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Acid-induced nitrite reduction of nonheme iron(II)nitrite: mimicking biological Fe–NiR reactions†

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Nitrite reductase (NiR) catalyzes nitrite (NO₂⁻) to nitric oxide (NO) transformation in the presence of an acid (H⁺ ions/pH) and serves as a critical step in NO biosynthesis. In addition to the NiR enzyme, NO synthases (NOSs) participate in NO production. The chemistry involved in the catalytic reduction of NO₂⁻, in the presence of H⁺, generates NO with a H₂O molecule utilizing two H⁺ + one electron from cytochromes and is believed to be affected by the pH. Here, to understand the effect of H⁺ ions on NO₂⁻ reduction, we report the acid-induced NO₂⁻ reduction chemistry of a nonheme Fe^{II}-nitrito complex, [(12TMC) Fe^{II}(NO₂⁻)]⁺ (Fe^{II}-NO₂⁻, **2**), with variable amounts of H⁺. Fe^{II}-NO₂⁻ upon reaction with one-equiv. of acid (H⁺) generates [(12TMC)Fe(NO)]²⁺, {FeNO}⁷ (**3**) with H₂O₂ rather than H₂O. However, the amount of H₂O₂ decreases with increasing equivalents of H⁺ and entirely disappears when H⁺ reaches \cong two-equiv. and shows H₂O formation. Furthermore, we have spectroscopically characterized and followed the formation of H₂O₂ (H⁺ = one-equiv.) and H₂O (H⁺ \cong two-equiv.) and explained why bio-driven NiR reactions end with NO and H₂O. Mechanistic investigations, using ¹⁵N-labeled-¹⁵NO₂⁻ and ²H-labeled-CF₃SO₃D (D⁺ source), revealed that the N atom in the {Fe^{14/15}NO}⁷ is derived from the NO₂⁻ ligand and the H atom in H₂O or H₂O₂ is derived from the H⁺ source, respectively.

Nitric oxide (NO), a critical biological component, participates in numerous bio-physiological processes such as neurotransmission, vascular regulation, inhibiting platelet aggregation, and immune response to multiple infections at nanomolar concentration.¹ Also, NO is known to be involved in plant growth and development.² NO meagerness may cause pathogenic effects such as atherosclerosis, diabetic hypertension, *etc.*³ However, at micromolar concentrations, NO is highly toxic and utilized for immune defense against harmful pathogens,⁴ in addition to its oxidized species, *i.e.*, peroxynitrite (ONOO⁻)⁵ or/nitrogen dioxide (`NO₂).⁶ In contrast to the immune response towards pathogens, oxidized NO species also show various toxicological actions in biological systems.^{56,7}

Hence, sensible production of NO is required to maintain physiological homeostasis and is usually achieved by two metalloenzymes, *i.e.*, NO synthases (NOSs)⁸ and/or nitrite reductases (NiRs).^{8a,9} NOS enzymes are heme-proteins that generate NO by catalyzing the conversion of L-arginine to L-citrulline under aerobic conditions.^{8b,c} However, under ischemia and hypoxic conditions, the suppression of NOS activity results in the decrease of NO generation. Under such conditions, NO_2^- works as an active NO source in biological systems, generating NO in acid-induced NO2reduction reactions.¹⁰ Sometimes, under abnormal conditions, biochemical dysfunction may cause NO overproduction by NiRs or NOSs. Under such conditions, NO dioxygenase (NOD) enzymes available in vivo convert excess NO to biologically benign nitrate (NO₃⁻).¹¹ NO₃⁻, the product of the NOD reaction, serves as a critical component of NO₂⁻ generation and a precursor to the biological NO cycle.12 In humans, commensal bacteria in the oral cavity play a vital role in converting NO_3^- to $NO_2^{-.12a}$ Bacteria reduce NO_3^- to NO_2^- via an OAT reaction mediated by molybdenum-based NR enzymes.13 The interconversion of NO3 to/NO₂^{-/to} NO (or vice versa) is the critical step of the denitrification process.¹⁴ In vivo studies have proven that NO_2^{-} is a fundamental source of NO in mammalian or bacterial systems, an intermediate species of the biological nitrogen cycle (NO₃⁻ \rightarrow $NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ ¹⁴ At the bio-physiological level, $NO_2^$ gets reduced to NO, primarily by globins¹⁵ or by acid-catalyzed NO2⁻ reduction in the stomach^{16,17} or by Fe/Cu-NiR enzymes/ cytochrome c oxidase (CcO)/xanthine oxidase,18 which reduces NO_2^- to NO in the presence of two-equiv. of H⁺ ions, *i.e.*,^{8a,9,15}

