A dinuclear peroxo-vanadium(V) complex with coordinated tripeptide. Synthesis, spectra and reactivity in bromoperoxidation

J. Chem. Research (S), 2001, 172–174
J. Chem. Research (M), 2001, 0536–0551

Swapnalee Sarmah and Nashreen S. Islam*

Department of Chemical Sciences, Tezpur University, Tezpur 784 028, India

Synthesis of a triglycine peroxo-vanadate complex, $[V_2O_2(O_2)_3(Gly\text{-}Gly\text{-}Gly)_2].H_2O$ by the reaction of V_2O_5 with H_2O_2 and the tripetide at pH 2 is described; the peroxo-bridged complex is highly effective in generating a bromination competent intermediate at physiological pH.

Keywords: triglycine peroxo-vanadate complex

Introduction

Vanadium is a trace metal that influences a variety of biological functions.[1,2] Bromoperoxidase, involved in the biosynthesis of naturally occurring brominated products, contains vanadium that is essential for its activity.[1,2]

By itself, H_2O_2 is capable of oxidising bromide in highly acidic medium (pH<3) but is ineffective in solution at pH>5.0. This reaction is catalysed by $VOSO_4$[8] and V_2O_5.[9,10] Initially, the mono and diperoxovanadates (MPV and DPV) formed in solution containing vanadate and excess H_2O_2, with DPV predominating at pH > 5.0, were proposed to be the oxidants of bromide.[9] However, synthetic DPV and MPV could not substitute for the V_2O_5 and H_2O_2 mixture.[16] The proposal was then modified to include a dioxotriperoxodivanadate intermediate, presumed to form by combination of DPV and MPV,[17] but this dimeric species was found only in highly acidic medium. The need for the presence of vanadyl or excess vanadate[10] for effective bromination in the early experiments remained unexplained.

The requirement of free vanadate for the DPV-dependent bromination at pH > 5, implied that inactive DPV gains oxidant activity by forming a peroxo-bridged intermediate $[OVOOV(O_2)]^{3+}$, proposed as a proximate oxidant of bromide.[18] Support for such an intermediate as the bromide oxidant came from our studies on a synthetic compound with a "VOOV" bridge,[21] which could produce a bromination competent intermediate at physiological pH. In order to provide further evidence in confirmation of this potential of bromide oxidation and also in view of the paucity of information on peroxo-bridged divanadate compounds, we were interested in synthesising newer members of such complexes stabilised by biogenic heteroligands, *viz*, di- and tri-peptides. The information derived regarding structure, bonding and reactivity of such small peptide-vanadium systems can be expected to provide further insight into the vanadium-protein interaction in biochemical processes. In this paper, the synthesis and characterisation of a novel dinuclear tripeptide peroxo vanadate with a μ-peroxide group, and its activity in bromoperoxidation of organic substrates is reported.

Experimental

Synthesis of $[V_2O_2(O_2)_3(Gly\text{-}Gly\text{-}Gly)_2].H_2O$: V_2O_5 (0.25g, 1.37 mmol) was mixed with triglycine (0.76g, 4.02mmol), maintaining the molar ratio of V: triglycine at 1:1.5. To this 30% H_2O_2 (15 ml, 132.3 mmol) was added gradually with constant stirring. The mixture was cooled in an ice-bath and kept stirred for ~ 15 min by which time the solid dissolved, yielding a red-coloured solution. The pH of the solution was recorded to be 2. On adding pre-cooled ethanol (~ 50ml) to the above solution under continuous stirring, an orange coloured, pasty mass separated. After standing for ~ 15 min in the ice-bath, the supernatent liquid was decanted, and the residue was treated repeatedly with acetone:ethanol mixture under scratching until it became a microcrystalline solid. The product was separated by centrifugation, washed with ethanol and dried *in vacuo* over conc. H_2SO_4.

Anal. calc. for $[V_2O_2(O_2)_3(Gly\text{-}Gly\text{-}Gly)_2].H_2O$: V, 16.29; O_2^{2-},15.34; C, 23.0; H, 3.51; N, 13.42. Found: V, 16.6; O_2^{2-},15.25; C, 23.4; H, 4.0; N, 13.9.Yield: 52%.

IR (cm^{-1}): 952 [ν(V=O)], 845 [ν(O–O)], 805[ν(O–O)], 619[ν(V–O_2), ν_2], 560[ν(V–O_2), ν_3], 1678[ν(CO), amide I], 1610[ν_{as}(COO)], 1389[ν_s[COO)], 3255[ν(N–H)], 1564 (N–H deformation).

