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LETTERS

Revisiting the Putative Ferryl Tilting Mode of Oxidized Cytochrome *c* Oxidase with Density Functional Vibrational Analyses of Model Complexes

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Full vibrational analyses have been carried out on Fe(IV)═O model complexes using energy second derivatives obtained with nonlocal density functional theory. The calculations are consistent with a controversial proposal that a 356 cm^{-1} band in the resonance Raman spectrum of oxidized cytochrome *c* oxidase, which exhibits an ^{18}O downshift of -14 cm^{-1}, is a ferryl tilting mode.

Cytochrome *c* oxidase (CcO) is a terminal enzyme of the respiratory chain of aerobic organisms.[1] It catalyzes the reduction of molecular oxygen to water, a process that is coupled to proton translocation across the mitochondrial inner membranes. The enzyme contains four redox-active metal centers, organized into two functionally distinct groups. The cytochrome *a* part consists of heme A (Fe-*a*) and copper A and is involved in electron transfer from cytochrome *c* to the catalytic site. The cytochrome a_3 part consists of heme a_3 (Fe-a_3) and copper B and serves as the site of dioxygen reduction.

Spectroscopic characterization, especially resonance Raman spectroscopy,[2] of various Fe-a_3−oxygen intermediates has been an important tool for unraveling the mechanism of this enzyme. Five oxygen isotope sensitive bands at 804, 785, 571, 450, and 356 cm^{-1} have been identified for CcO. The bands at 804 and 785 cm^{-1}, which exhibit ^{18}O isotope shifts of -40 and -35 cm^{-1}, have been assigned to the Fe(IV)═O stretch, in good agreement with experiments on model complexes and theoretical[3] studies. There is also general consensus that the 571 and 450 cm^{-1} bands are the Fe(III)−O_2^- (or equivalently, Fe(II)−O_2) and Fe(III)−OH^- stretches. In recent work,[2a−c] the 356 cm^{-1} band, which has an ^{18}O isotope shift of -14 cm^{-1} but is insensitive to D_2O as solvent, has been interpreted as the histidine-Fe(IV)═O bend (or a ferryl tilt) However, this last assignment remains doubtful and perhaps controversial, which has caused some hindrance to mechanistic studies of CcO using time-resolved resonance Raman spectroscopy.

For a C_{4v} metal−oxo porphyrin complex, the metal−oxo tilting mode is of *E* symmetry and Raman-forbidden. Although the axial ligand in enzymatic and most synthetic metal−oxo complexes lowers the molecular symmetry to below C_{4v}, the selection rule holds effectively, consistent with the fact that the ferryl tilting mode has not been observed for any Fe(IV)═O system, enzymatic or synthetic, with the exception of CcO. Kitagawa and co-workers have suggested[2c] that the Fe(IV)═O group in CcO is tilted, i.e., deviates significantly from the normal upright orientation, possibly due to hydrogen bonding with a protein residue, which results in a relaxation of the selection rule. It has not been possible to verify this suggestion independently, since a bent ferryl group has no precedent among either synthetic or biological systems. Moreover, it may be argued that a bent ferryl group may be strained and therefore should have a low Fe(IV)═O stretching frequency, whereas this stretch is particularly high for CcO. In addition, no complete vibrational analyses of Fe(IV)═O model complexes are available that can permit a theoretical examination of the ferryl tilting mode. Here we report such vibrational analyses based on nonlocal (NL) density functional theory (DFT), describe the ferryl tilting mode and its isotope shifts, and comment on the implications of the results for CcO. Aside from their relevance to CcO, these calculations are part of our ongoing[3,4] efforts to characterize