$$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O \tag{1}$$



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However, few functional mimicking models were developed and investigated in vivo/in vitro to explore the mechanistic insight of microbial NiR enzymatic chemistry. Brooks and coworkers proposed the NiR activity of mammalian hemoglobin (Hb) protein under anaerobic conditions, which converts NO₂⁻ to NO with the formation of metHb.¹⁹ E. T. Papish et al. explored the Cu-NiR chemistry and showed NO production with H₂O as a side product *via* a Cu^{I} -NO⁺ \leftrightarrow Cu^{II} -NO intermediate in the reaction of Cu-NO2⁻ with two-equiv. of H⁺.²⁰ A heme-Fe/Cu assembly model has been developed to mimic the cytochrome c oxidase, illustrating the reversible conversion of NO₂⁻ to NO.²¹ For the first time, Murphy and coworkers explored the explained Cu-NO₂⁻ reduction reaction to release NO via the {CuNO} intermediate and characterized it structurally.9b,22 Patra and coworkers have mimicked NO2⁻ reduction reactivity using $Cu^{II}-NO_2^{-}$ with two-equiv. of H⁺ and one e⁻, leading to NO and H₂O molecule formation.²³ Lehnert and coworkers have explored electrocatalytic reduction using Cu^{II}-NO₂⁻ producing NO in aqueous media²⁴ and compiled the electronic structure and reactivity of the biologically relevant coordination chemistry of iron and NO.15 In addition to acid-encouraged NO₂⁻ reduction to NO in different model systems. Various models have been explored for the reduction of metal-bound NO_2^- to NO via (i) oxygen atom transfer (OAT) caused by (R₂S)²⁵/thiol (RSH)}^{18a,26}/triphenylphosphine (PPh₃)²⁷/vanadium chloride (VCl₃)²⁸ and (ii) photo-induced reactions²⁹ of metalbound nitrite (M–NO₂⁻).

In addition to the developments on biomimetic synthetic modeling of M-NOs/or active sites associated with NiR and/or NOS.11d,30 Recently, Lehnert and coworkers have established the synthetic strategy for Fe-NOs,31 and Nam and coworkers have explored the photo-induced NiR reactivity of Fe-NO2⁻ to generate Fe-NOs and also stabilize Co-NOs.32 Ford and coworkers continuously discover the NiR chemistry of various heme systems.33 Although NiR is the key source of NO in the biological system, such reactions are not investigated extensively to characterize the intermediates and transition states of NO₂⁻ reduction reactions. Hence, several research groups are working to understand the proper NO2⁻ reduction reaction mechanism. There are only very few reports on acid-induced NO₂⁻ reduction reactions to mimic NiR enzymatic reactions and understand the mechanistic aspects.9b,29,34 In this investigation, we intend to characterize different intermediates of H⁺induced NO₂⁻ reduction in Fe^{II}-NO₂⁻ and its reaction products and then explore its mechanistic aspects. This report will focus on how different amounts of acid (H⁺ ion) affect the reaction mechanism and regulate the side products in addition to NO.

