A bridged di-iron porphyrin hyponitrite complex as a model for biological N₂O production from hyponitrite

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Abstract: Heme-hyponitrites are intermediates that form at the bimetallic active sites of bacterial nitric oxide reductases. To probe a possible effect of the Fe-Fe distance on hyponitrite stability, we prepared a bridged bis-porphyrin Fe-hyponitrite compound, namely [(OEP-CH₂)Fe]₂(μ₂,η^I,η^I-ONNO). Its ν_{NO} of 992 cm⁻¹ (ν_{15NO} of 976 cm⁻¹) is close to the ν_{NO} of 983 cm⁻¹ reported previously by us for the crystallographically characterized [(OEP)Fe]₂(μ₂,η^I,η^I-ONNO) compound. The bridged bis-porphyrin Fe-hyponitrite complex is unstable with respect to N₂O production, supporting the role of the bis-Fe porphyrin system in hyponitrite conversion to N₂O. The preparation and crystallographic determination of the bridging sulfato derivative is also reported.

Keywords: *hyponitrite, iron, porphyrin, sulfate, infrared*

Introduction

The mechanism by which bacteria utilize bimetallic heme/non-heme active sites to detoxify NO remains an active area of research. Crystallographic [1-3] and density functional theory calculations [4-6] have provided significant insight into the likely pathways by which the bimetallic active site of bacterial enzymes responsible for the coupling of two molecules of NO to form a hyponitrite intermediate en route to the non-toxic N_2O product that is a greenhouse gas [7]. Several mechanisms have been proposed for the bacterial NO coupling reaction [6, 8, 9], some of the most widely cited are shown as **A**, **B**, and **C** in Figure 1.

Figure 1. Commonly proposed hyponitrite intermediates in bimetallic heme active sites.

The hyponitrite intermediates **D** (Figure 1; $\text{M} = \text{Fe}, \text{Cu}$) have been characterized based on resonance Raman spectroscopy for a bacterial NO reductase [10] and *caa*₃ oxidoreductase [11]. The involvement of protons in hyponitrite formation, and hyponitrite decomposition to N_2O in the presence/absence of protons remain an intense area of study.

It is interesting to note that although hyponitrite complexes of coordination compounds are known [12, 13], only two heme model-hyponitrite compounds have been reported to date [14, 15], and only one has been structurally characterized. The first isolated hyponitrite-bridged iron heme model complex $[(\text{OEP})\text{Fe}]_2(\mu_2, \eta^I, \eta^I\text{-ONNO})$ was reported by us [14]. This

compound releases N_2O gas upon reaction with the protic acid HCl , but also converts slowly at room temperature to the known $(\text{OEP})\text{Fe}(\text{NO})$ nitrosyl. Importantly, the $(\text{OEP})\text{Fe}(\text{NO})$ does not self-couple to give the bimetallic hyponitrite species [14,15]. The molecular structure of $[(\text{OEP})\text{Fe}]_2(\mu_2, \eta^l, \eta^l\text{-ONNO})$ revealed that the bridged *trans*-hyponitrite ligand binds to two Fe centers through the hyponitrite O atoms (Figure 2).

Figure 2. The molecular structure of $[(\text{OEP})\text{Fe}]_2(\mu_2, \eta^l, \eta^l\text{-ONNO})$ [14].

The non-bonding distance between the two Fe centers is ~ 6.7 Å, which is significantly longer than those in the bimetallic heme/non-heme active sites of NO reductases and cytochrome oxidases exhibiting NOR activity. For example, the distance between the heme-Fe and nonheme-Fe in the NO reductase from *Pseudomonas aeruginosa* is 3.8 Å [1]; the related distance between the heme-Fe and active-site Cu center in *T. thermophilus* cytochrome ba_3 is 4.4 Å [16]. The long Fe–Fe distance in $[(\text{OEP})\text{Fe}]_2(\mu_2, \eta^l, \eta^l\text{-ONNO})$ (Figure 2) may well help favor the *O,O*-bonding mode of the hyponitrite bridge. The closely related iron porphyrin hyponitrite model compound $[(\text{PPDME})\text{Fe}]_2(\mu_2, \eta^l, \eta^l\text{-ONNO})$ was also reported by us [15].

To improve upon our approach to study heme-hyponitrite binding and reactivity, we sought to prepare di-Fe heme-hyponitrite model compounds with potentially shorter Fe–Fe

distances similar to those present in the bacterial NO reductases (~ 4 Å). In this paper, we report the preparation and thermal reaction of such a bridged porphyrin-hyponitrite complex.

