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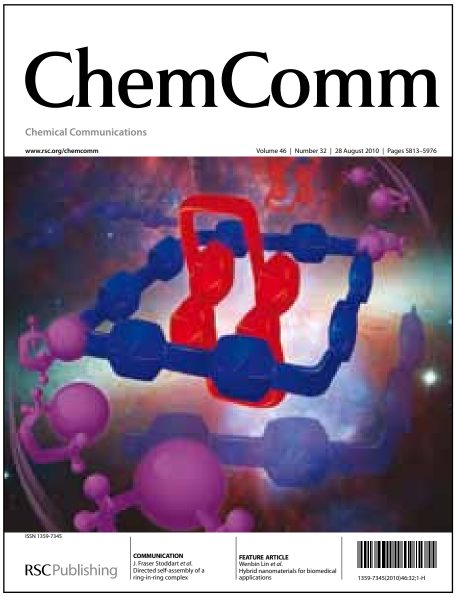

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An isoelectronic NO dioxygenase reaction using a nonheme iron(III)-peroxo complex and nitrosonium ion†

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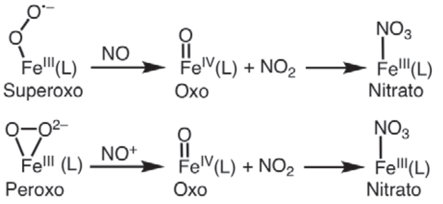
Reaction of a nonheme iron(III)-peroxo complex, [Fe^{III}(14-TMC)(O$_2$)]$^+$, with nitrosonium ion (NO$^+$), a transformation which is essentially isoelectronic with that for nitric oxide dioxygenases [(Fe(III)(O$_2^{\bullet-}$) + NO], affords an iron(IV)-oxo complex, [Fe^{IV}(14-TMC)(O)]$^{2+}$, and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

Metal ion/nitric oxide (NO) interactions are of great interest since nitric oxide plays important roles in physiological processes such as a signaling agent and in the mammalian immune response.[1] However, overproduction of NO can be lethal poison and lead to toxicological processes in aconitase[2] and/or cytochrome *c* oxidase[3] that affect respiration. One manner to maintain the proper NO levels *in vivo* is a detoxification of NO by enzymes called 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; Fe(II) + O$_2$ → Fe(III)(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, have been reported to react with NO to give metal-peroxynitrite (M^{n+}-OON=O, PN)[4,6] intermediate.[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 ([Cr^{IV}(12-TMC)(O$_2$)(Cl)]$^+$) reacted with NO to form a Cr(III)-nitrato complex ([Cr^{III}(12-TMC)(NO$_3$)(Cl)]$^+$),[10] whereas a Cr(III)-superoxo complex ([Cr^{III}(14-TMC)(O$_2$)(Cl)]$^+$) and NO gave a Cr(IV)-oxo complex ([Cr^{IV}(14-TMC)(O)(Cl)]$^+$) and NO$_2$ *via* the formation of a presumed Cr(III)-peroxynitrite intermediate ([Cr^{III}(14-TMC)(OON=O)(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 metal-nitrosyl complexes in a reaction with O$_2$. However, considering the reaction between a metal(III)-superoxo complex and NO depicted in 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 isoelectronic transformation and thus represents potentially another manner in which to generate PN species.

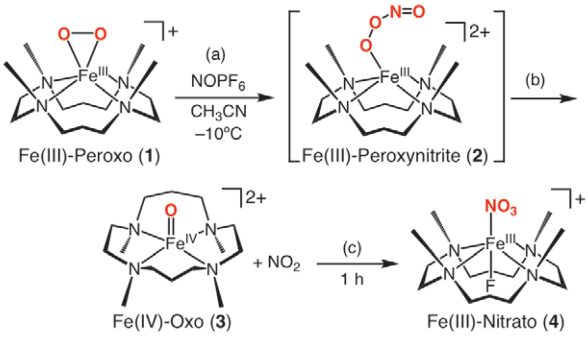


(1)

(2)

We describe herein the reactivity of an Fe(III)-peroxo complex ([Fe^{III}(14-TMC)(O$_2$)]$^+$, **1**),[12] with nitrosonium ion (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 NO$_2$ *via* formation of a putative Fe(III)-PN intermediate species **2** (Scheme 1, reactions (a) and (b)). The Fe(IV)-oxo complex **3** and NO$_2$ slowly reacted to form an Fe(III)-nitrato complex ([Fe^{III}(14-TMC)(NO$_3$)(F)]$^+$, **4**) (Scheme 1, reaction (c)). This is the first biomimetic example of a NOD reaction using a nonheme iron(III)-peroxo complex and nitrosonium ion.

