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Dioxovanadium(v) complexes with side chain substituted *N*-salicylidenehydrazides modelling supramolecular interactions in vanadium haloperoxidases

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Abstract

The water soluble ammonium salts of *cis*-dioxovanadium(v) complexes with side chain functionalized *N*-salicylidenehydrazides show hydrogen bonding interactions relevant for haloperoxidase enzymes. Under turnover conditions for the catalytic bromination of trimethoxybenzene, no substitution of the side chain hydroxy group is observed.

Keywords: Haloperoxidases; Vanadium complex; *N*-Salicylidenehydrazides; Crystal structure; Hydrogen bonding interaction

Vanadium haloperoxidases (V-HPO) are enzymes catalyzing the oxidation of halides to corresponding hypohalous acids, which then readily undergo halogenation of organic substrates or conversion of hydrogen peroxide to singlet oxygen and generation of halides [1]. The active site of vanadium chloroperoxidase (V-ClPO) isolated from the fungus *Curvularia inaequalis* and of vanadium bromoperoxidase (V-BrPO) from the red algae *Corallina officinalis* and seaweed *Ascophyllum nodosum* contains a five-coordinated vanadium(v) moiety with a proposed trigonal bipyramidal geometry that is directly bound to the protein by an axial histidine residue. This active site vanadate is embedded in the protein via extensive hydrogen bonding to several amino acid residues of the highly conserved primary protein shell [2–4]. In addition, this active site architecture is also found for certain acid phosphatases [5], but with subtle distinctions concerning the relative positions of the amino acid residues [6], in particular those potentially hydrogen bonded toward the equatorial oxygen atoms of the vanadate moiety (i.e., lysine, arginine and serine). It has been proposed that the haloperoxidase enzymes first react with hydrogen peroxide to form an intermediate with a side-on bound peroxide in an equatorial position that is hydrogen bonded to the lysine residue, followed by attack of a halide ion and uptake of a proton which leads to the generation of hypohalous acid (HOX) and regeneration of the vanadate moiety in the enzyme active site [3,7]. The catalytic cycle is suggested to proceed through hydrogen bonding network, e.g. the peroxide intermediate activated by protonation which increases the electrophilicity on the oxygen atoms of the peroxo group and thus makes attack by halide more favorable [8]. This mechanistic feature is also found for the heterolytic cleavage of O–O bonds in heme based peroxidase enzymes [9]. Based on this aspect of the biological function of vanadate in V-HPO, the synthesis of peroxovanadate complexes as structural models

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with relevant hydrogen bonding interaction of the peroxo group has been reported [10].

Besides the lysine another equatorial amino acid residue, namely the serine, has become a matter of considerable interest recently, which is based on EXAFS studies of V-BrPO from *A. nodosum*, suggesting that the serine plays a role in catalysis as the actual site of bromination by forming a carbon–bromine bond during turnover [11]. This proposed essential role of the serine residue could not be verified by site-directed mutagenesis experiments performed on the V-ClPO from the fungus *C. inaequalis* [12]. Although, the basic activity of the enzyme is retained by the mutation of the active site serine to alanine, a significant decrease of the enzyme activity can be observed for both the chlorination and the bromination activity of the enzyme to about 4% and 20%, respectively. This indicates that the serine residue, even though not crucial, nevertheless plays an important role for the reactivity of the active site.

Recently, we reported the ammonium salt of a dioxovanadium(v) complex derived from the *N*-salicylidene-hydrazide ligand system that exhibits extensive hydrogen bonding [13,14]. We propose to probe the role of the amino acid residues involved in the hydrogen bonding network of the protein bound vanadate in haloperoxidases by modification of the ligand side chain in the *N*-salicylidene-hydrazide ligand system with relevant functional groups, e.g., a hydroxy group.

The new Schiff base ligand (Fig. 1) derived from 5-hydroxy pentane acid hydrazide and salicylaldehyde (H_2salhyhp) reacts with ammonium vanadate in refluxing methanol solution to afford the yellow ammonium salt of the dioxovanadium(v) complex $NH_4[VO_2$(salhyhp)] [15]. For prolonged reaction times the primarily formed ammonium salt was found to be converted to the corresponding neutral complex [VO_2(Hsalhyhp)] under release of ammonia. In addition, the reconversion to the ammonium salt is possible by deprotonation of the neutral complex in liquid ammonia. This reversible protonation seems to be a general feature for vanadium(v) complexes of carbonic acid hydrazide ligands [13,14].