Herein, we report the NO₂⁻ reduction chemistry of a nonheme FeII–NO₂⁻ complex, $[(12TMC)Fe^{II}(NO_2^{-})]^+$ (2), bearing the 1,4,7,10-tetramethyl-1,4,7,10-tetraazacyclododecane (12TMC) ligand (Scheme 1, reaction I). Complex 2 reacts with one-equiv. of triflic acid (HSO3CF3, H⁺ source) and generates the corresponding nonheme Fe–nitrosyl complex, [(12TMC)Fe(NO)]²⁺({FeNO}, 3), and H₂O₂ (Scheme 1, reactions II & III) in CH3CN at 233 K. However, upon reaction with a base (OH⁻), 2 does not form 3. Mechanistic investigations using ¹⁵N-labeled-¹⁵ NO₂⁻ demonstrated explicitly that the N atom in the NO moiety of 3 is derived from the NO₂⁻ anion and H₂O₂ by the protonation of the O atom of the NO₂⁻ moiety. Conversely, an increased H⁺ concentration showed a significant fall in H₂O₂, which disappeared completely when the H⁺ ion quantity was \cong two-equiv. with the simultaneous formation of a substantial amount of H₂O (Scheme 1, reaction IV). To the extent of our knowledge, the present work reports the very first comparative study for the reaction of an Fe^{II}–NO₂⁻ complex with varying H⁺ concentrations and the evidence showing the formation of H₂O₂ (one-equiv. of H⁺) and H₂O (\cong two-equiv. of H⁺), illustrating a new approach for NiR enzyme activity (Scheme 1).

Results and discussion

Preparation of the Fe^{II}-nitrito complex, [(12-TMC)Fe^{II}(NO₂⁻)]⁺ (2)

The initial FeII-NO₂⁻ complex, $[(12TMC)Fe^{II}(NO_2^{-})]^+$ (2), was prepared by the addition of one equivalent of NaNO₂ in the presence of a 15-crown-5 to Fe^{II}-complex, [(12TMC) $Fe^{II}(NCCH_3)^{2+}$ (1), in CH3CN at 298 K (Scheme 1 & reaction I; also see the ESI and Experimental section (ES)). Complex 2 was further characterized by various spectroscopic measurements, including the determination of the single-crystal X-ray structure. A UV-vis absorption band ($\lambda_{max} = 325 \text{ nm}$ and $\varepsilon = 356 \text{ M}^{-1}$ cm^{-1}) was formed upon adding an equivalent amount of NaNO₂ to the CH₃CN solution of 1, which corresponds to 2 (Fig. 1a). A characteristic peak for metal-bound NO₂⁻ stretching at 1270 cm^{-1} was observed in the FT-IR spectrum of 2, which shifted to 1247 cm⁻¹ when 2 was prepared using ¹⁵N-labeled-nitrite (¹⁵N¹⁶O₂⁻) (inset: Fig. 1a andS1; ESI[†]).³⁵ The electrospray ionization mass spectrum (ESI-MS) recorded for 2 showed a prominent ion peak at m/z 330.1, which shifted to m/z 331.1 when prepared with ¹⁵N-labeled Na¹⁵N¹⁶O₂, and their mass and isotope distribution pattern corresponds to [(12TMC) $Fe(NO_2^{-})^+$ (calc. m/z 330.1) and $[(12TMC)Fe(^{15}NO_2^{-})]^+$ (calc. m/z331.1), respectively (Fig. 1b and S2; ESI[†]). The ¹H-NMR spectrum of 2 showed fairly clean paramagnetic proton signals for the protons of the 12TMC ligand (Fig. S3a[†]), suggesting a magnetically active Fe center. The spin-state of the Fe center in 2 was determined by calculating the magnetic moment of the Fe^{II} center by Evans' method and found to be 5.19 BM, suggesting a high spin Fe^{II} ion (S = 2) in complex 2 (ESI,† ES, Fig. S3b). The electrochemical measurement of 2 showed





Fig. 1 (a) UV-visible spectra of 1 (0.50 mM, black line) and 2 (0.50 mM, red line) in CH₃CN under Ar at 298 K. Inset: IR spectra of $2^{-14}NO_2^{-}$ (red line) and $2^{-15}NO_2^{-}$ (blue line) in KBr. (b) ESI-MS spectra of 2. The peak at 330.1 is assigned to [(12TMC)Fe^{II}(NO₂⁻)]⁺ (calcd *m/z* 330.1). Inset: isotopic distribution pattern for $2^{-14}NO_2^{-}$ (red line) and $2^{-15}NO_2^{-}$ (blue line).

a reversible cyclic voltammogram (redox potential + 0.56 V vs. Ag/AgNO₃⁻) (ESI, Fig. S4a†). In addition to the above spectral measurements, the structural details of 2 were obtained by its single-crystal X-ray structure determination (Fig. 2). The Fe^{II} center of 2 was found to have O, O[/]-chelated bi-dentate NO₂⁻ anions in a distorted octahedral geometry (ESI,† ES, Fig. S5 and Tables 1 & 2).

