNO synthesis appear to be low, this does not diminish the utility of LiNO as a source of oxonitrate(1−) anion.

$$\mathrm{Fe(TPP)Cl + LiNO \rightarrow Fe(TPP)NO + LiCl} \quad (5)$$

LiNO is very stable at room temperature under an argon atmosphere and no evidence of decomposition was observed over many months. However, upon exposure to O_2, the salts slowly turn from white to pale yellow. LiNO is not soluble in organic solvents and is only slightly soluble (and/or slowly decompose due to trace water) in polar aprotic solvents such as DMSO and DMF. LiNO is slightly soluble in basic aqueous solutions (pH > 13) and decomposes in more acidic media. Addition of Li^+ specific crown ether (12-crown-4) did not solvate LiNO into organic solvents. Also, the reaction of N-hydroxybenzenesulfonamide with 12-crown-4/phenyllithium complex did not yield LiNO. Perhaps the stability of LiNO is in part due to its insoluble nature as we propose that LiNO exists as a catenated polymeric species, as organolithium compounds are known to behave in this manner [24].

3.2. Physical characterization of LiNO

Oxonitrate(1−) is isoelectronic with O_2 and therefore is predicted to have a triplet ground state. However like O_2, both the singlet and triplet states may be accessible for NO^-. The triplet state of NO^- is ≈17 kcal/mol lower in energy than the singlet state and predicted to be a paramagnetic triplet ground state species [25]. SQUID magnetization experiments on a solid sample of LiNO measured in the temperature range 5–300 K at a field strength of 0.15 kOe show an inverse relationship between magnetic moment and temperature in good agreement with Curie–Weiss paramagnetism (Supplemental Fig. 2). The molar magnetic susceptibility, χ_M, versus temperature



Scheme 2. Synthesis of LiNO by deprotonation of N-hydroxybenzenesulfonamide.

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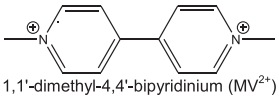
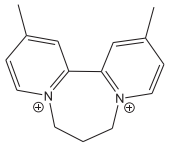
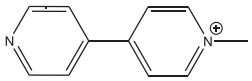
plot (Fig. 1A) shows that χ_M increases as temperature decreases until the Neel temperature is reached ($T_N = 25$ K), characteristic of antiferromagnetic interactions. The plot of $1/\chi_M$ versus T shows that LiNO exhibits a negative value Weiss constant ($\theta = -61.7$ K) also a feature of antiferromagnetism (Fig. 1B). Furthermore, the solid state EPR spectrum of LiNO clearly shows a paramagnetic species (Supplemental Fig. 3). The presence of a paramagnetic species with antiferromagnetic properties is strong evidence that $^3NO^-$ has been synthesized.

The one-electron reduction potential for the $NO/^3NO^-$ couple is −0.8 V (vs. normal hydrogen electrode (NHE)) and therefore LiNO was predicted to be an electron-donor [20]. To examine the oxidation potential of LiNO, the relative rate of viologen reduction was measured (Table 1). The reduction of MV^{2+} by LiNO was exceptionally fast while the reduction of $V2^{2+}$ was relatively slow. These data suggest that LiNO has an oxidation potential greater than +0.7 V. Furthermore LiNO did not react with MMV+ indicating that the oxidation potential of LiNO is not greater than +0.8 V. The reduction of these viologens by LiNO is similar to the results obtained from the decomposition of N-hydroxymethanesulfonamide in basic conditions, further validating $^3NO^-$ synthesis.

Table 1
Reaction of LiNO with viologen electron acceptors.

Viologen	E° (V)[a]	Reaction time[b]
 1,1'-dimethyl-4,4'-bipyridinium (MV^{2+})	−0.440	<1 s
 7,8-dihydro-2,12-dimethyldipyrido[1,2-a:2',1'-c][1,4]diazepiinedium ($V2^{2+}$)	−0.690	~50 min
 1'-methyl-4,4'-bipyridinium (MMV^+)	−0.805[c]	*No reaction*

[a] Reduction potentials are relative to NHE, pH 7.
[b] The reaction consisted of the addition of 3 mL of 1.4 mM viologen in 1.0 M NaOH to 20 mg LiNO.
[c] The reduction potential of MMV+ in 1.0 M NaOH is more negative than −0.805 V due to the unprotonated pyridyl lone pair.

