Electrospray Ionization Mass Spectral Characterization of Transient Iron Species of Bioinorganic Relevance

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Strategies have been developed to obtain electrospray ionization mass spectral data on short-lived intermediates derived from the reactions of non-heme iron complexes with peroxides. The molecular composition of a transient green intermediate generated from $[Fe_2O(5\text{-}Me_3\text{-}TPA)_2(OH)(H_2O)](ClO_4)_3$ with H_2O_2 in CH_3CN at −40 °C was determined by introducing the solution via a precooled syringe to the inlet of the mass spectrometer. The observation of prominent ion clusters in both positive and negative mass regions, together with isotope distribution patterns characteristic of the number of associated ClO_4 ions, allows its formulation as $[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_3$. The molecular composition of a transient blue species derived from the reaction of $[Fe_2O(TPA)_2(H_2O)(ClO_4)](ClO_4)_3$ with excess benzyl alcohol and ^tBuOOH at −40 °C was also determined. Due to its limited stability even at −40 °C, the blue intermediate was generated in a cooled mixing tee from its precursor components and directly introduced into the mass spectrometer. Its formulation as $\{[Fe(TPA)(OO^tBu)(ROH)](ClO_4)\}^+$ ($R = H$ or CH_2Ph) is based on the masses observed, isotope distribution patterns, the observation of expected shifts in the mass values by appropriate substitutions, and tandem mass spectral data. These transient species relate to possible intermediates of non-heme iron enzymes.

Electrospray ionization mass spectrometry (ESI-MS) has found extensive application in the characterization of high molecular weight biopolymers because of the mildness of the ionization method.[1] This technique has also been useful for the characterization of multiply charged metal complexes,[2] which have proved difficult to study by fast atom bombardment and laser desorption methods. Recently, ESI-MS has been applied to the identification of metastable species. By this method, an intermediate in the reaction of the antitumor drug Fe-bleomycin with O_2, "activated bleomycin", has been characterized as a (hydroperoxo)iron(III) complex,[3] demonstrating that ESI-MS can be useful for solving problems in bioinorganic chemistry. As part of our effort to understand the mechanisms of non-heme iron enzymes, we recently focused on the reactions of synthetic non-heme iron complexes with dioxygen[4] or peroxides.[5] In the course of this work, we have uncovered conditions under which a number of novel transient species can be generated. We have found that ESI-MS can be useful in establishing the molecular composition of these species and detail two pertinent examples in this paper, the characterization of a high-valent $Fe_2(\mu\text{-}O)_2$ complex and an (alkylperoxo)iron(III) species, to illustrate the strategies we have used and their utility.

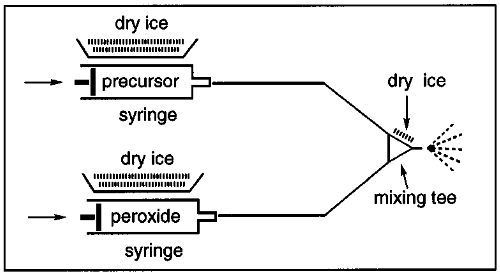

Figure 1. Schematic diagram of the low-temperature mixing apparatus for mass spectrometry with an electrospray ion source, by which intermediate **3** was analyzed.

Experimental Section

Sample Preparation: $[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_3$ (5-Me$_3$-TPA = tris(5-methyl-2-pyridylmethyl)amine) (**1**)[5a] and $[Fe_2O(TPA)_2(H_2O)(ClO_4)](ClO_4)_3$[6] (TPA = tris(2-pyridylmethyl)amine) were prepared as described previously. For the mass spectral experiment, a 0.7 mM solution of **1** was prepared in CH_3CN at −40 °C and transferred to a syringe precooled with dry ice immediately before injection. Intermediate **3** was prepared using the mixing apparatus illustrated in Figure 1. A pair of Harvard Apparatus (South Natick, MA) Model 22 syringe pumps were used to deliver the reactant solutions to an Upchurch Scientific (Oak Harbor, WA) metal-free static mixing tee. Solutions in the syringes and in the mixing tee were cooled by placing powdered dry ice in Al foil boats at these locations. The temperatures inside the mixing tee and the syringes were estimated to be −35 and −40 °C,

