Vanadium(IV) Complexes of an Active-Site Peptide of a Protein Tyrosine Phosphatase

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Phosphate-mediated intracellular signal transduction is important for biological regulation[2] and has been associated with cancer[3] and diabetes.[4] Key proteins involved in this process are the protein tyrosine kinases (PTKs)[5] and protein tyrosine phosphatases (PTPs),[6] which catalyze the specific phosphorylation and dephosphorylation of tyrosine residues, respectively. The insulin receptor is a transmembrane PTK, the action of which is mediated by a PTP. The phosphate cascade initiated by the PTK results in various insulin responses such as glucose uptake into cells. Oral administration of vanadium(IV)[7] or vanadium(V)[8] to lab animals which do not produce insulin results in alleviation of the diabetic symptoms. The mechanism of insulin mimicry is of interest since a detailed understanding of phosphate signal transduction may lead to an oral treatment for diabetes and a general approach to drug development.[9] Herein is reported the coordination chemistry of vanadium(IV) with VHCSAG–NH_2 (single letter amino acid nomenclature), an active-site polypeptide[10] from PTP 1B, the first structurally characterized PTP.[11] The histidine (H) and cysteine (C) residues in this peptide are conserved across the PTP family of proteins and the cysteine thiolate is essential for activity.

In the absence of coordinating ligands vanadium(IV) is EPR silent at physiological pH. In the presence of coordinating ligands such as those provided by VHCSAG–NH_2, monomeric vanadium(IV) species give rise to axial EPR spectra as shown in Figure 1A. The spectral parameters (g and A) for these vanadium-peptide complexes are sensitive to pH as is apparent in Figure 1B which highlights the $M_I = {}^7/_{2_{x,y}}$ and $M_I = {}^3/_{2_z}$ transitions. Addition of vanadyl sulfate (100μM) to a solution of VHCSAG–NH_2 (1mM) and adjusting to pH 5 yields a solution that is EPR active as shown in the bottom trace of Figure 1. This spectrum has been attributed to the presence of the monomeric complex $V^{IV}O(H_2O)_5$, $\mathbf{V^{IV}(aq)}$, and a new vanadium-peptide complex, **1**, which dominates the spectrum at pH 6. At pH 7 a second vanadium-peptide species, **2**, is formed. The concentration of **2** increases at pH 8 and dominates the spectrum at pH 9.

To determine which heteroatoms of the peptide interact with the metal in **1** and **2**, vanadyl complexes of the peptides VHCGAG–NH_2, VHMSAG–NH_2, and VFCSAG–NH_2 were examined using EPR spectroscopy at pH 7.3 ± 0.2 where both