Experimental Section

All reactions were performed under an atmosphere of nitrogen using standard Schlenk glassware and/or in anaerobic and moisture-free glove box. Solutions for spectral studies were also prepared under a nitrogen atmosphere. Solvents were dried by passage through alumina (using a Pure Solv 400-5-MD system) and deaerated just prior to use. The $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ oxo-dimer (**1**) [17] and hyponitrous acid ($\text{H}_2\text{N}_2\text{O}_2$) [18] were prepared according to literature procedures. The ^{15}N -labeled $\text{H}_2^{15}\text{N}_2\text{O}_2$ was prepared using similar procedures as used for the unlabeled analog. Infrared spectra were recorded on a Bio-Rad FT-155 FTIR spectrometer.

Preparation of $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ (2**):** To a CH_2Cl_2 solution (10 mL) of $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**; 8 mg, 0.006 mmol) was added an aqueous H_2SO_4 solution (0.25 mL, $\sim 25\times$ excess). The color of the stirred solution changed from green to brown overnight. The resulting dark brown powder (6 mg) was collected by filtration and dried in vacuo. The IR spectrum of the resulting solid indicated that it was a mixture of the product $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ (**2**) and the precursor $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**). The characteristic bands of sulfate anion [19, 20] in the $980\text{-}1140\text{ cm}^{-1}$ range are obscured by the porphyrin bands. X-ray diffraction-quality crystals were grown at room temperature from a CH_2Cl_2 /acetonitrile (1:2) solution.

Preparation of $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO})$ (3**):** To a CH_2Cl_2 solution (10 mL) of $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**; 15 mg, 0.011 mmol) was added dropwise an excess of $\text{H}_2\text{N}_2\text{O}_2$ (~ 0.15 mmol in Et_2O). The color of the stirred solution changed from green to brown-green over a 30 min

period. The solution was reduced to ~2 mL, and hexane(10 mL) was added to precipitate the product. IR (NaCl disk): $\nu_{\text{NO}} = 992 \text{ cm}^{-1}$. The ^{15}N -labeled $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}^{15}\text{N}^{15}\text{NO})$ analog was prepared similarly using the $\text{H}_2^{15}\text{N}_2\text{O}_2$ reagent. IR (NaCl disk): $\nu_{\text{NO}} = 976 \text{ cm}^{-1}$.

X-ray diffraction data for compound **2** were collected at 100(2) K using Mo $\text{K}\alpha$ ($\lambda = 0.71073 \text{ \AA}$) radiation. Tables of bond lengths and angles are contained in the Supporting Information. CCDC 1428275 (compound **2**) contains the supplementary crystallographic data. These data can be obtained free of charge from the Cambridge Crystallographic Data Center via <http://www.ccdc.cam.ac.uk/conts/retrieving.html>.

Results and Discussion

The iron porphyrin oxo-dimer precursor, namely $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**), was prepared as previously reported [17] and crystallized from a slow evaporation from CH_2Cl_2 /ether/hexane (2:1:1) solution of the complex. The molecular structure of this oxo-dimer precursor, reported previously by Rath [17] and reproduced by us, is shown in Figure 3, and confirms the presence of the ethane bridge and the "single atom" oxo bridge.

Figure 3. The molecular structure of $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**) [17].

The Fe–Fe distance of ~ 3.4 Å [17] is shorter than the related distances in the active sites of the bacterial NOR proteins (see above). Flexibility in this Fe–Fe distance is evidenced by the Fe–Fe distance of 3.627(1) Å reported by Rath [17] for the structure of a μ -hydroxo derivative. In addition, longer Fe–Fe distances of ~ 5.5 Å have been observed in two di-Zn complexes containing relatively bulky bidentate diamine ligands bound to the two Zn centers [21, 22]. To further examine this flexibility, we prepared the μ -sulfato derivative $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ (**2**) containing a "three atom" $[-\text{O}-\text{S}-\text{O}-]$ bridge, shown in Figure 4. Here, the Fe–Fe distance is 5.56 Å, confirming this conformational flexibility. We note that only a handful of diporphyrin μ -sulfato crystal structures have been reported in the literature, namely those of $[(\text{TPP})\text{Fe}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ [23, 24], $[(\text{TPP})\text{Mo}(=\text{O})]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ [25], $[(\text{TPP})\text{Mn}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ [26], and $[(\text{TPP})\text{Hf}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)_{1,2}$ [27].

