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An isoelectronic NO dioxygenase reaction using a nonheme iron(III)-peroxo complex and nitrosonium ion†

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Abstract

Reaction of a nonheme iron(III)-peroxo complex, $[\text{Fe}^{\text{III}}(14\text{-TMC})(\text{O}_2)]^+$, with NO^+ , a transformation which is essentially isoelectronic with that for nitric oxide dioxygenases $[\text{Fe}^{\text{III}}(\text{O}_2^{\bullet-}) + \text{NO}]$, affords an iron(IV)-oxo complex, $[\text{Fe}^{\text{IV}}(14\text{-TMC})(\text{O})]^{2+}$, and nitrogen dioxide (NO_2), followed by conversion to an iron(III)-nitrate complex, $[\text{Fe}^{\text{III}}(14\text{-TMC})(\text{NO}_3)(\text{F})]^+$.

Metal ion/nitric oxide (NO) interactions are of great interest since NO plays important roles in physiological processes as a signaling agent and in the mammalian immune response.¹ However, overproduction of NO can be lethal and lead to toxicological processes by inhibiting aconitase² and/or cytochrome *c* oxidase,³ that affect respiration. One manner to maintain the appropriate NO levels *in vivo* is detoxification of NO by nitric oxide dioxygenases (NODs); microbial or mammalian heme protein NODs (*e.g.*, hemoglobins or myoglobins) catalyze the reaction of NO and O_2 (*via* the formation of an Fe^{III} -superoxo species; $\text{Fe}^{\text{II}} + \text{O}_2 \rightarrow \text{Fe}^{\text{III}}(\text{O}_2^{\bullet-})$) to yield the biologically benign nitrate ion (NO_3^-).^{4,5} In biomimetic studies, mononuclear metal- O_2 complexes, best described as metal-superoxo species, are reported to react with NO to give metal-peroxynitrite ($\text{M}^{n+}\text{-OON=O}$, PN)^{4,6} intermediates.^{7–9} To mimic NOD reactivity, we recently reported the conversion of NO and metal- O_2 species to NO_3^- and metal-oxo species, respectively, using peroxide or superoxide complexes of chromium-TMC (TMC = *N*-tetramethylated cyclam); a Cr(IV)-peroxo complex ($[\text{Cr}^{\text{IV}}(12\text{-TMC})(\text{O}_2)(\text{Cl})]^+$) reacted with NO to form a Cr(III)-nitrate complex ($[\text{Cr}^{\text{III}}(12\text{-TMC})(\text{NO}_3)(\text{Cl})]^+$),¹⁰ whereas a Cr(III)-superoxo complex ($[\text{Cr}^{\text{III}}(14\text{-TMC})(\text{O}_2)(\text{Cl})]^+$) and NO gave a Cr(IV)-oxo complex ($[\text{Cr}^{\text{IV}}(14\text{-TMC})(\text{O})(\text{Cl})]^+$) and NO_2 *via* the formation of a presumed Cr(III)-peroxynitrite intermediate ($[\text{Cr}^{\text{III}}(14\text{-TMC})(\text{OON=O})(\text{Cl})]^+$).^{‡11}

So far in biomimetic chemistry, many studies have been focused on the formation of a peroxynitrite (PN) species using metal-superoxo complexes reacting with NO or employing

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‡Abbreviations used: 12-TMC, 1,4,7,10-tetramethyl-1,4,7,10-tetraazacyclododecane; 14-TMC, 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane.

metal-nitrosyl complexes in a reaction with O₂. However, considering the reaction between a metal(III)-superoxo complex and NO according to eqn (1), it is notable that a reaction between a metal(III)-peroxo complex and nitrosonium ion [NO⁺; a nitrite analog] (eqn (2)) is essentially an iso-electronic transformation and thus represents potentially another manner in which to generate PN species.

We describe herein the reactivity of an Fe(III)-peroxo complex ([Fe^{III}(14-TMC)(O₂)]⁺, **1**)¹² with NO⁺ as an approach for generating PN species; the reaction of **1** with NO⁺ generated an Fe(IV)-oxo species ([Fe^{IV}(14-TMC)(O)]⁺, **3**)¹³ and NO₂ *via* formation of a putative Fe(III)-PN intermediate species **2** (Scheme 1, reactions (a) and (b)). Complex **3** and NO₂ slowly reacted to form an Fe(III)-nitrato complex ([Fe^{III}(14-TMC)(NO₃)(F)]⁺, **4**) (Scheme 1, reaction (c)). This is the first biomimetic example of a NOD reaction using a nonheme iron(III)-peroxo complex and NO⁺.