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respectively, using a thermocouple. The calculated dead volume in the mixing tee was about 3.1 μL. All connections were made using fused-silica capillary tubing (0.076 mm i.d.). The flow rates of both syringes were equal and could be varied from 10 to 50 μL/min. The effluent from the mixing tee entered the ionization chamber within 3 s. To obtain the mass spectrum of intermediate **3**, an CH_3CN solution of 1.0−2.0 mM $[Fe_2O(TPA)_2(H_2O)(ClO_4)](ClO_4)_3$ in the presence of alcohol (0.1−0.2 M) was mixed with an CH_3CN solution of 5−100 mM alkyl hydroperoxide in the tee. The solutions were precooled in a cold bath (−40 °C) before being transferred into each syringe. The mixing apparatus was flushed with cold CH_3CN (−40 °C) immediately before each run.

Instrumentation. Electrospray ionization mass spectra were acquired using a PE Sciex (Sciex, Thornhill, Ontario, Canada) API III triple quadrupole mass spectrometer. The flow rate of the coaxial nebulizer gas (air) was 0.4 L/min and that of the nitrogen curtain gas was 0.8 L/min. Spectra were acquired, using a step size of 0.2 D, 5−10 ms dwell per step. Typical operating voltages were 5000 V needle voltage (ISV) and 35 V orifice voltage (OR) for positive ions and −3500 V ISV and −35 V OR for negative ions.

Tandem mass spectra (MS/MS) were obtained by collision-induced dissociation of precursor ions selected in the first quadrupole. The argon pressure in the collision cell was adjusted to reduce the precursor ion intensity by about 50%, which corresponded to a collision gas thickness (CGT) of $(1.7-3.1) \times 10^{14}$ molecules/cm^2. Precursor translational ion energy ($R_0 - R_2$, where R_n is the quadrupole rod offset voltage) was varied from 50 to 110 eV (laboratory reference frame). Instrument settings, data acquisition, and data processing were controlled by a Macintosh computer. Spectra were signal-averaged by acquisition in multichannel analyzer (MCA) mode.

Results and Discussion

A number of transient species can be observed in the reactions of synthetic non-heme iron complexes with peroxides.[4,5,7] By suitable manipulation of the ligands and going to low temperatures (−40 °C), these species can be generated with sufficient lifetimes to allow their characterization by a number of spectroscopic methods, including electrospray ionization mass spectrometry (ESI-MS). As the first example of our experimental strategies, we discuss our studies on an $[Fe_2(\mu\text{-}O)_2]^{3+}$ species, which may serve as a model for high-valent intermediates in the oxygen activation chemistry of non-heme diiron enzymes.[8] Thus treatment of $[Fe_2O(5\text{-}Me_3\text{-}TPA)_2(OH)(H_2O)](ClO_4)_3$ with H_2O_2 in CH_3CN at −40 °C affords a transient green intermediate with novel spectroscopic properties that are associated with a bis(μ-oxo)diiron(III,IV) complex, $[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_3$ (**1**).[5a] **1** is stable for weeks at −40 °C but decomposes within minutes at room temperature. When introduced via a precooled syringe to the inlet of the mass spectrometer, **1** affords an ESI-MS spectrum that unambiguously establishes its molecular composition (Figure 2). Prominent ion clusters can be observed in both positive and negative mass regions at *m*/*z* values corresponding to the molecular ions $\{[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_2\}^+$ and $\{[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_4\}^-$, providing strong evidence that the parent complex has the formula $[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_3$. The molecular compositions of the ions are further corroborated by the appearance of isotope distribution patterns that are characteristic of the deduced formula of each of the ions observed. The use of ClO_4 as the counterion with its characteristic 3:1 ^{35}Cl:^{37}Cl isotope distribution affords a complex pattern that allows us to determine unambiguously the number of perchlorates associated with the ion and thus infer the charge of the complex cation and the formal oxidation states of the metal centers. Thus the ESI-MS results and those of other spectroscopic techniques[5a] combine to afford the conclusion that **1** contains the novel bis(μ-oxo)diiron(III,IV) core structure. It represents the first example of a high-valent non-heme iron−oxo complex relevant to the mechanisms of methane monooxygenase[9] and ribonucleotide reductase.[10]