The nitrite reduction reaction of the $Fe^{II}-NO_2^-$ complex (2)

To further investigate the NO₂⁻ reduction chemistry of FeII-NO₂⁻ (2), we explored its reaction with different equivalents of acid (H⁺ ions). When 2 was reacted with H+, we observed a visible color change from yellow to green and a new absorption band ($\lambda_{max} = 350$ nm and $\varepsilon = 1450$ M⁻¹ cm⁻¹), characteristic of a new species (3), formed over ~2 minutes in CH3CN under Ar at 233 K (Fig. 3a; ESI,† ES, and Fig. S6).^{8a,9} Complex 2 was found to be very stable in CH₃CN and at 298 K as it did not



Fig. 2 Displacement ellipsoid plot (15% probability) of 2 at 100 K. Disorder C atoms of TMC, anions and H atoms have been removed for clarity.



Fig. 3 (a) UV-visible spectral changes of 2 (0.50 mM, black line) upon addition of H⁺ (one-equiv.) in CH₃CN at 233 K. Black line (2) changed to a red line (3) upon addition of H⁺. Inset: IR spectra 3-¹⁴NO (red line) and 3-¹⁵NO (blue line) in KBr. (b) ESI-MS spectra of 3. The peak at 463.1 is assigned to [(12TMC)FeII(NO)(OTf)]+ (calcd *m/z* 463.1). Inset: isotopic distribution pattern for 3-14NO (red line) and 3-15NO (blue line). (c) Time-dependent EPR spectra of the generation of 3 (red line) in the reaction of 2 and H⁺ (one-equiv.) in CH₃CN at 77 K.

show any spectral variations in the absence of H^+ (ESI,† Fig. S7a). Complex 2 was also found inert towards OH⁻ as it does not indicate any change in UV-vis spectra when treated with tetrabutylammonium hydroxide (ESI, Fig. S7b†), suggesting that Fe- NO_2^- reacts only with H⁺. The amount of H⁺ required to reduce the NO₂⁻ moiety was determined by spectral titration, which confirmed the ratio-metric equivalent of 2 with H^+ as 1 : 1 (ESI,† Fig. S8). The compound 3, obtained in the reaction of 2 with H+ was determined to be an Fe-nitrosyl complex, {FeNO}⁷, based on various spectroscopic characterization techniques (vide infra). The other product of the NO₂⁻ reduction using oneequiv. of H^+ was determined to be H_2O_2 , in contrast to previous reports on biological NiR and NO2⁻ reduction chemistry, via a proposed thermally unstable ONOH intermediate as reported in the literature (Scheme 1, reactions II & III).36 However, when reacted with more than one-equiv. of H^+ (\cong two) 2 generated 3, but the amount of H2O2 decreased gradually with

increasing H^+ . This suggests the decomposition of H_2O_2 or utterly new chemistry in the presence of more than one-equiv. of H^+ ; the new product was confirmed to be H_2O by using various spectral measurements (Scheme 1, reaction IV). To the best of our knowledge, this work reports the first-ever study where the side products of NO_2^- reduction are regulated by different amounts of H^+ , which opens a new pathway of acidinduced NO_2^- reduction chemistry to the scientific community.

