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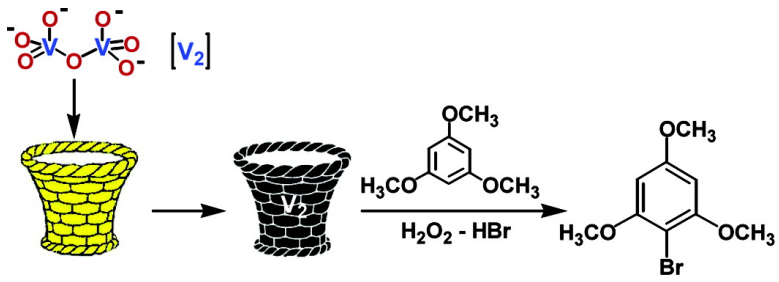

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A Supramolecular Fluorescence Sensor for Pyrovanadate as a Functional Model of Vanadium Haloperoxidase

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In recent years, remarkable progress has been achieved on the development of artificial receptors and sensors for anions,[1] the transition metal oxoanions, such as vanadate; however, it received little attention,[2] in contrast to other anions of biological importance, such as phosphate or pyrophosphate.[3] Vanadate is not only the structural analogue of phosphate in tetrahedral coordination but also a transition state analogue of phosphate in the pentacoordinated form; accordingly, vanadate interacts with a broad range of phosphate-metabolizing enzymes.[4] Vanadate was also found as the cofactor of vanadium haloperoxidases (V−HPOs),[5] which are important enzymes catalyzing the halogenation of natural compounds.[6] The X-ray structures of the enzymes revealed that the anionic cofactor (HVO_4^{2-}) is mainly bound via electrostatic interaction and a hydrogen-bond network from the positively charged amino acid residues, except of a single coordinating bond from Nϵ2 of a histidine to the metal center.[7] We recently reported the first supramolecular tris(guanidinium) receptor **1** and its corresponding HVO_4^{2-} complex as a structural model of V−HPO.[8] We present herein a modified receptor which preferentially binds pyrovanadate ($V_2O_7^{4-}$).

Introducing pyrene moieties at each N-terminal of the guanidinium substructures of **1** gives rise to a modified receptor, tris-(2-(*N*′-pyren-1-ylmethylguanidinium)ethyl)amine **2** (for synthesis, see Supporting Information). The stacking tendency of pyrene units was expected to hold the three guanidinium moieties in close proximity, rendering the molecule a more preorganized basket-shaped receptor to facilitate the binding of suitable anions. Moreover, compared to **1**, pyrenes increase the solubility of **2** in organic solvent, in which the electrostatic interaction and hydrogen bonds with anions should be enhanced as compared to those in water. Further, the $-CH_2-$ link between the guanidinium units and the pyrenes allows for the photoinduced electron transfer (PET) from the anion binding substructure to the excited fluorophore.[9]

The fluorescence spectrum of **2** in acetonitrile exhibits characteristic emission bands of the pyrene monomer at 376 and 396 nm and a dominant band at 474 nm assigned to the emission of the pyrene excimer,[10] which confirms the stacking of pyrenes. Upon titration with vanadate, the fluorescence of both monomer and excimer was significantly quenched (Figure 1). The interaction of vanadate with **2** raises the HOMO of the guanidinium units above the HOMO level of the fluorophore, and hence, upon excitation, electron transfer from guanidinium to pyrene occurs, quenching the normal emission process. The complexation with vanadate was also observed in the absorption spectra (Figure 2), and the pyrene transition bands became red shifted (i.e., $\Delta\lambda = 6$ nm for ^1L_a band) and broader (the broadening is qualitatively indicated by the ratio of peak-to-valley intensities, P_a), implying that the pyrenes of **2** become more tightly packed on binding vanadate.[10]

The titration curve derived from fluorescence data reveals a sharp break-point at 2 equiv of monovanadate ($\mathbf{V_1}$); consequently, the Job plot gives a maximum at 1/3 for the molar fraction of **2**