3.3. Chemical characterization of LiNO

3.3.1. Reactivity with H^+ and O_2

HNO is not stable with respect to dimerization and decomposition to nitrous oxide (N_2O) and water (Reaction (1)). We predicted that protonation of the NO^- anion would result in the formation of N_2O. The addition of HCl, dissolved in either water or diethyl ether, to a solid sample of LiNO resulted in the generation of N_2O as detected by gas chromatography. N_2O is also detected upon dissolving LiNO in deoxygenated pH 7 phosphate buffer. The yields of N_2O generation from the reaction of excess HCl with LiNO are typically ca. 20% in agreement with the yields described above. Furthermore, the ^{1}H-NMR of LiNO dissolved in D_2O was identical to benzenesulfinate as predicted in Scheme 2 (data not shown). The significant production of N_2O from the protonation of LiNO and the presence of benzenesulfinate strongly supports the claim that NO^- has been isolated as a component of the salt mixture.

Triplet NO^- reacts with O_2 with a second-order rate constant on the order of 10^9 $M^{-1}\cdot s^{-1}$ to yield oxoperoxonitrate(1−) (Reaction (6)) [17].

$$^3NO^- + {}^3O_2 \rightarrow {}^1ONOO^- \quad (6)$$

Addition of LiNO to 1.0 N NaOH caused the solution to become yellow in the presence of O_2, suggesting the formation of $ONOO^-$ (Reaction (6)).

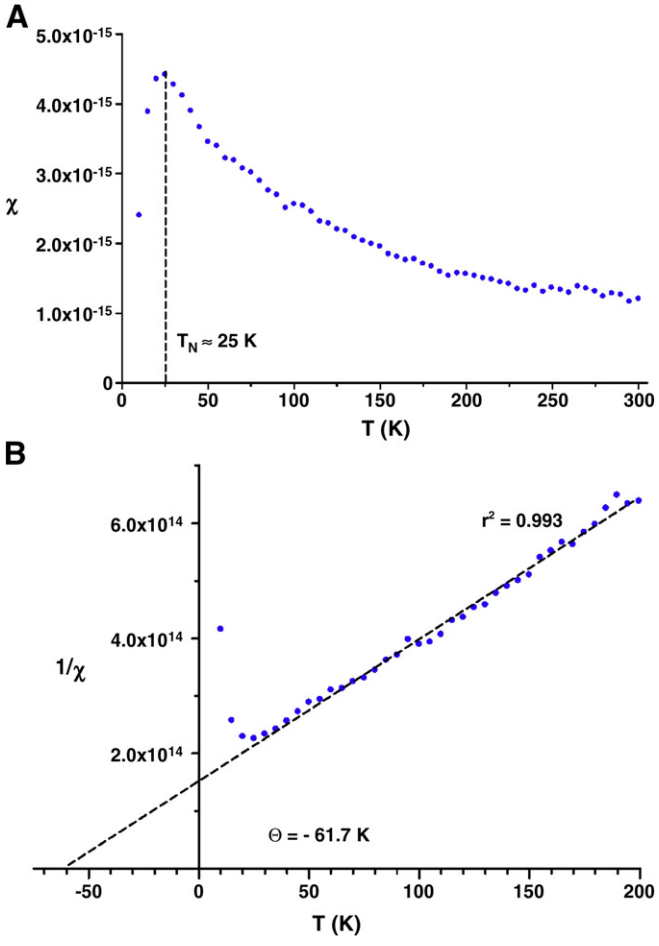


Fig. 1. Magnetic susceptibility of LiNO. LiNO molar magnetic susceptibility (A) and inverse molar magnetic susceptibility (B) versus temperature. SQUID measurements were recorded from 5 to 300 K at 0.15 kOe.