Measurement of bromination activity: The method of de Boer *et al*.[29] of introducing four bromine atoms into the molecule of phenol red to form bromophenol blue was used to measure bromination activity. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (2M) and phenol red (20 μM). The reaction was started by adding a weighed amount of the solid compound (0.14 mg/ml), and was monitored by the increase in absorbance at 592 nm of bromophenol blue.

Instant bromination activity: 65 μM bromine transfer per mM compound.

Secondary rate of bromination: 8 μM Br transfer/min with 1 mM compound.

Results and discussion

One of the essential parameters for achieving success of the synthesis of the peroxo-bridged complex, $[V_2O_2(O_2)_3(Gly\text{–}Gly\text{–}Gly)_2].H_2O$, was the use of an acidic medium. The pH value of 2 attained spontaneously during the reaction was not raised. The low pH probably favoured the co-ordination of the triglycine to V(V) in its zwitterionic form thereby stabilising the $V_2O_2(O_2)_3$ moiety and leading to the synthesis of the desired molecular complex.

From elemental analysis, a ratio of 2:3 was obtained for V: O_2^{2-} which suggested a dimeric nature for the complex, presumably involving a bridging peroxide group.

Appearance of two ν(O–O) bands in the IR spectrum of the complex, one at 845 cm^{-1} and another at 805 cm^{-1} with some broadening, indicated the presence of two structurally different peroxo groups, the terminal chelated and the bridging type. A chelated peroxo bonded terminally to V(V) exhibits a strong ν(O–O) band[35] at 870 cm^{-1}. Bands due to ν_2 and ν_3 modes of V–O_2 vibrations and the terminally bonded V = O group were observed as expected.

Three distinct bands were observed for the complex at 1678 cm^{-1}, 1610 cm^{-1} and 1389 cm^{-1} representing the carbonyl (amide)[37,39(b)] and carboxyl groups,[39(b)] respectively. The shift in ν_{as}(COO) (1610 cm^{-1}) are typical of a carboxylate group bonded in a unidentate zwittrionic fashion.[39(a)] The small shift of the ν(CO) (amide) to a lower frequency with some broadening, as compared to free triglycine (1684cm^{-1}) was probably due to the participation of one of the carbonyl groups in co-ordination. Other characteristics of the spectrum were the observance of the N–H stretching (3255–3083 cm^{-1}) and N–H deformation modes (~ 1560 cm^{-1}) due to the amide and N^+H_3 groups and the indication of the presence of lattice water. Based on these observations, it may be inferred that the

* To receive any correspondence. E-mail:nsi@agnigarh.tezu.ernet.in

triglycine ligands, occurring as zwitterions, co-ordinate to V(V) through O (carboxylate). Co-ordination of one of the carbonyl (amide) groups of the peptide chain probably completes the hexa co-ordination of vanadium in the complex leading to the formation of a seven-member ring around each vanadium. A provisional structure of the type shown in Fig. 1 has been envisaged for the complex. The second amide group in the peptide side chain is not shown in the structure for simplicity. It is possible that hydrogen bonding between the peptide side chains stabilise the molecule.

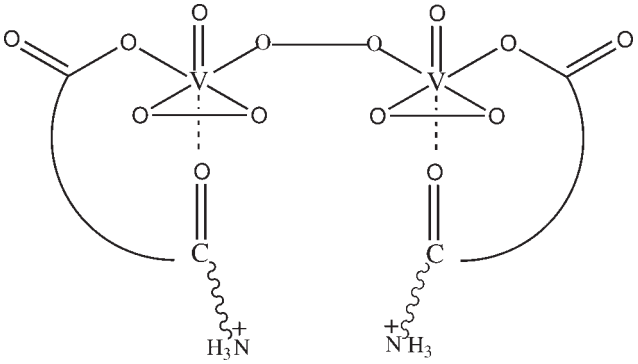

Fig. 1 Proposed structure of the peroxovanadate compound, $[V_2O_2(O_2)_3(Gly\text{-}Gly\text{-}Gly)_2].H_2O$. The second amide group in the peptide chain is not shown.

On dissolving the compound in water partial loss of peroxide occurred, as indicated by bubbles of dioxygen coming out. The electronic spectrum of the compound recorded at this stage displayed a single broad LMCT band at 328nm ($\varepsilon = 720$) originating from co-ordinated peroxide. On adding the enzyme catalase to the solution, further releases of dioxygen was observed which was measured with an oxygraph. The rate of dioxygen release (12.1μM/min) paralleled the residual peroxide concentration.

