Binding of Oxovanadium(IV) to Dipeptides Containing Histidine and Cysteine Residues

Eugenio Garribba,*[a] Elzbieta Lodyga-Chruscinska,[b] Giovanni Micera,*[a] Angelo Panzanelli,[a] and Daniele Sanna[c]

Keywords: Vanadium / Peptides / Bioinorganic chemistry / EPR spectroscopy / Coordination modes / Bond theory

The complexation of the oxovanadium(IV) ion with five dipeptides containing L-histidine or L-cysteine (GlyHis, HisHis, HisGly, CysGly, GlyCys) was studied. L-histidinamide ($HisNH_2$) was assumed as a model system for dipeptides with L-histidine in the N-terminal position. The study was performed in aqueous solution through the combined application of potentiometric and spectroscopic (electronic absorption and EPR) techniques. The results indicate that simple dipeptides lacking a strong anchoring group can form mono- and bischelated complexes with the $V^{IV}O$ ion if a suitable "donor" is present in the chain. The ligands behave like amino acids in the acidic and neutral pH range, inhibit the precipitation of hydroxides and suppress the formation of hydrolytic species if at least a fivefold molar excess of ligand is used. In alkaline media all the ligands, except CysGly, promote the deprotonation and N-coordination of the amide group. CysGly forms a bischelated complex with a [2 × (NH_2, S^-)] donor set. The contribution of the deprotonated amide group to the ^{51}V hyperfine coupling constant, A_z, as a function of the total equatorial charge of oxovanadium(IV) ion, is discussed. The results have general validity and are useful to predict the geometry and donor set of complexes involving the bonding of the $V^{IV}O$ ion to the deprotonated amide group.

Introduction

Vanadium is an important element for higher animals and essential for certain organisms including tunicates, some fungi and bacteria.[1] It elicits a number of physiological responses in human organism, e.g. the inhibition of ATPases,[2] phosphotyrosine phosphatase[3] and so forth. One of the most relevant properties of vanadium is its insulin-mimetic activity.[4] To clarify its biological role, it is of primary importance to understand the mechanisms of reaction (ligand-exchange and redox processes) and complexation of vanadium in organisms and to find the target biomolecules. Among the biomolecules present in intra- and extra-cellular fluids, proteins have a special importance because of their high amount in the cellular environment and their possible interaction with metal ions through a number of active sites. For instance, glutathione plays an important role in the processes of reduction of vanadium(V) to oxovanadium(IV) and in the following complexation reactions.[5a,5b]

Detailed studies on synthetic models of the interaction of vanadium with proteins can contribute greatly to our knowledge of its biological activity. Oligopeptides are among the models most closely related to proteins. These ligands can interact with metal ions through various donor groups: terminal amino and carboxylate groups, intermediate peptide groups and side-chain donor groups, e.g. imidazole-N or thiolate-S. Usually, these groups are not strong enough to keep metal ions in solution at the physiological pH. However, they can play the role of "anchoring group" and promote the deprotonation of the amide bond and its coordination in the $-N^-$ form. Various metal ions exhibit this ability, e.g. Pt^{II}, Pd^{II}, Cu^{II}, Ni^{II} and, in some cases, Zn^{II} and Co^{II}.[6]

Recently, the role of anchoring donor groups was studied with $V^{IV}O$ and it was found that their effectiveness in promoting peptide amide deprotonation and coordination follows the order: phenolate-O^- < alcoholate-O^- < thiolate-S^- < carboxylate-COO^- < NH_2.[7]

The interaction with oligopeptides in the physiological pH range, through the coordination of a deprotonated peptide group, was first proved to involve vanadium(V).[8] The amide coordination of a few synthetic ligands to $V^{IV}O$ ion was observed in the solid state too.[9] Costa Pessoa and coworkers suggested the deprotonation and coordination of $V^{IV}O$ by glycine/alanine dipeptides in aqueous solution.[10a,10b] However, the first unambiguous proof for $V^{IV}O$-amide deprotonation and coordination, favoured by

[a] Department of Chemistry, University of Sassari,
Via Vienna 2, I-07100 Sassari, Italy
Fax: +39-079-212069
E-mail: garribba@uniss.it; micera@uniss.it
[b] Institute of General Food Chemistry, Technical University of Lodz,
ul. Stefanowskiego 4/10, PL-90924 Lodz, Poland
[c] Istituto C. N. R. di Chimica Biomolecolare,
Trav. La Crucca 3, 07040 Li Punti, Sassari, Italy

an anchoring donor like the "hard" phenolate group, was obtained with salicylglycine.[11]

The solution speciation and the characterisation of the $V^{IV}O$ complexes of five dipeptides (GlyHis, HisGly, HisHis, GlyCys and CysGly) are reported in this work. Their structures are displayed in Scheme 1. A model system for the dipeptides with L-histidine in the N-terminal position, L-histidinamide ($HisNH_2$), has been included. Though in aqueous solution terminal $–NH_2$ is not a particularly good anchoring donor for the $V^{IV}O$ ion,[7] additional donors, like the imidazole nitrogen and the thiolate sulfur atoms, favour the binding of the ligands.

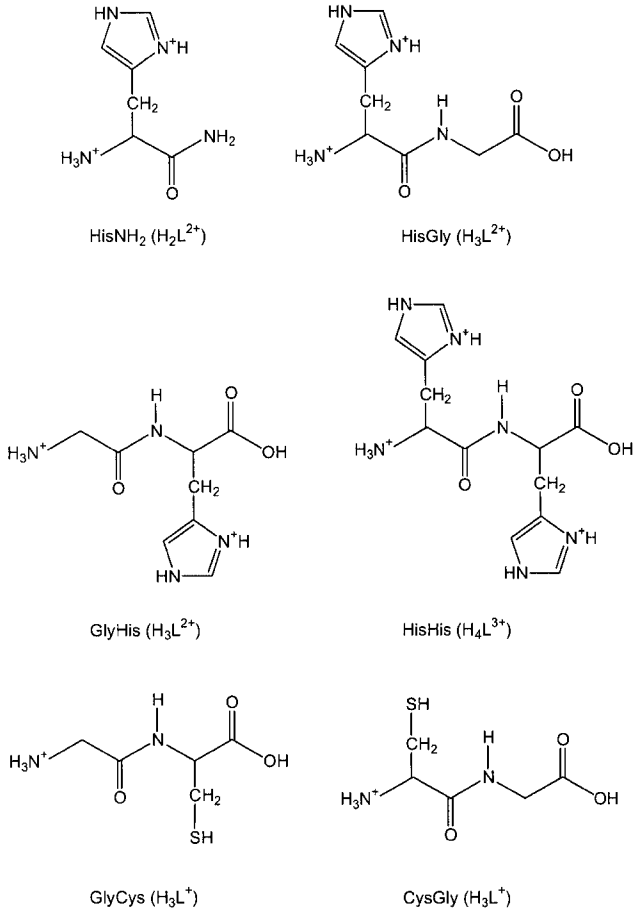
Results

L-Histidinamide ($HisNH_2$)

The aromatic nitrogen of imidazole is a major binding site of metalloenzymes. The imidazole nitrogen of L-histidine can compete with the amino acid site and significantly enhance the stability of the complexes. Therefore, imidazole side chains play a relevant role in complex formation. However, the effect depends critically on the position of the histidyl residue.

To model peptides with histidine in the N-terminal position, we examined L-histidinamide. The potentiometric titrations of the ligand (H_2L^{2+}, Table 1 and Scheme 1) indicate two deprotonation steps with pK_a of 5.39 ($–N_{im}H^+$) and 7.49 ($–NH_3^+$). The values are in good agreement with those of the literature. For instance, Michailidis and Martin reported values of 5.85 and 7.78 at 0.5 M ionic strength.[12] $HisNH_2$ forms mono- and biscomplexes with the $V^{IV}O$ ion (Table 1). The concentration distribution curves of the $V^{IV}O$ complexes as a function of pH are depicted in Figure 1.

The detection of a series of $V^{IV}O$ complexes at a ligand-to-metal molar ratio (L/M) as low as 7 is in contrast to the findings for other simple amino acids systems. For instance, L/M values of 77 are needed with glycine.[13a,13b] This substantiates a stronger binding mode, due e.g. to a tridentate behaviour for $HisNH_2$ with the involvement of the imidazole nitrogen in metal coordination. The speciation process was inferred by a pH-potentiometric titration of the metal-ligand system (see Experimental Section). Complex formation starts with a VOL species, which predominates at pH 4–5. The biscomplexes VOL_2H and VOL_2 are major



Scheme 1. Structures of the ligands in the fully protonated forms.

Table 1. Protonation constants (log K) and oxovanadium(IV) (log β_{pqr}) stability constants for the studied ligands and complexes at 25.0 ± 0.1 °C and I = 0.20 mol dm^{-3} (KCl).

Log K / log β_{pqr}	$HisNH_2$	HisGly	HisHis	GlyHis	GlyCys	CysGly
–COOH		2.82	2.61	2.51	2.73	3.13
$–N_{im1}H^+$	5.39	5.94	5.68	6.78	–	–
$–N_{im2}H^+$	–	–	6.85	–	–	–
$–NH_3^+$	7.49	7.69	7.79	8.24	8.04	6.94
–SH	–	–	–	–	9.48	9.37
$VOLH_3$			23.67(4)			
$VOLH_2$			20.82(1)	18.00(3)	21.29(2)	
VOLH			16.57(3)			
VOL	7.15(2)	7.00(5)				9.31(2)
VOL_2H_4					41.09(3)	
VOL_2H_3				31.89(3)	36.84(1)	
VOL_2H_2			28.97(3)	27.16(2)	32.14(2)	
VOL_2H	17.48(2)	17.91(4)	22.47(2)	22.10(3)	26.84(2)	
VOL_2	11.42(1)	12.00(4)	15.53(2)	15.56(3)	19.57(3)	16.60(2)
VOL_2H_{-1}	4.22(4)	4.74(4)	7.48(2)	7.04(3)	11.70(4)	
$VOLH_{-2}$					–1.60(3)	

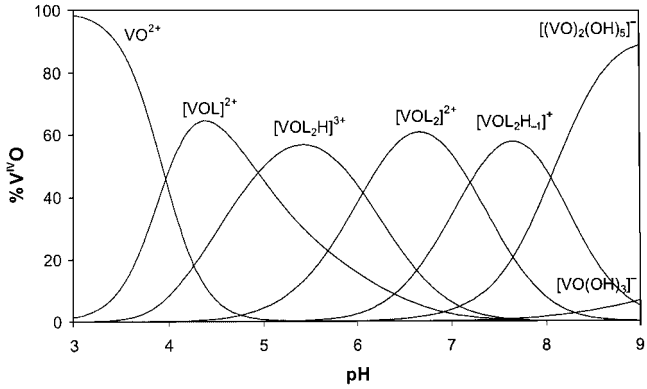


Figure 1. Species distribution for the $V^{IV}O$-$HisNH_2$ system at a metal-to-ligand molar ratio of 1:7 and a $V^{IV}O$ concentration of 1 mM.

species in the pH range 5–7. The log K values for VOL and VOL_2 formation are 7.15 and 4.27, respectively. The "basicity-adjusted" stability constants for the reactions $VO^{2+} + HL^+ \rightarrow [VOL]^{2+} + H^+$ and $[VOL]^{2+} + HL^+ \rightarrow [VOL_2]^{2+} + H^+$ are –0.34 and –3.22, which are greater by about three orders and one order of magnitude, respectively, than those of $V^{IV}O$ complexes of simple amino acids (cf. –3.05 and –4.25 for glycine [14]).