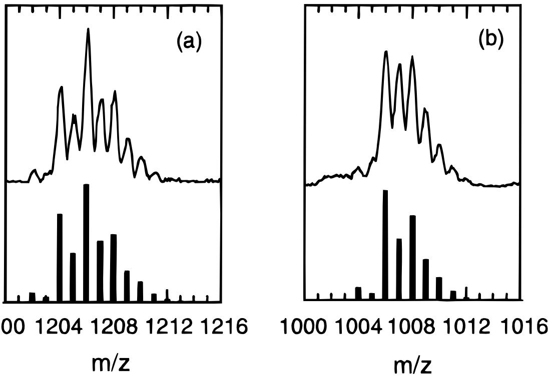

Figure 2. Negative (a) and positive ion (b) electrospray ionization mass spectra of **1**. The upper traces show the experimental data, while the bars below represent the isotopic distributions calculated for $\{[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_4\}^-$ (a) and $\{[Fe_2(O)_2(5\text{-}Me_3\text{-}TPA)_2](ClO_4)_2\}^+$ (b).

ESI-MS has also proven useful in the characterization of a transient blue species that has been shown to oxidize alcohols directly and is proposed to be involved in the oxidation of alkanes in $[Fe^{III}{}_2O(TPA)_2]^{4+}$/ROOH systems.[5c] EPR studies show that the reaction of $[Fe_2O(TPA)_2(H_2O)(ClO_4)](ClO_4)_3$ with excess benzyl alcohol in CH_3CN affords a mononuclear complex **2**. Complex **2** gives rise to ESI-MS signals at m/z 560, 552, and 470, with mass values and isotopic distribution patterns which indicate that **2** is best formulated as an equilibrium mixture of two species $[Fe(TPA)(OCH_2Ph)(ROH)](ClO_4)_2$ (R = H, CH_2Ph). The ions observed derive from loss of neutral $HClO_4$ or ROH from the parent ion, $\{[Fe(TPA)(OCH_2Ph)(ROH)](ClO_4)\}^+$, as shown in Scheme 1.

Addition of ^tBuOOH to **2** at −40 °C affords blue intermediate **3**, which has a limited lifetime even at −40 °C.[5c] Thus introduction of a solution of **3** via a precooled syringe, as previously done for the $Fe_2(\mu\text{-}O)_2$ complex, affords only ESI-MS signals associated with **2** due to decomposition of **3** back to **2** under the conditions of the experiment. However new positive ion signals at m/z 542, 534, and 452 can be observed

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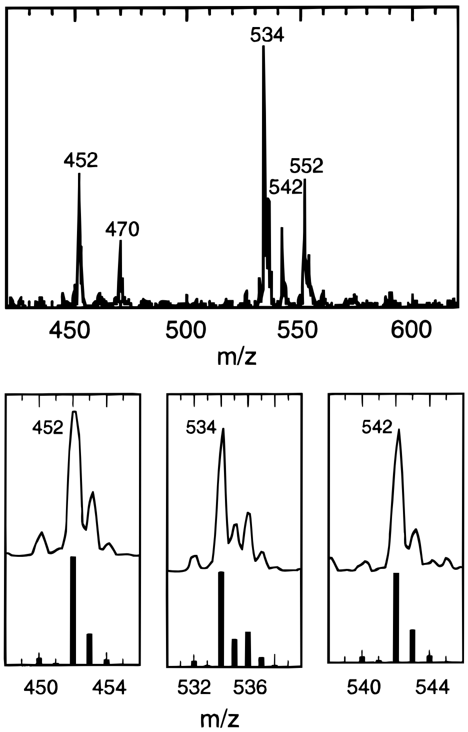

Figure 3. Top: Mass spectrum of **3** generated from the reaction of **2** and ^{t}BuOOH. Peak assignments are indicated. The *y* axis indicates relative peak intensity. Bottom: Observed and calculated isotopic distribution patterns for the three major features in the mass spectrum of **3**.