The ^{51}V-NMR spectrum of the complex displayed five product signals. The peaks observed at –424, –509 and –525 ppm correspond to decavanadate[14] formed as a result of depletion of the peroxovanadate in solution. The signal at –694 ppm indicated the formation of DPV.[14] The two resonances observed near –714 ppm were probably due to the co-ordination of peptides through carboxylate oxygen.[3] Instability of the complex in water and degradative loss of the peroxide groups were implicit. Thus no direct information regarding the nature of the original solid could be obtained from the NMR studies.

During the transient period before degradation, the solid compound was able to oxidise bromide and transfer bromine atoms to an acceptor molecule, phenol red, at physiological pH. Addition of the solid complex to bromide solution instantly produced a 262 nm absorbing compound that converted phenol red (a trap) to its 592-absorbing bromoderivative (Fig. 2a). After the instant activity, a slow increase in A_{592} (Fig. 2b) indicated a secondary rate of bromination (ΔA_{592}/min = 0.03/min) and the product incresased in amount (Fig. 2c). These results showed that the compound has both instant and secondary bromination activity. Of the expected bromination, 16% was realised as instant activity. The complex, a peroxo-bridged dimer, must possess an active group that readily oxidises bromide giving a large increase in A_{592}. It is obvious that the degredation products formed in solution *viz*: DPV and vanadate together, give a secondary rate of bromination. Under these conditions DPV was inactive and in the presence of vanadate showed the secondary rate but not the instant activity. The need for a "VOOV" type intermediate is thus apparent.

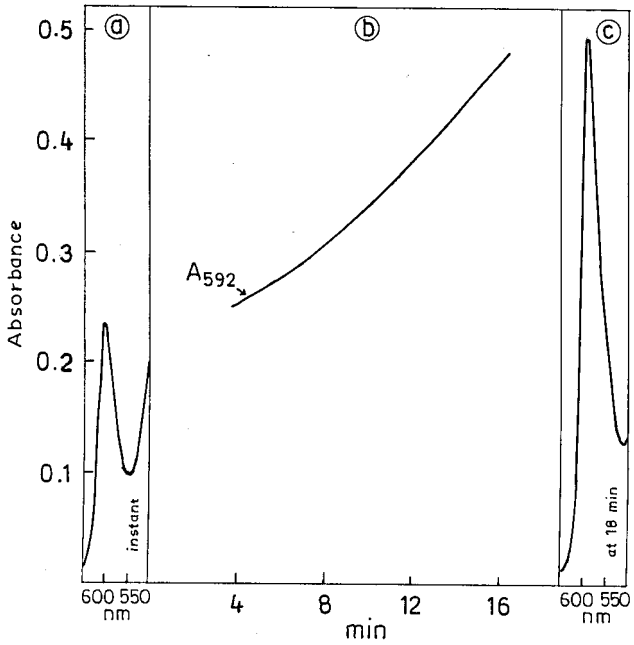

Fig. 2 Bromination activity with the vanadium compound: (a) spectrum taken immediately after adding the compound (0.14 mg/ml) to the reaction mixture showing the instant activity; (b) the increase in A_{592} indicating the secondary rate; (c) the peak at 592 nm at 18 min of the reaction.

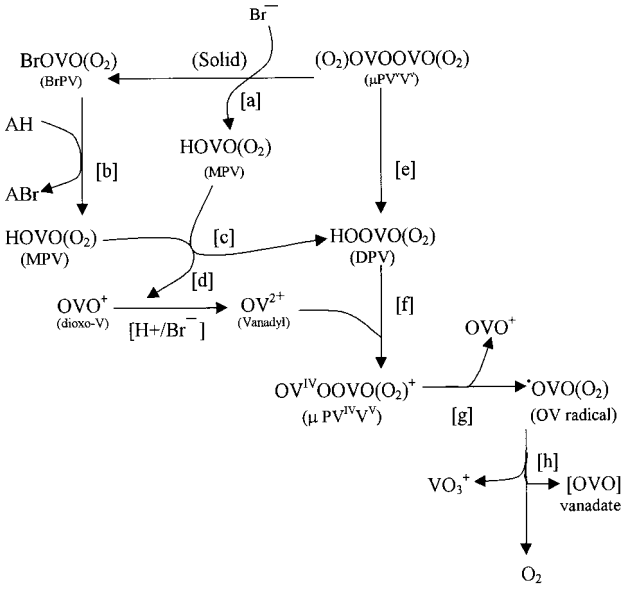

Fig. 3 Schematic representation of reactions occurring with peroxo-bridged divanadate compounds: (a) formation of active bromine compound BrPV and MPV when solid is added to a bromide solution; (b) transfer of bromine to acceptor AH (phenol red); (c) dismutation of MPV to DPV and vanadate; (d) reduction of vanadate to vanadyl by acid and bromide; (e) separation of DPV and MPV on adding solid compound to water (in absence of bromide); (f) formation of a μ-peroxo compound from DPV + vanadyl; (g) breakdown of the μ-peroxo group; (h) dismutation of OV radical releasing O_2. The triglycine ligands in the compound are not shown. Valency state of reduced vanadium is shown as V^{IV} and all others are V^{V}. No attempt is made to show the exact stoichiometry of reactions.