The findings substantiate the involvement of the imidazole nitrogen atom in the coordination of vanadium in VOL. The ligand presumably adopts an equatorial-axial-equatorial (*eq-ax-eq*) binding mode (the two external donor atoms are in the equatorial and the third in the axial position) and this increases the stability of the species. At a pH higher than 5, according to the potentiometric and spectroscopic measurements, a second ligand replaces two water molecules and forms first VOL_2H and then VOL_2. Only two positions of the equatorial plane are available for the second ligand. The pK_a of 6.06 for the VOL_2H to VOL_2 deprotonation, which is very close to that of the free ligand, suggests that the aromatic nitrogen atom of the second ligand molecule does not participate in the coordination. Finally, VOL_2 loses a proton with pK_a = 7.20 to form VOL_2H_{-1} before the start of the processes leading to $[(VO)_2(OH)_5]^-$ and $[VO(OH)_3]^-$. The formation of these hydrolytic species characteristic of $V^{IV}O$ is supported by the potentiometric titrations and by the progressive disappearance of the EPR signal at pH > 8.

The EPR parameters are: g_z = 1.946, A_z = 169×10^{-4} cm^{-1} for VOL, and g_z = 1.950, A_z = 164×10^{-4} cm^{-1} for both VOL_2H and VOL_2 (Table 2). The data are similar to those of the mono- and bischelated complexes of $V^{IV}O$ with L-histidine methyl ester (HisOMe),[15] g_z = 1.946, A_z = 170×10^{-4} cm^{-1} and g_z = 1.950, A_z = 164×10^{-4} cm^{-1}, respectively. Moreover, we compared the EPR spectra with those of the $V^{IV}O$/L-glycine methyl ester (GlyOMe) system at L/M = 500. The stepwise formation of the two species was observed with g_z = 1.946, A_z =

Table 2. EPR parameters and donor sets for oxovanadium(IV) complexes.

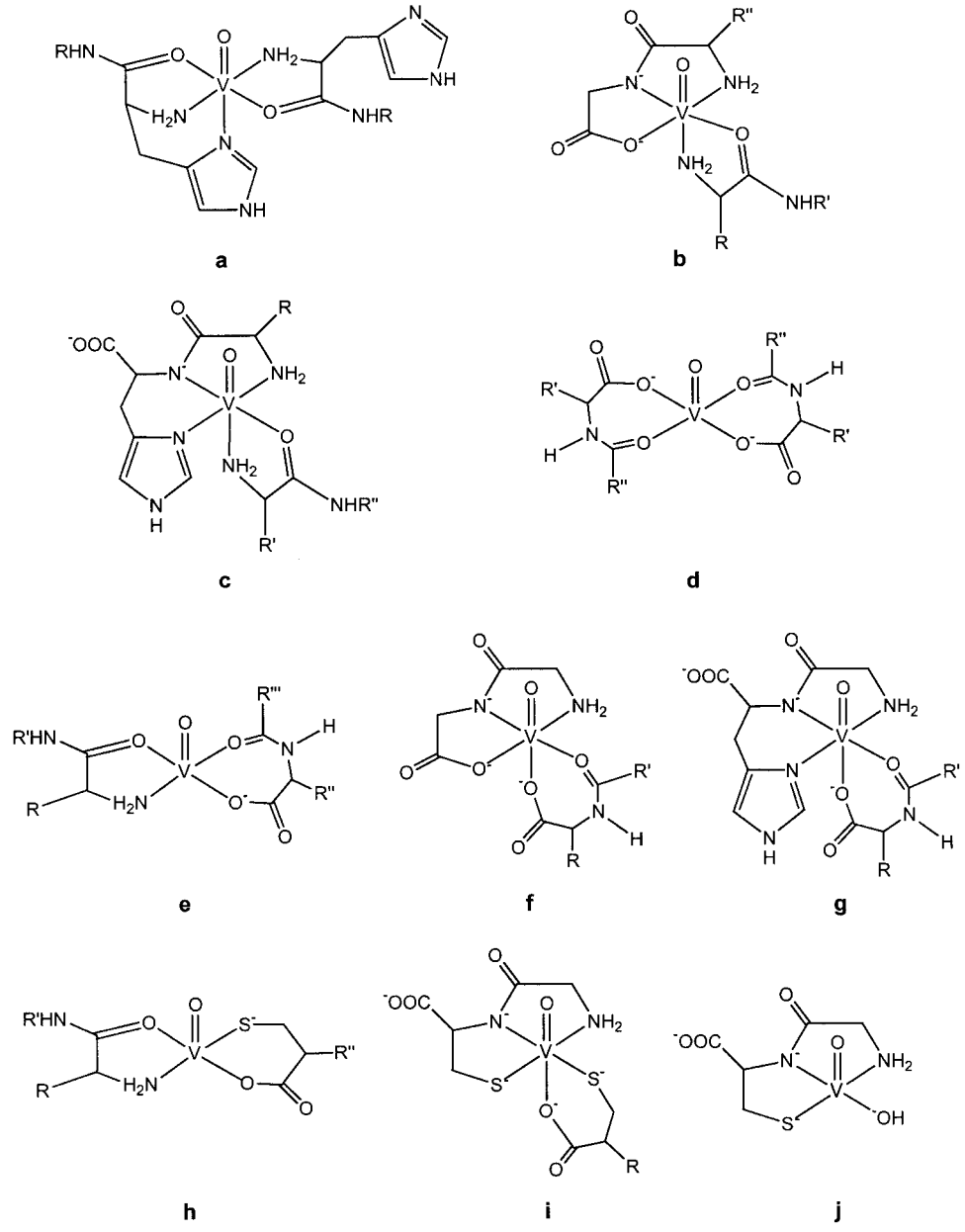
Ligand	Complex	g_z	A_z[a]	Donor set
$HisNH_2$	VOL	1.946	169	(NH_2, CO, N_{im}^{ax})
	VOL_2H	1.950	164	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2	1.950	164	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2H_{-1}	1.956	160	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO^-)]
HisGly	VOL	1.946	168	(NH_2, CO, N_{im}^{ax})
	VOL_2H	1.951	165	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2	1.951	165	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2H_{-1}	1.955	160	[(NH_2, CO, COO^-); (CO, NH_2^{ax})]
HisHis	$VOLH_3$	1.934	178	(COO^-)
	$VOLH_2$	1.947	169	(NH_2, CO, N_{im}^{ax})
	VOLH	1.947	169	(NH_2, CO, N_{im}^{ax})
	VOL_2H_2	1.950	164	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2H	1.950	164	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2	1.950	164	[(NH_2, CO, N_{im}^{ax}); (NH_2, CO)]
	VOL_2H_{-1}	1.957	157	[(NH_2, N^-, N_{im}); (CO, NH_2^{ax})]
GlyHis	$VOLH_2$	1.935	177	(COO^-, CO)
	VOL_2H_3	1.938	172	[2×(COO^-, CO)]
	VOL_2H_2	1.943	166	[(NH_2, CO); (COO^-, CO)]
	VOL_2H	1.958	161	[(NH_2, N^-, COO^-); (CO, COO^{-ax})]
	VOL_2	1.959	157	[(NH_2, N^-, N_{im}); (CO, COO^{-ax})]
	VOL_2H_{-1}	1.959	157	[(NH_2, N^-, N_{im}); (CO, NH_2^{ax})]
GlyCys	$VOLH_2$	1.935	176	(COO^-, CO)
	VOL_2H_4	1.940	172	[2×(COO^-, CO)]
	VOL_2H_3	[b]	[b]	[(NH_2, CO); (COO^-, CO)]
	VOL_2H_2	1.950	164	[2×(NH_2, CO)]
	VOL_2H	1.953	160	[(S^-, COO^-); (NH_2, CO)]
	VOL_2	1.959	155	[2×(S^-, COO^-)]
	VOL_2H_{-1}	1.968	144	[(NH_2, N^-, S^-); (S^-, COO^{-ax})]
	$VOLH_{-2}$	1.956	152	[(NH_2, N^-, S^-); OH^-]
CysGly	VOL	1.951	162	(NH_2, S^-)
	VOL_2	1.966	145	[2×(NH_2, S^-)]

[a] A_z measured in 10^{-4} cm^{-1} units. [b] Parameters not measurable because of the low concentration of the species.

168×10^{-4} cm^{-1} and g_z = 1.951, A_z = 163×10^{-4} cm^{-1}, respectively. They were identified as VOL and VOL_2 with (NH_2, CO) and [2×(NH_2, CO)] coordination modes, respectively. The features suggest that in the monochelated complex, $HisNH_2$ coordinates the metal ion through amino nitrogen and carbonyl oxygen donors in the equatorial plane and the imidazole nitrogen atom in the apical position, which enhances the stability of the species. The resulting donor set is (NH_2, CO, N_{im}^{ax}). The presence of an imidazole nitrogen atom in the axial position does not significantly affect the A_z value, according to the "Chasteen additivity rule", which assumes that only equatorial donors contribute to the parallel hyperfine ^{51}V constant.[16] The second ligand is bound through the (NH_2, CO) donor set: the resulting coordination mode is [(NH_2, CO, N_{im}^{ax}); (NH_2, CO)], as reported in Table 2. The structure is indicated as '**a**' in Scheme 2 with R = H. This scheme explains the rather high stability of the monochelated complex involving a tridentate ligand, compared with the bischelated species (in which a molecule is tridentate and another bidentate).

Potentiometric and spectroscopic studies on the $V^{IV}O$/L-histidine system in aqueous solution indicate that the VOL and VOL_2 species are formed with (NH_2, COO^-, N_{im}^{ax}) and [(NH_2, COO^-, N_{im}^{ax}); (NH_2, COO^-)] donor sets, respectively. The EPR parameters are g_z = 1.945, A_z = 170×10^{-4} cm^{-1} and g_z = 1.944, A_z = 163×10^{-4} cm^{-1}, respectively.[17a,17b]

At pH values higher than 6.5–7, a VOL_2H_{-1} species of $HisNH_2$ is detected by EPR spectroscopy with g_z = 1.956,



Scheme 2. Structures of the oxovanadium(IV) complexes.