Scheme 1

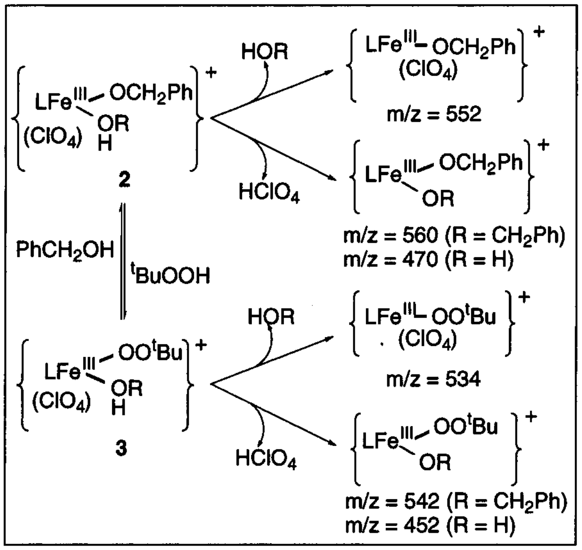

Table 1. Electrospray Mass Spectral Data of **2** and **3**

R'	R''	R	{[(TPA)Fe(OR')(OR)$_m$](ClO$_4$)$_n$}$^+$		{[(TPA)Fe(OOR'')(OR)$_m$](ClO$_4$)$_n$}$^+$	
			m=1, n=0	m=0, n=1	m=1, n=0	m=0, n=1
CH$_2$Ph	^{t}Bu	R'	560 (*602*)^a	552 (*594*)^a	542 (*582*)^a	534 (*576*)^a
		H	470 (*512*)^a		452 (*494*)^a	
	cumyl	R'	560	552	604	596
		H	470		514	
CH$_3$	^{t}Bu	R'	484	476	466	534
		H	394		376	

[a] Value observed when TPA is replaced with (5-Me)$_3$TPA.

(Figure 3) when precooled solutions of the precursor components are combined in a mixing tee (Figure 1) and the effluent from the tee is introduced directly into the mass spectrometer. The intensities of these signals increase relative to those associated with **2**, as the rate of mixing is increased, consistent with the metastable nature of **3**. The mass values and isotope distribution patterns observed for these ions formulate **3** as an equilibrium mixture of two species, [Fe(TPA)(OO^{t}Bu)(ROH)](ClO$_4$)$_2$ (R = H, CH$_2$Ph), with the observed ions derived from the loss of either HClO$_4$ or ROH from the parent ion {[Fe(TPA)(OO^{t}Bu)(ROH)](ClO$_4$)}$^+$ as shown in Scheme 1. Negative ion clusters at *m/z* 650 and 732 can also be observed with appropriate isotope distribution patterns that correspond to the ions {[Fe^{III}(TPA)(OH)(OO^{t}Bu)](ClO$_4$)$_2$}$^-$ and {[Fe^{III}(TPA)(OO^{t}Bu)](ClO$_4$)$_3$}$^-$, respectively. Further corroboration of the proposed formulation can be obtained by observing expected shifts in the mass values when substitutions are made (Table 1). For example, when 5-Me$_3$-TPA is used in place of TPA, the mass values of the three positive ions associated with **3** upshift by 42 mass units. When cumene hydroperoxide is used in place of ^{t}BuOOH, the mass values of the appropriate ions all upshift by 62 mass units, the mass difference between a *tert*-butyl and a cumyl group. When methanol is used in place of benzyl alcohol, the mass values of the appropriate ions downshift by 76 mass units. These observations are all consistent with **3** having the formula [Fe(TPA)(OO^{t}Bu)(ROH)](ClO$_4$)$_2$.

