Oxovanadium(IV)-Promoted Peptide-Amide Deprotonation in Aqueous Solution

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Introduction

Vanadium is an important trace metal with a great variety of physiological effects. It can act, for instance, as an enzyme regulator in various phosphate-metabolizing reactions, suggesting binding between vanadium and the protein side chains.[1] In the oxidation states III, IV, and V, vanadium binds to transferrin, the essential metal ion transport protein in the serum.[2] Its interactions with oligopeptides in the physiological pH range, via coordination of the deprotonated peptide-N^- group, have been proved to involve vanadium in oxidation state V.[3−5]

$V^{IV}O$ forms complexes of fairly high stability with ligands containing O-donor atoms, but it binds more weakly to N- or S-donor containing biogenic ligands[6] in solution. The amide coordination of a few synthetic ligands to $V^{IV}O$ has been observed in the solid state.[7] Kabanos et al.[8] recently isolated the first $V^{IV}O$ complexes of dipeptides containing a deprotonated peptide-N^- group. In aqueous solution, however, no unambiguous proof has been obtained for $V^{IV}O$-promoted amide deprotonation and coordination, although this was strongly suggested in some glycine/alanine dipeptide complexes by CD and EPR measurements.[9] It has been found for many metal ions that the presence of a suitable anchoring donor which can bind metal ions strongly enough plays a crucial role in amide deprotonation.[10] In aqueous solution the terminal -NH_2 is not a particularly good anchoring donor for $V^{IV}O$, which is regarded as a hard metal ion. To make arrangement of the donor groups more favorable for $V^{IV}O$ binding, the terminal -NH_2 group was replaced by a hard phenolate as anchoring donor group: the solution speciation and solution structural characterization of the $V^{IV}O$ complexes of the dipeptide analogue 2-OH-hippuric acid ($HOC_6H_4C(O)NHCH_2COOH$, salicylglycine, SalGly) are reported here.

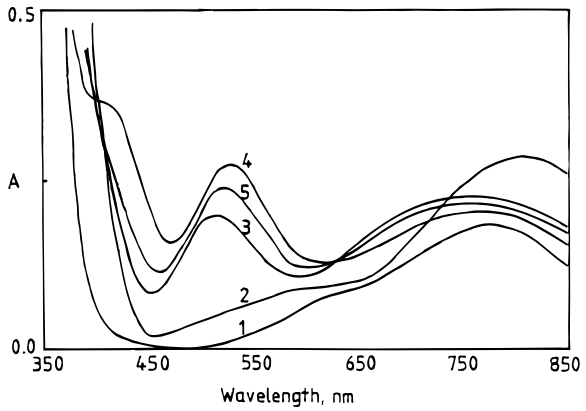

Figure 1. Electronic absorption spectra of $V^{IV}O$−SalGly at 1:2 metal-to-ligand ratio and at different pH values: (1) 2.60, (2) 4.25, (3) 6.30, (4) 7.70, (5) 10.80; $c_{VO} = 0.01$ mol dm^{-3}.

Results and Discussion

SalGly contains two protons that dissociate off the ligand in the measurable pH range. A value of log $K_{HA} = 8.16$ can be ascribed to the less acidic phenolic-OH, and of log $= 3.37$ to the terminal carboxylic function. These data are in reasonably good agreement with those reported earlier,[11,12] and correspond well to the characteristic acidity of the carboxylic groups of dipeptides (log $K_{COOH} \sim 3.3$)[13] and of salicylaldehyde (log $K_{OH} = 8.13$, $I = 0.15$) or salicylamide (log $K_{OH} = 8.89$, $I = 3.0$).[13]

A light-blue solution is obtained when SalGly is mixed with VO^{2+} in any metal ion-to-ligand ratio (pH $\sim$ 3). When the pH is increased, the color deepens; the solution remains blue up to pH $\sim$ 5. Other $V^{IV}O$−dipeptide systems start to form a hydroxide precipitate at around this pH even at a 180-fold excess of ligand,[9] whereas SalGly is able to keep even an equivalent quantity of $V^{IV}O$ in solution, and the color turns pinkish-red, with hardly any subsequent change up to pH $\sim$ 12. The electronic absorption spectra of the $V^{IV}O$−SalGly system at different pH values are depicted in Figure 1.