$A_z = 160 \times 10^{-4}$ cm^{-1}. In this pH range, potentiometry indicates a deprotonation with a pK_a of 7.20. We can exclude the deprotonation of an equatorial water molecule because all the five coordination sites are occupied by ligand molecules, one bound in *eq-ax-eq* mode and another in *eq-eq* mode. The spectral parameters do not support a complexation scheme similar to that of L-histidine, which forms a mixed species with a [(NH_2, COO^-, N_{im}^{ax}); (NH_2, N_{im})] donor set and $g_z = 1.955$, $A_z = 157 \times 10^{-4}$ cm^{-1}. Therefore, the deprotonation can be ascribed to the amide group, whereas donor sets [(NH_2, CO^-, N_{im}^{ax}); (NH_2, CO)] or [(NH_2, N^-, N_{im}^{ax}); (NH_2, CO)] are equally probable for the complex. Only an X-ray determination could solve the ambiguity. Attempts to obtain single crystals of the complex are in progress. However, the coordination of the amide group through a deprotonated nitrogen atom is most likely if a second chelated ring can be closed, as it happens with dipeptides.[6]

Pecoraro and coworkers demonstrated that the $V^{IV}O$ coordination of a ligand with a terminal amide group, e.g. H_2ada = *N*-(carbamoylmethyl)iminodiacetic acid, takes place through the carbonyl oxygen atom of the deprotonated amide group.[18] This is not surprising if the preference of the $V^{IV}O$ ion for oxygen rather than nitrogen donors is considered. Moreover, Kabanos et al. showed that a dipeptide like Hpycan [Hpycan = *N*-(2-nitrophenyl)pyridine-2-carboxamide] coordinates the VO^{2+} ion in a bidentate mode by adopting the (N_{py}, CO_{amide}) rather than the (N_{py}, N_{amide}) donor set.[19] Analogous results were obtained with vanadium(V).[20] Therefore, we can assume that the first coordination mode is more likely.

Glycyl-L-Histidine (GlyHis), L-Histidylglycine (HisGly), and L-Histidyl-L-histidine (HisHis)

The complex formation of dipeptides containing L-histidine depends on the "hardness" of the metal ion.[6] In N-terminal histidyl peptides, the amino and imidazole nitrogen atoms can give rise to a stable six-membered chelated ring. Histamine-like complexes are formed with "soft" ions, like Cu^{2+}. Histamine-like coordination is less favoured with $V^{IV}O$; suitable donors, possibly oxygen atoms, should be involved in anchoring the metal ion.

In simple dipeptides lacking strong anchoring groups in the N-terminal position, the carboxylate group in the C-terminal position and the amide carbonyl group can interact with $V^{IV}O$ at acidic pH values to form a seven-membered (COO^-, CO) chelated ring.[7,21] This favours the deprotonation and coordination of the amide group at higher pH values.

For HisGly (H_3L^{2+}, Table 1 and Scheme 1), three pK_a values were measured: 2.82 (–COOH), 5.94 ($-N_{im}H^+$) and 7.69 ($-NH_3^+$), which are comparable to those reported by Sóvágó et al.[22] The $V^{IV}O$ complexation scheme is very similar to that of $HisNH_2$ and involves the formation of VOL, VOL_2H and VOL_2 below pH 7 (Figure 2). In the pH range 4–7, EPR spectroscopy shows two species with $g_z = 1.946$, $A_z = 168 \times 10^{-4}$ cm^{-1} and $g_z = 1.951$, $A_z = 165 \times 10^{-4}$ cm^{-1}, values comparable with those measured for $HisNH_2$. This confirms that VOL has a (NH_2, CO, N_{im}^{ax}) coordination (**I** in Figure 3) and VOL_2H, a [(NH_2, CO,N_{im}^{ax}); (NH_2, CO)] donor set (**IIa** in Figure 3 and **a** in Scheme 2). The imidazole nitrogen atom of the second ligand molecule is still protonated in VOL_2H. The proton dissociation yielding VOL_2 (**IIb** in Figure 3) takes place with a pK_a value of 5.91, which is in excellent agreement with the value of the free ligand. In this case too, the deprotonation does not affect the coordination sphere of the vanadium atom. The "basicity-adjusted" stability constants for mono-and bischelated species are –0.69 and –2.69, respectively, which means that the monochelated complex is a little less stable and the bischelated species a little more stable than the corresponding species of $HisNH_2$.

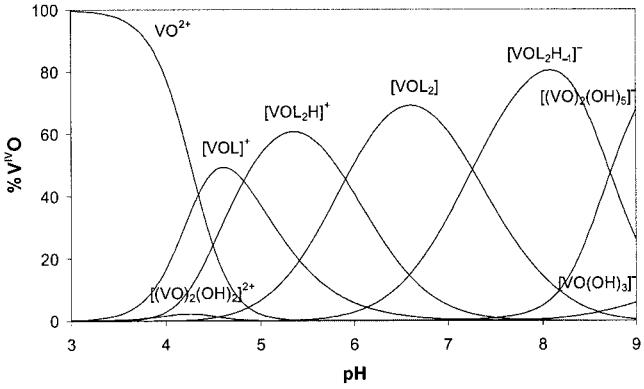


Figure 2. Species distribution for the $V^{IV}O$-HisGly system at a metal-to-ligand molar ratio of 1:10 and a $V^{IV}O$ concentration of 1 mM.

VOL_2 undergoes deprotonation with a pK_a of 7.26 to form a VOL_2H_{-1} species characterised by $g_z = 1.955$, $A_z = 160 \times 10^{-4}$ cm^{-1} (**III** in Figure 3). Also in this case the deprotonation can be attributed to the ligand and we can assign it to the peptide group. However, in contrast with $HisNH_2$, the coordination of a deprotonated nitrogen atom makes the closure of a second chelated ring possible through a bond between the terminal carboxylate group and vanadium. It is commonly accepted that a simple dipeptide coordinates with all the three donor atoms in the equatorial position of a metal ion.[6] Thus, the second molecule of HisGly must change its arrangement from *eq-eq* to *eq-ax*. Since histamine-like coordination is not favoured with the $V^{IV}O$ ion,[17b] we expect that the fourth and fifth coordination sites are occupied by the carbonyl oxygen atom and the amine nitrogen of the N-terminal part, respectively. Therefore, both the donor sets [(NH_2, N^-, COO^-); (CO, NH_2^{ax})] or [(NH_2, N^-, COO^-); (NH_2, CO^{ax})] are acceptable. The following discussion will demonstrate that the first structure, indicated as '**b**' in Scheme 2, is more suitable (vide infra).

HisHis (H_4L^{3+}, Table 1 and Scheme 1) shows four deprotonation steps in the pH range 2–10 with pK_a of 2.61 (–COOH), 5.68 ($-N_{im1}H^+$), 6.85 ($-N_{im2}H^+$) and 7.79 ($-NH_3^+$).[23] The complexation scheme is very similar to

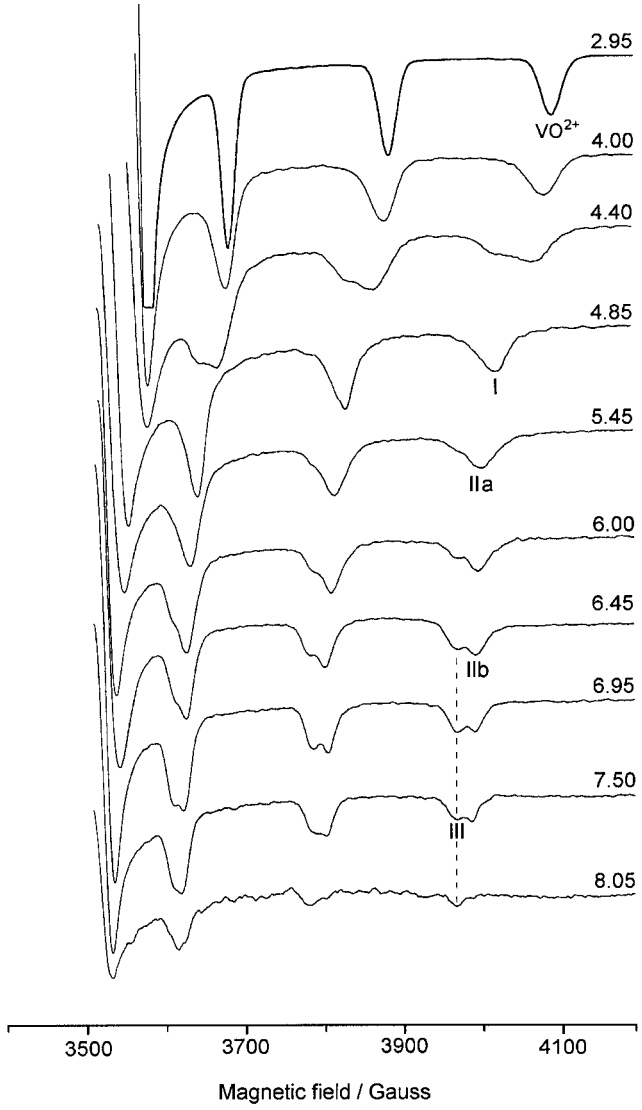


Figure 3. High-field region of the X-Band EPR spectra recorded at 140 K as a function of pH on aqueous solutions of $V^{IV}O$ and HisGly at a metal-to-ligand to molar ratio of 1:10 and a $V^{IV}O$ concentration of 4 mM. The dotted line indicates the high-field resonance of the complex with the deprotonated amide group.

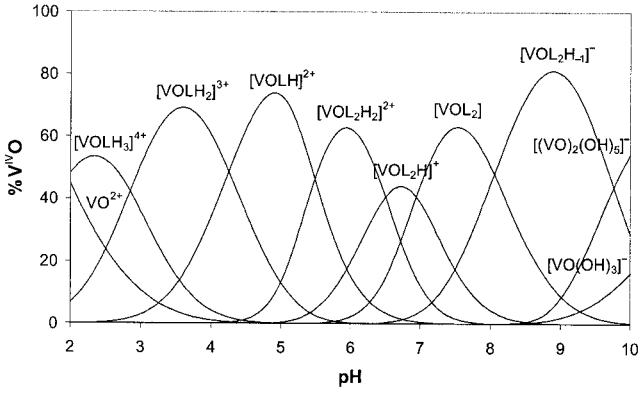


Figure 4. Species distribution for the $V^{IV}O$-HisHis system at a metal-to-ligand molar ratio of 1:3 and a $V^{IV}O$ concentration of 1 mM.