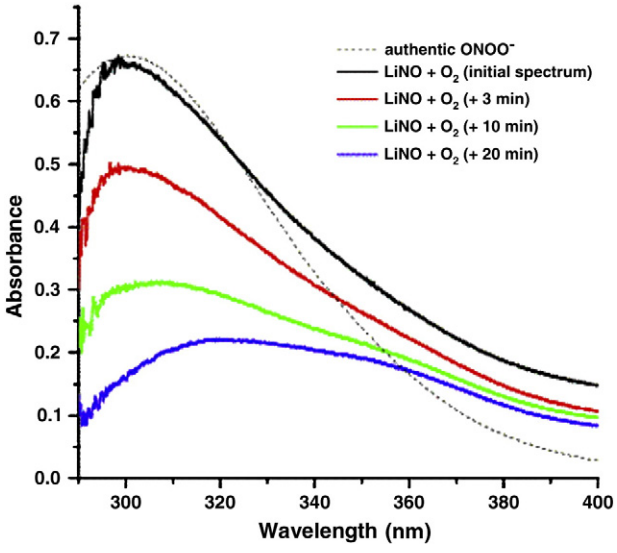


Fig. 2. Reaction of LiNO with O_2. UV–visible spectra of LiNO reacting with O_2 at indicated times with the absorption peak decreasing with time. A spectrum of authentic ONOO− is shown for reference.

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The initial UV spectrum was similar to authentic $ONOO^-$ (Fig. 2), however this spectrum decreased within minutes, indicating that $ONOO^-$ is being consumed. A similar decrease in absorption was observed upon the reaction of authentic $ONOO^-$ with LiNO, suggesting that $ONOO^-$ reacts with NO^- to yield two equivalents of nitrite (NO_2^-) (Reaction (7)).

$$ONOO^- + NO^- \rightarrow 2NO_2^- \quad (7)$$

The reaction of $ONOO^-$ with NO^- has not yet been examined, however the two reactants are thermodynamically poised to conproportionate to nitrite. This is supported by ^{15}N-NMR experiments using $Li^{15}NO$ (Supplemental Fig. 4). Reaction of O_2 with $Li^{15}NO$ in 1.0 N KOD forms NO_2^- by ^{15}N-NMR, while NO_3^- was never observed. The consumption of O_2 by LiNO is strong evidence that $^3NO^-$ has been isolated. Furthermore, the formation of $ONOO^-$, albeit transient, and the eventual production of nitrite can be explained by the initial reaction of O_2 with $^3NO^-$.

Upon the addition of a solid sample of LiNO to an air saturated pH 14 aqueous solution, O_2 consumption was measured with a Clark-type oxygen electrode. The rate consumption of O_2 by LiNO was predicted to be highly pH-dependent. As the pH of the reaction is lowered from 14, the rate of O_2 consumed decreased (Fig. 3). Based on the pH profile of O_2 consumption, it appears that the pKa of the reactive species in LiNO is between 13 and 14, and is further evidence that the reactive species is $^3NO^-$, which has a pKa of 11.6 (+/-3.4) [20].

3.3.2. Tetracyanonickelate(2 −)

A diagnostic reaction of HNO is the formation of tricyanonitrosylnickelate (2−) [$Ni(CN)_3NO^{2-}$] from tetracyanonickelate(2−) [$Ni(CN)_4^{2-}$] (Reaction (8)) [26,27].

$$HNO + Ni(CN)_4^{2-} \rightarrow Ni(CN)_3NO^{2-} + H^+ + CN^- \quad (8)$$

This reaction is unique for HNO/NO^-, as the metal is formally reduced by two electrons ($Ni^{2+} \rightarrow Ni^0$) while the nitrogen oxide is formally oxidized by two electrons ($NO^- \rightarrow NO^+$). Therefore formation of $Ni(CN)_3NO^{2-}$ from the reaction of LiNO with $Ni(CN)_4^{2-}$ would further confirm the existence of NO^- as a component of LiNO. LiNO reacted with $K_2Ni(CN)_4$ in either 1.0 N NaOH or DMF to yield $Ni(CN)_3NO^{2-}$ ($\lambda_{max} = 498$ nm) as observed by visible spectroscopy (Supplemental Fig. 5). The product of these reactions are identical to the complex formed from the reaction of $K_2Ni(CN)_4$ with N-hydroxymethanesulfonamide in 0.1 N NaOH, indicating that NO^- is present in the LiNO salt mixture.