These findings reveal a significantly stronger $V^{IV}O$-binding capability of SalGly as compared with simple dipeptides and

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Table 1. Oxovanadium(IV) (log β) Stability Constants and Spectral Parameters for the Complexes of 2-Hydroxyhippuric Acid at 25.0 °C and $I = 0.20$ mol dm^{-3} (KCl)[a]

species[b]	log β	g_o	A_o (10^{-4}·cm^{-1})	$g_{\|\|}$	$A_{\|\|}$ (10^{-4}·cm^{-1})	$g_\perp$	$A_\perp$ (10^{-4}·cm^{-1})	λ_{max} [nm] (ϵ [mol^{-1} cm^{-1} dm^3])
VOAH	10.24(3)							
VOA	7.05(2)	1.964	101	1.938	175			804 (28), 600 (sh)
$VOAH_{-1}$	2.29(2)	1.971	92	1.949	165	1.980	57	755 (22), 512 (20)
$VOAH_{-2}$	−5.28(3)	1.971	89	1.951	163	1.980	55	770 (20), 526 (27), 404 (37)
VOA_2H_{-1}	5.55(6)	–	–	–	–			–

[a] The fitting parameter, the average difference in the calculated and experimental titration curves expressed in cm^3 of the titrant, was 0.0068 cm^3, 241 titration points were used. Other chemically reasonable species such as VOA_2 or VOA_2H_{-2} were also assumed in speciation calculation but were rejected by the computer program. [b] The negative sign of the stoichiometric number for proton indicates that the proton dissociates only when the complex is formed and does not dissociate in the absence of the metal ion or the ligand.

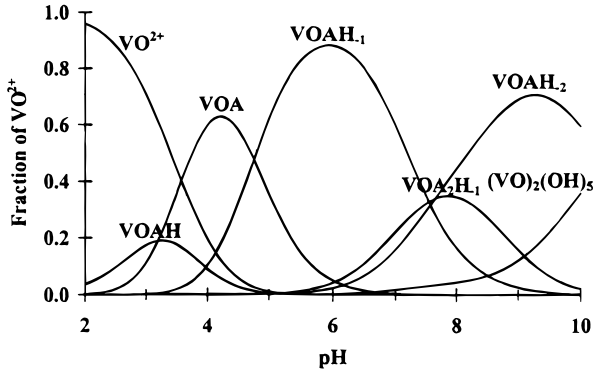


Figure 2. Speciation curves of the $V^{IV}O-$SalGly system at 1:2 metal-to-ligand ratio $c_{VO} = 0.004$ mol dm^{-3}.

suggest the participation of the amide-N$^-$ in the metal binding (vide infra). SalGly has been shown to form complexes of MAH_{-1} stoichiometry containing coordinated amide-N$^-$ with other transition metal ions, such as Cu(II),[11,12] but not with Ni(II) or Zn(II).[11]

The pH-metric titration curves could be evaluated with the speciation model and stability constants given in Table 1. No other chemically relevant and reasonable models gave better fit with the experimental titration data. The concentration distribution curves of the complexes formed as a function of pH are depicted in Figure 2. The high-field region of the anisotropic EPR spectra at different pH values is shown in Figure 3.

The pH-metric speciation curves (Figure 2) indicate that complex formation starts at pH ∼ 3 with a protonated species VOAH. In this species, either the carboxylic or the phenolic function could be protonated. In the former case, a complex involving chelation through the phenolate and the peptide carbonyl of the ligand would be formed, maintaining the terminal carboxylic function protonated (structure **I**, Chart 1). Alternatively, coordination of the $V^{IV}O$ ion to the carboxylate function may be assumed, this being the only negatively charged group at this pH (with possible chelation via the peptide carbonyl) (structure **II**). This latter binding mode is more reasonable, inasmuch as only minor spectral differences, compared to the aquaion, are observed in the EPR and visible spectra recorded at pH ∼ 3 (see the first spectrum in Figure 3). Three protons are liberated from this complex, with stepwise pK values of 3.19, 4.76, and 7.57, respectively. The pK of 3.19 is attributable to the phenolic-OH group of the metal-bound ligand. This suggest a rearrangement to (O$^-$, =O)-coordination, as supported by the spectral changes. Both UV (the significant increase in absorption at <400 nm is due to the shift of phenolic-OH band when the group deprotonates) and EPR parameters (see Table 1) are characteristic of pure O-coordination and correspond well with those for other O-coordinated complexes,