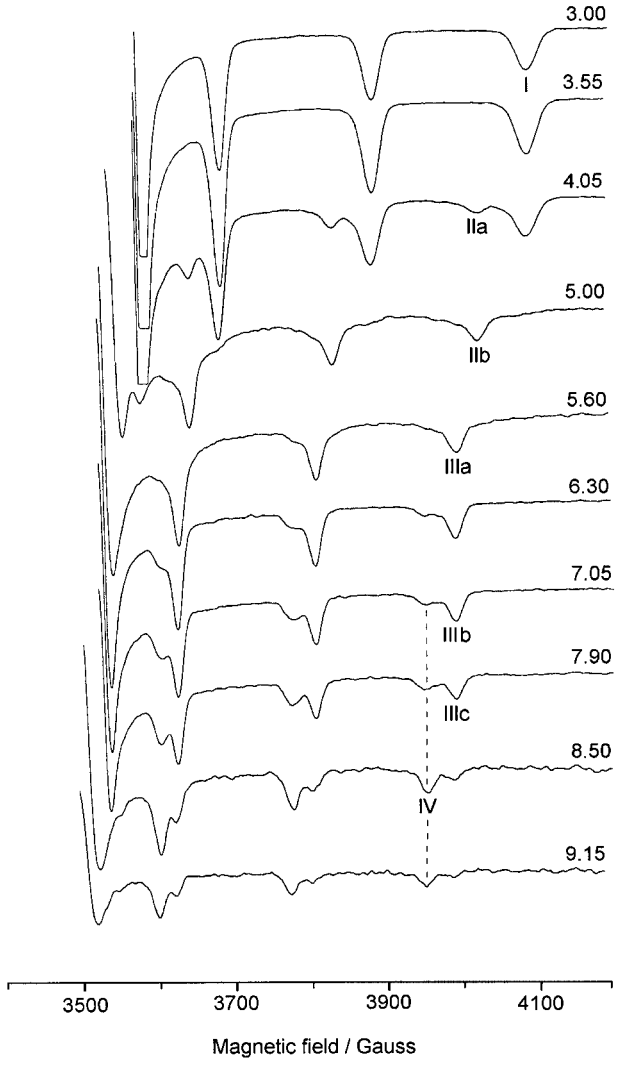


Figure 5. High-field region of the X-Band EPR spectra recorded at 140 K as a function of pH on aqueous solutions of $V^{IV}O$ and HisHis at a metal-to-ligand to molar ratio of 1:3 and a $V^{IV}O$ concentration of 4 mM. The dotted line indicates the high-field resonance of the complex with the deprotonated amide group.

that of $HisNH_2$ and HisGly if the presence of the imidazole ring on the C-terminal part of the dipeptide is taken into account. The distribution curves for this system are shown in Figure 4 and the EPR spectra in Figure 5.The complexation process starts to give $VOLH_3$ through the coordination of the weak carboxylate group (the stronger donor groups, i.e. the two imidazole and the amine nitrogen atoms, are still protonated). EPR and electronic absorption spectra are distinctive of this coordination mode. Indeed, the formation of carboxylate complexes does not change the EPR parameters very significantly (Table 2) relative to the aqua ion, $g_z = 1.934$, $A_z = 178 \times 10^{-4}$ cm^{-1} (**I**, Figure 5). As usual, in the absorption spectra, only a minor shift of the d-d band around 770 nm is observed, accompanied by a better resolution of the shoulder around 640 nm.

$VOLH_2$ and VOLH (**IIa** and **IIb** in Figure 5) exhibit $g_z = 1.947$, $A_z = 169 \times 10^{-4}$ cm^{-1} indicative of a (NH_2, CO, N_{im}^{ax}) coordination of HisHis; they have two and one imidazole rings still protonated, respectively. Analogously, VOL_2H_2 (**IIIa** in Figure 5 and **a** in Scheme 2) shows the [(NH_2, CO, N_{im}^{ax}); (NH_2, CO)] donor set displayed by $HisNH_2$ and HisGly, and keeps two imidazole residues protonated. The findings are confirmed by EPR ($g_z = 1.950$, $A_z = 164 \times 10^{-4}$ cm^{-1}). The deprotonation of the imidazole groups takes place in distinct steps with pK_a values of 6.50 and 6.94, similar to those of the free ligand, to afford VOL_2H and VOL_2 (**IIIb** and **IIIc** in Figure 5). The "basicity-adjusted" stability constant for the formation of VOL_2H_2 complex through the reaction $[VOLH]^{2+} + LH_2^+ \rightarrow [VOL_2H_2]^{2+} + H^+$ is –2.24, which is comparable to that of the complex of HisGly.

At a pH above 6.5, VOL_2H_{-1} (**IV** in Figure 5 and **c** in Scheme 2) is formed from VOL_2 with a pK_a of 8.05. The species is distinguished by $g_z = 1.957$, $A_z = 157 \times 10^{-4}$ cm^{-1}. The higher g_z and lower A_z values, in comparison with the analogous complex of HisGly, suggest that the imidazole nitrogen atom at the C-terminal part takes part in the coordination to the VO^{2+} ion rather than the weaker carboxylate group. Also for this complex as well as for HisGly, two different donor sets, [(NH_2, N^-, N_{im}); (CO, NH_2^{ax})] or [(NH_2, N^-, N_{im}); (NH_2, CO^{ax})], could be proposed; the first one with the carbonyl group in equatorial position is most probable (vide infra).

GlyHis (H_3L^{2+}, Table 1 and Scheme 1) shows three pK_a values in the titratable pH range: 2.45 (–COOH), 6.85 ($-N_{im}H^+$) and 8.33 ($-NH_3^+$).[22] If histidine is in the C-terminal position of a dipeptide, the complexation scheme is completely different to that of $HisNH_2$, HisGly and HisHis. The species distribution curve, shown in Figure 6 for a molar ligand excess of 10, shows the species $VOLH_2$ and VOL_2H_3 in the pH range 2–5. The $VOLH_2$ complex exhibits $g_z = 1.935$, $A_z = 177 \times 10^{-4}$ cm^{-1}. In less acidic solution, the EPR components shift continuously until pH 5 is reached, with a change of A_z from 178×10^{-4} to 173×10^{-4} cm^{-1}, which supports the coordination of a carboxylate group to the $V^{IV}O$ ion. Similar changes were observed with carboxylic[13b] and amino acids[13a] at high ligand excess, also suggesting COO^- and/or (COO^-; COO^-) coordination. The stability constants of these complexes are somewhat higher than expected for pure carboxylate coordination[24] and indicate the binding of extra donors, presumably the amide carbonyl. A (NH_2, CO) donor set can be excluded because the EPR parameters expected for this coordination mode are $g_z = 1.946$, $A_z = 168 \times 10^{-4}$ cm^{-1} (vide supra). Moreover, the exclusive coordination of a monodentate histidine residue in the N-terminal position can be ruled out because in such a case L/M ratios as high as 500 are necessary to suppress the hydrolysis of the vanadyl ion. This suggests that (COO^-, CO) is the coordination set. In $VOLH_2$, the amino and imidazole nitrogen atoms are still protonated. VOL_2H_3 is characterised by $g_z = 1.938$, $A_z = 172 \times 10^{-4}$ cm^{-1} and can be described as a bischelated complex of the [2×(COO^-, CO)] type, see '**d**' in Scheme 2 and Table 2. The amine and imidazole nitrogen atoms in one ligand are still protonated, whilst in another ligand, only the amino nitrogen atom is protonated. The attributions and the EPR parameters are in agreement with recent contributions,[21,25–27] which report EPR hyperfine coupling constants in the range 175–179×10^{-4} cm^{-1} for monochelated and 171–174×10^{-4} cm^{-1} for bischelated species.

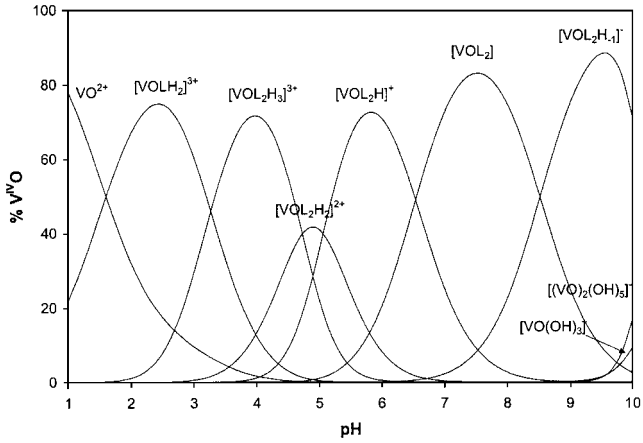


Figure 6. Species distribution for the $V^{IV}O$-GlyHis system at a metal-to-ligand molar ratio of 1:10 and a $V^{IV}O$ concentration of 1 mM.

Upon deprotonation of the first amino group, the dipeptide switches its coordination mode from (COO^-, CO) to (NH_2, CO) and forms a minor VOL_2H_2 species with a [(NH_2, CO); (COO^-, CO)] mixed donor set (**e** in Scheme 2). The EPR parameters ($g_z = 1.943$, $A_z = 166 \times 10^{-4}$ cm^{-1}), intermediate between those of [2×(COO^-, CO)] and [2×(NH_2, CO)], support the coordination mode, whereas potentiometry indicates the presence of this species at around pH 5 (Figure 6).

The coordination of a ligand molecule through (NH_2, CO) hinders hydrolysis and favours the subsequent deprotonation of the amide group to form a VOL_2H species with donor set [(NH_2, N^-, COO^-); (CO, COO^{-ax})], **f** in Scheme 2, which exhibits $g_z = 1.958$, $A_z = 161 \times 10^{-4}$ cm^{-1}. The imidazole and amino groups of the second ligand are still protonated. They undergo deprotonation with pK_a 6.54 and 8.52, respectively; these are both a little higher than for the free ligand. Two new species VOL_2 and VOL_2H_{-1} are formed, and differ only with respect to the protonation of an amino group. Since the same EPR parameters ($g_z = 1.959$, $A_z = 157 \times 10^{-4}$ cm^{-1}) are measured, we suggest a [(NH_2, N^-, N_{im}); (CO, COO^{-ax})] donor set for VOL_2 (**g** in Scheme 2) and [(NH_2, N^-, N_{im}); (CO, NH_2^{ax})] for VOL_2H_{-1} (**c** in Scheme 2). In both species, a carbonyl oxygen atom of the second ligand occupies the equatorial position (vide infra). The coincidence of the hyperfine coupling constants for HisHis and GlyHis complexes supports our conclusion.

Glycyl-L-cysteine (GlyCys), L-Cysteinylglycine (CysGly)

The "soft" deprotonated thiol group of cysteine is very effective as a metal-binding site for transition-metal ions.[6]

Also in this case, the complexation processes depend on the position of the residue in the peptide and on the "hardness" of the metal ion.[6]

With GlyCys the soft Ni^{2+} and Pd^{2+} ions promote the coordination of the deprotonated amide.[28,29] Instead, Co^{2+}, Zn^{2+} and Cd^{2+}[30,31] prefer the coordination of thiol and carboxylate groups in bischelated complexes with various protonated forms because of the presence of the free terminal amino group.