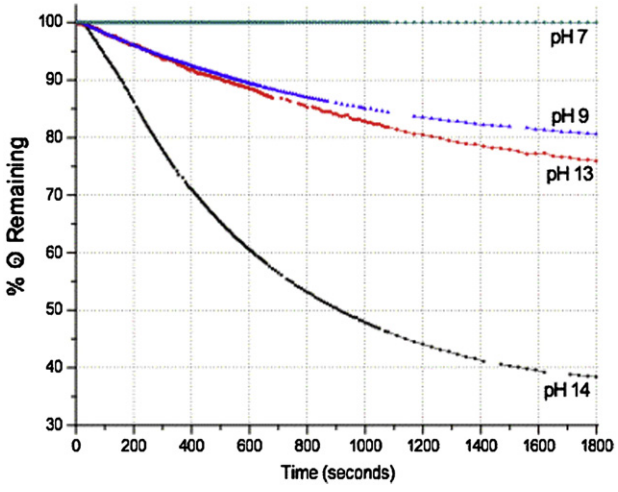

Fig. 3. LiNO reacts with O^2 in a pH-dependent manner. The rate of O^2 consumption by LiNO increases with pH, consistent with the reported pKa of NO^- (11.6±3.4). Data shown are from a representative experiment.

3.3.3. Ferric porphyrin complexes

HNO reacts with ferric hemoproteins [28] and ferric porphyrins [29] in a one-step reductive nitrosylation reaction to give the corresponding ferrous nitrosyl complex (Reaction (9)).

$$(porphyrin)Fe^{3+} + HNO \rightarrow (porphyrin)Fe^{2+}\text{–}NO + H^+ \quad (9)$$

To validate the existence of NO^- in the LiNO salt mixture, *meso*-tetraphenylporphyrin iron(III) chloride, Fe(TPP)Cl, was reacted with LiNO in argon saturated, anhydrous diethyl ether to produce the predicted ferrous nitrosyl complex, Fe(TPP)NO. The reaction was monitored by UV–visible spectroscopy (Fig. 4) and reveals two pairs of isosbestic points (522/495 and 520/492 nm), indicating a two-step mechanism to the final observed product Fe(TPP)NO. A potential mechanism for this reaction is initial reduction of the ferric complex followed by coordination of NO to form the final product. To examine this possible mechanism, LiNO and Fe(TPP)Cl were reacted in anhydrous pyridine. The bispyridine ferrous complex, $Fe(TPP)(py)_2$, was formed (data not shown), indicating that the initial step is reduction of the ferric complex followed by the coordination of the solvent. Similarly, LiNO reacted with Fe(TPP)Cl in the presence of 1 atmosphere CO yielded the ferrous carbonyl porphyrin, not the nitrosyl product. This proposed mechanism is not consistent with the one-step reductive nitrosylation reported for the reactions of HNO with ferric porphyrin systems and can be rationalized by the ground electronic states of ^1HNO versus $^3NO^-$.

LiNO also reacts with metmyoglobin in pH 10 carbonate buffer to yield ferrous nitrosyl myoglobin, indicating that LiNO can serve as an HNO donor for biochemical studies. In addition to the exploration of the unique chemistry of NO^-, the results presented here have biological implication as HNO can be generated without the use of donor compounds. The emerging field of HNO biochemistry and pharmacology relies on donor compounds that release HNO at physiological pH. However an obstacle often encountered when using a donor compound is deciphering which species (HNO, the donor molecule itself or another nitrogen oxide) is responsible for the biological activity observed. An additional potential bioactive molecule produced from the decomposition of using Angeli's salt is nitrite, which can interfere with the interpretation of data as nitrite has biological effects [30]. The use of LiNO as an HNO source can be used as an independent verification of chemical or biological activity attributed to HNO. Another advantage to using LiNO as an HNO source is the lack of a slow

