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Compound I of Naked Heme (Iron Protoporphyrin IX)

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Gaseous iron protoporphyrin IX (heme) ions, $Fe(PP\text{-}IX)^+$, obtained by electrospray ionization of a methanol solution of hemin chloride, are allowed to react with ozone, forming a species that is tentatively assigned the structure of an oxo complex, namely, an oxo iron(IV) protoporphyrin IX radical-cation species, $(PP\text{-}IX)^{\bullet+}Fe^{IV}{=}O$. This species, representing the naked core of the putative active oxidant (compound **I**) of heme enzymes, is characterized by its reactivity behavior in Fourier transform ion cyclotron resonance mass spectrometry, performing as an active O-atom donor. A quite distinct reactivity is displayed by an isomeric species, holding the additional oxygen on the porphyrin frame, $Fe(PP\text{-}IX(O))^+$. This isomer undergoes a ligand addition process, as was previously observed for $Fe(PP\text{-}IX)^+$.

A variety of heme-containing enzymes performing essential oxidation reactions are known. In a continuous effort to ascertain the significant steps of their catalytic cycles and to develop selective oxidants, several mechanistic and spectroscopic probes have been devised and synthetic metalloporphyrin models have been explored.[1−3] The putative active oxidants are thought to be oxo iron(IV) porphyrin radical-cation species, so-called compound I (Cpd I). Cpd I transients are observed in peroxidases, in catalases, and, recently, in cytochrome P450, a family of enzymes performing dioxygen activation and transfer of an O-atom to compounds of broad chemical diversity.[4,5] A dominant feature of these processes is the dramatic change in the product patterns and selectivities that Cpd I of cytochrome P450 may exhibit under the influence of reaction conditions.[6,7] Computational studies revealed that two closely lying spin states of Cpd I may account for a two-state reactivity, whose regioselectivity is tuned by the protein environment.[8] We now report the synthesis of an ion corresponding to the naked core of Cpd I by the reaction of iron protoporphyrin IX (heme) ions, $Fe(PP\ IX)^+$ (**1**), with ozone in the gas phase, namely, in a dielectric medium resembling to some extent the low-polarity environment surrounding the prosthetic group within a hemeprotein. The formation of a species that is tentatively assigned the structure of an oxo complex, a gaseous oxo iron(IV) protoporphyrin IX radical-cation species, $(PP\text{-}IX)^{\bullet+}Fe^{IV}{=}O$ (**2**), allows a viable entry to a species that proves to be elusive in solution, where it evolves presumably by activating the rapid growth of degradation products. The reactivity properties of **2** with gaseous molecules L, chosen among naturally occurring substrates of hemeproteins and model compounds, are presently explored by electrospray ionization (ESI) coupled with Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry.[9] FT-ICR mass spectrometry allows the kinetic study of ion molecule reactions in a dilute gas phase, in the absence of interfering effects due to the matrix or solvent. In this medium, the reactivity properties of ions **2** are found to match the ones expected from a genuine Cpd I lacking an axial ligand and any protein superstructure. Salient features are their ability to effect O-atom transfer to a wide variety of compounds and their stability toward unimolecular dissociation.

Figure 1 is a high-resolution mass spectrum of the ionic mixture that is obtained when a methanol solution of hemin

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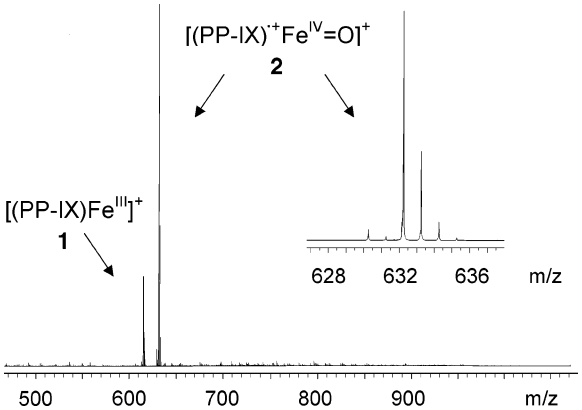

Figure 1. ESI FT-ICR mass spectrum from a methanol solution of (PP-IX)FeCl (10 μM), admitting O_3/O_2 in the capillary/skimmer interface of the ESI source. The inset is an enlargement of the spectrum displaying signals characteristic of (PP-IX)$^{\bullet+}$Fe^{IV}=O ions (**2**).

