A New Cu(II) Side-on Peroxo Model Clarifies the Assignment of the Oxyhemocyanin Raman Spectrum

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Coupled binuclear copper proteins perform a number of biological functions. Hemocyanin reversibly binds dioxygen for transport in the hemolymph of arthropods and molluscs. Tyrosinase binds O_2 and reductively cleaves the O−O bond with corresponding hydroxylation of monophenolic substrates and oxidation of *o*-diphenols to *o*-quinones.[1] Catechol oxidase reacts only with *o*-diphenols. The *o*-quinones produced rapidly polymerize to form melanins believed to be involved not only in pigmentation but also in sclerotization in arthropods and wound protection in the browning reaction of fruits and vegetables. Spectroscopic studies indicate that, despite these different reactivities, the active site structures of the coupled binuclear copper proteins are remarkably similar. The oxygenated form of these proteins contains two Cu(II) ions separated by 3.6−3.8 Å and bridged by a peroxo in a μ-η^2:η^2 ("side-on") configuration.[2−4] Each metal ion is additionally coordinated by three histidine residues from the protein backbone, but with a long Cu(II)−$N(His)_{axial}$ bond of 2.40 Å.

Model studies reproduce the electrophilic reactivity of tyrosinase and can also show H atom abstraction.[5] Recent findings have further shown that with certain ligand systems it is possible to obtain a rapid equilibrium between the $Cu(II)_2$ side-on peroxo complex and its $Cu(III)_2$ bis-μ-oxo isomer.[5d,6] Thus, there has been much interest in quantitating the presence of the two isomeric species to determine which correlates with electrophilic vs H atom abstraction reactivity.

Resonance Raman spectroscopy is an excellent method for determination of the composition of these mixtures in solution. The $Cu(III)_2$ bis-μ-oxo isomer is characterized by an extremely intense (ν_{Cu-O}) stretch at ca. 600−620 cm^{-1} that shifts down in frequency by 20−28 cm^{-1} upon $^{18}O_2$ substitution. The $Cu(II)_2$ side-on peroxo isomer, in contrast, is characterized by the (ν_{O-O}) stretch at 730−760 cm^{-1} (lower in some models) with an $^{18}O_2$ shift of −40 cm^{-1} as well as an intense feature at 267−296 cm^{-1} which does not show a detectable isotope shift.[3] However, it is often quite difficult to detect the ∼740 cm^{-1} band of the side-on peroxo isomer. This feature is extremely weak in comparison to the band at ∼290 cm^{-1}, and in some ligand systems (e.g., L^1 = *N*,*N*′-di-*tert*-butyl-*N*,*N*′-dimethyl-1,2-ethanediamine [Scheme S1, Supporting Information]) an intense ligand band overlaps, obscuring this stretch. Therefore it is attractive to use the more intense 290 cm^{-1} feature as diagnostic of the presence of the side-on peroxo isomer. However, the identity of the 290 cm^{-1} band is controversial, as there have been two very different assignments of this feature. A normal coordinate analysis by our group led to assignment of this band as an a_g "accordion" mode of the Cu_2O_2 core involving predominantly Cu−Cu motion, with very little oxygen motion mixed in.[3] An alternate assignment presented for the 290 cm^{-1} feature is as a Cu(II)−$N(His)_{axial}$ stretching mode.[7] Interestingly, the formal modal description of this band qualitatively matches that of an isotope-independent band observed at ∼118−133 cm^{-1} in the $Cu(III)_2$ bis-μ-oxo species composed predominantly of Cu−Cu motion.[8] This modal correspondence does not hold, however, for the side-on peroxo ν(O−O) = 750 cm^{-1} and the bis-μ-oxo ν(Cu−O) = ∼610 cm^{-1}.