Scheme 1



Addition of 1.2 equiv of nitrosonium hexafluorophosphate (NOPF$_6$) to a solution of **1** (1 mM) in CH$_3$CN at –10 °C under Ar immediately generated a 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, 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 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 mass-to-charge ratio (*m*/*z*) of 393.1 is assigned to [Fe^{III}(14-TMC)(NO$_3$)(F)]$^+$ (calcd. *m*/*z* of 393.2) (Fig. 1b). Considering peaks assigned to Fe(TMC) complexes with fluoride

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† Electronic Supplementary Information (ESI) available: experimental details and Figure S1-S10, See http://dx.doi.org/10.1039/b000000x/

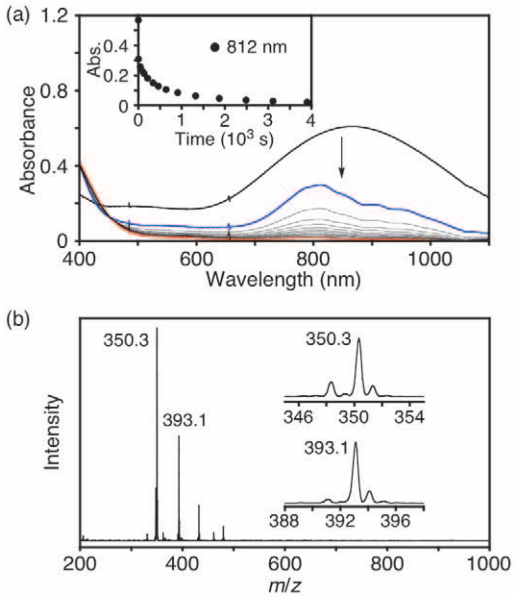


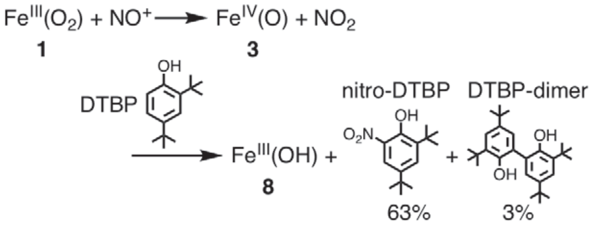
Fig 1. (a) UV-vis spectral changes of **1** (1 mM) upon addition of 1.2 equiv of NO^+ in CH_3CN at –10 °C under Ar. The initial black line spectrum (**1**) changed immediately to the blue line spectrum (**3**) upon addition of NO^+, followed by conversion to the red solid line spectrum (**4**) over 1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

ions (F^-), especially the main peak cluster detected at *m/z* of 350.3 in Fig. 1b ($[Fe^{III}(14\text{-}TMC)(F)_2]$, calcd. *m/z* of 350.3), the hexafluorophosphate ion (PF_6^-), which was the counter cation of NO^+, caused side reactions leading to the fluoride ion 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 Electronic Supplementary Information (ESI), Fig. S1), which are indicative of a d^5 electron configuration typically observed for high-spin (S = 5/2) iron(III) species.[14] The yield of **4** was estimated to be ~67%.§

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 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., $Fe(III)(O_2^{2-}) + NO^+ \rightarrow Fe(III)(O_2^{\bullet-}) + {\bullet}NO$], which then combine to form the Fe(III)-PN intermediate; note the high reduction potential of NO^+ (E = 0.87 V *vs* Fc/Fc^+ in CH_3CN).[15]