We have performed various spectral measurements to track the products of H⁺ (or D⁺)-induced reduction of Fe-bound ^{14/} $^{15}NO_2^{-1}$ in 2. The FT-IR spectrum of 3 showed a characteristic peak for Fe-bound nitrosyl stretching at 1783 cm⁻¹ ({Fe¹⁴NO}⁷), which shifted to 1755 cm⁻¹ ({Fe¹⁵NO}⁷) when 3 was prepared by the reaction of ¹⁵N-labeled-nitrite (2-¹⁵NO₂⁻) with one-equiv. of H⁺ (inset, Fig. 3a and S9; ESI[†]). This shifting in NO stretching frequency ($\Delta = 28 \text{ cm}^{-1}$) indicates that the N atom in NO moiety is derived from the ${}^{14/15}NO_2^{-1}$ ligand of 2. Similarly, the ESI-MS spectrum of 3 showed a prominent peak at m/z 463.1, [(12TMC) $Fe(NO)(OTf)^{+}$ (calc. *m*/*z* 463.1), which shifted to 464.1, [(12TMC) $Fe(^{15}NO)(OTf)]^+$ (calc. *m/z* 464.1), when $Fe^{II}-^{15}NO_2^-$ was reacted with H⁺ (Fig. 3b and S10; ESI[†]), specifying clearly that NO in 3 is derived from the NO₂⁻ moiety. The ¹H-NMR spectrum of 3 showed shifting in the ¹H-signals of the 12TMC ligand framework suggesting a paramagnetic system (ESI, Fig. S11a[†]).^{32a,b} We determined the spin-state of the Fe centre in 3 by calculating its magnetic moment using Evans' method and found it to be 2.3 BM, suggesting a low-spin Fe center in 3 (S = 1/2) for the complex 3 (ESI, ES, and Fig. S11b[†]).³⁷ Additionally, timedependent EPR measurements were followed for the generation of 3 in the reaction mixture of $2 + H^+$. EPR measurements (77 K), performed at different time intervals, showed the formation of a new species (g = 2.04) (Fig. 3c), which is characteristic of the EPR signal of isolated species {FeNO}⁷ (Scheme 1, reaction V), confirming the formation of low-spin 3 in the above reaction (ESI, Fig. S11c⁺). The electrochemical measurement of 3 showed a reversible cyclic voltammogram (redox potential + 0.36 V vs. Ag/AgNO₃⁻) (ESI, Fig. S4b⁺). Additionally, we have determined the binding constants $K_{\rm b}({\rm Fe}^{\rm II}-{\rm NO_2}^{-})$ and $K_{\rm b}{\rm \{Fe(NO)\}}^7$ using the Benesi-Hildebrand equation^{28,38} for the generation of 2 and 3 in the reaction of [(12TMC)Fe^{II}(CH₃CN)]²⁺ with NO₂⁻ and NO. The values were $K_{\rm b}({\rm Fe^{II}-NO_2}^-) = 4.7 \times 10^2$ $M^{-1} \& K_{b} \{ (FeNO) \} = 8.4 \times 10^{2} M^{-1} (ESI, \dagger ES, and Fig. S12),$ which also supports the forward reaction. In addition, the yield for the formation of 3 was calculated by comparing the UV-vis absorption spectra of 3 formed in the reaction of 2 with oneequiv. of H^+ with the authentic Fe-nitrosyl complex ({FeNO}⁷), prepared in a separate reaction of [(12TMC)Fe^{II}(CH₃CN)]²⁺ + NO, and was found to be 95%. However, the yield decreased to 85% when the reaction was carried out using two-equiv of H⁺ (ESI, ES, and Fig. S13[†]). Furthermore, the structural details of 3, obtained in the reaction of 2 and H⁺, were obtained by its singlecrystal X-ray structure determination (ESI, ES & Fig. 4). The NO moiety showed the coordination via the N atom to the Fe center of 3 with the a angle of 168° (ESI, ES Fig. S14;† & Tables T1 and T2). This arrangement suggests a neutral 'NO moiety with an Fe^{II} center^{37,39} (further supported by BVS calculation from the crystal parameters of 3, ESI,† ES) and can be formulated as



Fig. 4 Displacement ellipsoid plot (50% probability) of **3** at 100 K. Disordered C atoms of TMC, anions and H atoms have been removed for clarity.

 $[(12TMC)Fe^{II}(NO)]^{2+}$; however, in this manuscript, we used Enemark–Feltham notation for 3 ({FeNO}⁷).

