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Characterization of a Synthetic Peroxodiiron(III) Protein Model Complex by Nuclear Resonance Vibrational Spectroscopy

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Abstract

The vibrational spectrum of an η^1, η^1 -1,2-peroxodiiron(III) complex was measured by nuclear resonance vibrational spectroscopy and fit using an empirical force field analysis. Isotopic $^{18}\text{O}_2$ labelling studies revealed a feature involving motion of the $\{\text{Fe}_2(\text{O}_2)\}^{4+}$ core that was not previously observed by resonance Raman spectroscopy.

To understand better the molecular mechanisms of O_2 activation by carboxylate-bridged diiron enzymes,^{1,2} it is desirable to determine the structures of intermediates that form in the process. Exposure of the Fe^{II}_2 cores of carboxylate-bridged diiron proteins to O_2 often generates transient peroxodiiron(III) intermediates.^{3–9} Chart 1 depicts the possible coordination modes of a bridging O_2^{2-} ligand at a dinuclear iron center.¹ Studies of such peroxo units in the R2 subunit of ribonucleotide reductase (RNR),^{10,11} soluble methane monooxygenase hydroxylase (sMMOH),^{12,13} toluene 4-monooxygenase hydroxylase (T4moH),¹⁴ and Δ^9 desaturase¹⁵ have suggested that the reduced O_2 molecule is bound to the diiron core in an η^1, η^1 -1,2 fashion. Recent quantum mechanical/molecular mechanics (QM/MM) investigations of the peroxo intermediates in the T201S mutant¹⁶ of toluene/o-xylene monooxygenase hydroxylase (ToMOH)^{17,18} favor formation of both η^1, η^1 -1,2- and η^1, η^1 -1,1-peroxodiiron species upon reaction with O_2 . The peroxo ligand in the latter structure is believed to be protonated and further stabilized by hydrogen bonding to a nearby threonine residue, perhaps with an intervening water molecule. Attempts to characterize the ToMOH intermediates by resonance Raman (rR) spectroscopy or X-ray crystallography have not yet been successful, however. Although QM/MM theoretical studies have provided some insight into the nature of these $\{\text{Fe}_2(\text{O}_2)\}^{4+}$ units,^{16,19–23} new methods are required to study the protein intermediates directly.

Nuclear resonance vibrational spectroscopy (NRVS)^{24–27} is a valuable methodology recently applied in bioinorganic chemistry. For example, NRVS has been used to assign

metal-ligand vibrational modes of diatomic molecules coordinated to porphyrins^{26,28,29} and to detect nitrosylated iron-sulfur clusters in proteins.^{30,31} NRVS and density functional theoretical (DFT) studies of mononuclear Fe(III)–OOH³² and Fe(IV)=O^{33,34} compounds have provided insight into their distinctive chemical properties. In the present communication we describe the results of a study to evaluate NRVS as a means to interrogate the binding modes of peroxide ion at diiron centers in oxygenated protein intermediates by investigating a well-defined *cis-η¹,η¹*-1,2-peroxodiiron(III) protein model complex, [Fe₂(μ-O₂)(*N*-EtHPTB)(PhCO₂)](BPh₄)₂ (**1**·O₂, where *N*-EtHPTB = anion of *N,N,N',N'*-tetrakis(2-benzimidazolyl-methyl)-2-hydroxy-1,3-diaminopropane) (Chart 2).^{35–37}

To determine iron-ligand modes that arise from the *N*-EtHPTB and benzoate groups, the parent diiron(II) complex³⁶ [Fe₂(*N*-EtHPTB)(PhCO₂)](BPh₄)₂ (**1**) was studied by NRVS. As shown in Fig. 1A (blue), polycrystalline **1** exhibits intense features in the 150 to 350 cm^{−1} region of the spectrum. Because of the mixed ligand environment of **1**, this large envelope contains several overlapping Fe–N and Fe–O vibrations arising from benzimidazole, amine, alkoxide, and carboxylate units that are coordinated to the iron atoms. When a solution of **1** in tetrahydrofuran is exposed to dioxygen, a deep blue color rapidly develops, indicating generation of the peroxodiiron(III) complex **1**·O₂. The NRVS of **1**·¹⁶O₂ and **1**·¹⁸O₂ displayed intense bands between 150–300 cm^{−1} (Fig. 1B and 1C, respectively, blue), assigned to iron-ligand (*N*-EtHPTB/PhCO₂[−]) modes. In addition to these features, higher frequency bands at 338 and 467/480 cm^{−1} were observed for **1**·¹⁶O₂, where the latter two peaks are attributed to Fermi splitting.³⁶ By comparison to the spectrum of **1** (Fig. 1A, blue), these higher energy modes of **1**·O₂ are probably due to motions involving the {Fe₂(O₂)⁴⁺} unit. Upon ¹⁸O₂-isotopic labelling, these features shifted to 311 and 446/458 cm^{−1}, respectively.