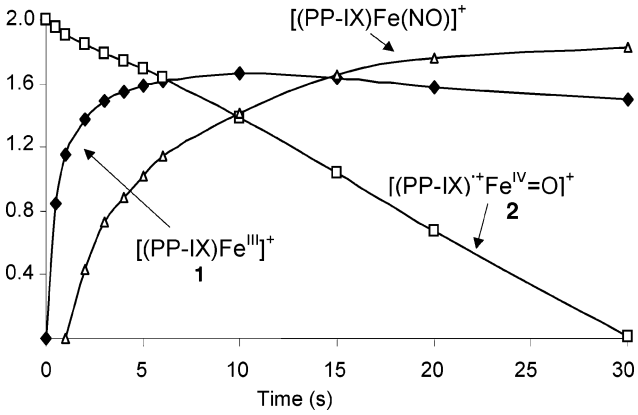

Figure 2. Plot of the logarithm of the relative ion abundances (%) versus time for the reaction of (PP-IX)$^{\bullet+}$Fe^{IV}=O with NO at 3.0×10^{-8} mbar.

chloride, (PP-IX)FeCl, is submitted to ESI and the so-formed ions **1** are exposed to a stream of a O_3 diluted in O_2 in the ionic path leading the ions into the FT-ICR cell.

Any attempt to induce the reaction with O_3 within the cell failed because of the decay of ozone in the metal-lined inlet path. Figure 1 shows ions **1** with their characteristic isotopic pattern, centered at *m/z* 616.201 (calculated value *m/z* 616.177), together with a similar cluster, centered at *m/z* 632.195 (calculated value *m/z* 632.172; calculated ratios of isotopic abundances 6.3/2.4/100/41.3/9.6/1.6 at *m/z* 630, 631, 632, 633, 634, and 635, respectively), which is indicative of the formation of **2**. When **2** is mass selected and allowed to react in the FT-ICR cell with neutrals (L) at a constant pressure in the range of $10^{-8}-10^{-7}$ mbar, an O-atom is released and the reduced species **1** is formed (eq 1).[10]

$$\mathbf{2} + \mathrm{L} \rightarrow \mathbf{1} + [\mathrm{LO}] \qquad (1)$$

The reaction of **2** responds to pseudo-first-order kinetics, and an exponential decay is typically obeyed over several half-lives. Figure 2 illustrates the reaction with NO.

The formation of the reduced species **1**, together with NO_2 as the likely neutral product, is followed by an addition reaction, giving (PP-IX)Fe(NO)$^+$ as the ultimate product ion. The latter addition reaction is amply documented and conforms to the well-established ability of iron porphyrin

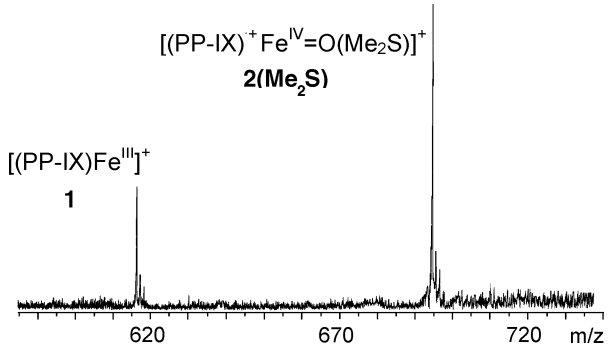

Figure 3. ESI FT-ICR mass spectrum reporting the formation of Fe(PP-IX)$^+$ ions at *m/z* 616 when mass-selected **2(Me$_2$S)** ions at *m/z* 694 are submitted to low-energy CID. Dimethyl sulfoxide is conceivably the neutral fragment released in the dissociation process.

ions with vacant axial sites to undergo ligand addition in the gas phase (eq 2).[11] The so-formed adduct ions (**1L**) revert to **1** upon low-energy collision-induced dissociation (CID) by loss of neutral L.