Mechanistic investigation of NO₂⁻ reduction

In vivo and in vitro biomimetic studies on acid-induced NO₂reduction reactions produce NO with H₂O (as explained in the biological NiR chemistry)34a,40 or in some cases 'OH (H2O2)36 or a metal hydroxide⁴¹ and should be accomplished via the proposed ONOH intermediate, as reported by Murphy et al.,9b and Rose et al.^{40g} for the biological Cu-NiR chemistry. Similarly, Fujii and coworkers also proposed a Cu(ONOH) intermediate in the acid-induced biomimetic NO2⁻ reduction on the Cu^I center.^{40h} Meanwhile, Shigeta et al.40i & Chen and coworkers40j theoretically established the presence of a Cu(ONOH) intermediate in acid-induced NO₂⁻ reduction. The present work elucidated how varying equivalents of H⁺ ions determine the side products of Fe^{II}bound NO₂⁻ reduction chemistry in addition to NO and should be accomplished by a similar proposed ONOH intermediate. In this regard, we proposed the reaction sequences, where the preliminary step of the NO2⁻ reduction reaction consists of an electrophilic addition of H⁺ to the NO₂⁻ anion of 2 and generating the suggested [Fe-ONOH]²⁺ intermediate species (Scheme 2, reaction I), as proposed previously.^{36,41} The presumed [Fe-(ONOH)]²⁺ intermediate is believed to produce {FeNO}⁷ via the homolytic cleavage of the ON-OH moiety, as reported in NO2⁻ reduction on



the Fe^{II} center and Cu-NiR,^{36,41} and 'OH ($^{1}/_{2}$ H₂O₂)⁴² (Scheme 2, reaction II). In contrast, NO₂⁻ reduction in the presence of \approx two-equiv. or more H⁺ produced 3 with H₂O as a side product in a multiple-step reaction (Scheme 2, reaction III). This reaction is believed to occur *via* the reduction of the NO₂⁻ anion of 2 in the presence of two H⁺, as reported in biological NiR^{9b,40g} and biomimetic NO₂⁻ reduction^{40h-j,43} reactions. The H₂O molecule may be generated either by (a) step-wise protonation of NO₂⁻ species of 2 as observed in biology,^{40a,b} (b) acidic decomposition of H₂O₂,⁴⁴ or (c) by auto-decomposition of H₂O₂.⁴⁵

To validate our proposed H⁺-induced NO₂⁻ reduction chemistry mechanism, we have reacted 2 with different equivalents of H⁺ and characterized all the products formed in the reaction mixture. In both the acid-induced reactions, we observed the formation of 3. However, the side product of NO₂⁻ reduction changed to H₂O instead of H₂O₂ when the H⁺ amount was \geq two-equiv. (ESI,[†] ES). H₂O₂/& H₂O formed in the NO₂⁻ reduction reaction was followed/characterized and quantified using ¹H-NMR spectroscopic measurements. A characteristic signal for H₂O₂ (8.66 ppm, ESI,† Fig. S15a)⁴⁶ was observed in the ¹H-NMR spectrum of 2 with one-equiv. of H⁺ in CD₃CN. Our proposal of H₂O₂ formation in one-equiv. of H⁺ induced NO₂⁻ reduction was authenticated by comparing this spectrum with those of the authentic samples: (i) H₂O₂ plus 3 (8.66 ppm; ESI,† Fig. S15b) and (ii) H₂O₂ only (8.66 ppm; ESI,† Fig. S15c).⁴⁶ The amount of H₂O₂ in the above reaction was confirmed to be more than 50% (defining $\frac{1}{2}$ equivalent of H₂O₂ relative to 2 as 100% yield) from ¹H-NMR spectral measurements and using benzene as the internal standard (ESI,† ES, and Fig. S15a).46 Time-based ¹H-NMR spectral measurements for the above reaction showed the gradual formation of H_2O_2 (8.66 ppm), which starts decreasing after reaching its maxima, suggesting the decomposition of H₂O₂ to H₂O (Fig. 5a).^{44,45} In addition to ¹H-NMR, iodometric titration likewise confirmed H₂O₂ formation in the reaction of 2 with one-equiv. of H⁺ which was determined to be \sim 65% (ESI, ES, and Fig. S16a⁺) (defining 1/2 equivalent of H₂O₂ relative to 2 as 100% yield). However, no H₂O₂ was observed in iodometric titration when the reaction was carried out in the presence of two-equiv. of H⁺ (ESI, ES, and Fig. S16b⁺).⁴⁷



Fig. 5 1H-NMR spectrum of (a) H_2O_2 formation and (b) H_2O formation in the reaction of 2 with one-equiv. & two-equiv. of H⁺ in CD₃CN recorded at different times, respectively.

