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QM/MM investigation of structure and spectroscopic properties of a vanadium-containing peroxidase

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ABSTRACT

We present a comprehensive analysis of the most likely ground state configuration of the resting state of vanadium dependent chloroperoxidase (VCPO) based on quantum mechanics/molecular mechanics (QM/MM) evaluations of ground state properties, UV–vis spectra and NMR chemical shifts. Within the QM/MM framework, density functional theory (DFT) calculations are used to characterize the resting state of VCPO via time-dependent density functional theory (TD-DFT) calculations of electronic excitation energies and NMR chemical shifts. Comparison with available experimental data allows us to determine the most likely protonation state of VCPO, a state which results in a doubly protonated axial oxygen, a site largely stabilized by hydrogen bonds. We found that the bulk of the protein that is beyond the immediate layer surrounding the cofactor, has an important electrostatic effect on the absorption maximum. Through examination of frontier orbitals, we analyze the nature of two bound water molecules and the extent to which relevant residues in the active site influence the spectroscopy calculations.

1. Introduction

The bioinorganic chemistry of vanadium haloperoxidases (VHPOs) has received considerable interest in recent years due to its fundamental role in the halogenation of organic compounds [1–4]. Vanadium haloperoxidases (VHPOs) are enzymes that catalyze the oxidation of halide ($X^- = Cl^-$, Br^-, or I^-) ions by hydrogen peroxide, producing as a result the corresponding hypohalous acids which, in turn, are capable of halogenating organic substrates [5–9]. The overall reaction catalyzed by the enzyme active site is given by Eq. (1).

$$H_2O_2 + H^+ + X \rightarrow H_2O + HOX \quad (1)$$

The identity of "HOX" is dependent upon both the pH of the reaction and the halide involved [6]. VHPOs are universally present in marine algae, terrestrial fungi, and in some lichen. There is much interest in understanding the structure and function of haloperoxidases, not only due to their role in the industrial production of halogenated compounds [10], but also because they may help in the design of biomimetic vanadium complexes used for biocatalysis applications and as lead compounds with pharmacotherapeutic potential [11–15], in particular for the treatment of diabetes. A number of X-ray crystal structures of VHPO have been obtained [16–19]. Two crystal structures of VCPO were determined from the fungus *Curvularia inaequalis* in both the native state and the stable intermediate with vanadate in the peroxo form [16]. In the native state of VCPO, the vanadate cofactor (VO_4^{3-}) is bound to the protein through a single coordinate covalent bond extending from the vanadium atom to the Nε2 of His496 (see Fig. 1). The active site of VCPO is a paradigm of active sites largely stabilized by hydrogen bonds. The negative charge on the equatorial oxygens is compensated by a number of protonated amino acids in the active site (Lys353, Arg360, and Arg490), which donate hydrogen bonds to the oxygen atoms of the cofactor. Ser402 and Gly403 also form hydrogen bonds with the equatorial oxygens of the vanadate cofactor. The apical oxygen has been assigned as a hydroxide group [16], and it is hydrogen-bonded to His404. This residue is proposed to activate the hydroxide in the deprotonation of H_2O_2 before binding of the peroxide to the vanadium center [20].

A detailed molecular picture of the catalytic mechanism of VCPO first requires a clear understanding of the structure and the relevant interactions between the cofactor and the protein cavity. However, due to the inherent limitations in resolution, the presence and positions of hydrogen atoms could not be unambiguously determined from the crystal structure. The distinction between oxide and hydroxide moieties was inferred by slight differences in ligand–metal bond distances [21], which were of the order of the uncertainty in the atomic positions. The role of the protein cavity in the catalytic activity of the vanadate cofactor is still not completely understood, although it has been the subject of continued interest [7,22–25]. In particular, mutagenesis studies in which key residues surrounding the cofactor were mutated to alanine, have provided some insight into the relative importance

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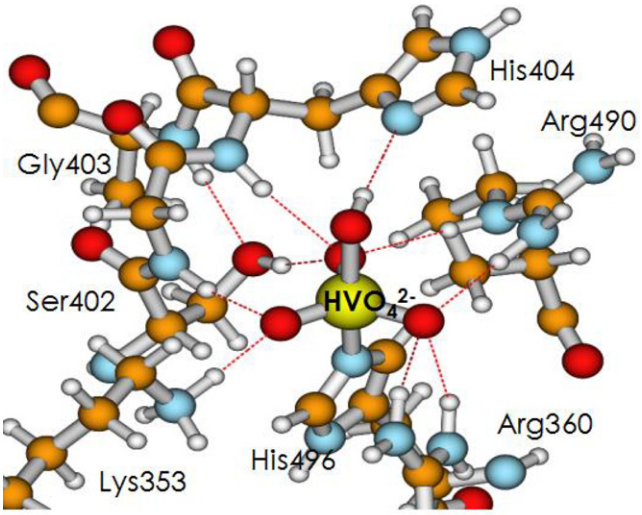

Fig. 1. Crystal structure of the vanadium chloroperoxidase from the fungus *C. inaequalis* with an arbitrary protonation state on the vanadate. Hydrogens were added using the application pdbxyz from the program TINKER.

of various active site residues in the catalytic activity [19,25,26]. Optical absorbance studies have also been carried out on the native and mutant VCPOs to characterize the binding site and correlate the role of certain residues to activity [23,24]. The most important feature of the optical absorption of native VCPO is a peak at 315 nm, attributed not to the free vanadate, but rather to the spectral tuning caused by the enzyme cavity [23]. Macedo-Ribeiro et al. [19] shown in their crystallographic studies carried out at a high concentration of vanadate (1 mM) that for the mutant His496Ala the cofactor is still present in the active site, despite the absence of the covalent bond. This mutation, however, does not result in the formation of the absorption band in the 300–400 nm region.

