Synthesis, Structure, and Biological Activity of a New Insulinomimetic Peroxovanadium Compound: Bisperoxovanadium Imidazole Monoanion

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Recent studies using vanadium compounds as antidiabetic agents in humans have increased interest in both vanadium coordination chemistry and in the biological mechanism of action of vanadium compounds.[1−5] To design vanadium compounds with enhanced insulinomimetic properties, we have focused on the peroxovanadium compounds[6−8] and now report the synthesis and characterization of a new bisperoxovanadium compound with equal or greater insulinomimetic potency than described for previously characterized peroxovanadium compounds or bismaltolatooxovanadium.[9,10] Further, the compound described in this paper is of chemical interest since it is one of the first structurally characterized vanadium imidazole compounds (and the first vanadium(V) imidazole complex).[11,12] Vanadium imidazole complexes have received significant interest given the fact that both haloperoxidases[13] and various phosphorylases[14] contain histidine residues that coordinate to vanadium atoms.

The bisperoxovanadium imidazole complex (**1**) was prepared as an imidazolium salt. To a cold solution of 1.82 g (10.0 mmol) of V_2O_5 in 15 mL of 30% H_2O_2 was added 2.80 g (41.1 mmol) of imidazole. The yellow solution was stirred for 4 h at ambient temperature at which time acetone was added until the solution was about to turn cloudy. The resulting solution was kept at −20 °C for 1 week, and yellow crystals suitable for X-ray diffraction studies precipitated. The crystals were filtered, washed with 3 mL of cold water and 10 mL of cold acetone (3 times), and dried on a filter paper. The isolated yield was 70%. Compound **1** was characterized[15] and found to be stable as a solid at 4 °C for periods beyond 6 months.

Single-crystal X-ray diffraction was used to obtain the structure of the compound, using a Siemens P4 diffractometer (see ref 16) for the experimental parameters. The molecular structure and numbering scheme of the complex anion of **1** is shown in Figure 1, with selected bond lengths and angles given in the caption. Crystals also contain at least one water molecule and the imidazolium counterion. The coordination environment of the vanadium atom approximates a pentagonal pyramid, with the oxo group occupying the axial position. Basal positions are occupied by the imidazole nitrogen atom and the two peroxo groups. The V═O, V−O (peroxo), and V−N bond lengths are similar to those in other vanadium complexes.[6,8,17,18] The most unusual feature of this complex is the six-coordinate ion, since vanadium atoms are seven-coordinate in most peroxovanadium complexes.[19] Only one peroxovanadium complex exhibiting similar coordination has been reported previously ($(NH_4)[VO(O_2)_2NH_3]$).[17] It is interesting to note that the V−N bonds in **1** and $(NH_4)[VO(O_2)_2NH_3]$ do not differ within experimental error, despite the difference in hybridization of the coordinating nitrogen atoms in these two complexes. The vanadium atom is raised approximately 0.5 Å above the basal pentagon in the six-coordinate complex compared to approximately 0.3 Å in seven-coordinate bisperoxo complexes.[19−21]

The solution properties of **1** were examined in both stock solution and solutions of relevance to the *in vitro* cellular phosphatase assay. The ^{51}V NMR chemical shift for **1** in D_2O at neutral pH is −744 ppm; it is one of the lowest chemical shifts observed for peroxo complexes. A decomposition product, presumably $[H_2VO_2(OO)_2]^-$,[22] is observed at −686 ppm at pH <6. The ^{1}H NMR spectrum of **1** shows the peaks from the coordinated imidazole as well as the signals from the imidazolium counterion. Two bands, one at 325 nm ($\pi_v^* \rightarrow d\sigma^*$) and one at 203 nm ($(\sigma)\pi_v^* \rightarrow d\sigma^*$), were observed in the UV−vis spectrum as expected for peroxo complexes.[23,24] Compound **1** is stable in aqueous solution in the presence of Hepes buffer (pH 7.0) and up to the 5.0 mM EDTA tested.

