Vanadium-Bromoperoxidase-Mimicking Systems: Direct Evidence of a Hypobromite-Like Vanadium Intermediate

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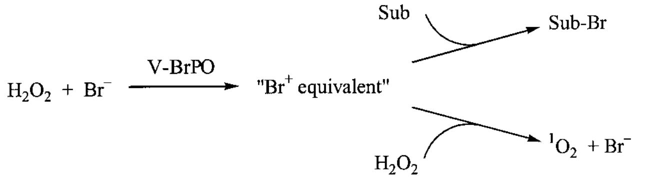
Here we report a direct observation, by electrospray ionization mass spectrometry, of the intermediate $[VO(OH_2)(OH)(OBr)]^+$, which may be involved in the catalytic cycle of the V-HalPO enzymes. Ab initio calculations also support the stability of such a species.

Vanadium-dependent bromoperoxidases (V-BrPOs) catalyze the oxidation of bromide ion by hydrogen peroxide.[1−3] Kinetic studies on V-BrPOs indicate a mechanism where V^V ion binds H_2O_2 to produce a peroxo complex that reacts with the Br^- ion to generate an oxidized "bromine equivalent" species, able either to halogenate organic substrates or to react again with H_2O_2 to form singlet oxygen[1−3] (Scheme 1).



Scheme 1. General reactivity for vanadium haloperoxidases

The nature of the intermediate (e.g. HOBr, Br_2, Br_3^-, V_{enz}-OBr, enz-Br), however, is still a matter of some controversy.[1−4] With the aim of shedding light on this point several functional models of V-BrPOs have been reported.[1−4] These, however, fail to reproduce the chemistry of V-BrPOs for different reasons. A two-phase system has also been proposed as a mimicking prototype of the enzymatic activity on the basis of similar reactivity found during peroxidative bromination[5] (Scheme 2).

In this latter case, kinetic data and a product distribution analysis supported the occurrence of a V-containing hypobromite-like intermediate as the "bromine equivalent", although without direct evidence. This proposal was further justified by ab initio calculations which showed that the suggested intermediate lies at a relative minimum of potential energy in comparison with the reactants $[OV(O_2)^+]$/HBr and the couple $[O_2V(H_2O)^+]$/HOBr.[6]

An analysis of the system behaviour[5,6] as a function of the aqueous phase pH and H_2O_2 concentration was performed with styrene as the organic substrate. With this substrate, in our two-phase system, formation of two products has been observed: the corresponding bromohydrin and the dibromide. These crucial results are reported in Table 1.

Variation of the above mentioned parameters produces the selective formation of the mono- or the diperoxovanadium derivative in the aqueous phase, as indicated by ^{51}V NMR analysis of the aqueous phase of the reaction mixture.[4] In particular, at pH 1.1 ($HClO_4$) equal concentrations of NH_4VO_3 and H_2O_2 give rise to more than 95% of the monoperoxo derivative (reaction 1 in Table 1). Upon decreasing the $[NH_4VO_3]/[H_2O_2]$ ratio to 0.5, in otherwise identical conditions, mono- and diperoxovanadium complexes are formed in roughly a 1:1 ratio (reaction 2). By increasing the pH (≥ 3) the almost exclusive formation of the diperoxo derivative is obtained; in such conditions the efficiency of the reaction strongly decreases (reactions 3−5).[4]

Interestingly, the two reactions (1 and 2) carried out at the same pH (1.1) afforded an almost identical amounts of products. This fact indicates that the dimeric triperoxovanadium derivative, invoked as the active species in the oxybro-

