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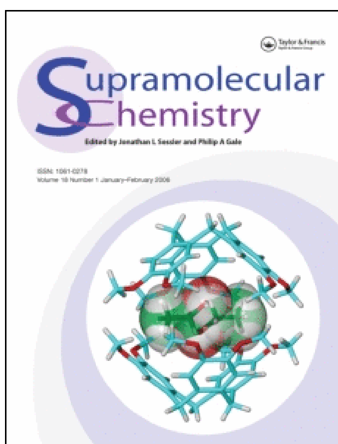

Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
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To cite this Article Tapper, Sophia , Littlechild, Jennifer A. , Molard, Yann , Prokes, Ivan and Tucker, James H. R.(2006) 'Anion Binding Tripodal Receptors as Structural Models for the Active Site of Vanadium Haloperoxidases and Acid Phosphatases', Supramolecular Chemistry, 18: 1, 55 — 58
To link to this Article: DOI: 10.1080/10610270500368840
URL: http://dx.doi.org/10.1080/10610270500368840

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Supramolecular Chemistry, January–February 2006 Vol. 18 (1), pp. 55–58

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Anion Binding Tripodal Receptors as Structural Models for the Active Site of Vanadium Haloperoxidases and Acid Phosphatases

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Received (in Southampton, UK) 21 August 2005; accepted 16 September 2005

In an attempt to mimic the active sites of the anion-binding enzymes vanadium haloperoxidase and acid phosphatase, two tripodal receptors have been shown to bind phosphate and vanadate anions in organic solvents through H-bonding interactions.

Keywords: Anion binding; Enzyme models; Vanadate; Phosphate; Vanadium haloperoxidase

INTRODUCTION

Anion binding by supramolecular receptors is an important topic within supramolecular chemistry [1], partly due to the fact that anions are often found at the active site of enzymes, where they are bound through non-covalent interactions. Two enzymes that bind anions as co-factors at their active sites are the vanadium haloperoxidases [2–7] (VHPO) and bacterial acid phosphatases (AP) [8]. Despite these enzymes having relatively low amino acid sequence identity, their three dimensional structures are similar and, as described below, the residues that bind the anion, either phosphate or vanadate, are conserved. Several crystal structures of the VHPOs, that catalyse the oxidative halogenation of aromatic compounds, have now been solved including one chloroperoxidase [5] and two bromoperoxidases [6,7]. These reveal virtually identical active sites, where the V(V) vanadate anion, HVO_4^{2-}, is bound through an array of hydrogen bonds and just one direct V–N bond from a histidine residue (Fig. 1a). It is quite remarkable that the crystal structure of the acid phosphatase from *Escherichia blattae* [8], which catalyses phosphomonoester hydrolysis, reveals a phosphate anion bound by identical H-bonding amino-acid residues to those in the VHPOs. However, no His-P bond is observed, although it is likely that one is formed as an intermediate during the catalytic cycle. Given these findings, it is not surprising that vanadate can replace phosphate and vice-versa in such enzymes [9], affecting or inhibiting their activities.

With one notable exception [10], previous examples of small molecule models of the VHPOs have not involved binding of the vanadate anion; instead vanadium is bound directly by a chelating (e.g. Schiff-base) ligand through coordinate bonds [11–14]. However, given that phosphate can be bound by tripodal supramolecular receptors through H-bonding interactions [15], we decided to investigate the potential of these receptors as simple models for the VHPOs and APs (Fig. 1b), in the expectation that a closer structural (and consequently functional) model for these enzymes could be established.

RESULTS AND DISCUSSION

Compounds **1** [15] and **2** were synthesised in one step from their corresponding tetraamines, tris(2-aminoethyl)amine and tris(3-aminopropyl)amine [16] respectively. Binding studies were carried out using the tetrabutylammonium salts of dihydrogenphosphate and dihydrogenvanadate as guest species; the latter was synthesized using a literature procedure [17] and characterized by elemental

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ISSN 1061-0278 print/ISSN 1029-0478 online © 2006 Taylor & Francis
DOI: 10.1080/10610270500368840