These results are consistent with previously reported resonance Raman spectra of **1**·O₂.^{35,36} Most notably, the rR spectrum of **1**·¹⁶O₂ exhibits bands at 466/474 cm^{−1} that shift to a single peak at 452 cm^{−1} upon substitution of ¹⁶O₂ with ¹⁸O₂ (Fig. S1A). The 300–350 cm^{−1} region of the rR spectrum does not show any resonance enhanced vibrations at the accessible excitation wavelengths (Fig. S1B). The ability of NRVS to reveal a distinctive {Fe₂(O₂)⁴⁺} mode at 338 cm^{−1}, not previously observed for **1**·¹⁶O₂ and shifting to 311 cm^{−1} for **1**·¹⁸O₂, illustrates the utility of this spectroscopic method.

To obtain a qualitative description of the modes that display significant ¹⁶O₂/¹⁸O₂ isotopic shifts in the NRVS, normal coordinate analyses were performed for **1**·¹⁶O₂, and **1**·¹⁸O₂ using VIBRATZ.^{38,39} To test the validity of the VIBRATZ simulations, the NRVS of **1** was calculated using the Cartesian coordinates of the diiron core from its X-ray crystal structure.³⁵ As shown by the red trace in Fig. 1A, the calculated spectrum reproduces the experimental one (blue) to a satisfactory first approximation. A complete assignment of this spectral region is beyond the scope of this study. Most importantly, the simulated NRVS of **1** does not show any peaks at energies greater than 350 cm^{−1}.

Because an X-ray structure of **1**·O₂ is not available, the geometry of its primary coordination sphere was modeled using the Cartesian coordinates from the X-ray structure of [Fe₂(μ-O₂)(Ph-bimp)(PhCO₂)]²⁺ (**2**·O₂, Chart 2),⁴⁰ which has a ligand environment similar to that of **1**·O₂.³⁷ Although the structure of [Fe₂(μ-O₂)(*N*-EtHPTB)(OPPh₃)₂]³⁺ (**3**·O₂) is known,⁴¹ it has two triphenylphosphine oxide ligands rather than a benzoate group. The NRVS of **1**·¹⁶O₂ and **1**·¹⁸O₂ were simulated using the {Fe₂N₆O(μ-O₂)(μ-PhCO₂)²⁺} core of **2**·O₂ as a structural model (Fig. 1B and 1C, respectively, red traces). In agreement with experiment, the computed difference spectrum (**1**·¹⁸O₂ minus **1**·¹⁶O₂) showed that ¹⁸O₂ substitution should result in isotopically shifted peaks in the ~300–600 cm^{−1} region (Fig. S2). An

additional isotope-sensitive mode was also calculated at 898 cm^{-1} for $1\text{-}^{16}\text{O}_2$ and 847 cm^{-1} for $1\text{-}^{18}\text{O}_2$, frequencies outside the window that was measured.