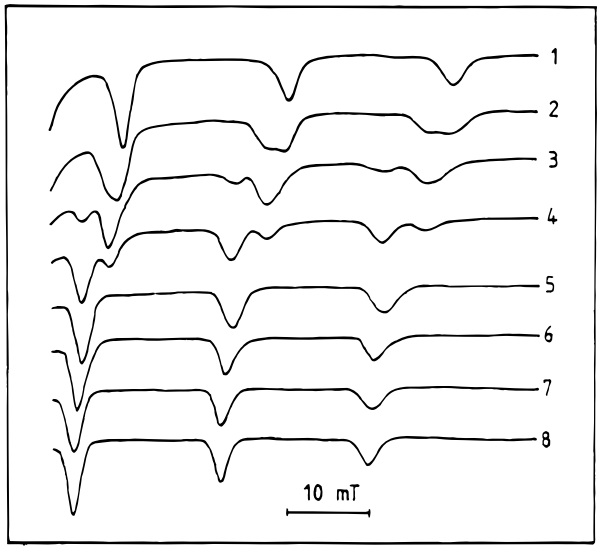
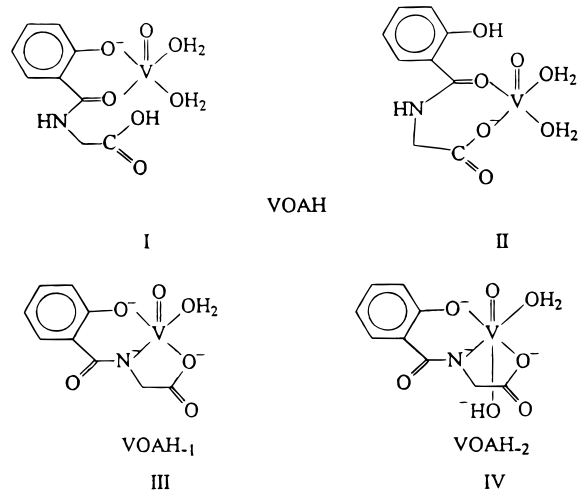


Figure 3. Parallel region of the EPR anisotropic spectra of VO−SalGly system at 1:1 metal ion-to-ligand ratio at 170 K, at pH values (1) 2.95, (2) 3.70, (3) 4.50, (4) 5.00, (5) 6.20, (6) 7.55, (7) 9.05, and (8) 10.60; $c_{VO} = 0.004$ mol dm^{-3}.

Chart 1



such as aromatic hydroxycarboxylic acids (e.g., $g_o = 1.967$, $A_o = 98$, $g_{||} = 1.942$, $A_{||} = 173$, $\lambda_{max}(\epsilon) = 795$ (26) and 575 (8) for 2,4-dihydroxybenzoic acid).[14] Such a considerable shift in the deprotonation pH range of a phenolic-OH due to metal ion coordination is not exceptional with $V^{IV}O$ or other hard metal

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ions.[14] The violet-blue solid isolated at pH 4.5 and formulated as $[VO(SalGly)(H_2O)_2]$ possibly corresponds to the stoichiometry VOA present in aqueous solutions. IR measurements indicate no amide involvement in the coordination in this complex (see Experimental Section).

The process with pK = 4.76 can be ascribed to deprotonation of the amide group, accompanied by a change in binding mode to furnish a (O^-, N^-, COO^-) (6+5)-membered joint chelate system (structure **III**). In principle, this process could also be ascribed to the ionization of a metal ion-bound water molecule. However, this would mean a significant acidification of the water molecule in the complex as compared to a water in the coordination sphere of the free $VO(H_2O)_5^{2+}$ (= 5.96), and this is not reasonable. In addition, as well-known from literature data, a monohydroxo complex of $V^{IV}O$ formed with a bidentate chelating ligand would easily yield polynuclear hydroxo-bridged species, EPR silent at room temperature. The spectral changes (visible and EPR) indicate more covalent bonding in the equatorial plane (see Table 1). The A|| value obtained from the EPR spectrum (165×10^{-4} cm^{-1}) is in good agreement with that estimated for structure **II** on use of the contributions expected for the equatorial donors present ($162-164 \cdot 10^{-4}$ cm^{-1}).[8,9,15] (The (NH_2, N^-, COO^-) binding mode of dipeptides is typical for metal ions which prefer the terminal -NH_2 group as the anchoring donor.[10]) The third proton of VOAH is released from the water molecule at the fourth equatorial site of $V^{IV}O$. Therefore, $VOAH_{-2}$ is a mixed hydroxo complex (structure **IV**). This process is seen in the electron absorption spectra with a third absorption at ~400 nm, as is usual in the visible spectra of $V^{IV}O$ complexes with strong donors. The negligible change in the EPR parameters suggest, however, that proton is liberated from the axial water and not from the equatorial one.

The potentiometric titration curves fitted significantly better when the formation of a bis complex VOA_2H_{-1} was also assumed. This formed in parallel with the species $VOAH_{-2}$, but could not be detected by EPR even at a 20-fold ligand excess. In the bis complex, the second ligand should coordinate to the complex $VOAH_{-1}$ either in a monodentate way, by displacing the water molecule in the equatorial plane, or to form a chelate in an equatorial−axial way. It appears a reasonable assumption that coordination of a phenolate-O^- or a OH^- group at this equatorial site would not cause a change significant enough to be reflected in the EPR spectral parameters.