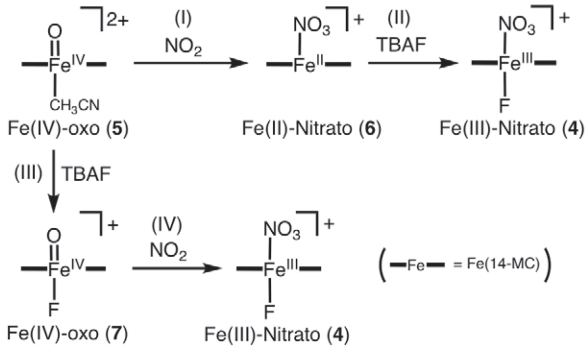
To ensure that NO_2 was truly generated in the reaction above (*vide supra*), a trapping experiment was carried out using 2,4-di-*tert*-butylphenol (DTBP).[16] After generating complex **3** and NO_2 as described in Scheme 2, 2 equiv of DTBP was added to this reaction solution and the reaction mixture was stirred and kept in a closed UV cuvette. UV-vis spectral changes for this reaction were monitored (SI, Fig. S2), and the 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 above was carried out with use of an open UV cuvette and gave the nitro-DTBP product in a lower yield (~38%), indicating the generation of 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 $[Fe^{III}(14\text{-}TMC)(OH)(F)]^+$ (**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 on 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.

Scheme 2



Further supplemental and systematic experiments were performed to clarify the reactivity of the Fe(IV)-oxo complex with NO_2 in the presense and absence of fluoride ion. We prepared an Fe(IV)-oxo complex ($[Fe^{IV}(14\text{-}TMC)(O)(CH_3CN)]^{2+}$, **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)-nitrato complex ($[Fe^{II}(14\text{-}TMC)(NO_3)]^+$, **6**) was observed (Scheme 3, reaction I; ESI, Figs. S4 and S5). This reaction includes one electron reduction on the iron centre (e.g., $Fe^{3+} + e^- \rightarrow Fe^{2+}$), as was also the case previously observed for the reaction of Fe(IV)-oxo species with NO (e.g., $Fe(IV)(O) + NO \rightarrow Fe(II) + NO_2^-$).[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 ion 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 on the iron centre, forming a fluoride coordinated Fe(III)-nitrato complex ($[Fe^{III}(14\text{-}TMC)(NO_3)(F)]^+$, **4**), which

Scheme 3



was the same product found in Scheme 1 (Scheme 3, reaction II; ESI, Figs. S5 and S6). In addition, we prepared a fluoride coordinated Fe(IV)-oxo complex ($[Fe^{IV}(14\text{-TMC})(O)(F)]^+$, **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)-nitrato complex **4** (Scheme 3, reaction IV; ESI, Figs. S8 and S9). The yields of nitrate ion in reactions I and IV in Scheme 3 were estimated to be ~67% for both.§ Therefore, from the systematic study described above, we found that the iron centre in a given Fe-nitrate complex chooses a ferric state in the presence of fluoride ion or a ferrous state in the absence of fluoride ion, respectively. In other words, the fluoride ion determines the oxidation state of iron centre in the Fe-nitrate complex. This result is consistent with the those described in Scheme 1.

Electrochemical measurements on **5** and **7** also help us to understand the definition of 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 $TBAPF_6$ were measured to investigate their redox properties, and we found that the coordination of fluoride ion to **5** causes a quite large negative shift ($\Delta E = -0.69$ V) in the Fe(IV)/Fe(III) reduction process (**5**, $E_{p.c.} = 0.10$ V *vs* SCE;[20] **7**, $E_{1/2} = -0.59$ 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 one electron reduction/oxidation process based on the experimental observations, we have not been able to pinpoint the exact source of the reducing equivalent, also as previously reported for the reaction between 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 (NO^+) and found that **1** rapidly reacts with NO^+ 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)-nitrato complex **4**. It is notable that this is the first obsevation of a nonheme iron(III)-peroxo complex showing step-wise and stoichiometric NOD reactivity. The new concept in this study, such as mimicing NOD reaction using a metal(III)-peroxo species and nitrosonium ion, provides a new approach that we may in other cases be able to design isoelectronic reactions for enzymatic reactions.

Notes and references

‡ 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.

§ The yield of nitrate ion (NO_3^-) was estimated by using QUANTOFIX® Nitrate/Nitrite Test Strips (see ESI, experimental section).

¶ The reaction was carried out in dry box under Ar atomospher with an open UV cuvette.

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