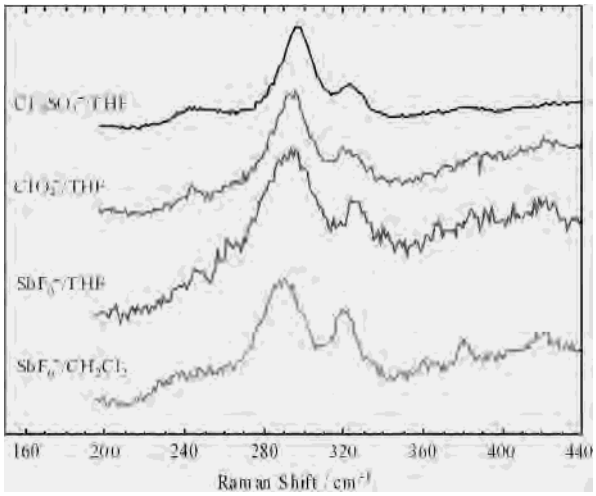

Figure 1. Resonance Raman spectra of $[(L^1Cu)_2O_2]X_2$. Counterions and solvents are listed to the left of each spectrum. All spectra were obtained with λ_{ex} = 363.8 nm except for X = SbF_6^- in CH_2Cl_2 (bottom), for which λ_{ex} = 379.5 nm was used.

Recently we reported that the use of the bidentate peralkylated diamine ligand L^1 results in an equilibrium mixture of $Cu(II)_2$ side-on peroxo and $Cu(III)_2$ bis-μ-oxo species.[5d] The side-on peroxo species produced lacks a third axial nitrogen ligand and is thus an ideal model for investigation into the nature of the vibration in the resonance Raman spectrum at 290 cm^{-1}.

The rR spectra of $[L^1Cu(II)]_2(O_2^{2-})$ are shown in Figure 1 (experimental details provided in Supporting Information). An intense band is observed at 296 cm^{-1} which is strongly enhanced

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by excitation into the 360 nm absorption band of this complex.[5d] (Note that there is a second, weaker peak at ~322 cm^{-1} which shifts in frequency and intensity with changes in the ligand system. We tentatively assign this mode as one involving $Cu-N_{eq}$ motion.) Retention of the 296 cm^{-1} feature despite the lack of a third axial nitrogen ligand supports assignment of this band as a Cu−Cu stretching mode. However, anions in solution could coordinate to the open axial positions of the coppers.[9] To assess the influence of such coordination on the 296 cm^{-1} stretch, the mass and coordinating ability of the counterion (X) employed in solution was varied. The spectra obtained utilizing $X = CF_3SO_3^-$, ClO_4^-, and SbF_6^- are also shown in Figure 1. The feature at 296 cm^{-1} shows little, if any, change over the range of counterions employed. A large shift in the frequency and/or shape of the 296 cm^{-1} band would be expected if it were due to a Cu(II)−axial anion stretch.

Finally, the possibility that a solvent molecule might coordinate in the axial position was considered. Fixing the counterion (SbF_6^-) and changing the solvent from THF to CH_2Cl_2 also resulted in no significant change in this feature (Figure 1, bottom two spectra). The observation of the 296 cm^{-1} stretch with a bidentate ligand system, as well as the lack of any measurable change in this band with solvent and counterion variation, allows a conclusive assignment of this vibration to the Cu_2O_2 core.

The Raman enhancement patterns of the two a_g modes of the Cu_2O_2 core of this model differ, but mirror the well-defined case of *Busycon canaliculatum* hemocyanin, which has Raman bands at 267 and 749 cm^{-1}.[3b] The 267 cm^{-1} feature is strongly enhanced by excitation into the 350 nm charge transfer (CT) band of oxyHc, but shows a relatively small enhancement upon excitation into the 570 nm CT band. In contrast, the 749 cm^{-1} stretch shows similar enhancement in both CT bands. The magnitude of the excited state distortions along these two modes in both of the CT excited states is correlated to the absorption bandwidths and the relative Raman intensities of both the 267 and 749 cm^{-1} modes using the equations given in the Supporting Information. Equation S2 allows evaluation of relative distortions along the 296 and 740 cm^{-1} modes for each CT excited state, while eq S1 allows comparison of the distortions along a given mode between the two different CT excited states. This analysis results in values of $|\Delta_{749}(350\text{ nm})| = 1.11$, $|\Delta_{749}(570\text{ nm})| = 3.23$, $|\Delta_{267}(350\text{ nm})| = 6.87$, and $|\Delta_{267}(570\text{ nm})| = 0.03$ for oxyhemocyanin. Thus the excited state of the 350 nm CT transition involves a much larger distortion along the 267 cm^{-1} vibrational mode than that of the 570 nm CT. Using the potential energy distribution from the normal coordinate analysis (NCA) of the side-on peroxo Cu_2O_2 core[3], these distortions may be projected onto the Cu−Cu and O−O vectors. The NCA results indicate that the 267 cm^{-1} mode is composed of 90% Cu−Cu motion, resulting in a very small calculated ^{18}O isotope shift (−0.2 cm^{-1}). The majority of O motion (90%) is localized in the 749 cm^{-1} mode, resulting in its large isotopic shift of −40 cm^{-1}. Thus, the distortion along the 267 cm^{-1} mode projects primarily as Cu−Cu motion, while the distortion along the 749 cm^{-1} mode projects as a change in the O−O bond length.