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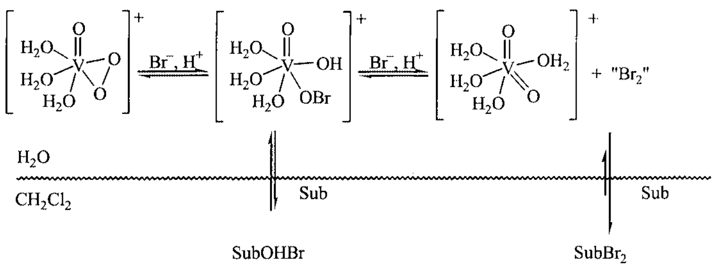


Scheme 2. Two-phase system for oxybromination of organic substrate catalyzed by V^V peroxo complexes in the presence of bromide ions

Table 1. Oxybromination reactions of styrene (1.6 mmol) with NH_4VO_3 (0.2 mmol) and H_2O_2 (0.4 mmol) in the presence of KBr (0.5 mmol) in $H_2O/CHCl_3$ 20:20 mL, T = 25 °C, stirring rate 800 rpm

	pH	H_2O_2 consumed (%)	Yield of bromohydrin (%)[b]	Yield of dibromide (%)[b]
1[c]	1.1	95	61.8	5.4
2	1.1	90	60.5	1.9
3	1.8	98	54.6	0.9
4	3.0	21	16.5	0.3
5	4.0	18	2.7	n.d.

[a] Products analysed after 4 hours. [b] Yield calculated on the basis of the consumed oxidant. [c] NH_4VO_3 0.4 mmol.

mination reaction, is not involved,[1] and probably that the only peroxo derivative implicated in the formation of the "bromine equivalent" species is the monoperoxo complex. Thus, either the reactivity of the diperoxo complex is markedly lower (reactions 4 and 5), or it acts as a reservoir of the active species.

It has been shown recently that electrospray ionization mass spectrometry (ESI-MS) in association with heteronuclear NMR spectroscopy and ab initio calculations is a powerful combination for the elucidation of solution structures of highly reactive intermediates.[7−9] Therefore, this approach has been applied to the $V/H_2O_2/H^+/Br^-$ system in order to obtain direct experimental information on the occurrence of the postulated "bromine equivalent" species.

The ESI-MS positive-ion mode full scan of solutions containing millimolar vanadate and H_2O_2 at a pH of about 1 ($HClO_4$) in 1:1 water/alcohol (MeOH or EtOH) mixtures shows the occurrence of different species identified as the monoperoxo derivatives $[OV(O_2)(H_2O)]^+$ (at *m*/*z* = 117), $[OV(O_2)(H_2O)_2]^+$ (at *m*/*z* = 135) and $[OV(O_2)(H_2O)(ROH)]^+$ (at *m*/*z* = 149 or 163), depending on the alcohol used, and the dimeric peroxo complexes $[\{OV(O_2)\}_2O(H_2O)_2(ROH)(H)_2]^{2+}$ (at *m*/*z* = 142 or 149) and $[\{OV(O_2)\}_2O(H_2O)(ROH)(H)]^+$ (at *m*/*z* = 265 or 279). Other significant peaks corresponding to the monomeric and dimeric non-peroxidic species are equally observed as well, in agreement with previously reported data.[7] The addition of a fivefold excess of Br^- to the infused H_2O/MeOH solution gives rise to the formation of a new vanadium species containing a single bromine atom, as demonstrated by the related isotopic cluster. The new complexes, although transferred in the gas-phase in low relative abundance (≤15%), were clearly detected as doublets at *m*/*z* = 179 ^{79}Br (181, ^{81}Br), 193 (195) and 197 (199) and tentatively identified as shown in Table 2.