Addition of 1.2 equiv. of nitrosonium hexafluorophosphate (NOPF₆) to a solution of **1** (1 mM) in CH₃CN at −10 °C under Ar immediately generated an Fe(IV)-oxo complex **3** with an electronic absorption band at λ_{max} = 820 nm (*i.e.*, spectral change from black line to blue line, in Fig. 1a).^{12,13} The yield of **3** was ~90% based on the reported spectroscopic absorptivity value for **3** (ε = 400 M^{−1} cm^{−1}).¹³ Following this, UV-vis spectral changes of **3** were observed with an isosbestic point at 460 nm (*i.e.*, spectral changes from blue line to red line in Fig. 1a), indicating that there was a subsequent conversion of **3** to another species. The electrospray ionization mass spectrum (ESI-MS) of the resulting final solution indicates the formation of an Fe(III)-nitrato complex **4**; the observed peak cluster at a mass-to-charge ratio (*m/z*) of 393.1 is assigned to [Fe^{III}(14-TMC)(NO₃)(F)]⁺ (calcd *m/z* of 393.2) (Fig. 1b). Considering peaks assigned to Fe(TMC) complexes with fluoride ions (F[−]), especially the main peak cluster detected at *m/z* of 350.3 as shown in Fig. 1b ([Fe^{III}(14-TMC)(F)₂], calcd *m/z* of 350.3), the hexafluorophosphate ion (PF₆[−]), which was the counter cation for NO⁺, caused side reactions leading to F[−] containing products (*vide infra*). An EPR spectrum of **4** revealed a rhombic signal with *g* values of 6.6, 5.0, and 1.97 (see ESI,† Fig. S1), which are indicative of a d⁵ electron configuration typically observed for high-spin (*S* = 5/2) iron(III) species.¹⁴ The yield of **4** was estimated to be ~67%.[§]

[§]The yield of the nitrate ion (NO₃[−]) was estimated by using QUANTO FIX[®] Nitrate/Nitrite Test Strips (see ESI,† experimental section).

Thus, we surmise that a sequence of reactions occurs as shown in Scheme 1: **1** interacts with NO^+ to produce an Fe(IV)-oxo complex **3** and NO_2 via formation of a putative Fe(III)-PN species which undergoes O–O bond homolysis. The mixture of **3** and nitrogen dioxide is then converts to an Fe(III)-nitrate complex **4**. Although no evidence was obtained in our experiments to provide details concerning the first reaction step and formation of the Fe(III)-PN species, it would probably occur via fast electron transfer between the Fe(III)-peroxo moiety and NO^+ to generate a short-lived Fe(III)-superoxo complex and NO [*i.e.*, $\text{Fe(III)(O}_2^{2-}) + \text{NO}^+ \rightarrow \text{Fe(III)(O}_2^{\bullet-}) + \bullet\text{NO}$], which then combine to form the Fe(III)-PN intermediate; note the high reduction potential of NO^+ ($E = 0.87 \text{ V vs. Fc/Fc}^+$ in CH_3CN).¹⁵

To ensure that NO_2 was truly generated in the above reaction (*vide supra*), a trapping experiment was carried out using 2,4-di-*tert*-butylphenol (DTBP).¹⁶ After the formation of complex **3** and NO_2 as described in Scheme 2, 2 equiv. of DTBP was added to this reaction solution and the reaction mixture was stirred and kept in a closed UV cuvette.[¶] UV-vis spectral changes for this reaction were monitored (ESI,[†] Fig. S2), and product analysis was performed by GC. As a result, 2,4-di-*tert*-butyl-6-nitrophenol (nitro-DTBP) and 3,3', 5,5'-tetra-*tert*-butyl-(1,1'-biphenyl)-2,2'-diol (DTBP-dimer) were detected in 63% and 3% yield, respectively (Scheme 2). Moreover, a supplemental experiment also supported the generation of NO_2 ; the same reaction was carried out using an open UV cuvette and gave the nitro-DTBP product in a lower yield (~38%), indicating the generation of the gaseous NO_2 molecule in the original reaction, but which could escape when the experiment was conducted openly. As for the iron species in this reaction, the observation of an ESI-MS peak at m/z of 348.3 corresponding to $[\text{Fe}^{\text{III}}(14\text{-TMC})(\text{OH})(\text{F})]^+$ (**8**) (calcd m/z of 348.2) indicates that a hydrogen atom abstraction reaction took place between **3** and DTBP (ESI,[†] Fig. S2).^{10,11} Additionally, an EPR spectrum of complex **8** showing a feature due to a d^5 high-spin ($S = 5/2$) Fe(III) species confirmed that one electron reduction occurred at the iron center in **3** through this reaction (ESI,[†] Fig. S3).^{14,17} Thus, NO_2 was successfully accounted for and recovered in good yield, and this result strongly supports our proposed reaction profile as given in Scheme 1.