The 350 nm CT band is assigned as a transition from a molecular orbital (MO) which is primarily peroxo π^*_σ and

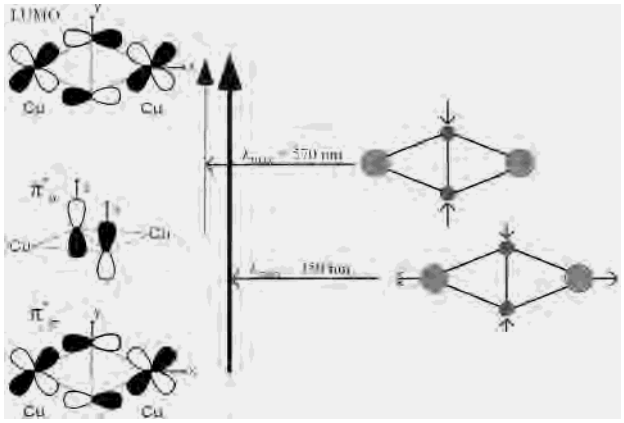

Figure 2. Donor and acceptor molecular orbitals for the 350 and 570 nm CT excited states in the $Cu(II)_2$ side-on peroxo species. The directions of the excited state distortions along the Cu−Cu and O−O vectors are shown to the right.

σ-bonding with the Cu(II) $d_{x^2-y^2}$ orbitals (Figure 2) to an MO which is primarily Cu(II) $d_{x^2-y^2}$ and σ-antibonding with the π^*_σ (LUMO).[3] The shift of electron density from a Cu/O σ-bonding to a σ-antibonding MO results in a lengthening of the $Cu-O_2$ (and Cu···Cu) distance. The 570 nm CT band is assigned as a transition from the primarily peroxo (out of plane) π^*_ν to the same antibonding LUMO. Since the π^*_ν donor orbital is effectively nonbonding with the Cu(II)'s, the distortion along the Cu−Cu vector is much smaller than for the 350 nm CT [$\Delta_{267}(570) = 0.03$ vs $\Delta_{267}(350) = 6.87$], consistent with the observed decrease in resonance enhancement of the 267 cm^{-1} mode at 570 nm. In contrast, the 749 cm^{-1} O−O stretch is not significantly enhanced in the 350 nm band as both the donor and acceptor orbitals contain π^*_σ character. Thus the intra O−O bonding is not significantly affected.[10]

In conclusion, we have shown that the large feature at 267−296 cm^{-1} in the rR spectrum of the $Cu(II)_2$ side-on peroxo species is a Cu−Cu stretch. Additionally, the modal description of this band clarifies why it undergoes a large excited state distortion in the 350 nm CT excited state, resulting in its high intensity in the UV rR spectrum. With the conclusive assignment of the ~290 cm^{-1} band as diagnostic of the side-on peroxo Cu_2O_2 core, it is possible to sensitively discriminate between the peroxo and bis-oxo isomeric forms using resonance Raman spectroscopy.

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Supporting Information Available: Experimental details on the sample preparation and collection of resonance Raman spectra, as well as the ligand structure and the equations used in calculation of the excited state parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The larger distortion of ν_{O-O} in the 570 nm band has been associated with the loss of an electron from the π^*_ν orbital, which is less covalent (and thus has more O−O antibonding character) than the π^*_σ (Larrabee, J. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4217−4223 and in ref 3).