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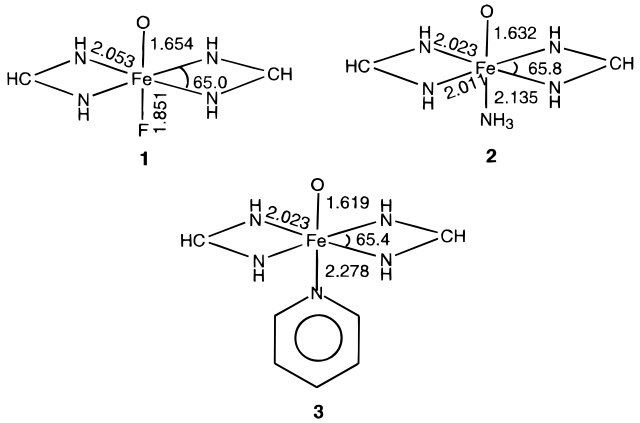


Figure 1. Molecules studied in this work along with key features of their optimized geometries (Å, deg).

the potential energy surfaces and vibrational properties of high-valent iron−oxo intermediates of enzymes and synthetic catalysts, a topic of considerable current interest.

The theoretical methods used consist of NL-DFT with the B3LYP functional, 6-311G* basis sets, spin-unrestricted calculations, full geometry optimizations, and analytical frequencies, as implemented in the Gaussian94 program system.[5] This level of theory is by now fairly standard for moderately accurate calculations of a variety of properties of transition-metal-containing molecules of moderate size. The model Fe(IV)═O complexes studied, **1**−**3**, are depicted in Figure 1 along with key features of their optimized geometries. A pair of equatorial formamidinate ligands have been used to model the porphyrin ligand in each of the molecules studied here. Formamidinate and vinylogous formamidinate ligands have been successfully used to simulate the effect of the porphyrin ligand in other studies of potential energy surfaces and vibrations of Fe(II)CO andFe(III)CN groups in hemes.[6−8]

Before proceeding to describe the ferryl tilting mode, we comment on some other results that serve to calibrate the performance of our calculations.

The optimized Fe−O bond lengths are 1.654, 1.632, and 1.619 Å for **1**, **2**, and **3**, respectively, all of which are in good agreement with previous local DFT (LDF),[3] CASSCF,[9] EXAFS,[10] preliminary small-molecule crystallography,[11] and protein crystallography[12] results on other Fe(IV)═O systems. The significant variation of the Fe−O bond length among **1**−**3** reflects the important role that the axial ligand plays in tuning the strength of the Fe−O bond. Structural information at this level of detail is not available experimentally, but, as will be discussed below, it is well-established that the ferryl stretching frequency varies significantly as a function of the axial ligand[13] and that stretching frequencies have a direct correlation with bond lengths.

For all three molecules **1**−**3**, the unpaired electron spin density is largely localized on the Fe(IV)═O units, with the gross atomic spin populations being about 1.3 for the iron atoms and 0.7 for the oxygen atoms. In other words, the Fe−O bond is highly covalent and the Fe(IV)═O unit can also be approximately described as Fe(III)−O$^{\bullet}$. These results are in good agreement with previous LDF calculations[3] although the oxygen spin population of 0.7 found here is significantly higher than the value of 0.5 reported originally on the basis of ENDOR measurements.[14] However, the ENDOR results have been recently reanalyzed[15] showing that the spin populations provided by DFT are actually consistent with the ENDOR results.

Figure 2 depicts various vibrations of **1**−**3** that can be described as ferryl stretching and tilting and their frequencies for various isotopomers.

The Fe−O stretching frequencies for **1**−**3** have been computed to be 863.8, 908.9, and 917.4 cm^{-1} for the normal isotopomers, with the ^{18}O downshifts being 34.7, 37.7, and 39.7 cm^{-1} and the ^{54}Fe upshifts being 3.8, 3.7, and 3.6 cm^{-1}, all of which are in good agreement with previous LDF results on PFe(IV)O (P = porphinato). The calculated Fe−O stretches are approximately 15% higher than experimental results on synthetic and enzymatic ferryl intermediates. LDF calculations have shown that anharmonic corrections should lower the calculated Fe−O stretching frequencies by about 15 cm^{-1}. The remainder of the discrepancy between theory and experiment must be ascribed to the well-known overestimation of force constants by common DFT methods. The isotope shifts found here are in excellent agreement with experiment.