The structure of $NH_4[VO_2$(salhyhp)] determined by X-ray crystallography features a dioxovanadium(v) moiety as depicted in Fig. 2 [16]. In the equatorial plane, the vanadium atom is coordinated by the oxo group O2 and the donor atoms N1, O3 and O4 of the tridentate chelate ligand. The apical position at

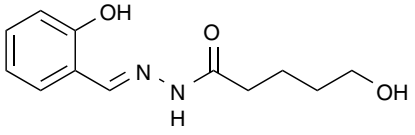

Fig. 1. New Schiff base ligand H_2salhyhp with hydroxy alkyl side chain.

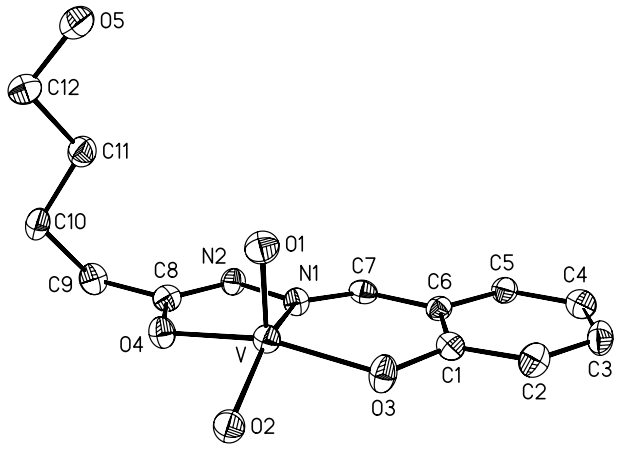

Fig. 2. Molecular structure of the complex anion $[VO_2$(salhyhp)]$^-$. Hydrogen atoms are omitted for clarity, displacement ellipsoids are at 50% probability level. Selected bond lengths (pm): V–O1 162.5(3), V–O2 164.7(3), V–O3 188.1(3), V–O4 196.4(3), V–N1 214.0(3), O4–C8 130.6(4), C8–N2 131.1(5), N1–N2 140.6(4). Selected angles (°): O1–V–O2 111.07(14), O3–V–O4 147.39(12), O1–V–N1 105.46(13), O2–V–N1 142.44(14).

the vanadium atom is occupied by the oxo group O1. Relative to the mean plane given by the chelate ligand system, the vanadium atom is displaced toward the apical oxo group O1 by 37 pm, whereas the oxo group O2 is slightly distorted to the opposite side by 39 pm. This leads to a τ value of 0.08 ($\tau = 0$ for ideal tetragonal pyramid; $\tau = 1$ for ideal trigonal bipyramid) consistent with a slightly distorted square pyramidal geometry at the vanadium atom. Nevertheless, it is known that the local geometry at a *cis*-dioxovanadium(v) moiety is rather flexible and can be varied by crystal packing effects [13,17]. The V=O (O1 and O2) as well as the V–N1 and V–O (O3 and O4) bond lengths are within the expected range for *cis*-dioxovanadium(v) complexes (cf. Fig. 2). The anionic nature of the complex and, therefore, the presence of the iminolate form of the ligand is consistent with the observed V–O4 and O4–C8 bond lengths of 196.4 and 130.6 pm, respectively. The flexible hydroxy alkyl side chain is almost perpendicular to the planar ligand system and oriented toward the same side as the apical oxo group O1, with an angle of 9° between the normal vector of the corresponding mean plane and the vector given by the atoms C9 and O5 of the side chain. As a result, the oxygen atoms O1 and O5 are perfectly arranged to accommodate the hydrogen bonded ammonium cation.

As depicted in Fig. 3, the X-ray structure analysis reveals that the ammonium cation is located in the vicinity of the anionic *cis*-dioxovanadium(v) moiety and hydrogen bonded to the apical oxo group O1 (N3···O1 291 pm) and the hydroxy oxygen atom O5 of the side chain (N3···O5 287 pm). In addition, the ammonium cation forms hydrogen bonds with the hydrazide nitrogen atom