[1] a) Recently, the Italian Chemical Society devoted the conference IASOC VIII to the topic of *Friendly Processes: A Recent Breakthrough in Organic Chemistry* (Ischia Porto, Italy, 1998). The *First Summer School on Green Chemistry* (Venice, Italy, 1998) was supported by the European Commission within its program *Training and Mobility of Researchers*; b) *Green Chemistry* is a new international journal published by the Royal Society of Chemistry.
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Phosphate and Vanadate in Biological Systems: Chemical Relatives or More?

Winfried Plass*

The biological function of vanadium is well established and has been documented in several review articles.[1, 2] Of particular importance is the ability of vanadium to influence phosphate-metabolizing systems and the fact that vanadium is an inherent part of enzymatic active sites. For both types of vanadoenzymes known today, the vanadium-containing haloperoxidases and the vanadium nitrogenases from the nitrogen-fixing bacteria *Azotobacter*, functional analogues are found in nature which are either more widely spread or more efficient, for example the heme-containing haloperoxidases and the conventional nitrogenases with molybdenum cofactor, respectively. This immediately leads to the question of how these enzyme systems evolved and in particular whether the vanadium-containing enzymes known today are retained functional analogues, which withstood evolutionary forces. New insight concerning these questions may be gained on the basis of newly found similarities for vanadate and phosphate in biological systems.

The widespread physiological effects of vanadium are mainly attributed to the similarity of vanadate(v) ions and phosphate ions. But there are also important differences between these two anions. At physiological pH values monovanadate is found as doubly protonated $[VO_2(OH)_2(H_2O)]^-$ species, whereas phosphate occurs in the monoprotonated form HPO_4^{2-}. This is also important for possible mechanisms of the transport systems for these two anions.[3–5] In addition vanadium is easily reduced under physiological conditions to yield cationic species. The third difference is given by the pronounced ability of vanadium to adopt higher coordination numbers. The higher coordinative flexibility of vanadium can be deliberately used for the structural characterization of phosphate-metabolizing enzymes.

Recently the crystal structures of several stable enzyme aggregates of phosphatases with vanadate as transition state analogue have been reported. Interesting examples are the protein tyrosine phosphatases,[6, 7] which are involved in signal transduction mechanisms for controlling and regulating intracellular processes (e.g. the insulin receptor system)—in

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this context it is worth noting that vanadate complexes show insulin-mimetic effects.[8] In these aggregates the vanadium center resides in a trigonal-bipyramidal environment and is linked to the protein through a single axially bound cysteine residue, whereas the oxygen atoms of the vanadate moiety are involved in a hydrogen-bonding network. A similar structure is found for the active site of rat prostatic acid phosphatase with the complexed transition state analogue vanadate[9] (Figure 1, top). In this case the vanadium is linked to the protein through an axially bound histidine residue.

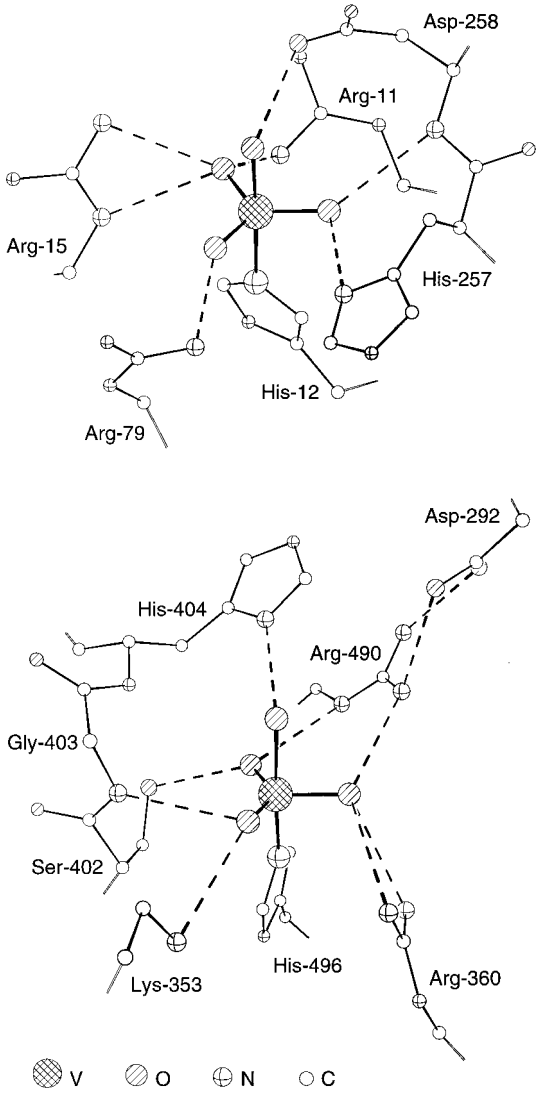

Figure 1. Top: Structure of the active site of the rat prostatic acid phosphatase with complexed vanadate.[9] Bottom: Structure of the active site of vanadium haloperoxidase from the fungus *Curvularia inaequalis*.[11] Hydrogen bonds are shown as broken lines.