Further supplemental and systematic experiments were performed to clarify the reactivity of the Fe(IV)-oxo complex with NO_2 in the presence and absence of fluoride ions. We prepared an Fe(IV)-oxo complex ($[\text{Fe}^{\text{IV}}(14\text{-TMC})(\text{O})(\text{CH}_3\text{CN})]^{2+}$, **5**)¹³ and reacted it with NO_2 in the presence and absence of TBAPF_6 . In both cases, the formation of an Fe(II)-nitrate complex ($[\text{Fe}^{\text{II}}(14\text{-TMC})(\text{NO}_3)]^+$, **6**) was observed (Scheme 3, reaction I; ESI,[†] Fig. S4 and S5). This reaction includes one electron reduction at the iron centre (*e.g.*, $\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$), as was also the case previously observed for the reaction of Fe(IV)-oxo species with NO (*e.g.*, $\text{Fe(IV)(O)} + \text{NO} \rightarrow \text{Fe(II)} + \text{NO}_2^-$).^{18,19} We could also deduce that the PF_6^- was not involved in this reaction, not generating any fluoride ion. Therefore, we presume that incorporation of fluoride ions, as shown in Scheme 1, took place through the reaction between residual NO^+ and PF_6^- via uncontrollable radical chain reaction chemistry and fluoride became an axial ligand in **4** due to its affinity to the iron centre. Addition of 1.2 equiv. of TBAF (*n*-tetrabutylammonium fluoride) to **6** resulted in one electron oxidation at

[¶]The reaction was carried out in a dry box under an Ar atmosphere using an open UV cuvette.

the iron centre, forming a fluoride coordinated Fe(III)-nitrate complex ($[\text{Fe}^{\text{III}}(14\text{-TMC})(\text{NO}_3)(\text{F})]^+$, **4**), which was the same product observed in Scheme 1 (Scheme 3, reaction II; ESI,† Fig. S5 and S6). In addition, we prepared a fluoride coordinated Fe(IV)-oxo complex ($[\text{Fe}^{\text{IV}}(14\text{-TMC})(\text{O})(\text{F})]^+$, **7**; Scheme 3, reaction III; ESI,† Fig. S7) and examined its reactivity with NO_2 ; **7** and NO_2 gave the fluoride coordinated Fe(III)-nitrate complex **4** (Scheme 3, reaction IV; ESI,† Fig. S8 and S9). The yields of nitrate ion in reactions I and IV shown in Scheme 3 were estimated to be ~67% for both.[§] Summarizing these systematic studies, we find that the iron centre in a given Fe-nitrate complex chooses a ferric state in the presence of a fluoride ion or a ferrous state in the absence of a F^- , respectively. In other words, the fluoride ion determines the oxidation state of the iron centre in the Fe-nitrate complex. These results are consistent with those described in Scheme 1.

Electrochemical measurements of **5** and **7** also help us to understand the oxidation state of iron: cyclic voltammograms (CV) of **5** and **7** in CH_3CN at -10°C under Ar in the presence of 0.1 M TBAPF₆ were measured to investigate their redox properties, and we found that the coordination of fluoride ion to **5** causes a quite large negative shift ($E = 0.96\text{ V}$) in the Fe(IV)/Fe(III) reduction process (**5**, $E_{\text{ox}} = 0.37\text{ V vs. SCE}$; ²⁰ **7**, $E_{1/2} = -0.59\text{ V vs. SCE}$; ESI,† Fig. S10). Thus, the coordination of a monoanion (fluoride ion) to the iron centre stabilizes its high-valent oxidation state, leading to the change of valency of the fluoride coordinated Fe-nitrate complex. However, although we summarized the reaction in Scheme 3 including the one electron reduction–oxidation process based on the experimental observations, we have not been able to pinpoint the exact source of the reducing equivalent, as also previously reported for the reaction between an Fe(IV)-oxo complex and nitric oxide.¹³

In summary, we have described the reactivity of an Fe(III)-peroxo complex **1** with nitrosonium ion to generate an Fe(IV)-oxo species **3** and nitrogen dioxide (NO_2). This Fe(IV)-oxo species and NO_2 reacted slowly to give an Fe(III)-nitrate complex **4**. It is notable that this is the first observation of a nonheme iron(III)-peroxo complex showing stepwise and stoichiometric NOD reactivity. The new concept in this study, such as mimicking the NOD reaction using a metal(III)-peroxo species and a nitrosonium ion, provides a new approach that we may in other cases use to design isoelectronic pathways for enzymatic reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

Fig. 1.

(a) UV-vis spectral changes of **1** (1 mM) upon addition of 1.2 equiv. of NO^+ in CH_3CN at -10°C under Ar. The initial black line spectrum (**1**) changed immediately to the blue line spectrum (**3**) upon addition of NO^+ , followed by conversion to the red solid line spectrum (**4**) over 1 h. Inset: time course of the absorbance change at 812 nm. (b) ESI-MS spectrum of **4** (calcd m/z of 393.2) and $[\text{Fe}^{\text{III}}(14\text{-TMC})(\text{F})_2]^+$ (calcd m/z of 350.3).

Scheme 2.

Scheme 3.