In the case of CysGly a stable cysteine-like (NH_2, S^-) coordination is favourable for a number of metal ions (e.g. Ni^{2+}, Pd^{2+}, Co^{2+}, Zn^{2+} and Cd^{2+}).[28–31] This means that the N-terminal cysteine residue prevents amide deprotonation, and biscomplexes $[MA_2]^{2-}$ are observed.

GlyCys (H_3L^+, Table 1 and Scheme 1) shows three pK_a values in the measured pH range: 2.73 (–COOH), 8.04 ($-NH_3^+$) and 9.48 (–SH).[30] The speciation scheme of GlyCys is more complex than for other systems. The species distribution curves depicted in Figure 7 indicate that at pH 2–4.5 $VOLH_2$ and VOL_2H_4 (**I** and **II** in Figure 8) are formed in strongly overlapping processes. Their EPR parameters are g_z = 1.935, $A_z = 176\times10^{-4}$ cm^{-1} and g_z = 1.940, $A_z = 172\times10^{-4}$ cm^{-1}, very similar to those of $VOLH_2$ and VOL_2H_3 of GlyHis and of $[VO\text{-}(mpg)]^+$ and $[VO\text{-}(mpg)_2]$, where H_2mpg is 2-mercaptopropionylglycine.[21] As for GlyHis, the features support (COO^-, CO) and [2×(COO^-, CO)] coordination modes (**d** in Scheme 2). In both complexes, the amino and thiol groups in each ligand are protonated.

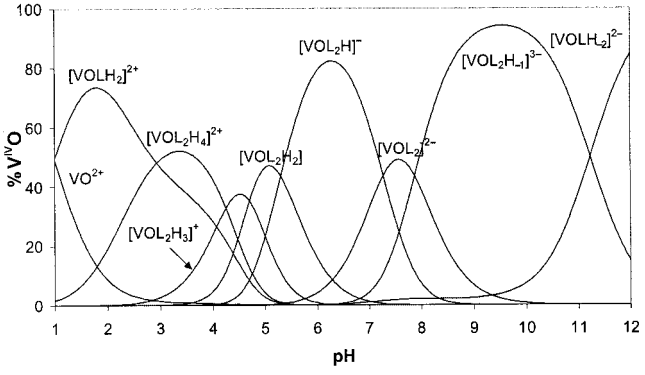


Figure 7. Species distribution for the $V^{IV}O$-GlyCys system at a metal-to-ligand molar ratio of 1:10 and a $V^{IV}O$ concentration of 1 mM.

A two-step deprotonation of the amino groups gives rise to VOL_2H_3 and VOL_2H_2 (**III** and **IV** in Figure 8) at pH 5–6. As suggested by EPR and pH-potentiometry, the first is a minor complex and it is, therefore, a rather hard task to accurately measure the spectral parameters. However, a mixed coordination [(NH_2, CO); (COO^-, CO)] could be proposed (**e** in Scheme 2). The second complex exhibits g_z = 1.950, $A_z = 164\times10^{-4}$ cm^{-1}, indicative of a [2×(NH_2, CO)] coordination.

With increasing pH the first –SH group deprotonates and VOL_2H_2 is replaced by VOL_2H (**V** in Figure 8 and **h** in Scheme 2), presumably a complex with a mixed donor set

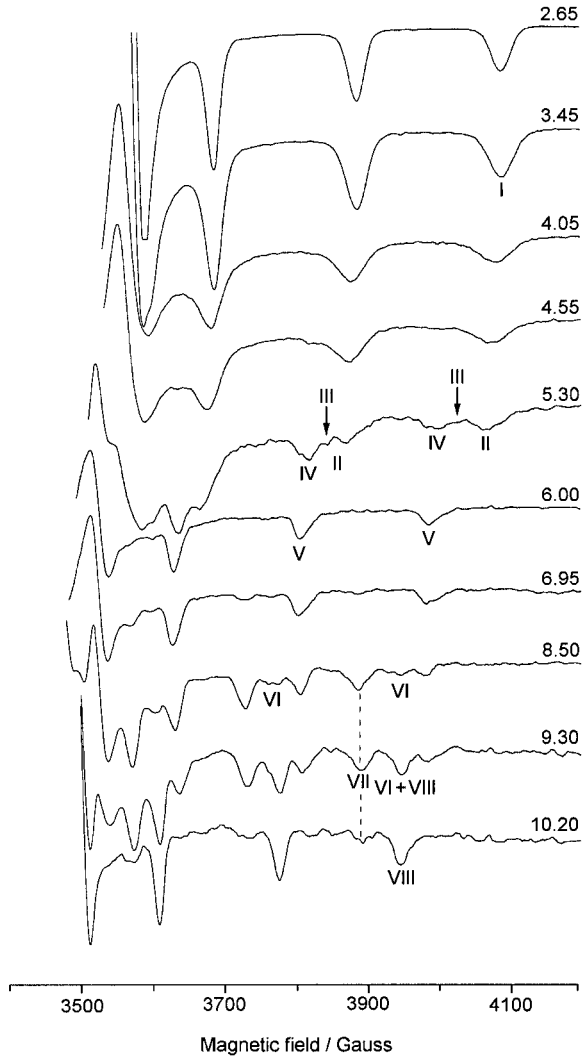


Figure 8. High-field region of the X-Band EPR spectra recorded at 140 K as a function of pH on aqueous solutions of $V^{IV}O$ and GlyCys at metal-to-ligand to molar ratio of 1:10 and a $V^{IV}O$ concentration of 4 mM. The dotted line indicates the high-field resonance of the complex with the deprotonated amide group.

[(S^-, COO^-); (NH_2, CO)]. The conclusion is supported by the EPR parameters, g_z = 1.953, $A_z = 160\times10^{-4}$ cm^{-1}, which are intermediate between those of the [2×(NH_2, CO)] and [2×(S^-, COO^-)] donor sets (g_z = 1.959, $A_z = 155\times10^{-4}$ cm^{-1} for N-acetyl-L-cysteine and g_z = 1.960, $A_z = 154\times10^{-4}$ cm^{-1} for 3-mercaptopropionic acid).[13b] The deprotonation of the thiol group of the second ligand yields VOL_2 (**VI** in Figure 8) with g_z = 1.956, $A_z = 155\times10^{-4}$ cm^{-1} and a [2× (S^-, COO^-)] coordination set.[13b]

At pH values higher than 8, VOL_2 is transformed into VOL_2H_{-1} (**VII** in Figure 8 and **i** in Scheme 2) with a pK_a of 8.43. The EPR parameters are g_z = 1.968, $A_z = 144\times10^{-4}$ cm^{-1}. To this species we attribute a coordination mode of the [(NH_2, N^-, S^-); (S^-, COO^{-ax})] type, according to the assignments for 2-mercaptopropionylglycine.[21]

Finally, at pH >10, a $VOLH_{-2}$ species (**VIII** in Figure 8 and **j** in Scheme 2) exhibits EPR parameters, g_z = 1.956, $A_z = 152\times10^{-4}$ cm^{-1}, which are very similar to those of VOL_2.

A species with donor set [2×(S⁻, COO⁻)] likely undergoes hydrolysis at pH >9.[13b] Thus, around pH 9, the two species **VI** and **VIII** with similar spectral parameters coexist, whereas only $VOLH_{-2}$ remains in solution at higher pH values. As suggested by potentiometry, most probably this is a hydrolytic species with donor set [(NH_2, N^-, S^-); OH^-], similar to that formed by 2-mercaptopropionylglycine with [(COO^-, N^-, S^-); OH^-] coordination.[21] The difference in the A_z value, 154×10^{-4} cm^{-1} versus 152×10^{-4} cm^{-1} (this work), is attributable to the replacement of a carboxylate by an amino group.

For CysGly (H_3L^+, Table 1 and Scheme 1), three pK_a values were observed by Sóvágó et al.: 3.13 (–COOH), 6.94 (–NH_3^+) and 9.37 (–SH).[30] The complexation behaviour is rather simple because the (NH_2, S^-) donor set can form very stable mono- and bischelated complexes. Besides the aqua ion $[VO(H_2O)_5]^{2+}$, we observe a VOL species around pH 5 with g_z = 1.951, $A_z = 162\times10^{-4}$ cm^{-1}, and another species VOL_2 above pH 6.0 with g_z = 1.966, $A_z = 145\times10^{-4}$ cm^{-1}. The EPR parameters of VOL_2 are very similar to those of the bischelated complexes of cysteamine (g_z = 1.973, $A_z = 144\times10^{-4}$ cm^{-1}) and L-cysteine (g_z = 1.967, $A_z = 144\times10^{-4}$ cm^{-1}) for which a [2×(NH_2, S^-)] donor set was proposed.[13b] To the best of our knowledge, a coordination mode (NH_2, S^-) was never observed: we expect EPR parameters intermediate between those of $[VO(H_2O)_5]^{2+}$ (g_z = 1.933, $A_z = 180\times10^{-4}$ cm^{-1}) and VOL_2. The spectra are consistent with that expected.

The "basicity-adjusted" stability constants for the mono- and bischelated species VOL and VOL_2 in the reactions $VO^{2+} + HL^- \rightarrow [VOL] + H^+$ and $[VOL] + HL^- \rightarrow [VOL_2]^{2-} + H^+$ are –0.06 and –2.08; these are significantly greater than the corresponding complexes of HisGly. This confirms that L-cysteyl is a more efficient anchoring residue than L-histidyl and can hinder the subsequent deprotonation of the amide group (Figure 9).

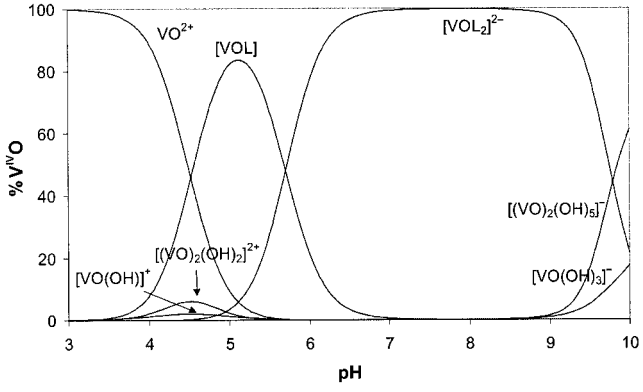


Figure 9. Species distribution for the $V^{IV}O$-CysGly system at a metal-to-ligand molar ratio of 1:10 and a $V^{IV}O$ concentration of 1 mM.