Striking similarities are observed for the only structurally characterized vanadoenzyme known today, the chloroperoxidase of the fungus *Curvularia inaequalis* (Figure 1, bottom).[10–12] As in the case of the rat prostatic acid phosphatase the vanadate is directly linked to the protein only through the axially bound histidine residue and is embedded in the protein through an extensive hydrogen-bonding network.

Vanadium-containing haloperoxidases (V-HPO) catalyze the two-electron oxidation of halide ions (X^-) to the corresponding hypohalous acids [Eq. (1)].[13] HOX may

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

further react with a broad range of nucleophilic acceptors to form a diversity of halogenated compounds. These haloperoxidases are named after the most electronegative halide they are able to oxidize, and thus a vanadium chloroperoxidase (V-CPO) is able to oxidize chloride, bromide, and iodide ions.

Based on the recently published crystal structures of the native and *apo* forms as well as the peroxide- and azide-bound derivatives of this chloroperoxidase[10–12] several interesting questions arise: 1) What is the electronic structure of the active site vanadate moiety, that is mono- or dioxovanadium(v) species (VO^{3+} or VO_2^+)? 2) How is the peroxo group or the chloride ion bound to the active site? 3) What is the influence of the protein environment upon the structure of the active site and the mechanism? 4) Does the apo-protein of this chloroperoxidase exhibit phosphatase activity as expected based on its structural similarities to phosphatases?

As Wever et al. recently showed, the apo-protein of the chloroperoxidase isolated from the fungus *Curvularia inaequalis* indeed exhibits phosphatase activity.[14] Although it is obvious from the kinetic data that the active site of this V-CPO is not optimized for phosphatase activity, it is nevertheless clear that within the same supramolecular environment it is possible to catalyze two very different reactions such as those of haloperoxidases and phosphatases. Consequently this structural motif should also be observed for the active sites of other V-HPOs and phosphatases. Moreover, this structural similarity should give rise to genetic relationships as well. According to the sequence alignment of relevant enzymes nearly all amino acid residues coordinating vanadate in V-CPO isolated from *Curvularia inaequalis* (Lys 353, Arg 360, Ser 402, Gly 403, His 404, Arg 490, and His 496 see Figure 1, bottom) are conserved in the V-HPOs sequenced thus far as well as within three families of acid phosphatases (Figure 2).[14] Within this active site architecture the fully conserved histidine residues play an important role. His 496 covalently links the vanadate to the protein, whereas His 404 is essential for catalysis and is proposed to function as an acid–base acceptor group. Together with the observed phosphatase activity of the apo-protein of the V-CPO this indicates a very similar architecture of the active sites of these V-HPOs and acid phosphatases.

A dendrogram (Figure 2), based on the sequence alignment of 14 phosphatases and V-HPOs, indicates that these enzymes have divergently evolved from a common ancestor. In combination with the observed stability of V-CPO from *Curvularia inaequalis* to high concentrations of the substrate (H_2O_2) and the product (HOX), which would readily inactivate the heme-containing HPOs, this contradicts the introductory hypothesis that V-HPOs are retained enzymatic systems that have withstood evolutionary forces. This is further evidenced by the optimized functionality of V-CPO from *Curvularia inaequalis*, which is given by its recently proposed putative role as source for the starting material for

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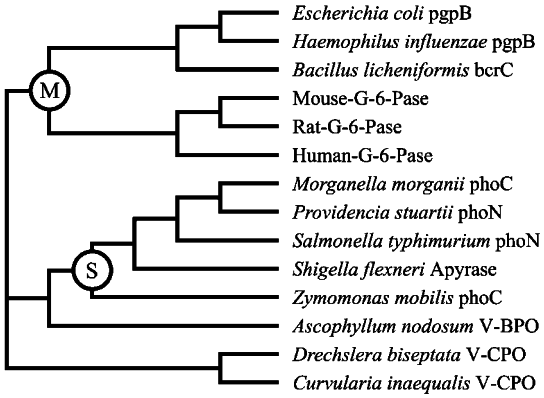