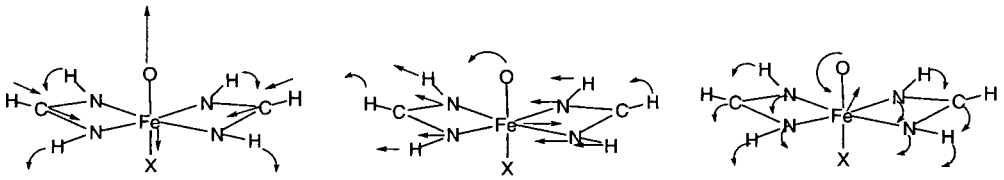
As for the Fe−O bond lengths, the Fe−O stretching frequencies vary over a significant range for compounds **1**−**3**. As mentioned above, the same effect is observed experimentally: the ferryl stretching mode varies sensitively as a function of the axial ligand. For example, the Fe−O stretch for five-coordinate (TPP)FeO[16] is at 852 cm^{-1} and that for horseradish peroxidase compound II,[17] which has an imidazolate axial ligand, is 787−789 cm^{-1} at pH 11. In contrast, substituents at the porphyrin periphery scarcely affect the ferryl stretching frequency. For instance, the ferryl stretching frequencies of the five-coordinate complexes (TPP)Fe(IV)═O (852 cm^{-1}) and (TPFPP)FeO (854 cm^{-1}) are very similar.[18]

For each of the compounds **1**−**3**, there are at least six vibrations with significant ferryl tilting character. As shown in Figure 2, the reason for the relatively large number of such modes is that these vibrations actually involve coupling of ferryl tilting with puckering of the equatorial ligands. As a result of this coupling, the frequencies of these ferryl tilting modes span a relatively wide range. Following are some comments on the individual modes.

The ferryl tilting mode of the highest frequency (δ_1 in Figure 2), which is above 400 cm^{-1} for all the molecules studied, involves significant motion of the iron and the ligand and relatively limited motion of the oxygen. For the C_{2v} molecules, it has b_2 symmetry. In general, the iron on the one hand and all the lighter atoms on the other hand move in roughly opposite directions. The iron isotope shifts are quite significant, and the oxygen isotope shifts are rather small. Thus, this mode does not correspond to the experimental 356 cm^{-1} mode, which has a large ^{18}O isotope shift.

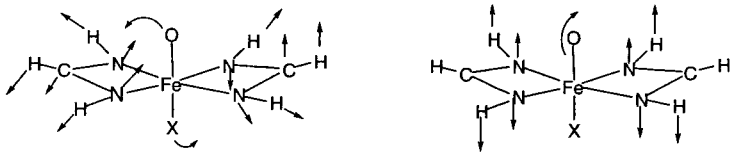
The second-highest bending mode, δ_2, may be described as the b_1 partner of the b_2 mode described above. Thus, the tilting of the ferryl group occurs in a different plane, and the ligand motions are different. The isotope shifts are similar to the mode described above and not to those observed for the experimental 356 cm^{-1} band.

The third bending mode around 350 cm^{-1}, δ_3, which has b_2 symmetry for the C_{2v} systems, involves significant motion of the oxygen and the other ligands but limited motion of the iron. This translates to relatively large ^{18}O isotope shifts of 8−10 cm^{-1} for molecules **1**−**3**. The calculated frequencies of this vibration are clearly very close to the experimental value of 356 cm^{-1}. The calculated isotope shifts are not as high as observed experimentally, viz. 14 cm^{-1}. Nevertheless, this vibration appears to provide the best theoretical description of the putative ferryl tilting mode observed experimentally. The smaller calculated ^{18}O isotope shifts can be reasonably attributed



