Spectroscopic Investigations of Vanadium Dependent Bromoperoxidases

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In the past, the vanadyl ion (VO^{2+}) has been used to probe spectroscopically silent metal binding sites in biological molecules (e.g. carbonic anhydrase, pyruvate kinase).[1] More recently, the active site structure of reduced vanadium bromoperoxidase [$V^{(IV)}$BrPO] has been of special interest to inorganic chemists.

Several chemical properties of this ion allow for spectroscopic techniques (EPR, ESEEM) to gain information on the coordinating atoms, as well as ligand orientation with respect to the vanadium-oxo bond. Properties include: I=7/2, S =1/2 and a $3d^1$ electron configuration where the lone d electron occupies the nonbonding d_{xy} orbital.[2] Typically, EPR spectra of this ion have distinct axial symmetry with well defined $A_{\|}$ and $A_{\perp}$ values.[3]

Vanadium bromoperoxidases were discovered from a marine brown algae species in the early 1980s, and the enzyme has recently been found to be prevalent in the marine ecosystem.[4] The active enzyme [$V^{(V)}$BrPO] contains the vanadate prosthetic group, $(VO_4)^{3-}$, and catalyzes the 2 e^- oxidation of Br^- to hypobromous acid (HOBr):[5]

$$H_2O_2 + Br^- + H^+ \rightarrow H_2O + HOBr$$

K-edge X-ray studies and EPR have shown that V(IV) or V(III) states are not produced during catalytic turnover. In addition, single electron reduction of the vanadate center results in subsequent inactivation of the enzyme.[6] The crystal structure of the reduced, inactivated enzyme [$V^{(IV)}$BrPO] has remained elusive to date.

Spectroscopic studies of the inactivated enzyme in the 1980s suggested the presence of the vanadyl ion, ligated by a mixed ligand field of oxygen and nitrogen donors.[7] The geometry of the reduced active site, as well as the type of ligating functional groups (e.g. carboxylate, imine) was not well understood. Insight into the type of ligands in the equatorial coordination sphere of the vanadyl ion in [$V^{(IV)}$BrPO] was gained through structural model studies and the application of the "additivity" principle in EPR. "Additivity" suggests that the parallel hyperfine coupling constant ($A_{\|}$) is equal to the individual hyperfine contribution of each ligand (A_{zi}) multiplied by the number of representative ligands in the equatorial plane: [3,8]

$$A_{\|} = \sum n_i A_{zi}$$

EPR spectra of several octahedral oxovanadium(IV) compounds with iminodiacetic acid derivatives (e.g. [$VO(H_2O)$ada]) illustrate that this principle was very useful in determining equatorial ligand types in solution. Thus, $A_{\|}$ from well characterized structural models as well as from the reduced enzyme allowed for the prediction the equatorial ligand field in [$V^{(IV)}$BrPO]. Pecoraro *et al.* suggested an equatorial ligand field of an alkoxide, two waters, one imidazole, and a O/N donor.[9] However, ambiguity in the hyperfine coupling values of equatorial imidazoles introduced room for speculation.

As the additivity principle has been used to elucidate equatorial ligands in oxovanadium(IV) complexes, ESEEM has been equally applicable in identifying ligands removed from the equatorial plane. Model complexes with an amine nitrogen *trans* to the oxo ligand (e.g. [VO(H_2O)nta]) resulted in modulations atypical of equatorial amines directly ligated to oxovanadium(IV) species.[10] This low frequency modulation found in the structural models,[7] was used to gain insight on a similar signal found in the reduced enzyme species.

Additional studies by Fukui *et al.* suggested that the ESEEM spectrum of a square pyramidal oxovanadium(IV) complex with a ligand containing carboxylic acid and imidazole functional groups [e.g.VO(Himac)$_2$] closely resembled that of the reduced [$V^{(IV)}$BrPO] spectrum.[11] It was suggested that [$V^{(IV)}$BrPO] had two histidine ligands in the equatorial plane, instead of one. Further EPR "additivity" studies of crystallized oxovanadium(IV) complexes suggested that equatorial, parallel oriented imidazoles contain nitrogen p oribitals that overlap more with the d_{xy} orbital of the vanadyl ion, resulting a lower $A_{||}$ value (Figure 1A). This resolved some of the ambiguity seen in EPR "additivity" studies of imidazole ligands, while also explaining the low frequency modulations in ESEEM studies. New interpretations of the imidazole orientation allowed for the proposal of a structural model that better fits the experimental data of the reduced vanadium bromoperoxidases.[1] (Figure 1B)

Future studies include pulsed ENDOR spectroscopy of [$V^{(IV)}$BrPO] in comparison with structural model spectra.[12]

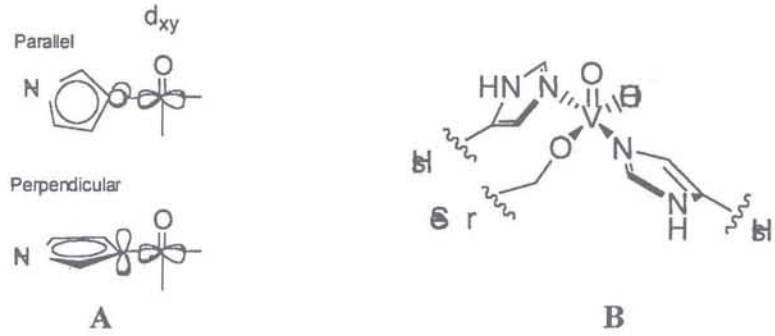

Figure 1

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