The results for the $V^{IV}O$ complexes of GlyCys and CysGly are similar to those for the Cu^{II} complexes of L-histidyl peptides. In the latter case, amide deprotonation and binding were prevented by an N-terminal side chain and promoted by a C-terminal side chain.[6]

Discussion

Chasteen introduced an "additivity rule" to estimate the parallel ^{51}V hyperfine constant of $V^{IV}O$ complexes, based on the contribution to A_z from each of the four equatorial donor groups.[16] The rule provides a valid criterion for the identification of equatorial donor atoms. Data for amine, carboxylate, alcoholate, phenolate, aromatic nitrogen, hydroxo ion and aliphatic and aromatic thiolate groups were listed by Chasteen (Table 3).[16] Kabanos et al. used values of 44.2 and 43.2×10^{-4} cm^{-1} for Cl^- and SCN^-, respectively.[32] Based on the model $[VO(oxalato)_2]^{2-}$ complex,[33] Kiss and Costa Pessoa changed the COO^- contribution from 42.7 to 41.8×10^{-4} cm^{-1} (Table 3).[21] The mean value for a neutral oxygen atom belonging to an amide CO group is 43.5×10^{-4} cm^{-1}.[18]

Table 3. Contribution of various groups to ^{51}V hyperfine coupling constants A_z.

Donor group	A_z expected[a]	Reference
H_2O	45.6	[16]
COO^-	41.8	[21]
CO	43.5	[18]
OH^-	38.7	[16]
Ar-O^-	38.9	[16]
R–O^-	35.3	[16]
Ar–S^-	35.3	[16]
R–S^-	31.9	[16]
NH_2	40.1	[16]
N(imino)	41.6	this work
N(aromatic)	40.7	[16]
Cl^-	44.2	[32]
SCN^-	43.2	[32]

[a] A_z measured in 10^{-4} cm^{-1} units.

Chasteen did not include the imino nitrogen atom, which, as noticed by Cavaco et al., is certainly different from an aromatic nitrogen atom, e.g. those of 2,2′-bipyridine.[34] On the basis of the EPR parameters of [VO(*N*-salicylidene-glycinato)(bipy)] and [VO(*N*-salicylidene-L-alaninato)(bipy)] Cavaco et al. calculated a value in the range $41.5–43.5\times10^{-4}$ cm^{-1}.[34] From the analysis of the hyperfine coupling constants of twenty three compounds,[34–35] we obtained a more accurate value of $A_z(N_{imino})$; A_z values in the range $38.1–43.7\times10^{-4}$ cm^{-1} were found,[36] with a mean value of 41.6×10^{-4} cm^{-1}, which is in good agreement with those reported by Costa Pessoa.[34,35d] The values used in this work are summarised in Table 3.

With respect to the amide contribution A_z(amide), the values, in the range $29–43\times10^{-4}$ cm^{-1},[37] are sensitive to the balance of the coordination sphere. The A_z(amide) value decreases with increasing donation of electron density from the ligands to the metal ion (Table 4). Tasiopoulos et al. listed the amide contribution for 15 $V^{IV}O$ compounds characterised by X-ray diffraction.[37] They found that A_z(amide) is affected by the charge of the other equatorial donor atoms. If the total charge of the donor atoms in the equatorial plane (TEC) is –2, –3 or 4 (including the –1 charge of the deprotonated peptide nitrogen), the mean A_z(amide) value is 32.2, 36.5 and 40.3×10^{-4} cm^{-1}, respectively.

Table 4. Contribution of deprotonated amide group to A_z value for oxovanadium(IV) complexes as a function of the equatorial donor set.

Charge	Ligand[a]	Equatorial donor set	g_z	A_z[b]	A_z(amide)[b]	Reference
–4	SalGly	O_{ar}^-, N^-, COO^-; OH^-	1.951	163	43.6	[11]
–4	H_2mpg	COO^-, N^-, RS^-; RS^-	1.968	146.0	40.4	[21]
–4	H_2mpg	COO^-, N^-, RS^-; OH^-	1.960	154.0	41.6	[21]
–4	SalGly-L-Ala	O_{ar}^-, N^-, N^-, COO^-	1.958	159	39.2	[27]
–4	GSH	N^-, RS^-, N^-, COO^-	1.959	154	40.2	[37]
–4	H_4m_2pc	COO^-, N^-, RS^-, RS^-	1.959	149.3	43.7	[37]
–4	H_4m_3pc	COO^-, N^-, RS^-, RS^-	1.959	148.4	42.8	[37]
–4	H_4hybeb	O_{ar}^-, N^-, N^-, O_{ar}^-	1.960	156.2	39.2	[38]
–4	H_4hymeb	RO^-, N^-, N^-, RO^-	1.964	145	37.2	[44]
–3	H_2mpg/phen	COO^-, RS^-, N^-; N_{ar}	1.957	151.1	36.7	[9]
–3	SalGly	O_{ar}^-, N^-, COO^-; H_2O	1.949	165	38.7	[11]
–3	H_2mpg	COO^-, N^-, RS^-; H_2O	1.959	157.0	37.7	[21]
–3	H_2Sal-RGlyGly	O_{ar}^-, N^-, COO^-; H_2O	1.953	164	37.7	[26]
–3	H_2Sal-RGlyGly	O_{ar}^-, NH, N^-; OH^-	1.955	158	40.3	[26]
–3	H_2Sal-RGlyGlyGly	O_{ar}^-, NH, N^-; OH^-	1.960	157	39.3	[26]
–3	SalGly-L-Ala	O_{ar}^-, N^-, CO, COO^-	1.945	165	40.8	[27]
–3	H_3hypyb	N_{ar}, N^-, N^-, O_{ar}^-	1.960	156.1	38.3	[38]
–3	SalenGlyGly	O_{ar}^-, N_{imino}, N^-; COO^-	1.950	160	37.7	[39]
–2	Gly-L-Ala	NH_2, N^-, COO^-; H_2O	1.955	161.7	34.2	[10b]
–2	L-AlaGly	NH_2, N^-, COO^-; H_2O	1.953	160.1	32.6	[10b]
–2	L-Ala-L-Ala	NH_2, N^-, COO^-; H_2O	1.955	161.5	34.0	[10b]
–2	GlyGly	NH_2, N^-, COO^-; H_2O	1.958	161.8	34.3	[10b]
–2	H_2capcah / Cl^-	N_{ar}, N^-, NH; Cl^-	1.946	164.5	39.5	[32]
–2	H_2capcah/SCN^-	N_{ar}, N^-, NH; SCN^-	1.946	160.0	36.0	[32]
–2	GlyGly/ phen	NH_2, N^-, COO^-; N_{ar}	1.952	160.0	37.4	[37]
–2	Gly-L-Ala/phen	NH_2, N^-, COO^-; N_{ar}	1.952	158.6	36.0	[37]
–2	H_2thipca	N_{ar}, N^-, N_{imino}, RS^-	1.965	150.6	33.0	[38]
–2	Gly-L-Val/phen	NH_2, N^-, COO^-; N_{ar}	1.951	159.1	36.5	[40]
–2	Gly-L-Leu/phen	NH_2, N^-, COO^-; N_{ar}	1.952	159.2	36.6	[40]
–2	Gly-L-Asp	NH_2, N^-, COO^-; H_2O	1.953	160.7	33.2	[41]
–2	L-AspGly	NH_2, N^-, COO^-; H_2O	1.950	165.0	37.5	[41]
–2	Gly-L-Phe/phen	NH_2, N^-, COO^-; N_{ar}	1.951	159.0	36.4	[42]
–2	Gly-L-Tyr/phen	NH_2, N^-, COO^-; N_{ar}	1.952	160.0	37.4	[42]
–2	H_2pycac	N_{ar}, N^-, NH, RO^-	1.956	151.4	35.3	[43]
–2	H_2pycbac	N_{ar}, N^-, NH, RO^-	1.957	152.4	36.3	[43]
–2	H_2bpb	N_{ar}, N^-, N^-, N_{ar}	1.955	145	31.8	[44]
–2	H_2phepca	N_{ar}, N^-, N_{imino}, O_{ar}^-	1.961	154	32.8	[44]

[a] SalGly = Salicylglycine; H_2mpg = 2-mercaptopropionylglycine; SalGly-L-Ala = Salicylglycyl-L-alanine; GSH = glutathione; H_4m_2pc = *N*-(2-*m*ercaptopropionyl)cysteine; H_4m_3pc = *N*-(3-*m*ercaptopropionyl)cysteine; H_4hybeb = 1,2-bis(2-hydroxybenzamido)benzene; H_4hymeb = 1,2-bis(2-hydroxy-2-methylpropanamido)benzene; H_2Sal-RGlyGly = *N*-salicylglycylglycine; H_2Sal-RGlyGlyGly = *N*-salicylglycylglycylglycine; H_3hypyb = 1-(2-hydroxybenzamido)-2-(2-pyridinecarboxamido)benzene; SalenGlyGly = *N*-salicylideneglycylglycine; phen = 1,10-phenanthroline; H_2capcah = *N*-{2-[(2-pyridylmethyl)amino]phenyl}pyridine-2-carboxamide; H_2thipca = *N*-[2-((2-thiophenoylmethylene)amino)phenyl]pyridine-2-carboxamide; H_2pycac = *N*-[2-(4-oxopent-2-en-2-ylamino)phenyl]pyridine-2-carboxamide; H_2pycbac = *N*-[2-(4-phenyloxobut-2-en-2-ylamino)phenyl]pyridine-2-carboxamide; H_2bpb = 1,2-bis(2-carboxamidopyridyl)benzene; H_2phepca = [*N*-(salicylideneamine)phenyl]pyridine-2-carboxamide. [b] A_z measured in 10^{-4} cm^{-1} units.

Unfortunately, they analysed only two data for the –3 charge and four for –4 charge, whereas no data for the –1 charge were available.

We included further four solid-state compounds and 18 complexes characterised in solution. Therefore, we have taken into account a total of 37 compounds, nine with a –4 charge, nine with –3, and finally, 19 with a –2 charge.[9,10b,11,21,26,27,32,37–44]

Among 25 $V^{IV}O$ structures involving the coordination of a deprotonated amide group found with a search in the Cambridge Structural Database,[45] we excluded only the octahedral complexes *trans*-[VOX(capca)], where Hcapca is *N*-{2-[(2-pyridylmethylene)amino]phenyl}pyridine-2-carboxamide and X^- (Cl^-, SCN^-, CH_3COO^- and $PhCOO^-$) is an axial ligand,[46] because the authors noticed an anomalous reduction of A_z of about 10% due to the effect of X^-.

On the whole, an evaluation of the contribution of a deprotonated amide group to the hyperfine constant along the *z* axis confirms the results of Kabanos and Deligiannakis.[37] However, the mean values for TEC = –2, –3 and –4 are slightly different: 35.5, 38.3 and 40.9×10^{-4} cm^{-1} in this work versus 32.2, 36.5 and 40.3×10^{-4} cm^{-1}, respectively. As shown in Figure 10, a linear relationship is observed with a correlation coefficient of 0.99. Based on these results, we can obtain the contribution of the amide group with a –1 charge by extrapolation. The calculated value is 32.7×10^{-4} cm^{-1}.