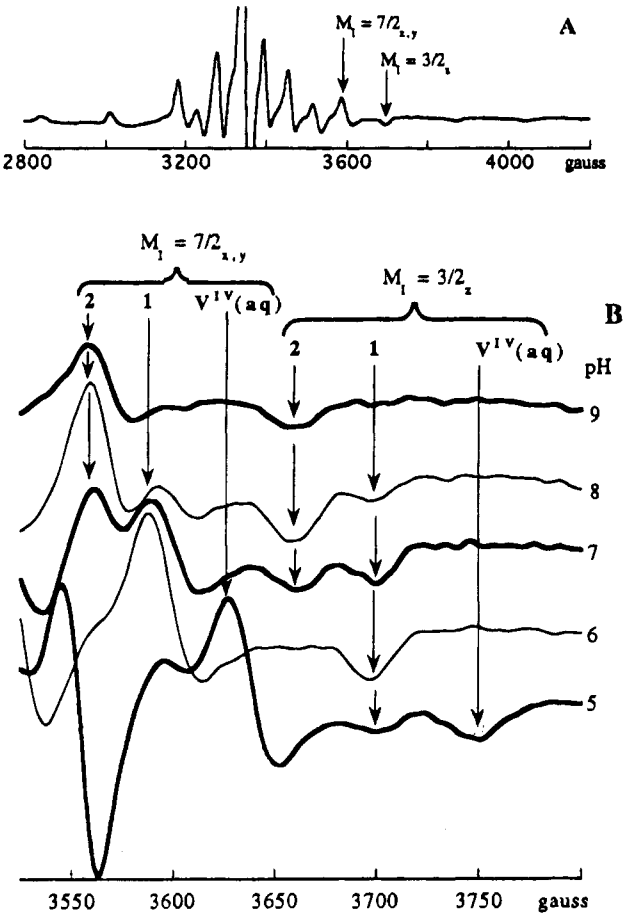

Figure 1. A. EPR spectrum of V^{IV} (100 μM) with VHCSAG–NH_2 (1 mM) at pH 6 (the intense central band has been truncated for clarity). B. EPR spectra of a similar solution between pH 5 and 9 (only $M_I = {}^7/_2$ (x,y) and $M_I = {}^3/_2$ (z) are shown).

1 and **2** are present. Figure 2 presents the high field region of the these spectra. Trace A results from vanadyl complexes of VHCSAG–NH_2 (as in Figure 1B, pH 7). There is no spectral change upon substituting a hydrogen for the hydroxymethyl side-chain of serine (S → G, Trace B), strongly suggesting that there is no interaction between the serine–OH and the vanadyl ion in **1** or **2**. Replacement of the thiol group of cysteine with the thioether of methionine (C → M, Trace C) eliminates the resonances of **2** indicating that the cysteine thiol is requisite for the formation of **2** but not **1**. Replacement of the imidazole group of histidine with a phenyl group of phenylalanine (H → F, Trace D) eliminates the resonances associated with **1** indicating that imidazole is requisite for the formation of **1** but not **2**. A third metal–peptide complex, **3**, is also present in vanadium(IV) solutions of VFCSAG–NH_2; however, **3** is not readily apparent in vanadium(IV) solutions of VHCSAG–NH_2.

The aqueous chemistry of the vanadyl ion between pH 5 and 9 is dominated by the formation of an EPR silent dimer ($K = 1.7 \times 10^{-7}$ M) and precipitation of vanadyl dihydroxide ($K_{sp} \approx 10^{-22}$ M^3).[12] The difficulties of quantitative EPR have precluded the direct determination of formation constants for these complexes. However, addition of the tripeptide glycyl–

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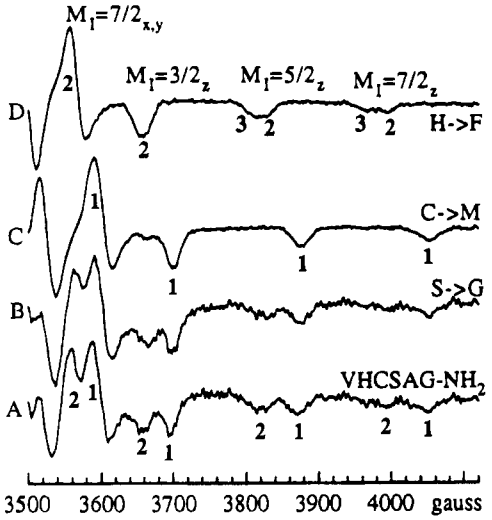
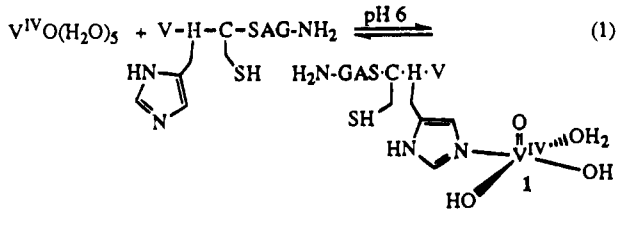


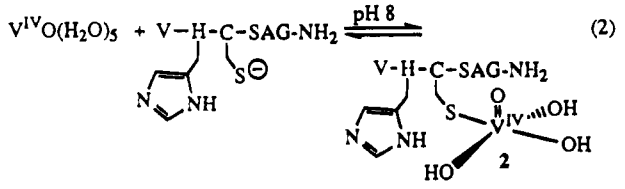
Figure 2. EPR spectra of 0.5 mM vanadium(IV) plus 5 equiv of (A) VHCSAG−NH_2, (B) VHCGAG−NH_2, (C) VHMSAG−NH_2, and (D) VFCSAG−NH_2. Only the high-field portion of the spectrum is shown. Acquisition parameters: ν = 9.42 GHz, modulation amplitude = 10 mT, power = 20 mW, center = 3475 G, and width = 2000 G. Spectral parameters (ref 14b): **1**, $g_x = g_y = 1.982$, $g_z = 1.958$, $A_x = A_y = 53 \times 10^{-4}$ cm^{-1}, $A_z = 159 \times 10^{-4}$ cm^{-1}; **2**, $g_x = g_y = 1.988$, $g_z = 1.972$, $A_x = A_y = 45 \times 10^{-4}$ cm^{-1}, $A_z = 147 \times 10^{-4}$ cm^{-1}.