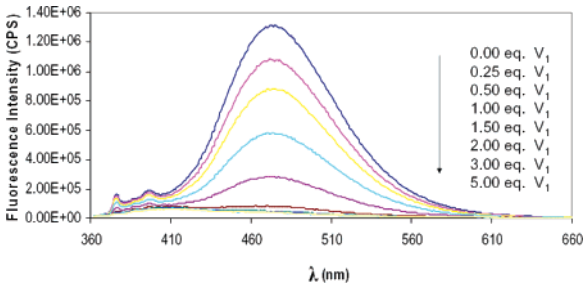

Figure 1. Fluorescence spectra of **2** (3 μM in acetonitrile) in the presence of different equivalents of vanadate; $\lambda_{ex} = 345$ nm, the isosbestic point in the corresponding UV spectra (Figure 2).

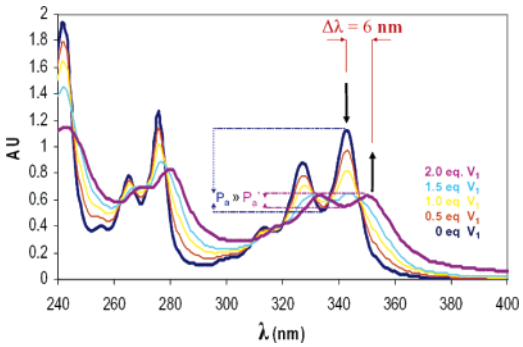

Figure 2. UV spectra of **2** (3 μM in acetonitrile) in the presence of different equivalents of vanadate.

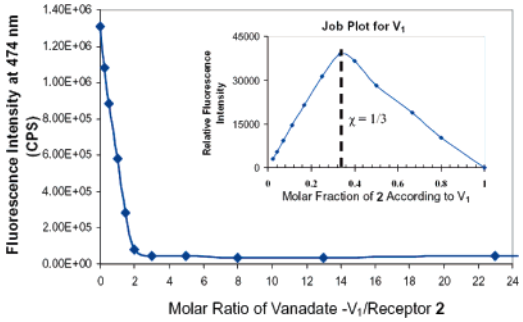

Figure 3. Fluorescence titration curve of **2** (3 μM in acetonitrile) with vanadate and the related Job plot (inset).

according to [HVO_4^{2-}] (Figure 3). The observed stoichiometry indicates that **2** preferentially binds pyrovanadate ($\mathbf{V_2} = V_2O_7^{4-}$) rather than monovanadate. Since a solution of vanadate[4a] contains monovanadate in equilibrium with its oligomers, the binding constant of $\mathbf{V_2}$ to **2** can only be estimated to be $K_a > 10^8$ M^{-1}. In contrast, phosphates, the structural analogues of vanadates, are kinetically inert with respect to oligomerization.[4a] Hence, the binding of pyrophosphate to **2** was used as a probe to confirm the estimation of K_a of $\mathbf{V_2}$. Indeed, very similar changes were observed in both UV and fluorescence spectra when **2** was titrated with

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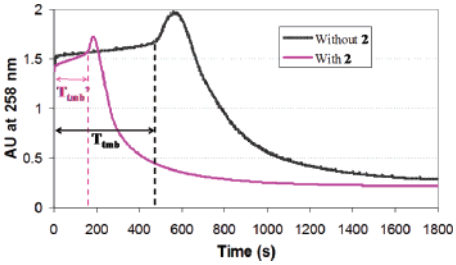
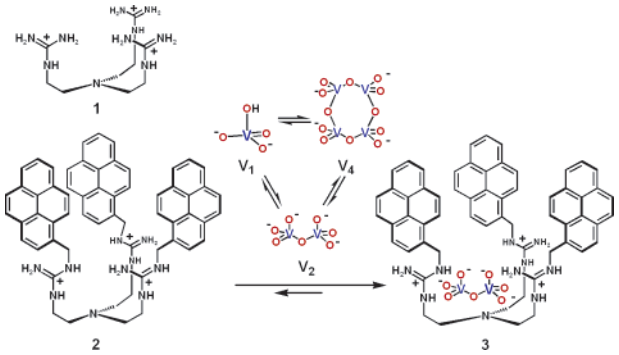


Figure 4. Competitive catalytic bromination of TMB/MCD with and without 3 μM **2**. T_{tmb} represents the duration of bromination of TMB. Intensity changes at 258 nm were recorded every 2 s. Reaction conditions: 6 μM vanadate (counted as $\mathbf{V_1}$), 0.12 mM MCD, 0.12 mM TMB, 0.5 mM H_2O_2, and 0.24 mM HBr.