The substitution of methanol with ethanol in the infusion solvent composition produces no significant changes in the full-scan mass spectrum, with the exception that the ionic species at *m*/*z* = 193 (195) is replaced by the homologous ethoxy-containing ion at *m*/*z* = 207 (209). Further support for the identification of the species was obtained by collision-induced decomposition (MS^2) spectra and the relevant results are collected in Table 2. For low energies (up to 0.5 V), the only peaks observed are those related to ion-molecule reactions of the selected species P with solvent molecules. At higher energies (1.5 V), decomposition takes place with loss of HBr, accompanied by addition of water molecule(s). Formation of dinuclear doubly charged species, i.e. *m*/*z* = 147 (149) $[V_2Br_2O_2H_2]^{2+}$ and $[V_2Br_2O_2C_2H_6]^{2+}$ 161 (163), formally deriving from expulsion of O_2, is also observed.[10]

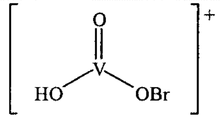
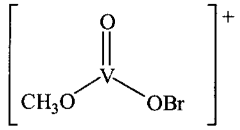
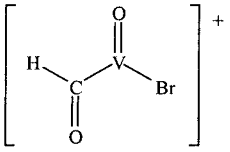
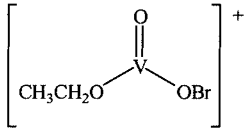
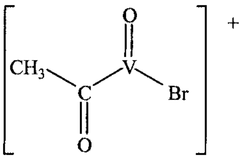
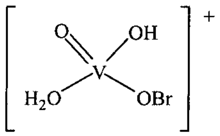
Of special interest is the formal loss of water found in the MS^2 spectra of the $-OCH_3$ [*m*/*z* = 193 (195)] and $-OCH_2CH_3$ [*m*/*z* = 207 (209)] containing derivatives. This process, which corresponds to an internal oxidative decomposition, formally produces ions where a direct coordination of a carbonyl moiety to the metal may be invoked.[4] This gas-phase chemistry has also been observed with other vanadium picolinate[11] and ruthenium[12] derivatives. Synthesis of stable alkyl-V^V complexes has also been reported.[13]

The involvement of fragmentations occurring on vanadium-bound ligands other than OBr confirms that bromide ion is not bound to the complex merely as an ionic couple, but through covalent bonds probably originating from the activation of the peroxidic O−O bridge.[14]

The analysis of the $V/H_2O_2/H^+$ system, upon addition of an excess of Br^-, was completed by extending the ESI-MS inspection to solutions containing a twofold excess of H_2O_2 with respect to vanadate, at a pH of about 2. According to previous studies,[7] the diperoxo complex is the dominant species. These solutions, analyzed both in positive- and negative-ion mode, did not reveal the activation of diperoxovanadium species by bromine ions, in agreement with the reactivity reported above (Table 1).

Further support for the rational formation of the vanadium-containing "bromine equivalent" species was ob-