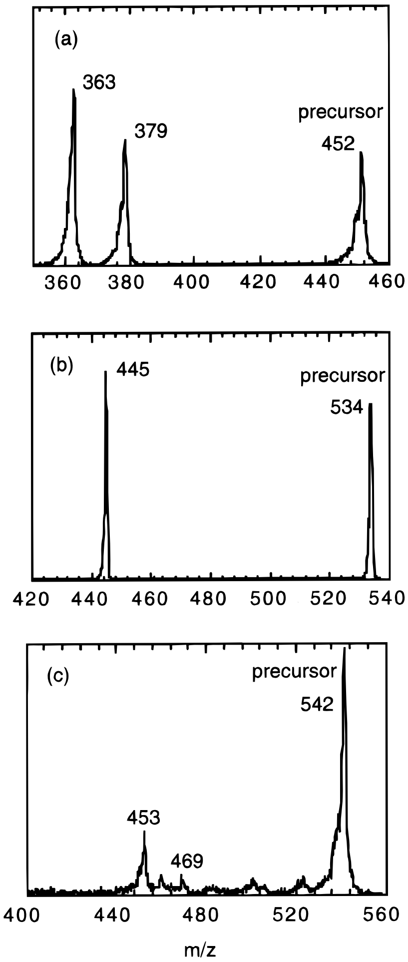

Figure 4. Tandem mass spectra of the signals at (a) *m/z* 452 (CGT, 3.1 × 10^{14} molecules/cm^2, R_0 = 30 eV, R_2 = −30 eV), (b) *m/z* 534 (CGT, 3.1 × 10^{14} molecules/cm^2, R_0 = 30 eV, R_2 = −20 eV), and (c) *m/z* 542 (CGT, 1.7 × 10^{14} molecules/cm^2, R_0 = 30 eV, R_2 = −80 eV). The precursor ion is indicated in each spectrum. The *y* axis indicates relative peak intensity.

An alternative formulation for **3** that is equally consistent with the mass spectral data discussed thus far is [Fe(TPA-O)(O^{t}Bu)(ROH)](ClO$_4$)$_2$, wherein the ^{t}BuOO$^-$ group has been reduced to $^tBuO^-$ and one of the pendant pyridines on TPA has been converted to its *N*-oxide. For example, the *m/z* 452 ion could also be formulated as $\{Fe^{III}(TPA\text{-}O)(O^tBu)(OH)\}^+$ instead of $\{Fe(TPA)(OO^tBu)(OH)\}^+$. We have thus obtained tandem mass spectral data (Figure 4) to determine whether the alkylperoxo moiety remains intact in **3** as suggested by our resonance Raman studies.[5c] Thus isolation of each of the three ions at *m/z* 542, 534, and 452 and observation of their individual fragmentations show that all three exhibit loss of a ^tBuOO fragment (89 mass units), corresponding to the homolysis of an $Fe-OO^tBu$ bond. Such a fragmentation mechanism would not be available for the alternative formulation. The fragmentation of the *m/z* 452 ion also shows the loss of ^tBuO (73 mass units) to form $\{Fe^{IV}(TPA)O(OH)\}^+$, but the *m/z* 534 ion does not. The loss of ^tBuO corresponds to the homolysis of the $FeO-O^tBu$ bond, and the difference in fragmentation behavior may be rationalized by the nature of the sixth ligand. The *m/z* 452 ion has a hydroxide ion, which can stabilize the higher-valent Fe^{IV} species and thus promote its formation; on the other hand, the *m/z* 534 ion contains perchlorate, which is much more weakly basic, and thus does not show evidence of loss of the ^tBuO fragment. The *m/z* 542 ion with an alkoxide as the sixth ligand would also be expected to form the higher valent Fe^{IV} species, and a signal at *m/z* 469 (M − 73) can be discerned just above the noise. The fragmentation of the *m/z* 452 ion to afford a mononuclear non-heme iron(IV)−oxo species is a rare instance of observing such a reactive intermediate; only under matrix isolation conditions has an analogous species been previously observed.[11]

The described electrospray ion source produces intense beams of iron complex ions. Both molecular weight and structural information can be obtained by using the special experimental setup which allows the transient species during the reaction at low temperature to be detected. We have been able to analyze the reaction mixture and observe mass spectra that correspond to the key intermediate in the proposed mechanism for the reaction. Many transient intermediates have been reported during the catalytic reactions with metalloenzymes and transition metal complexes, but they are difficult to characterize due to their instability under the reaction conditions. Therefore, this low-temperature application can make electrospray ionization mass spectrometry a valuable mechanistic tool for characterizing transient catalytic intermediates.

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