The rather low contribution for an amide group in complexes with TEC = –1 could partially explain the anomalous reduction of the parallel hyperfine coupling constant observed by Kabanos and coworkers and mentioned above.[46] They reported A_z values of $144–148\times10^{-4}$ cm^{-1}

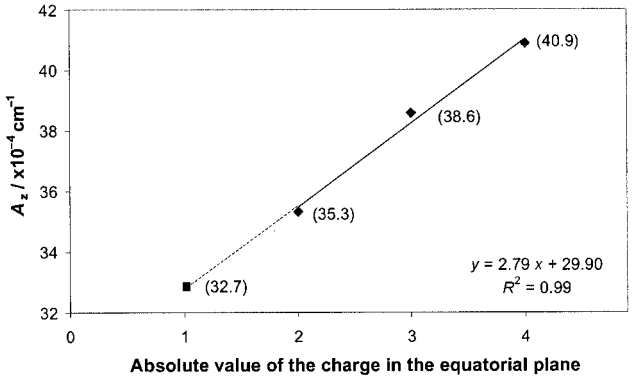


Figure 10. Contribution of a deprotonated amide group to A_z as a function of the absolute value of the charge in the equatorial plane for oxovanadium(IV) complexes.

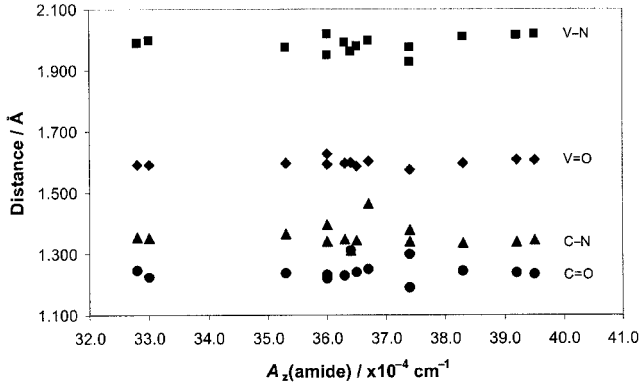
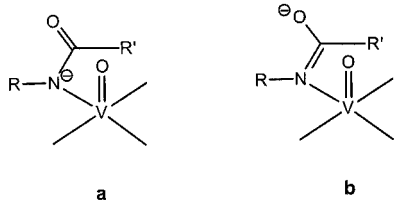


Figure 11. Lengths of V–N, V=O, C–N and C=O bonds as a function of the contribution of the deprotonated amide group to A_z for oxovanadium(IV) complexes. All the solid structures present in literature, for which EPR parameters are reported,[9,32,37,38,40,42–44] were examined.

for the complexes [VOX(capca)], where X^- is Cl^-, SCN^-, CH_3COO^- or $PhCOO^-$ and occupies the axial position, relative to 161×10^{-4} cm^{-1} predicted by Chasteen's rule for (N_{ar}, N_{imino}, N^-, N_{ar}) coordination. With the contribution proposed here for this type of complexes we calculate a value of 155.9×10^{-4} cm^{-1}, which is significantly closer to the experimental one.

The wide range of the A_z(amide) contribution was explained [37,44] by assuming two limit resonance structures for an amide group coordinated through the nitrogen atom: one "amide-like", with the nitrogen atom negatively charged and a double bond between the carbon and oxygen atoms of the carbonyl group (**a** in Scheme 3), and another "imine-like", with the carbonyl oxygen atom negatively charged and a double bond between the carbon and nitrogen atoms (**b** in Scheme 3). The predominance of one of these structures depends on the electronic density of the metal ion: particularly, the importance of the **a** form should increase with decreasing the density (and hence TEC value).



Scheme 3. Limit resonance structures for a deprotonated amide group bound to oxovanadium(IV) species: (a) "amide-like" and (b) "imine-like" form.

If this suggestion is correct, one might expect that the C–N bond is longer and the V–N and C=O bonds are shorter as the "amide-like" form becomes prevalent. In the meantime, the contribution of the deprotonated amide group to A_z should be reduced. A comparison of V–N, C–N and C=O bond lengths, shown in Figure 11, does not reveal any apparent correlation. Therefore, the cause for the difference in the contribution of a deprotonated amide group to A_z must be another one.

The hyperfine coupling constant for ^{51}V can be expressed in the following form:[47]

$$A_z = P_d\left[\beta^2\left(-\kappa - \frac{4}{7}\right) + (g_z - 2.0023) + \frac{3}{14}(g_x - 2.0023) + \frac{3}{14}(g_y - 2.0023)\right]$$

In the above equation $P_d = g_N\beta_N g\beta\langle r^{-3}\rangle$ is the dipolar interaction between the unpaired electron and the ^{51}V nucleus, and is determined by the spatial distribution of the d electron.[47] κ is the Fermi contact term, a measure of the unpaired s-electron spin density at the nucleus, and is influenced by the polarisation of the inner s electrons upon interaction with the unpaired electron. The β^2 factor is, to a good approximation, the population of the ground state d orbital containing the unpaired spin; for $V^{IV}O$ species with C_{2v} symmetry or higher, it measures the population of the d_{xy} orbital. A value of 1 for β^2 indicates that the unpaired electron is localised exclusively on the vanadium d orbital and that there is no delocalisation onto the ligands. On the other hand, values of $\beta^2 < 1$ indicate that a $(1 - \beta^2)$ fraction of the spin density is delocalised onto the ligands.

Since the κ term is considered constant for vanadyl complexes,[48] a reduction in the A_z value could be connected to a decrease of P_d or β^2 or both. According to Kabanos et al.,[32] a reduced P_d value implies a larger $\langle r^{-3}\rangle$ value, which means a radial expansion of the $3d_{xy}$ orbital and a reduced electron density on the metal. This reduced electron density is expected to decrease the V=O bond strength.[49] From Figure 11 it can be observed that no correlation between the A_z(amide) values and the length of V=O bond in vanadyl unit is tenable. Thus, this mechanism can be excluded. This confirms that P_d can also be assumed constant for compounds with similar donor sets, as stated by Pecoraro.[48]

Pecoraro et al. proposed that the overlap of the π orbitals of the ligand with the d_{xy} vanadium atomic orbital would increase the covalence of the metal–ligand bond and produce a decrease in the β^2 value.[48] However, this hypothesis too can be ruled out because the π orbitals of the dipeptides

Table 5. Coordination modes of $V^{IV}O$ complexes with a deprotonated amide group on the basis of the EPR data.

Ligand	Complex	g_z	A_z exptl.[a]	A_z calcd.[a]	Donor set	Structure[b]
GlyHis	VOL_2H	1.958	160.7	160.7	[(NH_2, N^-, COO^-); (CO, COO^{-ax})]	f
GlyHis	VOL_2	1.959	157.4	157.0	[(NH_2, N^-, N_{im}); (CO, COO^{-ax})]	g
GlyHis	VOL_2H_{-1}	1.959	157.4	157.0	[(NH_2, N^-, N_{im}); (CO, NH_2^{ax})]	c
HisGly	VOL_2H_{-1}	1.955	159.9	160.7	[(NH_2, N^-, COO^-); (CO, NH_2^{ax})]	b
HisHis	VOL_2H_{-1}	1.957	156.7	157.0	[(NH_2, N^-, N_{im}); (CO, NH_2^{ax})]	c
GlyCys	VOL_2H_{-1}	1.968	143.6	142.5	[(NH_2, N^-, S^-); (S^-, COO^{-ax})]	i
GlyCys	$VOLH_{-2}$	1.956	152.3	149.3	[(NH_2, N^-, S^-); OH^-]	j

[a] A_z measured in 10^{-4} cm^{-1} units. [b] See Scheme 2.

and d_{xy} of vanadium are not in a favourable position to overlap with each other.

The only mechanism that may reduce the A_z value is a strong covalent bond between the deprotonated nitrogen atom and the $V^{IV}O$ ion. We suggest that if the TEC of amidate complexes is –4, i.e. the electronic density around the vanadium atom is high, the V–N bond exhibits a minor amount of covalent character and remains close to the ionic limit: this means higher β^2 and A_z values. On the other hand, if the TEC of the complex is –1, the V–N bond is more covalent, and this results in the reduction in the electron density at the d_{xy} nonbonding orbital of vanadium, and therefore a decrease in the β^2 and A_z values is observed.

According to Kabanos and Pecoraro,[32,48] we assume $P_d = 125 \times 10^{-4}$ cm^{-1} and $\kappa = 0.85$. Values of β^2 of approximately 0.92 for TEC = –4, 0.86 for TEC = –3, 0.80 for TEC = –2 and 0.74 for TEC = –1 are thus obtained.

Based on the A_z contribution of the amide group calculated in the previous discussion, we can attempt to find the donor set and the geometry for the $V^{IV}O$ species that involves this group. The first obvious observation is that the experimental coupling constants for the VOL_2H_{-1} species formed by two dipeptides with L-histidine in the C-terminal position show similar A_z values (about 157×10^{-4} cm^{-1}), whilst for HisGly, VOL_2H_{-1} exhibits a value of about 160×10^{-4} cm^{-1}. This substantiates the involvement of the imidazole nitrogen atom in metal coordination. Instead, the very low value of A_z for GlyCys suggests the participation of S^- in the bonding. Therefore, three equatorial positions are filled by HisGly, GlyHis, HisHis and GlyCys through (NH_2, N^-, COO^-), (NH_2, N^-, N_{im}), (NH_2, N^-, N_{im}), and (NH_2, N^-, S^-), respectively. The last two remaining sites of the coordination sphere of the $V^{IV}O$ ion are completed, according to the data in Table 5, by the (CO, NH_2) donor set which acts as a moderately strong donor towards the $V^{IV}O$ ion. The comparison of the experimental with calculated values supports that the CO group is in the equatorial position and the amino group is in the axial position.

The structures of the complexes formed upon deprotonation of the amide group are marked with **b**, **c**, **f**, **g**, **i** and **j** in Scheme 2.

Conclusions

Simple dipeptides coordinate the $V^{IV}O$ ion without the assistance of strong "anchoring" groups. The ligands coordinate the metal ion with (NH_2, CO) or (COO^-, CO) donor sets in the acidic and neutral pH range, and avoid the precipitation of the hydroxide if at least a fivefold excess of ligand is used. The presence of L-histidine in the N-terminal position enhances the strength of the (NH_2, CO) donor set. In the alkaline pH range, all the ligands, except CysGly, promote the deprotonation of the amide group. The effect of additional donors, e.g. imidazole and thiolate, can be seen also in species containing deprotonated amide groups. The donor sets (NH_2, N^-, N_{im}) and (NH_2, N^-, S^-) are stronger than the simple (NH_2, N^-, COO^-) donor set.