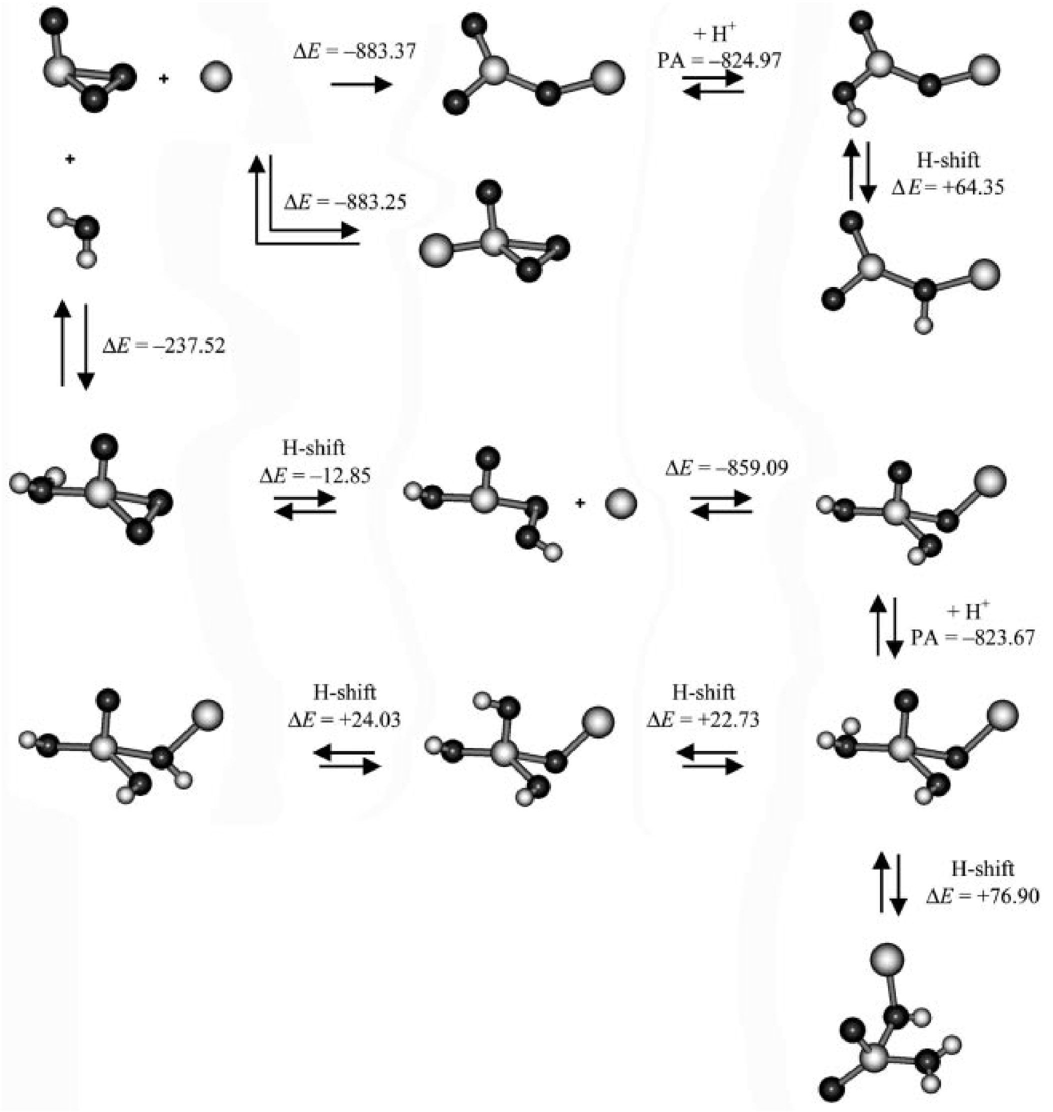
Table 2. MS^2 ions observed after collision-induced decomposition of the precursor ionic species (P) in mixed H_2O/CH_3OH (or CH_3CH_2OH) under ESI-MS conditions[a]

P m/z	P + CH_3OH m/z	P + H_2O m/z	P –HBr +H_2O m/z	P –H_2O m/z	P –HBr+2H_2O m/z	[b] m/z	[b] m/z
 179 (181)	211 (213)	197 (199)	----	----	135 (135)	147 (149)	161 (163)
 193 (195)	225 (227)	211 (213)	----	 175 (177)	149 (149)	147 (149)	161 (163)
 207[c] (209)	253[c] (255)	225 (229)	145 (145)	 189 (191)	------	147 (149)	161 (163)
 197 (199)	229 (231)	215 (217)	135 (135)	179 (181)	------	------	161[c] (163)

[a] The *m*/*z* values refer to the presence of ^{79}Br or ^{81}Br. [b] Doubly charged ions $[V_2Br_2O_2H_2]^{2+}$ [*m*/*z* = 147 (149)] and $[V_2Br_2O_2C_2H_6]^{2+}$ [*m*/*z* = 161 (163)], see text. [c] EtOH as the co-solvent.