The highest energy calculated feature, at $898(817)\text{ cm}^{-1}$, is primarily a symmetric O–O stretching mode (ν_1 , Fig. 2). Because ν_1 does not involve significant motion of the iron atoms, it is not likely to be very intense in the NRVS. The experimentally determined value for the symmetric $\nu(\text{O}–\text{O})$ mode of 1-O_2 by rR spectroscopy, $897(847)\text{ cm}^{-1}$ (Fig. S1A),^{35,36} is in good agreement with the calculated one. The second highest energy mode falls at $579(551)\text{ cm}^{-1}$ and is essentially the asymmetric O–O stretching/rotation of the peroxo ligand against the iron atoms (ν_2 , Fig. 2). Although the net stretching of the Fe–O(peroxo) bonds is minimal, ν_2 is expected to be observable by NRVS. The spectra of 1-O_2 , however, do not show any features in this region (Figs. 1B and 1C). It is possible that conformational heterogeneity or coupling with ligand vibrations, in a more complete model, may explain the absence of the feature at $579(551)\text{ cm}^{-1}$ in the NRVS. The weak bands at $513/532(500)\text{ cm}^{-1}$ in the rR spectrum³⁶ of 1-O_2 (Fig. S2A) might conceivably correspond to this ν_2 mode. The calculated peak at $471(452)\text{ cm}^{-1}$ (ν_3) is attributed to the symmetric Fe–O–O–Fe stretching motion. The frequency of this mode depends almost entirely on the Fe–OO force constant (Fig. 2). The theoretical frequency of ν_3 matches well those of 1-O_2 , observed at $467/480(446/458)\text{ cm}^{-1}$ in the NRVS (Figs. 1B and 1C) and at $466/474(452)\text{ cm}^{-1}$ in the rR spectra (Fig. S1A).³⁶ Lastly, the isotopically shifted peak with lowest energy was calculated at $325(313)\text{ cm}^{-1}$ (ν_4 , Fig. 2). In this mode, the O–O group moves as a unit parallel to the Fe–Fe vector and perpendicular to the pseudo-mirror plane that bisects the $\text{Fe}_2(\text{O}_2)$ atoms. It is possible that the ν_4 mode is absent in the rR spectrum because it is not strongly coupled to the electronic transition excited at 647 nm , the wavelength employed in the experiment.

In conclusion, the vibrational profile of a synthetic peroxodiiron(III) protein model complex has been revealed by nuclear resonance vibrational spectroscopy. Through $^{16}\text{O}_2/^{18}\text{O}_2$ isotopic labelling, the frequencies that correspond to motions of the $\{\text{Fe}_2(\text{O}_2)\}^{4+}$ unit in $[\text{Fe}_2(\mu\text{-O}_2)(N\text{-EtHPTB})(\text{PhCO}_2)]^{2+}$ have been assigned. Most notably, a lower energy mode at $\sim 338(311)\text{ cm}^{-1}$ involving parallel motion between the Fe–Fe and O–O groups has been detected by NRVS, a feature that was not previously observed by resonance Raman spectroscopy. Although a more comprehensive study is needed to correlate the vibrational characteristics of a peroxodiiron unit to its O_2 coordination geometry, these results demonstrate that synchrotron-based NRVS is a useful tool to interrogate the structure of oxygenated diiron protein intermediates. Such studies may help to clarify remaining questions regarding the mechanism of O_2 activation in carboxylated bridged diiron proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. ^{57}Fe partial vibrational density of states (PVDOS) for compounds **1** (A, 60 K, top), **1**· $^{16}\text{O}_2$ (B, 45 K, middle), and **1**· $^{18}\text{O}_2$ (C, 60 K, bottom) measured by NRVs. Panel A: Blue spectrum (selected peaks) = 182, 224, 240, 298, and 321 cm $^{-1}$. Red spectrum (selected peaks) = 192, 146, 195 cm $^{-1}$. Panel B: blue spectrum (selected peaks) = 190, 280, 338, 467, 480 cm $^{-1}$; red spectrum (selected peaks) = 176, 225, 264, 325, 471, 579 cm $^{-1}$. Panel C: blue spectrum (selected peaks) = 186, 241, 281, 311, 446, 458 cm $^{-1}$; red spectrum (selected peaks) = 175, 224, 262, 313, 452, 551 cm $^{-1}$. Color scheme: raw data in blue, empirical data fit in red, and individual eigenmode frequencies/intensities before broadening in black.

Fig. 2. Normal mode calculations for $\mathbf{1} \cdot \text{O}_2$ using the X-ray coordinates of $[\text{Fe}_2(\mu\text{-O}_2)(\text{Ph-bimp})(\text{PhCO}_2)_2]^{2+} (\mathbf{2} \cdot \text{O}_2)$, showing the vibrations that involve the Fe_2O_2 unit. The black arrows indicate the direction and relative degree of motion of the atoms to which they are attached. Color scheme: orange, iron; red, oxygen; blue, nitrogen; and gray, carbon.

Chart 1.
Possible geometries of the $(\mu\text{-peroxo})\text{diiron}$ unit.

Chart 2.

A proposed structure of $[Fe_2(\mu-O_2)(N-EtHPTB)(PhCO_2)]^{2+}$ ($1 \cdot O_2$, left). The benzoate ligand in $1 \cdot O_2$ may be coordinated in a terminal rather than bridging fashion.³⁷ The X-ray structure of $[Fe_2(\mu-O_2)(Ph-bimp)(PhCO_2)]^{2+}$ ($2 \cdot O_2$, right) has been determined.³⁹