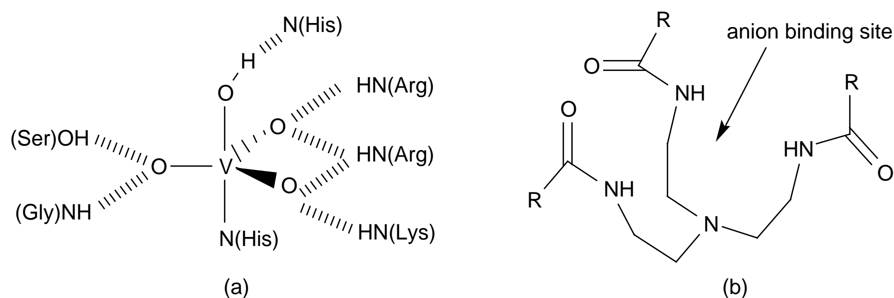
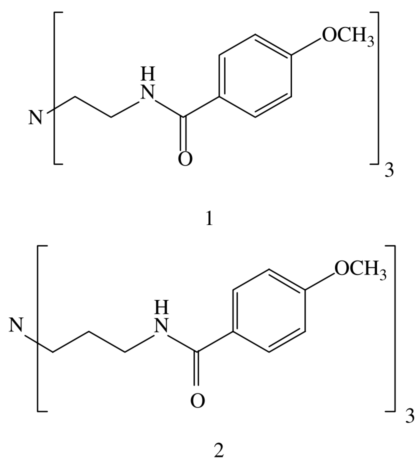


FIGURE 1 Schematic structures of (a) the active site of the VHPOs as determined by X-ray crystallography, and (b) a tripodal receptor as a simple model of the VHPOs and APs, showing the H-bonding anion pocket and a nitrogen atom at its apex for possible V–N and P–N bond formation, respectively.

analysis. The use of organic solvents for binding studies was not considered to be at odds with modelling the VHPOs, given that they are known to retain their catalytic activity in a range of organic solvents [18] and the intention to carry out catalytic studies on organic substrates in the future.



As expected from previous studies on this ligand [15], receptor **1** bound $H_2PO_4^-$ through H-bonding interactions in CD_3CN, as evidenced by the downfield shift of the amide resonance upon addition of excess guest (Fig. 2). The titration data were fitted using the computer program EQNMR [19] to give a 1:1 binding constant of $496\,M^{-1}$ ($\pm$10%), which was very similar to a literature value [15] of $510\,M^{-1}$ between these two species in acetonitrile. Studies were then repeated on **1** using the analagous vanadate salt, which produced very similar downfield shifts on the NMR spectrum and an almost identical binding curve (Fig. 2), consistent with a similar binding mode† through H-bonding interactions.

Although monomeric vanadates and phosphates are similar in terms of structure and chemical behaviour, (e.g. pK_a values) vanadates show a higher tendency to oligomerize in solution, forming dimers and higher aggregates in organic and aqueous media [20,21]. ^{51}V NMR is a useful tool for studying such effects. The spectrum of $(Bu)_4N^+H_2VO_4^-$ at 10 mM displayed peaks that corresponded to both monomeric and higher aggregates consistent with literature data for this compound in this solvent (see ESI) [20]. However at a lower concentration of 1 mM, only two major peaks were observed at -517 and -593 ppm, respectively, which have previously been assigned [20] to two monomeric species, the more upfield signal corresponding to either a peroxide or an acetonitrile adduct with vanadate. Upon addition of an excess amount (ca. 10 equivalents) of ligand **1** to the more concentrated (10 mM) solution of the guest, no significant changes were observed to the spectrum apart from a slight shift in the equilibrium towards monomeric species. Similar results were observed in the solvent mixture 2/1 CD_3CN/CD_2Cl_2 at a lower concentration of 1 mM (*vide infra*, also see ESI).

Binding studies were then repeated for the new, larger receptor **2**, which was studied in a 2/1 ratio of CD_3CN/CD_2Cl_2 for solubility reasons. At a total host concentration of 1 mM, the addition of phosphate induced a characteristic downfield shift in the signal for the three amide protons (see ESI), which was again consistent with an H-bonding interaction.

However the addition of vanadate produced a very different effect; in particular a net upfield shift to the amide NH signal was observed upon the addition of the first two equivalents of guest and at the same time, the amino-NCH_2 resonance underwent a sizeable upfield shift from 3.13 to 2.50 ppm (Fig. 3). An upfield shift in this resonance has been observed previously in the binding of vanadate in aqueous solution by a guanadium-based tripodal receptor [10] and is indicative of the formation of a

†A 1:1 binding constant of $540\,M^{-1}$ ($\pm$10%) was determined but this assumes the presence of negligible amounts of higher vanadate aggregates in solution over the concentration range used in the titration which is unlikely (see discussion on ^{51}V NMR studies).