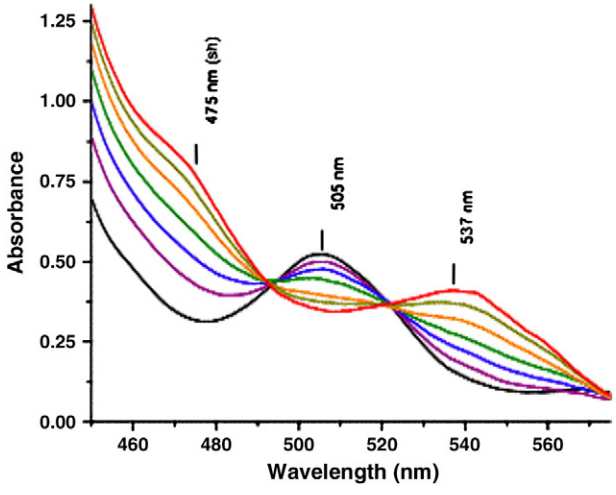

Fig. 4. LiNO reacts with Fe(TPP)Cl. Visible spectra of Fe(TPP)Cl reacting with LiNO in diethyl ether. The initial spectra (black, 505 nm peak) converts to Fe(TPP)NO (red, 537 nm peak and 475 nm shoulder) with 2 sets of isosbestic points (522/495 and 520/492 nm). Spectra were recorded every 10 min at room temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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half-life, or HNO release rate, associated with all donor compounds. As the conjugate base, the rate controlling step to HNO production from LiNO is the protonation reaction.

3.3.4. Nitrosobenzene

HNO, derived from either Angeli's salt or N-hydroxymethanesulfonamide, reacts with nitrosobenzene to form cupferron (Reaction (10)) [31].

$$Ph{-}NO + HNO \rightarrow Ph{-}N(NO)O^- + H^+ \quad (10)$$

The analogous reaction of nitrosobenzene with LiNO would further confirm the presence of NO^-. Nitrosobenzene in anhydrous THF was reacted with LiNO at room temperature for 12 h. The solvent was removed under reduced pressure to yield a yellow solid. The yellow solid was then treated with 2N HCl and was extracted with $CHCl_3$. An authentic sample of cupferron was treated in an identical fashion to verify suitable extraction. The crude product and authentic cupferron acids were analyzed by thin-layer chromatography. The reaction product had the same R_f as the authentic cupferron, confirming the presence of cupferron as a reaction product (data not shown). To further verify the production of cupferron from the reaction of LiNO and nitrosobenzene, the yellow crude product was dissolved in water and a precipitate was formed upon the addition of a concentrated cupric solution. This precipitate was filtered and dissolved in $CHCl_3$. The resulting UV–visible spectrum of the reaction product reveals that the Cu^{2+}–cupferron complex was formed from the reaction of LiNO with nitrosobenzene (Supplemental Fig. 6), further supporting the existence of NO^- as a component of the LiNO salt mixture.

4. Conclusion

The existence of oxonitrate(1−) anion ($^3NO^-$) in the salt mixture referred to as LiNO is supported by the results described here. Within the LiNO salt mixture is a species that 1) consumes O_2, 2) exhibits paramagnetic properties, 3) the paramagnetic properties are abolished in the presence of protons or O_2, and 4) N_2O is formed upon the addition of protons. This last property is not unique to oxonitrate, as $N_2O_2^{2-}$ also reacts with protons to yield N_2O, however $N_2O_2^{2-}$ is diamagnetic and is stable in air. The only plausible species that is explained by the above criteria is oxonitrate(1−). In addition, LiNO described here reacts identically as known HNO/NO^- donor compounds (Angeli's salts and Piloty's acid) in a number of established reactions. The combined results of the reactions studied strongly conclude that $^3NO^-$ has been rationally synthesized as the lithium salt. The synthesis of LiNO described here is a significant departure for the study of this diatomic anion as previous studies of this elusive molecule have been limited to theoretical calculations, low temperature inert gas matrices, and pulse radiolysis. The synthesis of stable salts of $^3NO^-$ detailed here allows for further study of this unique molecule. The synthesis of LiNO, however useful, is not without its problems. One of the major drawbacks is the presence of other salts in the product mixture. A method for purification of LiNO from the other salt products has not yet been developed, as the main obstacle to purification is that $^3NO^-$ is likely to decompose or react under separation and/or isolation conditions. The presence of other salts in the product mixture does not prohibit its use as a source of oxonitrate(1−) as the other salts, namely benzenesulfinate, do not alter the chemistry of $^3NO^-$.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jinorgbio.2012.09.022.

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