Scheme 1



pyrophosphate in acetonitrile. The nonlinear least-squares fitting reveals that the 1:1 complex is formed, and that **2** binds pyrophosphate with $K_a = 1.5 \times 10^7$ M^{-1}. In contrast, the affinity of **2** to monophosphate is much lower.

Supporting evidence for pyrovanadate binding to **2** was obtained from a ^{51}V NMR study in DMSO-d_6/D_2O (3:1). A 0.5 mM vanadate stock solution shows three peaks at −546, −574, and −585 ppm, which can be assigned to $\mathbf{V_1}$, $\mathbf{V_2}$, and $\mathbf{V_4}$, respectively (Scheme 1).[11] Upon the addition of **2**, the $\mathbf{V_1}$ and $\mathbf{V_4}$ peaks disappeared, while the resonance corresponding to $\mathbf{V_2}$ became dominant and significantly broader. This situation was reached above a 1.3-fold excess of **2**. It appears that the equilibrium of oligomeric vanadates shifts toward $\mathbf{V_2}$ that binds to **2**, albeit with a smaller K_a than in acetonitrile.

Many covalent vanadate complexes functionally mimic vanadium bromoperoxidases (V−BPO).[2,12] Butler reported that vanadate can mimic V−HPO in acidic aqueous solution, catalyzing the oxidation of $Br^- \rightarrow$ "Br^+" in the presence of H_2O_2, which can subsequently brominate organic substrates.[13] The active species was proposed to be a dimerized peroxovanadate $V_2O_2(O_2)_3$.[11a,13b] Our results show that the catalytic activity of vanadate is enhanced by changing water for acetonitrile.[11a,14] More interestingly, when vanadate forms a supramolecular complex with **2**, the catalytic efficiency is further enhanced significantly, as demonstrated by the following competition experiment (Figure 4).

1,3,5-Trimethoxybenzene (TMB), monochlorodimedone (MCD), H_2O_2, and **3** were premixed in acetonitrile. The reaction was initiated by addition of HBr (54 mM stock solution in acetonitrile) and followed by UV at 258 nm, the characteristic absorption of MCD.[15] Since TMB is much more reactive than MCD, it reacted preferentially with little change of UV at 258 nm during its bromination. However, as soon as the bromination of TMB was completed, MCD was brominated as indicated by the decreasing UV intensity at 258 nm. Hence, the period of UV-insensitive time corresponds to the total reaction time of TMB bromination. The bromination of TMB took about 480 s (Figure 4) when the reaction was catalyzed by vanadate alone; this reaction time was shortened to 150 s (turn over = 20) in the presence of **3**, which indicates that the catalyst is about 3 times more reactive with respect to the catalytic bromination of TMB. The increasing of UV intensity at 258 nm before the bromination of MCD is due to the formation of Br_3^- (λ_{max} = 268 nm in acetonitrile). Subsequent reaction with MCD led to the decrease of UV intensity at 258 nm. From the slopes, it can clearly be concluded that the catalytic bromination of MCD is also significantly faster in the presence of **3**. The catalytic system can be easily scaled up to a preparative scale for many organic substrates; for example, the bromination of TMB could be performed in gram-scale with 100 turnover within less than 10 min and quantitative yield.[14]

In conclusion, **2** can preferentially bind pyrovanadate ($\mathbf{V_2}$) over monovanadate ($\mathbf{V_1}$). The complexation of $\mathbf{V_2}$ by the host molecule was coupled with fluorescence quenching due to a PET-type mechanism,[1a,9] which renders **2** the first fluorescence probe for pyrovanadate. Moreover, the interaction of $\mathbf{V_2}$ with **2** significantly improves the catalytic activity of vanadate, generating the first supramolecular mimic of V−BPO.

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Supporting Information Available: Synthesis of **2**, UV and ^{51}V NMR titrations, and control experiments of catalytic bromination of MCD (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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