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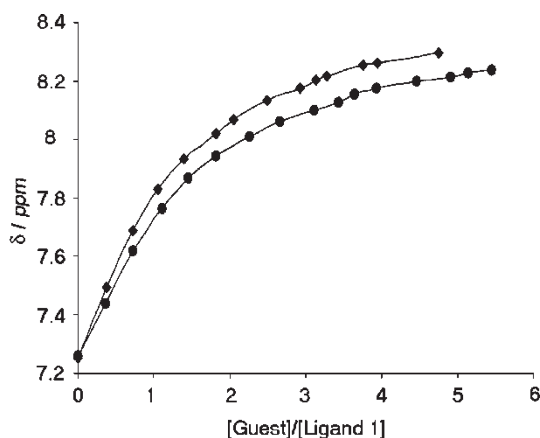

FIGURE 2 Titration in CD_3CN between ligand **1** and vanadate (◆) and phosphate (●) showing the change δ(NH) as a function of guest/ligand ratio $([1])_{initial} = 10$ mM).

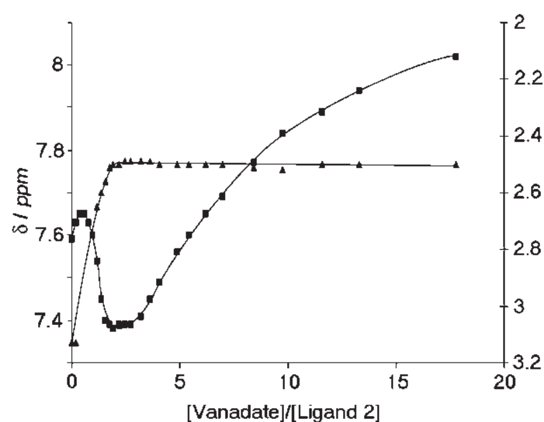

FIGURE 3 ^{1}H NMR titration between ligand **2** and vanadate in 2/1 CD_3CN/CD_2Cl_2. Triangles: δ(NCH_2), right-hand scale; Squares: δ(NH), left-hand scale; $[2]_{total} = 1$ mM.

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V–N bond. Once changes to the NCH_2 resonance were complete, the amide resonance underwent a gradual shift to lower field upon addition of further amounts of guest, in accordance with an H-bonding interaction (Fig. 3). These observations indicate that formation of a direct V–N bond from the central apical nitrogen in receptor **2**, as desired for a good structural model of the vanadate binding site in VHPO, occurs at the expense of any significant H-bonding interaction with the guest (evidence for coordination via this atom comes from the fact that only the NMR signal for the CH_2 group adjacent to it is significantly affected by complexation). However once V–N bond formation is complete, then a separate vanadate species binds to the receptor through H-bonding interactions. As a control, binding studies carried out on **1** in 2/1 CD_3CN/CD_2Cl_2 at 1 mM produced a titration curve indicative of amide H-bond formation only (see ESI), as observed in CD_3CN alone (Fig. 2), thus ruling out V–N bond formation being caused by a change in solvent.

The ^{51}V NMR spectrum of uncomplexed vanadate at 10 mM in 2/1 CD_3CN/CD_2Cl_2 gave a spectrum not dissimilar to that in CD_3CN alone at this concentration, indicating the presence of several aggregated species, although higher aggregates were more favoured presumably due to the poorer H-bonding ability of CD_2Cl_2. Interestingly, upon the addition of excess amount (ca. 10 equivalents) of ligand **2** to the solution, a new set of peaks appeared upfield (centred at ca. −570 ppm) in contrast to that observed with **1** where no changes were observed. Previous NMR studies have shown that donor atom coordination to vanadium is accompanied by a new ^{51}V signal [10,20]. The data therefore supports the notion that only ligand **2** forms a direct V–N bond; in support of this, the amino NCH_2 resonance on the ^{1}H NMR spectrum of **1** underwent only a very small shift (+0.05 ppm) upon addition of excess vanadate‡. At the lower anion concentration of 1 mM in CD_3CN/CD_2Cl_2, where the initial vanadate species was largely monomeric, a similar binding process was observed by ^{51}V NMR, that is the addition of excess **2** led to the emergence of a set of new upfield signals centred at ca. −570 ppm and the disappearance of peaks for free vanadate (see ESI). The fact that there is more than one vanadium signal indicates that under these conditions (i.e. with excess ligand in this solvent mixture) the interaction between **2** and vanadate leads to more than one type of complexed species in solution.