Figure 4. The molecular structure of $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}_2\text{SO}_2)$ (**2**). Selected bond lengths (Å) and angles ($^\circ$): Fe1–O1 = 1.901(2), Fe2–O2 = 1.898(2), O1–S1 = 1.524(2), O2–S1 = 1.529(2), S1–O3 = 1.435(2), S1–O4 = 1.439(2), Fe1–O1–S1 = 134.00(14), Fe2–O2–S1 = 135.89(14), O1–S1–O2 = 103.59(13).

We then made use of this flexible observed Fe–Fe distance in this ethane-bridged porphyrin system to prepare the bridged hyponitrite derivative. We reacted the oxo-dimer **1** in anhydrous CH₂Cl₂ with an ether solution of *in situ* generated hyponitrous acid for 4 h (eq 1).

IR spectrum of the resulting [(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO}) (**3**) product, as a dried sample on a NaCl disk, revealed a new band at 992 cm⁻¹ (Figure 5a) assigned to ν_{as} of the hyponitrite N–O group ($\nu_{\text{as}}(^{15}\text{NO})$ 976 cm⁻¹, Figure 5b). This band is similar to that reported for [(\text{OEP})\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO}) at 983 cm⁻¹ ($\nu_{\text{as}}(^{15}\text{NO})$ 973 cm⁻¹) [14], and lower than that for the H₂N₂O₂ reagent at 1014/1003 cm⁻¹ ($\nu_{\text{as}}(^{15}\text{NO})$ 991/980 cm⁻¹) [28]. The conformation of the hyponitrite bridge in metal complexes can be *cis* or *trans*. For the *cis*-hyponitrite metal complexes (Ni, Na), in addition to the ν_{NO} band at ~1000 cm⁻¹, the ν_{NN} is also active, showing a band at around 1300 cm⁻¹ [29-31], which is not observed in the IR spectrum of **3**.

Unlike its non-bridged OEP derivative reported earlier (Figure 1), the bridged porphyrin hyponitrite complex **3** is *unstable in the absence of added hyponitrous acid*. Indeed, our attempts to isolate **3** as a pure solid only resulted in the re-formation of starting complex **1** with generation of N₂O gas as described in eq 2.

The production of N₂O gas in the headspace of the reaction during the decomposition of **3** was confirmed by IR spectroscopy of the headspace. As shown in Figure 5c, new bands at 2236/2212 cm⁻¹ in the IR spectrum of the headspace due to N₂O were observed to form during the decomposition of **3**; these bands shifted to 2169/2144 cm⁻¹ when the ¹⁵N-labeled hyponitrous acid reagent was used.

Figure 5. IR spectra of the (a) unlabeled $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO})$ (**3**), and (b) ^{15}N -labeled $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-O}^{15}\text{N}^{15}\text{NO})$ hyponitrite complexes. IR spectra of the head space from the reactions of (c) $[(\text{OEP-CH}_2)\text{Fe}]_2(\mu\text{-O})$ (**1**) with unlabeled $\text{H}_2\text{N}_2\text{O}_2$, and (d) ^{15}N -labeled $\text{H}_2^{15}\text{N}_2\text{O}_2$.

Similar bands and an identical isotopic shift of 69 cm^{-1} have been reported for the unlabeled and ^{15}N -labeled N_2O in gas phase [32]. We note that the corresponding ν_s bands of N_2O at around $1298/1266\text{ cm}^{-1}$ were not observed due to the overlapping with the absorptions from the solvents or the occurrence outside the detection window.

Importantly, no N_2O (or $^{15}\text{N}_2\text{O}$) formation was observed to form from hyponitrous acid alone under our reaction conditions in the absence of the Fe porphyrin precursor. This observation is significant, as it supports the intermediacy of the intact di-Fe intermediate $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO})$ (**3**) for N_2O production from hyponitrite.

In summary, we have successfully prepared a hyponitrite adduct of an ethane-linked porphyrin di-Fe complex. This is only the third heme-model hyponitrite complex to be reported in the literature. Compared with the previously reported OEP derivative (Figure 1), the new $[(\text{OEP-CH}_2)\text{Fe}]_2(\eta^1, \eta^1\text{-ONNO})$ is unstable with respect to spontaneous N_2O production in the

absence of added protons, suggesting an important role of the Fe-Fe distances during the heme modeling of the bacterial NO reductase system.

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Supporting Information

Figures, crystal data, listing of bond lengths and angles for compound **2**.

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