The formation of the other bis complex, VOA_2H_{-2}, in which both ligands coordinate in a bidentate (O^-, N^-) way in the equatorial plane, was checked on. Such a complex has been be detected in numerous Cu(II)−dipeptide systems.[16] However, neither pH-metric nor spectral measurements revealed the formation of a new species, even at a 20-fold excess of ligand.

All the pH-metric and spectral data obtained in this work unequivocally prove that the metal ion-induced deprotonation and coordination of the peptide-amide occurs at pH ~ 4 in the $V^{IV}O$−SalGly system, in a pH range very close to that observed for the Cu(II)−SalGly system.[11] This means that the phenolic-OH is an efficient anchoring group for both $V^{IV}O$ and Cu(II) to promote amide deprotonation. Relative to the phenolic-OH group an amino group is a little more efficient for Cu(II) (e.g., deprotonation occurred with pK_{CuA} = 4.14 for GlyGly[17] and pK_{CuA} = 4.40 for SalGly[11]), but much less efficient for $V^{IV}O$ (pK_{VOA} ~ 8 for AlaAla[9]). The system examined in the present work is the most efficient one in which deprotonation and subsequent coordination to $V^{IV}O$ is observed for a peptide group. To date, this is the only aqueous system in which a $V^{IV}O$ complex involving a metal−peptide bond is the predominant species at physiological pH, even under equimolar conditions.

Experimental Section

Synthesis of $[VO(C_9H_7NO_4)]\cdot 2H_2O$. To a solution of 0.824 g (4.6 mmol) of SalGly and 0.626 g (4.6 mmol) of sodium acetate trihydrate in ethanol/water (10/10 cm^3) was added an aqueous solution of 0.750 g (4.6 mmol) of $VOSO_4$ dropwise. A few drops of an 1 mol dm^{-3} NaOH solution were added to afford pH 4.5. A week later, the resulting violet-blue solid was filtered off, washed with ethanol/water (2×5 cm^3) and diethyl ether (3×5 cm^3), and dried in vacuo. All manipulations were conducted under N_2. Yield 40%. Anal. Calcd for $C_9H_{11}NO_7V$: C, 36.50; H, 3.74; N, 4.73. Found: C, 36.7; H, 3.8; N, 4.5. IR (KBr disk, cm^{-1}): 3600−2900 (br) (O−H stretch, hydrogen-bonded), 3300 (w) ν(N−H), 1610 (s) ν(CO, amide I), 1580−1520 (s, br) $\nu_{asym}(CO_2)$ + ν(ring) + amide II band, 1410 (s) (ligand band), 1355 (m) $\nu_{sym}(CO_2)$, 973 (s) ν(V=O), 755 cm^{-1} (m−s) ν(C−H). Among others the relatively sharp band at 3300 cm^{-1}, which probably corresponds to stretching of the N−H bond, indicates that amide-N is not involved in the coordination. (The anionic complex $[VO(C_9H_6NO_4)]^-$ with coordinated amide-N^- could not be isolated so far.)

Magnetic Moments. The magnetic susceptibilities were measured by the Faraday method (magnetometer-susceptometer MANICS) in the range 6−290 K. The results can be fitted to $\chi = C/(T - \theta)$, the Curie−Weiss law, $\chi_d = -1.4 \times 10^{-4}$ emu·mol^{-1}, $\theta = -20.7$ K, and C = 0.420 emu·K·mol^{-1}. At 290.4 K, $\mu_{eff} = 1.78$ μ_B per V atom and the magnetic moments are approximately constant down to 6 K (μ_{eff} = 1.79 μ_B) with a slight minimum at ~60 K ($\mu_{eff} = 1.70$ μ_B). These results are consistent with the complex being monomeric, no significant interactions existing between the spins of neighboring molecules.

Solids obtained at higher pH were not stable in air, seemed to contain large amount of solvent in its structure, and were not suitable for structural studies. Further trials to isolate complex $VOAH_{-1}$ are in progress in our laboratories.

Measurements. The pH-metric titrations were made at two different ligand concentrations (0.004 and 0.002 mol dm^{-3}) and three different metal ion-to-ligand ratios (1:1, 1:2, and 1:3) to establish the solution speciation of the $V^{IV}O$−SalGly system. Concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[18] Other experimental conditions and details of the computer evaluation of the titration data were the same as described earlier.[14] X-band EPR spectra (9.15 GHz) were recorded at 120 K on aqueous DMSO solutions (80:20 v/v), using a Varian E-9 spectrometer. Spectral parameters were fit using the Bruker WIN-EPR SimFonia simulation program.[19] Absorption spectra were obtained on an HP 8452A diode array spectrometer.

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Supporting Information Available: Figure S1 showing the simulation EPR spectra of species $VOAH_{-1}$ and $VOAH_{-2}$ (1 page). Ordering information is given on any current masthead page.

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