$$\mathbf{1} + \mathrm{L} \rightarrow \mathbf{1L} \qquad (2)$$

Also, ion **2** may yield an adduct ion, (PP-IX)Fe(O)(L)$^+$ (**2L**), as a side product observed only with L = Me_2S, Me_2S_2, and $P(OMe)_3$ (eq 3).

$$\mathbf{2} + \mathrm{L} \rightarrow \mathbf{2L} \qquad (3)$$

When assayed by CID, **2L** releases **1** as the product ion, implying concomitant loss of LO, as shown in Figure 3 for L equal to Me_2S. One may thus infer that an O-coupling process has taken place between **2** and the O-acceptor L, appearing either as the result of a concerted O-atom-transfer process (eq 1) or from a stepwise addition−elimination process (eq 3 followed by CID). The bimolecular rate constants (k_{exp}) along with the relative efficiencies (Φ) and the branching ratio into the two reaction pathways depicted in eqs 1 and 3 are summarized in Table 1. The reaction efficiencies appear to increase with the oxophilic character of the active site of L (C < N < S < P). Overall, ozone results as viable oxidant[12] in the controlled conditions adopted in this study, via the intermediacy of **2**. At variance with

(10) The experiments were run on a Bruker BioApex 4.7T FT-ICR instrument with an Analytica of Branford ESI source. Pseudo-first-order rate constants, from the slope of the semilog decrease of the parent ion abundance, divided by the substrate concentration give second-order rate constants (k_{exp}). The reaction efficiencies (Φ) are percentages of the collision rate constant (k_{coll}) (Su, T.; Chesnavich, W. J. *J. Chem. Phys.* **1982**, *76*, 5183). The so-obtained second-order rate constants and the product distribution are found to be invariant with respect to the ligand pressure and to the presence of an added inert bath gas (Ar). The mild transfer of ions to the gas phase ensured by ESI and the production of **2** by the reaction of **1** with O_3 at relatively high pressure provide a basis for the formation of ground-state ions. Indeed, no evidence for the presence of excited species or multiple electronic states, possibly causing biphasic kinetic plots, is ever obtained in the present study.

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(12) The O-atom-transfer cascade from O_3 ($D(O_2-O)$ = 106 kJ mol^{-1}) via **2** to pyridine ($D(C_5H_6N-O)$ = 301 kJ mol^{-1}) suggests a Fe−O bond energy for **2** in the 106−301 kJ mol^{-1} range, accounting for the observed O-atom-transfer reactivity. (a) Holm, R. H. *Chem. Rev.* **1987**, *87*, 1401. (b) Deubel, D. V. *J. Am. Chem. Soc.* **2004**, *126*, 996.

Table 1. Gas-Phase Reactivity of Selected Substrates (L) with $(PP\text{-}IX)^{\bullet+}Fe^{IV}(O)^{+}$ (**2**) Formed by the Reaction of $Fe(PP\text{-}IX)^{+}$ (**1**) with O_3

L	k_{exp}[a]	Φ[b]	product ions **1**/(PP-IX)Fe(O)(L)$^{+}$
NO	3.0	4.2	100/0
NO_2	1.9	2.9	100/0
propyne	0.036	0.027	100/0
Me_2S	1.7	1.3	80/20
Me_2S_2	2.8	2.4	90/10
pyridine	2.4	1.7	100/0
$P(OMe)_3$	8.6	7.3	95/5

[a] k_{exp} in units of 10^{-11} cm^3 molecule^{-1} s^{-1} at 300 K. Estimated error ±30%. The following compounds failed to react with **2**: CO, $H_2{}^{18}O$, styrene, cyclohexene, and cycloheptatriene ($k_{exp} < 10^{-13}$ cm^3 molecule^{-1} s^{-1}). [b] $\Phi = k_{exp}/k_{coll} \times 100$. Φ is a measure of the collision events leading to products.

the reactivity behavior displayed by **2**, bare FeO^{+} does not oxygenate O- and S-based reductants in the gas phase.[13] The two species, though, are different in the coordination and electronic states of the metal, and distinct reactivity properties are to be expected.[3,8]