tained by performing ab initio calculations on the various species that can be obtained by reaction of the monoperoxo vanadium compound and bromine, both in the presence or in the absence of a molecule of solvent in the coordination sphere of the metal. Scheme 3 collects the results obtained by effecting geometry optimization of all the structures with density functional theory (DFT) calculations carried out with Becke's hybrid three-parameter functional with Lee−Yang−Parr nonlocal correlation (B3LYP).[15,16] Calculations were conducted with the (99,302) numerical integration grid[16] and full accuracy at all stages in order to achieve SCF convergence. The 6-311G+(d,f) basis set implemented in the Gaussian 98 suite of programs was used.[16] After the geometry optimisation step, the energy values weren evaluated through single point calculations (performed using the SCF=tight option in Gaussian 98) at the B3LYP/6-311G++(3df,3dp) level of theory.[16]

The most interesting feature of the results collected in Scheme 3 is the relative stability of the conjugated acids of the species $[OV(OH)_2(OBr)]$. Such a derivative presents three distinct protonation sites: a) the oxygen atom of the V=O group, b) the -OH oxygen atom, and c) the oxygen atom of the -OBr group. The corresponding protonated species are indicated in Scheme 3. Indeed, the structure with the proton located at the -OBr residue is the one located energetically uphill with respect to the other possible species protonated at the oxygen atom of the hydroxy moiety (ca. 47 kJ mol^{-1}), at the oxo moiety (ca. 24 kJ mol^{-1}) or at both hydroxy and oxo moieties (ca. 77 kJ mol^{-1}). This intermediate, $[OV(OH)_2(OHBr)]^+$, would be the species that releases HOBr — one of the postulated brominating species — in solution. Interestingly, the most stable protonated isomer corresponds to the $[OV(OH_2)(OH)(OBr)]^+$ structure. Following this result this species may be directly involved in the Br^+ transfer process. Furthermore, it should be noted that the thermodynamically feasible coordination of bromide ion to the cationic naked monoperoxo species, a process which does not involve oxidation of the halide, if it occurs in solution during the catalytic process, is probably not located along the reaction coordinate. According to this assumption, we decided to omit from our theoretical study all the possible species with the bromine moiety directly coordinated to the vanadium atom, such as $[OVBr(O_2)(OH_2)]$ and the related protonated derivatives. All these species can



Scheme 3. Ab initio calculations on the formation of the V-OBr intermediates; energy values are reported in kJ mol^{-1}; PA = proton affinity

be considered as real ionic couples, especially in the gas phase, where the electrostatic compensation between the negatively charged bromine ion and the positively charged peroxo-vanadium complexes might be the chemical explanation for their relatively high thermodynamic stabilities.

In conclusion we have reported a direct observation of the intermediate $[OV(OH_2)(OH)(OBr)]^+$ by ESI-MS spectrometry. Ab initio calculations also support the stability of such a species confirming that the choice of an appropriate model and its analysis with suitable techniques allows the collection of significant data for a better understanding of the mechanism of action of the V-HalPO enzymes.

Experimental Section

The ESI-MS measurements (positive-ion mode) were obtained using an LCQ instrument (Thermoquest, San Jose, CA). The peroxovanadium solutions were prepared by dissolving $n\mathrm{Bu_4NVO_3}$ (5×10^{-4} mol·L^{-1}) in MeOH/H_2O (or EtOH for the experiments conducted in this solvent) pH 1 $HClO_4$, in the presence of an equimolar amount of H_2O_2. In the experiments conducted in the presence of Br^-, a fivefold excess of KBr was used. Solution flow rate 8 μL·min^{-1}, capillary temperature 180 °C, spray voltage 2.5 kV, capillary voltage 5 V, tube lenses offset 15 V, nebulizing gas N_2 (40 units flow rate). The parameters related to octapoles and detector were achieved by the automatic set-up procedure. Collision-induced decompositions of selected ions were obtained by applying a supplementary r.f. voltage (tickle voltage) to the end-cap electrodes of the ion trap (resonance activation). For experimental details on the two-phase reactions reported in Table 1 see refs.[5,6]

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