A primary function of insulin is the regulation of glucose transport in insulin-responsive tissues.[25] This event is initiated by insulin binding to its cellular receptor and requires receptor autophosphorylation and activation of the endogenous receptor protein tyrosine kinase activity.[26] Vanadate has been shown to mimic the effects of insulin, resulting in increased glucose transport in isolated cells and normalization of blood glucose in several animal models of diabetes.[27] The insulinomimetic properties of vanadate may be linked to inhibition of protein tyrosine phosphatases, resulting in enhanced or sustained insulin

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(15) For **1**: ^{51}V NMR $\delta(D_2O/H_2O)$ −744; ^{1}H NMR $\delta(D_2O)$ 7.46 (2H, s), 8.63 (1H, s), 7.41 (1H, s), 7.49 (1H, s), 8.30 (1H, s); ^{13}C NMR $\delta(D_2O)$ 120.7, 121.5, 129.1, 136.0, 139.5; IR (KBr) 3141 (s), 1749 (m), 1651 (m), 1588 (m), 1543 (m), 1433 (m), 1329 (m), 1261 (w), 1184 (w), 1099 (w), 1071 (s), 950 (s), 875 (s), 757 (s), 654 (s), 377 (w). See text for additional characterization including UV−vis and X-ray crystallography.

(16) X-ray parameters for **2**: formula = $C_6H_{11}N_4O_6V$, fw = 286.1, monoclinic, space group $P2_1$, a = 6.454(3) Å, b = 13.083(6) Å, c = 6.5133(11) Å, β = 100.46(3)°, V = 540.8(3) Å^3, Z = 2, T = 173 K, R_1 = 0.023 ($F_o > 4\sigma(F_o)$, 1406 reflections); wR_2 = 0.071 (all reflections); goodness of fit 1.12.

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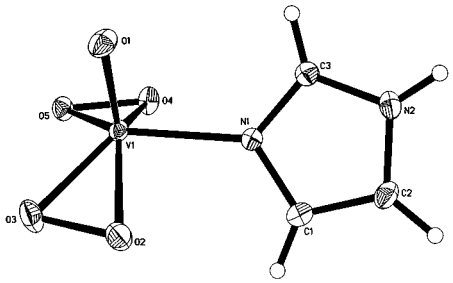

Figure 1. Structure and numbering scheme for the bisperoxovanadium imidazole monoanion, **1**. Hydrogen atoms are omitted, and 50% probability ellipsoids are shown. Selected interatomic distances (Å) are V(1)−O(1) 1.603(2), V(1)−O(2) 1.866(2), V(1)−O(3) 1.884(2), V(1)−O(4) 1.865(2), V(1)−O(5) 1.922(2), V(1)−N(1) 2.092(2), O(2)−O(3) 1.467(3), O(4)−O(5) 1.475(2).

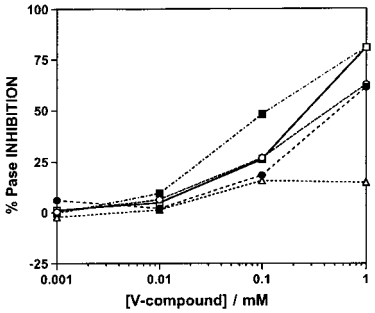

Figure 2. The total phosphatase activity[29] of bisperoxovanadium imidazole imidazolium salt (**1**) (□), vanadate (V_i) (●), bisperoxovanadium picolinic acid (**2**) (■), peroxovanadium imine diacetic acid (**3**) (○), peroxovanadium nitrile triacetic acid (**4**) (△) measured in H4 rat liver cell extracts. The assay buffer contained 50 mM HEPES (pH 7.5), 0.5 mM EDTA, 0.5 mg/mL BSA, and 0.5 mM DTT: 80 mL of this buffer was mixed with 20 mL of cell lysate and 10 mL of vanadium solution and incubated for 1 h at 30 °C after the addition of *p*-nitrophenol phosphate (PNPP). The SD values are less than 2% of values shown.

receptor autophosphorylation and tyrosine kinase activity.[28] Thus, to evaluate the insulinomimetic activities of the bisperoxovanadium imidazole imidazolium salt, we assayed its effects on total protein phosphatase (Pase) activity,[29] insulin receptor autophosphorylation, and skeletal muscle glucose transport. Total Pase activity[29] was measured in extracts of H4 rat liver cells in the presence of **1**, vanadate (V_i), bisperoxovanadium picolinic acid (**2**), peroxovanadium imine diacetic acid (**3**), and peroxovanadium nitrile triacetic acid (**4**) (Figure 2). V_i shows significant inhibition compared to the untreated control even though this assay is performed in the presence of 0.5 mM EDTA and 0.5 mM DTT. Although V_i forms a very stable complex with EDTA at low pH, at higher pH the complex is significantly less stable; the inhibition (and ^{51}V NMR studies) is consistent with the observation that V_i is not completely complexed under these assay conditions. DTT, under similar conditions, reduces 10% of V_i in 6 h.[30] As seen in Figure 2, at 1.0 mM complex **1** is equivalent to **2** and better than V_i, and **3**, and **4** in its ability to inhibit Pase in H4 cell lysates.