Due to their importance in biological, pharmacological, and industrial applications [22,27–32], there have been many experimental [7,10,31,33–35] and theoretical [28,36–40] investigations of structural analogues of the active site in VHPO. From the theoretical side, quantum chemical methods have proved to be useful for investigating structural, reactivity and spectroscopic properties of transition metal complexes [41] and models of the active site of metallo-enzymes [42]. A number of Density Functional Theory (DFT) studies of the active site of the vanadium chloroperoxidase have focused on reduced models of the protein active site [8,36,37,39,40,43,44]. Borowski et al. [36] carried out DFT calculations with the B3LYP (Becke, three-parameter, Lee-Yang-Parr) exchange-correlation functional [45] and basis set LanL2DZ [46], to study two model complexes and the active site of VCPO, and time dependent DFT (TD-DFT) to compute vertical energies and oscillator strengths for electronic transitions. It was found that His496 is indispensable for predicting the observed UV–vis absorption bands, in agreement with the experimental results [23]. Studies by Zampella et al. [8] focused on a systematic survey of a large number of small active site mimics, leading to the assignment of a doubly protonated vanadate as the most energetically stable state in the resting form. In later studies Zampella et al. [40] modeled native and peroxo forms of VCPO with the functionals BP86 and B3LYP, and TZVP as the basis set. They computed UV–vis spectra by the TD-DFT method. Although all these small vanadium complex models provided important insight, the role of the protein environment via supramolecular interactions [10], crucial in determining the stability of the active site and the enzymatic effect on reactivity, was not considered. To address the effect of the protein, recent studies have included the application of QM/MM approaches to analyze structure and spectroscopic properties of VCPOs [21,47,48]. Kravitz et al. [21] carried out a model calculation on a truncated protein where the active site was treated at the B3LYP level with basis set LACVP* [49] and the rest of the protein model by the OPLS 1999 force field [50]. It was proposed that the resting state is a hybrid of two configurations, one with an axially coordinated water molecule and another one with two hydroxyl groups, one apical and the other equatorial. More recently Waller et al. [48] reported a QM/MM simulation of protonation states for the active site of VCPO and a number of probable positional isomers for each of the protonation states. ^{51}V solid state NMR spectroscopy was used in conjunction with DFT methods as a guide to examine the most likely protonation state of the resting configuration of vanadium chloroperoxidase. These studies confirmed the resting state structure proposed in previous DFT and QM/MM studies [8,21].

In the present paper, a hybrid quantum mechanics/molecular mechanics (QM/MM) is used to study the geometries, energies, ^{51}V NMR chemical shifts and UV–vis spectroscopy of the resting state of the VCPO using an energy-minimized configuration starting from the X-ray structure. The study was performed taking fully account of the field of the entire protein. Differing from previous QM/MM studies, both the experiment data of ^{51}V NMR and UV–vis spectra are used to validate different structural models. To the best of our knowledge, this study is the first comprehensive QM/MM calculation to study the geometries, ground state energies, UV–vis spectra and NMR properties and use them as descriptors of the VCPO resting state. Furthermore, our study highlights the important role of the protein cavity, including bound water molecules, in tuning spectroscopic shifts, and confirms the valuable use of QM/MM schemes to interpret NMR and UV–vis properties of enzyme metal centers.

2. Computational details

All calculations were based on the crystal structure of the resting state of the enzyme from the fungus *Curvularia inaequalis* [16] (protein data bank entry: 1VNI). A crystal structure of similar resolution, derived in a previous study (access code 1IDQ) [16], resolves two water molecules bound to the vanadate cofactor via the axial oxygen. In contrast, the 1VNI structure shows only one water molecule bound to the vanadate axial oxygen. While the position of crystallographic waters must be always taken with caution, water molecules near the active site are sometimes essential to the mechanisms of enzyme reactivity. To determine whether these water molecules are a signature of the vanadate ion and to the interpretation of spectroscopy measurements, two QM/MM models were constructed, one without and one with these bound water molecules (herein referred as QM/MM set up **I** and QM/MM set up **II**, respectively). Thus, we will refer as QM/MM set up **I** the model with these two water molecules completely removed from the protein system, and QM/MM set up **II**, as a model with these two water molecules added to the system (in the QM layer). The notation "QM region **I**" and "QM region **II**" will be used indistinctly to the notation "QM/MM set up **I**" and "QM/MM set up **II**", respectively. In the ONIOM-Electronic Embedding [51–54] approach used in this study, the QM domain is treated at the DFT level while the rest of the protein is treated with molecular mechanics using the AMBER-99 [55] force field. In the first QM/MM set up, the QM region **I** included His496 and side chains of His404, Arg360, Arg490, and Lys353 (bound water molecules were excluded). This QM region had a total charge of +1. In the second QM/MM set up, QM region **II** included the same five residues plus the two resolved water molecules solved in 1IDQ (W1 and W2). The covalent links between the QM and the MM regions were