Furthermore, we have also established H₂O formation in the NO_2^- reduction reaction in the presence of two-equiv. of H⁺ by ¹H-NMR spectroscopic measurements (Fig. 5b and S15d, ESI[†]). To establish that H⁺ is only responsible for forming ¹H-NMR signals at 2.2 ppm, we have explored the same reaction using CF_3SO_3D (D⁺ source). Surprisingly, when the source was D⁺, we did not observe the formation of the H₂O peak (at 2.2 ppm); this clearly suggests that H^+ ions are responsible for the H_2O formation in the two-equiv. of H⁺ induced NO₂⁻ reduction (ESI, ES, and Fig. S17[†]). In addition, time-dependent ¹H-NMR spectral measurements for the reaction of 2 with two-equiv. of H⁺ showed an increment in the peak of H2O protons and a kind of first time-base measurement, further supporting our proposal of H₂O formation (Fig. 5b). However, our efforts to quantify the amount of H₂O formed in the reaction are futile; but they scientifically established the mechanistic aspect of acidinduced NO₂⁻ reduction in the presence of different equivalents of H⁺. These results are the only example where tracking H⁺-induced NO₂⁻ reduction products has confirmed that the variable amounts of H⁺ (pH/acidic conditions) generate NO with H_2O_2 (one-equiv. of H^+) or H_2O (\geq two-equiv. of H^+).

Furthermore, we attempted to characterize the proposed [Fe-ONOH]²⁺ intermediate to illustrate its conversion mechanism to 3. However, after several attempts, we failed to detect/ stabilize the intermediate even at low temperature (193 K) in UV-vis & FT-IR spectroscopic measurements, suggesting a kinetically driven reaction.48 Since metal-nitrous acid intermediates are known to be highly unstable intermediates,34b,36,40h-j there are only a few reports about the metal-bound nitrous acid species.34b,40h,40j,49 However, to support our mechanistic proposal for the formation of an 'OH radical (H₂O₂) via the homolytic cleavage of the N-O bond, we pursued the 'OH radical trapping experiment using 2,4-di-tert-butylphenol (2,4-DTBP).⁵⁰ In the one-equiv. of H⁺ induced NO₂⁻ reduction reaction, we have observed the formation of 3,5-Ditert-butylcatechol (3,5-DTBC, ~22%) and 2,4-DTBP-dimer (2,4-DTBP-D, \sim 8%) with a minimal amount of nitro-2,4-DTBP (NO₂-2,4-DTBP, ~6%) (ESI, ES, and Fig. S18 & S19[†]). The generation of 3,5-DTBC⁵¹ in the above experiments undoubtedly confirmed the 'OH formation via the N-O bond homolysis of the ON-OH moiety. Hence, the formation of 3,5-DTBC and other products confirms the reaction sequences (Scheme 3) and supports the



presence of the $[Fe-(ONOH)]^{2+}$ intermediate in the one-equiv. of H^+ induced NO_2^- reduction reaction.

The transformation of 2,4-DTBP in the presence of oneequiv. of H⁺ can be explained based on the radical coupling reaction.⁵¹ The sequences of the 2,4-DTBP conversion are believed to be (i) the generation of the phenoxyl radical and the release of Fe-NOs by the H-atom abstraction reaction of [Fe-ONOH²⁺ from DTBP (Scheme 3, reaction I & II). After that, the phenoxyl radical either (ii) dimerizes to give 2,4-DTBP-D (Scheme 3, reaction III) or (iii) produces 3,5-DTBC upon radical coupling with another molecule of [Fe-ONOH]²⁺ and releases 3 (Scheme 3, reaction IV). In some cases, NO₂-DTBP and 3 may generate in the presence of two molecules of [Fe-ONOH]²⁺ and a phenoxyl radical (Scheme 3, reaction V). Also, when the above radical trapping experiments were performed using CF₃SO₃D, we observed the generation 'OD-driven products of DTBC (ESI,† ES, and Fig. S20). In addition, we reacted 2-¹⁶O¹⁴N¹⁸O⁻ with one equiv. of H⁺ in the presence of 2,4-DTBP. Surprisingly, we observed the formation of 3,5-DTBC (¹⁸OH) with ¹⁶O¹⁴N¹⁸O-DTBP as a side product (ESI, Fig. S21[†]). These experiments support that one equiv. of H⁺ induced NO₂ reduction in 2 generates $3 + H_2O_2$ via the homolytic cleavage of the N-O bond of the ON-OH intermediate.36