The effects of the vanadium compounds on insulin receptor autophosphorylation were examined using the human liver cell line, HUH. HUH cells treated with increasing amounts of the compounds in the presence of submaximal doses of insulin and receptor autophosphorylation was evaluated using antiphosphotyrosine antibodies. As shown in a representative experiment in Figure 3, **1** enhanced insulin receptor autophosphorylation at all concentrations tested. Compounds **2**−**4** increased receptor

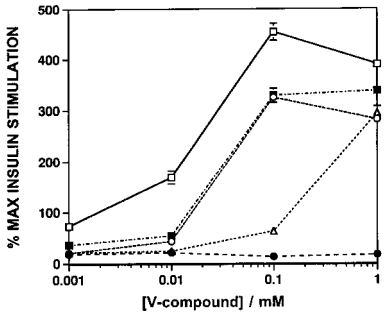

Figure 3. The effects on IR phosphorylation on the addition of **1** (□), vanadate (V_i) (●), bisperoxovanadium picolinic acid (**2**) (■), peroxovanadium imine diacetic acid (**3**) (○), peroxovanadium nitrile triacetic acid (**4**) (△). CHO-hIR cells were treated with vanadium compounds at 1.000, 0.100, 0.010, and 0.001 mM. 10 min prior to stimulation for 5 min at 37 °C with submaximal insulin (3 nM). Maximal insulin response was determined by stimulation of cells for 5 min at 37 °C with 100 nM insulin. Error bars represent SEM, $n = 3$.

Table 1. Glucose Uptake in Rat Adipocytes and Rat Epitrochlearis Muscle[a]

compound	rat adipocytes (%)	rat epitrochlearis muscle (%)
1	191(±7)	208(±28)
V_i	82(±41)	173(±33)
2	211(±27)	182(±13)
3	165(±13)	164(±17)
4	137(±11)	151(±11)

[a] Freshly prepared adipocytes were incubated in the presence of 1.0 μM V compound, [^{3}H]glucose, 30 pM insulin, and KRP/BSA (20 mM Hepes, pH 7.4, 128 mM NaCl, 5.13 mM KCl, 1.3 mM $MgSO_4$, 12.5 mM HPO_4^{2-}, 0.7 mM bacitracin, 1 mM $CaCl_2$, and 1% BSA) for 2 h at 37 °C. Glucose uptake in isolated rat epitrochlearis muscle was performed as previously described,[31] using 1.0 mM V compound in the presence of submaximal insulin (2.16 ng/mL, 360 pM). Results are presented as percent of the submaximal insulin response. Results are shown with SEM, $n = 4$.

phosphorylation but were less potent than **1**. Vanadate (V_i) did not increase insulin receptor phosphorylation above the level observed with submaximal insulin alone.

Given the effects of **1** on insulin receptor phosphorylation, we explored further the effects of this compound on glucose uptake in isolated rat adipocytes and in isolated rat epitrochlearis muscle.[31] In adipocytes in the presence of submaximal insulin, **1** increased glucose transport above the level observed in the presence of submaximal insulin alone (Table 1). Compounds **2** and **3** also significantly increased glucose transport, whereas V_i and **4** had minimal effect on glucose transport. In muscle, **1** enhanced glucose uptake 208% compared to 173% by V_i. Compounds **2**−**4** were also observed to enhance glucose uptake but to a lesser extent than observed with **1**. Thus, **1** is more effective than the other V compounds in enhancing insulin-stimulated glucose transport in muscle.

In summary, bisperoxovanadium imidazole (**1**) is a new vanadium complex that has interesting insulinomimetic properties; it is as good or better than previously reported compounds. In addition, **1** is of interest for its unusual structure since there has only been one previous report of a peroxovanadium compound containing six-coordinate vanadium and no previous reports of a vanadium(V) imidazole complex without other stabilizing organic ligands.

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Supporting Information Available: X-ray crystallographic details for 1 (6 pages). See any current masthead page for ordering and Internet access instructions.

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