glycyl−glycine ($K_f \approx 40\,000$ M^{-1} by enzymatic assay[13]) has no effect on the EPR spectra of solutions containing **1** and **2**. Thus, $K_f \geq 10^4$ M^{-1} is not unreasonable.

The EPR spectra of Figures 1 and 2 indicate that two EPR active vanadium−peptide complexes, **1** and **2**, are formed between pH 5 and 9. Complex **1** is dominant at lower pH where one would expect a neutral imidazole ligand and a protonated thiol group, while complex **2** is dominant at higher pH where one would expect the thiol group to be deprotonated. This is consistent with the data for the peptide derivatives (Figure 2) which imply the coordination of imidazole (but not thiolate) in complex **1**, and thiolate (but not imidazole) in **2**. Equations 1 and 2 summarize these findings.



(1)



(2)

EPR spectroscopy of vanadium(IV) has been used extensively as a probe of the metal binding sites of proteins[14] since the parallel coupling constant, A_z, can be related to the specific ligands of the equatorial coordination sphere according to eq 3 (n_i is the number of equatorial ligands of type i and A_i is the isolated contribution from each equatorial ligand of type i.).[16]

$$A_z = \sum_i n_i A_i \tag{3}$$

An A_z range of 120×10^{-4} cm^{-1} to 180×10^{-4} cm^{-1} is observed in most biological matrices and the accuracy of calculated A_z is estimated as $\pm 3 \times 10^{-4}$ cm^{-1}.[14a]

The structures for **1** and **2** proposed in eqs 1 and 2 are consistent with predictions based on eq 3 (161×10^{-4} cm^{-1} for **1** and 147×10^{-4} cm^{-1} for **2**). The low parallel coupling constant for **2** is similar to that observed for the crystallographically characterized complex $V^{IV}O[H_2NCH(CH_2S)CO_2CH_3]_2$ ($A_z = 143.6 \times 10^{-4}$ cm^{-1}).[17] The vanadyl ion in this complex is coordinated to two thiolate sulfur atoms and two amino nitrogen atoms, yielding from eq 3 $A_{z,calcd} = 144 \times 10^{-4}$ cm^{-1}, in excellent agreement with the experimental result.

The extent of water/hydroxide coordination can be studied by observing the line narrowing upon deuterium substitution.[18] We observe only a small (<3 G) narrowing upon deuterium substitution of **1** and no changes upon deuterium substitution of **2** in spite of the three hydroxide ligands proposed for **2**. It is probable that other factors, such as strong (yet unresolved) coupling between the electron spin and nuclear spin of the β-methylene protons, may dominate the linewidths in these complexes. Another possibility is that a deprotonated amide nitrogen (^{14}N, $I = 1$) may coordinate to form a six membered ring in **1** and a five-membered ring in **2**.[19]

Coordination of the thiolate sulfur to vanadium(IV) could account for the insulin-mimetic effect of vanadium(IV) *via* inhibition of PTPs that regulate the insulin receptor PTK. This mode of inhibition may well complement inhibition by vanadium(V) which probably interacts with the phosphate binding loop immediately downstream of VHCSAG as suggested by the recent crystal structures of the molybdate derivatives of two PTPs.[11,20] On the basis of these crystal structures, it is unlikely that imidazole from the conserved histidine interacts with vanadium(IV) due to a H-bonding network involving the imidazole protons. However, the work presented here clearly shows that the cysteine thiol of the PTP active site can directly coordinate to a *vanadium(IV)* ion and thus inhibit the cleavage of the phosphate esters of PTKs.

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