Figure 2. Dendrogram based on the alignment of 14 acid phosphatases and vanadium haloperoxidases (according to reference [14]). The group of membrane-bound phosphatases is marked with an M, and the group of soluble phosphatases is marked with an S. Abbreviations: pgpB, phosphatdiyl glycerophosphate phosphatase B; bcrC, gene product from *Bacillus licheniformis*, no function has been assigned yet; G-6-Pase, glucose-6-phosphatase; phoC and phoN, class A bacterial acid phosphatase; Apyrase, ATP-diphosphohydrolase; V-BPO, vanadium bromoperoxidase; V-CPO, vanadium chloroperoxidase.

the production of a chemical weapon, the hydroxyl radical [Eq. (2)]. The hydroxyl radical in turn can be used by the fungus to damage the protective lignocellulose of plant tissues allowing the penetration of the plant cell for nutrient acquisition.[15]

$$HOCl + (O_2)^{\cdot -} \longrightarrow (OH)^{\cdot} + Cl^- + O_2 \qquad (2)$$

It is remarkable, however, that for another class of acid phosphatases, that is exemplified by the rat prostatic acid phosphatase (see Figure 1, top), no sequence similarity is found with the enzymes given in the dendrogram of Figure 2. Nevertheless the observed structural similarity of the active site, obvious from Figure 1, indicates that a convergent evolution seems to have occurred with respect to these enzyme systems.

Besides the interesting evolutionary aspects of these enzymes, there are also important implications for both research in the acid phosphatase and in the V-HPO field. A first remarkable example for this—in particular from an anthropocentric point of view—is the structure of mammalian glucose-6-phosphatase (G-6-Pase). This enzyme catalyzes the last step in both gluconeogenesis and glycogenolysis and as such it is the key enzyme in glucose homeostasis. G-6-Pase deficiency is the cause of glycogen storage disease type 1 (von Gierke disease) and is characterized by severe clinical manifestations such as hypoglycemia, kidney enlargement, or growth retardation, which usually cause an early death of the patients.

The recently published model for the membrane topology of human G-6-Pase[16] is not consistent with the results outlined above and had to be modified accordingly as depicted in Figure 3.[17] The active site residues of G-6-Pase identified by sequence alignment are all situated on the same side of the membrane and are located within the helices II – V. In this new model the histidine residue His 176 is the nucleophile that forms the phosphohistidine enzyme-substrate intermediate. The phosphate moiety is positioned by interaction of the negatively charged oxygen atoms with the positively charged amino acids Lys 76, Arg 83, and Arg 170. In analogy to the active site of V-CPO the residues Ser 117 and Gly 118 may also donate hydrogen bonds and the histidine group His 119 may provide the proton needed to liberate the glucose moiety.

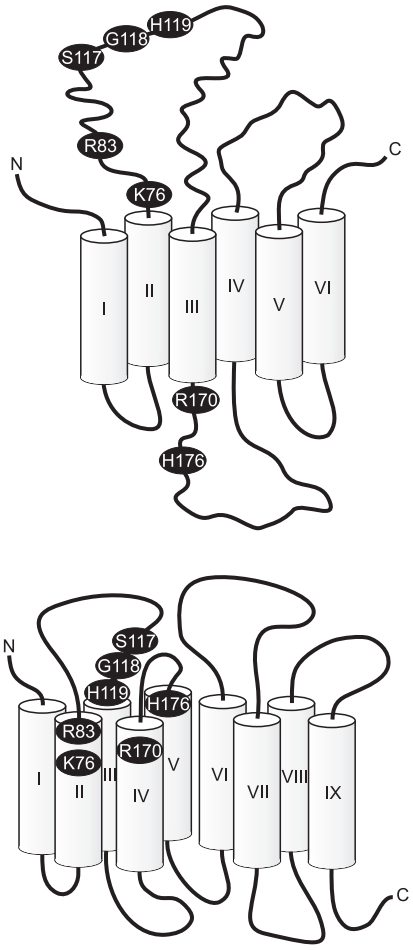

Figure 3. Membrane topology models for glucose-6-phosphatase. Top: Current six transmembrane-helix topology model.[16] Bottom: Newly proposed nine transmembrane-helix topology model.[17] Putative G-6-Pase active site residues are depicted as black ovals.

As shown by this first example, the common architecture of the active sites of the vanadium-containing peroxidases and the aligned acid phosphatases has important implications that even reach into seemingly independent fields of research.

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Stichwörter: bioinorganic chemistry · haloperoxidases · phosphatases · phosphorus · vanadium

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