Conclusion

The mechanistic investigation of acid-induced NO₂⁻ reduction became an important research topic in modern-day chemistry as it deals with NO2⁻ to NO transformation, an essential signaling molecule in biosystems.^{40b,52} The mechanistic aspects of NiR chemistry mediated by H⁺ are still challenging to the scientific community and yet to be resolved as two different side products have been proposed to form in vivo and in vitro studies.^{8a,9,16,25,30} Also, the pH/or H⁺ ion concentration effect is yet to be confirmed as it affects the reaction mechanism and the side products of NO₂⁻ reduction reactions.⁴⁸ In this report, we have shown the reduction of NO₂⁻ in a nonheme Fe^{II}-nitrito complex, $[(12TMC)Fe^{II}(NO_2^{-})]^+$ (2), to an Fe-nitrosyl complex $[(12TMC)Fe(NO)]^{2+}$, $\{FeNO\}^7$ (3), in the presence of different equivalents of H⁺ (CF₃SO₃D, D⁺ ion source), a biomimetic functional model of NiR. The structural details of {FeNO}⁷ showed an axially coordinated NO moiety to the Fe center. In addition, $^{15}\mbox{N-labeled}$ $^{15}\mbox{NO}_2^{-}$ experiments confirm that the N atom of the NO moiety in 3 is derived from the NO_2^- anion of 2. Acid-induced NO₂⁻ reduction of 2 showed the formation of ${\rm FeNO}^{7}$ along with H₂O₂ or H₂O as a side product when treated with different ratios of H^+ , one-equiv. or \geq two-equiv., respectively. Reports on acid-induced biomimetic NO₂⁻ reduction40h,j,43 and biological NiR reactions9b,40g,41 suggested a metal-ONOH intermediate before NO formation; hence, we believe that the H^+ -induced NO_2^- reduction on the Fe^{II} center in 2 should generate 3 via the proposed [Fe–ONOH]²⁺ intermediate and follow the NiR chemistry. The N-O bond homolysis of the proposed ONOH intermediate was supported by the observation of 3,5-DTBC-16 OH(18 OH) in 'OH radical trapping experiments using 2,4-DTBP⁵¹ in the reaction of 2-ON¹⁶O₂⁻⁽¹⁶ON¹⁸O⁻⁾ with one equiv. of H⁺. Also, the observation of DTBC(OD) in the

presence of D⁺ further supports the acid-induced reduction of NO₂⁻. In addition, a significant amount of H₂O₂ formation was also confirmed using ¹H-NMR/or UV-vis iodometric titration along 3.46 However, the generation of the H₂O molecule was believed to occur either (i) by NO₂⁻ reduction in the presence of two-equiv. of H⁺ and an electron^{9b,40c,40g,40h,40j} or (ii) by the acidinduced decay of H2O2 or auto-decomposition of H2O2.44,45 The redox potential of 2 was higher than that of the enzymatic iron-site, making 2 more prone to reduction.53 At this time, we are not sure about the source of another electron; however, we are currently exploring various NO_2^- bound $Cu^{I/II}$ & Fe^{II/III} complexes to understand the reaction sequences and track the electron source using the known electron donor species. These results provide entirely new reaction sequences for acidinduced NO₂⁻ reduction chemistry, a functional model of biomimetic NiR chemistry, and show how the H⁺ ion concentration determines H_2O_2 or H_2O as a side product along with NO.

Experimental Section

For the experimental details, see the ESI.†

Data availability

All the required data is already provided in the ESI[†] and manuscript.

Author contributions

PKK discovered /conceptualized the initial project. Kulbir carried out most of the experiments and gathered the data. PKK, SG & TD helped in interpreting the experimental results. SCS, Kulbir & SD worked on growing the crystals and recording the crystallographic data. Kulbir and SD write the first draft of the article. PKK & TD have corrected the manuscript, finalized the final draft, and guided during the revision. PKK followed and guided the whole project work.

Conflicts of interest

There are no conflicts to declare.

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