It appears that in the highly reactive compound the triglycine ligands stabilise the μ-peroxo group between the two peroxo-vanadate moieties through hydrogen bonding formed between the peptide side chains, leading to its isolation in the solid state. On adding the compound to water the hydrogen bonding weakens, the dimer separates and instant bromination activity vanishes. These observations strengthen the earlier proposal that a "VOOV" intermediate is the active group and is the likely primary oxidant of the bromide in phosphate buffered medium.[21,44] Based on these results, a scheme of reactions involving mono-, di- and μ-peroxo and bromoperoxo forms of vanadate is proposed for vanadium catalysed bromoperoxidation (Fig. 3)

The active site in the proteins having a vanadium dimer appeared possible, since crystal structures of two of the enzyme proteins[2,43] showed dimeric subunits with a vanadium atom per subunit. We are however, restrained to suggest any involvement of the reactions discussed above in the action of the enzymes, mainly because peroxo groups are not yet located in these enzyme structures. But from reports available, there appears to be a definite potential of the peroxovanadate intermediates (Fig. 3) in oxidative modifications in biological systems.

Financial assistance from the Council of Scientific and Industrial Research, New Delhi, is gratefully acknowledged. We express our sincere gratitude to Prof. T. Ramasarma, Department of Biochemistry, Indian Institute of Science, Bangalore, for valuable discussion and for allowing us to use the oxygraph. We thank Aparna V.S. Rao (IISC) for performing the oxygen release measurements and Prof. K. Ramanathan of Sophisticated Instrumental Facility (IISC) for the NMR spectra.

Techniques used: IR, UV-Vis, ^{51}V-NMR spectroscopy and Gilson Oxygraph.

References: 44

Received 20 November 2000; accepted 2 April 2001
Paper 00/627

References cited in this synopsis

1 E. de Boer, Y. Van kooyk, M.G.M. Tromp and R. Wever, *Biochem. Biophys. Acta*, 1986, **869**, 48.
2 H. Vilter and D. Rehder, *Inorg. Chim. Acta*, 1987, **136**, L7.
3 D. Rehder, *Coord.Chem.Rev.*, 1999, **182**, 297.
8 H. Sakurai and K. Tsuchiya, *FEBS Lett*, 1990, **260**, 109.
9 R.I. De la Rosa, M.J. Clague and A. Butler, *J. Am. Chem. Soc.*, 1992, **114**, 760.
10 M. Bhattacharjee, *Polyhedron*, 1992, 2817.
14 O.W. Howarth and J.R. Hunt, *J. Chem. Soc. Dalton Trans.*, 1979, 1388.
16 M. Bhattacharjee, S. Ganguly and J. Mukherjee, *J. Chem. Res. (S)*, 1995, 80.
17 M.J. Clague and A. Butler, *J. Am. Chem. Soc.*, 1995, **117**, 3475.
18 A.V.S. Rao, H.N. Ravishankar and T. Ramasarma, *Arch. Biochem. Biophys.*, 1996, **334**, 121.
21 A.V.S. Rao, N.S. Islam and T. Ramasarma, *Arch. Biochem. Biophys.*, 1997, **342**, 289.
29 E. de Boer, H. Plat, M.G.M. Tromp, R. Wever, M.C.R. Franssen, H.C. van der Plas, H.C. Meijer and H.E. Schoemaker, *Biotech. Bioeng.*, 1987, **30**, 607.
35 N.J. Campbell, A.C. Dengel and W.P. Griffith, *Polyhedron*, 1989, **8**, 1379.
37 T. Miyazawa and E.R. Blout, *J. Am. Chem. Soc.*, 1961, **83**, 712.
39 K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds.* Part B, 5th Edn, J. Wiley and Sons, New York, 1997: (a) p. 60; (b) p. 71.
43 M.N. Isupov, A.R. Dalby, A.A. Brindley, Y. Izumi, T. Tanabe, G.N. Murshudov and J.A. Littlechild, *J. Mol. Biol.*, 2000, **299**, 1035.
44 H.N. Ravishankar, M.K. Chaudhuri and T. Ramasarma, *Inorg. Chem.*, 1994, **33**, 3788.