Among the tested hydrocarbons, only propyne does react, albeit with very low efficiency ($\Phi = 0.027\%$). This finding is in line with the reactivity behavior of oxometalloporphyrins that are about 50-fold less reactive with typical olefins relative to dialkyl sulfides in solution reactions. Quite in contrast, NO and NO_2 are noticeably reactive, in line with their acknowledged interaction with Cpd I in nature. In the gas phase, the intermediacy of a transient species analogous to **2** has been invoked to account for the formation of a nitrosyl adduct from the reaction of **1** with NO_2.[14] However, **2** fails to yield nitrosylation products by reaction with NO_2, rather releasing **1** and NO/O_2 as the likely neutral products (eq 4).

$$\mathbf{2} + NO_2 \rightarrow \mathbf{1} + [NO + O_2] \qquad (4)$$

The structural features assigned to ion **2** are further substantiated if one compares its gas-phase bimolecular reactivity with the reactivity behavior of an oxo iron(IV) porphyrin radical-cation complex that can be accessed in solution. The $(TPFPP)^{\bullet+}Fe^{IV}{=}O$ ion, where TPFPP is the *meso*-tetrakis(pentafluorophenyl)porphinato dianion, bears electron-withdrawing pentafluorophenyl substituents on the meso positions, making it a long-lived species that can be formed by oxidation of (TPFPP)FeCl in solution. The ESI FT-ICR study of its gas-phase reactivity with a variety of compounds, including the substrates listed in Table 1, has disclosed competitive pathways quite analogous to the ones described by eqs 1 and 3.[15] As described in detail in a previous report,[15] product ions are either the reduced species, $Fe(TPFPP)^{+}$, or an adduct ion that releases an oxygenated neutral when submitted to low-energy CID. The close similarity in the reactivity behavior displayed by $(TPFPP)^{\bullet+}$-$Fe^{IV}{=}O$ and **2** provides support for the oxo−iron character of **2**. On quantitative grounds, $(TPFPP)^{\bullet+}Fe^{IV}{=}O$ is endowed with higher reactivity (for example, Φ is equal to 3.5 and 6.1 for reactions with Me_2S and pyridine, respectively),[15] as was expected in view of its electronic activation.[2a,16] However, **2** displays a distinct bias toward NO and NO_2, with Φ values of 4.2 and 2.9, respectively (Table 1), that can be compared with the Φ values of 2.5 and 2.0 reported for the reactions of $(TPFPP)^{\bullet+}Fe^{IV}{=}O$.[15]

In order to provide further grounds to the assignment of an oxo−iron structure to the sampled ionic species, routes were explored for forming a distinct isomer holding the additional O atom on the porphyrin frame. Such a species can be obtained by controlled oxidation of hemin chloride using iodosylbenzene in methanol or, more easily, by treating an aerated solution of hemin with dithiothreitol.[17] Isobaric ions at *m*/*z* 632, as obtained by ESI, were led into the FT-ICR cell and sampled for their gas-phase chemistry, which was found to conform to the reactivity behavior expected for a porphyrin-oxidized species, $Fe(PP\ IX(O))^{+}$ (**3**). Thus, the reaction with NO yields exclusively an addition product, in marked contrast with the O-atom-transfer reactivity displayed by **2**. The kinetics of the NO addition reaction yield a Φ value of 4.6, not far from the Φ value of 3.0 characterizing the addition of NO to **1**.[11a,b] This common ligand addition reactivity displayed by **3** and **1** suggests that NO is reacting with two similar active sites. This feature holds true if the entering ligand interacts with iron(III) in a coordination environment differing only for the presence of an additional O atom on the porphyrin ligand. In a similar way, the reaction of **3** with pyridine yields an adduct ion that is found to release the intact neutral ligand when submitted to CID. Under no circumstances is any O-atom-transfer reactivity by ion **3** ever observed.

The reported gas-phase chemistry of **1** and **2** can be envisioned as an approach to the stoichiometric oxidation of organic and inorganic molecules by O-atom transfer from ozone. Notably, intervention of ozone as a novel reactive oxygen species in metabolism has been recently invoked.[18] Circumstantial evidence has been obtained about the formation of a species, **2**, described as an oxo iron(IV) porphyrin radical-cation complex, displaying appropriate O-atom-transfer reactivity. Ultimately, the present study is aimed at providing a simplified model of enzyme chemistry in the gas phase.

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