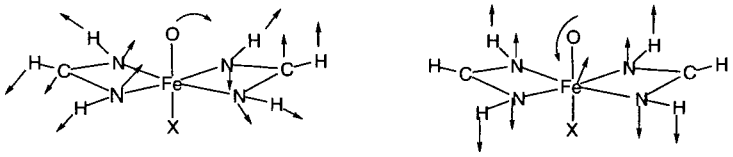
Fe-O stretch δ_1 δ_2

X =	F	NH_3	pyridine	F	NH_3	pyridine	F	NH_3	pyridine
Symm.	a_1	a'	a_1	b_2	a'	b_2	b_1	a"	b_1
$^{16}O^{56}Fe$	863.8	908.9	917.4	421.9	463.1	463.4	363.0	368.2	401.5
$^{18}O^{56}Fe$	829.1	872.2	877.7	418.4	461.0	462.1	361.3	367.6	401.0
$^{16}O^{54}Fe$	867.6	912.6	921.0	426.6	467.5	468.8	367.1	371.3	405.2



δ_3 δ_4

X =	F	NH_3	pyridine	F	NH_3	pyridine
Symm.	b_2	a'	b_2	b_1	a"	b_1
$^{16}O^{56}Fe$	354.1	349.1	356.3	254.6	228.7	254.6
$^{18}O^{56}Fe$	346.5	340.0	346.5	253.4	224.4	247.6
$^{16}O^{54}Fe$	353.9	349.1	356.3	254.4	228.7	254.7



δ_5 δ_6

X =	F	NH_3	pyridine	F	NH_3	pyridine
Symm.	b_2	a'	b_2	b_1	a"	b_1
$^{16}O^{56}Fe$	265.9	227.8	255.6	226.4	190.9	204.1
$^{18}O^{56}Fe$	263.5	226.2	252.2	220.9	193.9	203.9
$^{16}O^{54}Fe$	265.9	227.9	255.6	226.3	194.7	204.5

Figure 2. Schematic view of calculated ferryl stretching and bending vibrations with frequencies in cm^{-1}.

to the difference between the porphyrin ligand in CcO and the formamidinate ligands chosen in this model study. One can argue that a mode with a purer ferryl tilting character would exhibit an ^{18}O shift that is greater than what we have found here.

The fourth bending mode is the b_1 partner of the b_2 mode described immediately above and has a significantly lower frequency in the range 220−260 cm^{-1}. The ^{18}O isotope shifts for this mode are somewhat smaller than the b_2 mode, which is at least partly due to the lower absolute frequencies of the b_1 mode. The relatively large difference in frequency between this pair of b_1 and b_2 modes merely reflects the fact that the equatorial ligand set employed here deviates considerably from the 4-fold symmetry of a porphyrin.

There is another pair of b_2 and b_1 modes of frequencies roughly around 200 cm^{-1}. Figure 2 shows that this (b_1, b_2) pair of vibrations is closely related to the (b_1, b_2) pair described immediately above. The two pairs are simply "in-phase" and "out-of-phase" combinations of the same type of ferryl and ligand motions.

Finally, we have evaluated the energetics of tilting of the ferryl group using a series of single-point calculations to scan the energy (E) of molecule **3** as a function of the OFeC angle (θ), where C refers to the carbon atom in one of the formamidinate ligands. Only the oxygen atom was moved in these calculations, and all other atoms and internal coordinates were frozen. The ferryl tilting force constant obtained by least-squares fitting of the $E(\theta)$ data by a polynomial has a value of 6.3×10^{-2} kcal/deg^2 or 1.4 mdyn·Å/rad^2, which corresponds to a moderately stiff tilting potential. Tilting of the ferryl group from its upright orientation by 5, 10, 15, and 20° costs 1.3, 5.2, 12.1, and 22.6 kcal/mol, respectively, when all other internal coordinates are fixed at their equilibrium values. Coupling with ligand puckering should bring down these energies to some extent, and a 10° deviation of the ferryl group from its upright

orientation in response to hydrogen bonding with the protein matrix is not an unreasonable proposition.

In conclusion, the suggestion that the 356 cm^{-1} band of CcO is a ferryl tilting mode does not conflict with NLDFT vibrational analyses of various isotopomers of Fe(IV)═O model complexes. However, the present results should not be regarded as a firm assignment of the 356 cm^{-1} band. There may well be other species to consider that could give rise to this band. For instance, a vibrational analysis of an Fe(III)OOH intermediate also reveals a number of oxygen isotope sensitive modes in the 300−350 cm^{-1} interval.[19]

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