Despite their structural similarities and their similar binding behaviour towards phosphate, it is clear that **1** and **2** bind vanadate in strikingly different ways. This effect must be related to the ease with which each receptor is able to form a stable V–N bond involving the apical nitrogen atom. Since tributylamine was not found to bind vanadate by either ^{51}V or ^{1}H NMR under similar conditions, it is reasonable to assume that a chelating binding mode, involving one or more amide units and the apical nitrogen atom, stabilises the V–N in bond **2**, which, as evidenced by two equivalents of guest being required to obtain a full shift in the NCH_2 resonance (Fig. 3), might allow dimeric vanadate to bridge between the donor atoms. However such a process does not appear to prevent a subsequent H-bonding interaction at the amide units involving a separate vanadate species. In contrast, no stable chelating mode is possible for **1** due to its shorter spacer unit, or indeed for **1** and **2** in the case of

‡Shifts in the NCH_2 ^{1}H NMR resonance upon addition of excess phosphate: Receptor **2** (in 2/1 CD_3CN/CD_2Cl_2): 3.14–2.80 ppm; Receptor **1** (in CD_3CN): 2.70–2.73 ppm.

phosphate resulting in simple H-bonded complexes in these cases.

CONCLUSIONS

In summary, we have demonstrated the first successful complexation of vanadate by simple tripodal receptors in organic solvents and the first direct evidence of its binding by H-bonding interactions, as similarly observed at the active sites of the VHPO enzymes. Further work will involve designing receptors that allow the simultaneous H-bonding and V–N coordination of vanadate and an investigation of the activity of H-bonded anion complexes in reactions catalysed by the VHPOs and APs. The recent characterization of the halide binding site of the VHPOs in close proximity to the vanadate site [22] offers further scope for receptor design.

EXPERIMENTAL

General

Reagent grade reactants and solvents were used as received from chemical suppliers. The synthesis of all compounds was carried out under nitrogen unless specified otherwise. ^{1}H and ^{13}C NMR spectroscopy were performed on a Bruker AC300 or a Bruker Advance DRX 400 spectrometer. ^{51}V spectroscopy was performed on a Bruker Advance DRX 400 spectrometer and spectra were referenced to sodium orthovanadate (Na_3VO_4) as an internal standard. Chemical shifts are reported in ppm (δ). IR spectra were recorded on a Nicolet 550 spectrometer. Compound **1** [15], tris(2-cyanoethyl)amine [23], tris(3-aminopropyl)amine [16] and tetrabutylammonium dihydrogenvanadate [17] were prepared using literature procedures. Elemental analysis for $C_{16}H_{38}NO_4V.1H_2O$, found: C, 50.93; H, 10.58; N, 3. 69%. Calcd.: C, 50.92; H, 10.68; N, 3.71%. Titrations were carried out by adding aliquots from an anion stock solution to a receptor solution in an NMR tube. Except for those titrations depicted in Fig. 2, the anion stock solution also contained dissolved receptor at the same concentration as that of the receptor solution so that the total receptor concentration (bound and unbound) remained constant over the course of the titration.

Tris(4-methoxybenzamido-3-propyl)amine 2

Tris(3-aminopropyl)amine (1.32 g, 0.007 mol) in dichloromethane (100 ml) was added to triethylamine (2.79 ml, 0.20 mol), cooled in an ice-bath. 4-Methoxybenzoyl chloride (3.47 g, 0.02 mol) in dichloromethane (50 ml) was then added drop-wise to the cooled solution over one hour. The resulting mixture was poured into 200 ml of ice-cold deionised water and extracted with dichloromethane (2 × 100 ml) and dried with $MgSO_4$. Following removal of the solvent, the crude product was purified by column chromatography on silica (30% methanol in dichloromethane) to give **2** as a white solid (1.65 g, yield 40%), m.p = 154–155°C. The compound could be isolated in crystalline form by recrystallization from dichloromethane/diethyl ether. ^{1}H NMR, 400 MHz, ($CDCl_3$): 2.59 (6H, t, $J = 6.0$ Hz, NC$\boldsymbol{H}_2$), 3.48 (6H, m, NCH$_2$C$\boldsymbol{H}_2$), 3.80 (6H, t, $J = 9.0$ Hz, NCH$_2$CH$_2$C$\boldsymbol{H}_2$NH), 3.80 (9H, s, OC$\boldsymbol{H}_3$), 6.88 (6H, d, $J = 8.8$ Hz, ArH), 7.06 (3H, s, N***H***CO), 7.80 (6H, d, $J = 8.8$ Hz, ArH). ^{13}C NMR ($CDCl_3$) 400 MHz: 23.82, 37.09, 50.63, 55.73, 114.13, 125.79, 129.61, 162.80, 168.19 IR (KBr): v_{max}/cm^{-1}: 1548 (C=C Aromatic), 1631 (C=O), 2958br (C–H), 3299 (N–H); Elemental analysis for $C_{33}H_{42}N_4O_6.0.5H_2O$, found: C, 66.21%; H, 7.00%; N, 9.08%. Calcd.: C, 66.09%; H, 7.23%; N, 9.34%.

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