The composition of the complexes of GlyCys and CysGly depends on the location of the thiolato group. In GlyCys the coordination mode is (NH_2, N^-, S^-) and the C-terminal carboxylate does not take part in complexation. On the other hand, N-terminal cysteine peptides can form a stable five-membered chelated ring with the participation of the amino group, which gives rise to stable bischelated complexes without amide binding.

According to Kabanos and Cornman[37,44] the total charge on the equatorial plane of vanadium(IV) affects the bond type of the deprotonated amide group and determines the contribution of the nitrogen atom to the ^{51}V hyperfine coupling constant. We have demonstrated that the reduction of A_z(amide) observed when the TEC changes from –4 to –1 does not depend on the "shift" of "imine-like" to "amide-like" coordination. Instead, it is attributable to an increase in the covalence of the V–N bond accompanied by a decrease in the electron density at vanadium. Thus, it could be misleading to take into account a mean value for the contribution of the deprotonated amide groups without considering the effect of the total equatorial charge.

A direct application of these theoretical results allows the prediction of the geometry and donor set of the compounds involving the coordination of the amide group examined in this study.

Experimental Section

Chemicals: The ligands were Aldrich products of puriss. quality. Their purity and concentration were determined by the Gran method.[50] VO^{2+} solutions were prepared following literature methods.[51] The concentration of the metal ion was determined by $KMnO_4$ titration and that of H^+ by a potentiometric titration using the appropriate Gran function. The ionic strength was adjusted to 0.1 M with KNO_3. The temperature was 25.0 ± 0.1 °C.

Potentiometric Measurements: The protonation constants for the ligands and the stability constants for the $V^{IV}O$ complexes were determined by pH-potentiometric titrations of 2.0 mL samples. The ligand-to-metal molar ratio was in the range 1:1–5:1. The VO^{2+} concentration was 0.001 M. Titrations were performed by adding 0.1 M NaOH with a calibrated syringe under purified argon. The pH values were recorded with a combination glass electrode (Russel CMAWL/S7) calibrated for hydrogen concentration by the method of Irving et al.[52] The reproducibility of the titration points included in the evaluation was within 0.005 pH units in the whole pH range examined (2–12). As usual, the stability of the complexes is reported as the logarithm of the overall formation constant $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$, where M stands for the metal ion, H is the proton, and L is the deprotonated form of the ligand in the absence of metal coordination and was calculated with the aid of the SUPERQUAD program.[53] Standard deviations were calculated by assuming random errors. The conventional notation has been used. Negative indices for protons indicate either the proton dissociation of groups which do not deprotonate in the absence of coordination of the metal ion, or hydroxo ligands. Hydroxo complexes of VO^{2+} were taken into account in the calculations. The following species were assumed: $[VO(OH)]^+$ ($\log \beta_{1-1} = -5.94$), $[(VO)_2(OH)_2]^{2+}$ ($\log \beta_{2-2} = -6.95$), and the stability constants were calculated from the data of Henry et al.[54] and corrected for the different ionic strengths by use of the Davies equation,[55] $[VO(OH)_3]^-$ ($\log \beta_{1-3} = -18.0$) and $[(VO)_2(OH)_5]^-$ ($\log \beta_{2-5} = -22.0$).[56]

Spectroscopic and Analytical Measurements: Anisotropic EPR spectra were recorded on aqueous solutions with an X-band (9.15 GHz) Varian E-9 spectrometer in the temperature range 120–140 K. As usual for low temperature measurements, a few drops of DMSO were added to the samples to ensure good glass formation. The spectra were simulated with the computer program Bruker WinEPR SimFonia. Electronic spectra were recorded with a Beckman Acta MIV spectrophotometer.

[1] D. Rehder, in *Metal Ions in Biological Systems* (Eds.: A. Sigel, H. Sigel), Marcel Dekker, New York, **1995**, vol. 31, p. 1–43.
[2] B. R. Nechay, *Annu. Rev. Pharmacol. Toxicol.* **1984**, *24*, 501–524.
[3] G. Swarup, K. V. Speeg, S. Cohen, D. L. Garbers, *J. Biol. Chem.* **1982**, *257*, 7298–7301.
[4] D. Rehder, J. Costa Pessoa, C. F. G. C. Geraldes, M. M. C. A. Castro, T. Kabanos, T. Kiss, B. Meier, G. Micera, L. Pettersson, M. Rangel, A. Salifoglou, I. Turel, D. Wang, *J. Biol. Inorg. Chem.* **2002**, *7*, 384–396.
[5] a) M. Garner, J. Reglinski, W. E. Smith, J. McMurray, I. Abdullah, R. Wilson, *J. Biol. Inorg. Chem.* **1997**, *2*, 235–241; b) D. C. Crans, M. Mahroof-Tahir, A. D. Keramidas, *Mol. Cell. Biochem.* **1995**, *153*, 17–24.
[6] I. Sóvágó, in *Biocoordination Chemistry* (Ed.: K. Burger.), Ellis Horwood, New York, **1990**, ch. 4, 135–184.
[7] T. Kiss, T. Jakusch, J. Costa Pessoa, I. Tomaz, *Coord. Chem. Rev.* **2003**, *237*, 123–133.
[8] V. M. Fritzsche, V. Vergopoulos, D. Rehder, *Inorg. Chim. Acta* **1993**, *211*, 11–16.
[9] A. J. Tasopoulos, A. T. Vlahos, A. D. Keramidas, T. A. Kabanos, Y. G. Deligiannakis, C. P. Raptopoulou, A. Terzis, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2531–2533.
[10] a) J. Costa Pessoa, S. M. Luz, R. Duarte, J. J. G. Moura, R. D. Gillard, *Polyhedron* **1993**, *12*, 2857–2867; b) J. Costa Pessoa, S. M. Luz, R. D. Gillard, *J. Chem. Soc. Dalton Trans.* **1997**, 569–576.
[11] T. Kiss, K. Petrohan, P. Buglyó, D. Sanna, G. Micera, J. Costa Pessoa, C. Madeira, *Inorg. Chem.* **1998**, *37*, 6389–6391.
[12] M. S. Michailidis, R. B. Martin, *J. Am. Chem. Soc.* **1969**, *91*, 4683–4689.
[13] a) J. Costa Pessoa, L. F. Vilas Boas, R. D. Gillard, R. J. Lancashire, *Polyhedron* **1988**, *7*, 1245–1262; b) A. Dessì, G. Micera, D. Sanna, *J. Inorg. Biochem.* **1993**, *52*, 275–286.
[14] I. Fábián, I. Nagypál, *Inorg. Chim. Acta* **1982**, *62*, 193–199.
[15] E. Garribba, G. Micera, D. Sanna, unpublished results.
[16] N. D. Chasteen, in *Biological Magnetic Resonance* (Eds.: L. J. Berliner, J. Reuben), Plenum Press, New York, **1981**, vol. 3, p. 53–119.
[17] a) L. D. Pettit, J. L. M. Swash, *J. Chem. Soc. Dalton Trans.* **1976**, 588–594; b) D. Sanna, G. Micera, M. G. Molinu, *J. Chem. Research (S)* **1996**, 42–43.
[18] B. J. Hamstra, A. P. L. Houseman, G. J. Colpas, J. W. Kampf, R. LoBrutto, W. D. Frasch, V. L. Pecoraro, *Inorg. Chem.* **1997**, *36*, 4866–4874.
[19] T. A. Kabanos, A. D. Keramidas, A. Papaioannou, A Terzis, *Inorg. Chem.* **1994**, *33*, 845–846.
[20] A. D. Keramidas, S. M. Miller, O. P. Anderson, D. C. Crans, *J. Am. Chem. Soc.* **1997**, *119*, 8901–8915.
[21] T. Jakusch, P. Buglyó, A. I. Tomaz, J. Costa Pessoa, T. Kiss, *Inorg. Chim. Acta* **2002**, *339*, 119–128.
[22] I. Sóvágó, E. Farkas, A. Gergely, *J. Chem. Soc. Dalton Trans.* **1982**, 2159–2163.
[23] C. E. Livera, L. D. Pettit, M. Bataille, B. Perly, H. Koszlowski, B. Radomska, *J. Chem. Soc. Dalton Trans.* **1987**, 661–666.
[24] A. Lorenzotti, D. Lorenzotti, A. Cingolani, P. DiBernardo, *Inorg. Chim. Acta* **1981**, *52*, 149–152.
[25] J. Costa Pessoa, I. Tomaz, T. Kiss, P. Buglyó, *J. Inorg. Biochem.* **2001**, *84*, 259–270.
[26] J. Costa Pessoa, I. Correia, T. Kiss, T. Jakusch, M. M. C. A. Castro, C. F. G. C. Geraldes, *J. Chem. Soc. Dalton Trans.* **2002**, 4440–4450.
[27] T. Jakusch, A. Dörnyei, I. Correia, L. M. Rodriguez, G. K. Tóth, T. Kiss, J. Costa Pessoa, S. Marcão, *Eur. J. Inorg. Chem.* **2003**, 2113–2122.
[28] H. Kozlowski, B. Decock-Le Révérend, D. Ficheux, C. Loucheux, I. Sóvágó, *J. Inorg. Biochem.* **1987**, *29*, 187–197.
[29] K. Cherifi, B. Decock-Le Révérend, K. Várnagy, T. Kiss, I. Sóvágó, H. Kozlowski, *J. Inorg. Biochem.* **1990**, *38*, 69–80.
[30] I. Sóvágó, T. Kiss, K. Várnagy, B. Decock-Le Révérend, *Polyhedron* **1988**, *7*, 1089–1093.
[31] H. Kozlowski, I. Sóvágó, J. Spychala, J. Urbanska, K. Várnagy, A. Kiss, K. Cherifi, *Polyhedron* **1990**, *9*, 831–837.
[32] E. J. Tolis, V. I. Teberekidis, C. P. Raptopoulou, A. Terzis, M. Sigalas, Y. Deligiannakis, T. A. Kabanos, *Chem. Eur. J.* **2001**, *7*, 2698–2710.
[33] P. Buglyó, E. Kiss, I. Fàbiàn, T. Kiss, D. Sanna, E. Garribba, G. Micera, *Inorg. Chim. Acta* **2000**, *306*, 174–183.
[34] I. Cavaco, J. Costa Pessoa, D. Costa, M. T. Duarte, R. D. Gillard, P. Matias, *J. Chem. Soc. Dalton Trans.* **1994**, 149–157.
[35] a) K. P. Callahan, P. J. Durand, *Inorg. Chem.* **1980**, *19*, 3211–3217; b) J. A. Bonadies, C. J. Carrano, *J. Am. Chem. Soc.* **1986**, *108*, 4088–4095; c) J. C. Dutton, G. D. Fallon, K. S. Murray, *Inorg. Chem.* **1988**, *27*, 34–38; d) I. Cavaco, J. Costa Pessoa, M. T. Duarte, R. T. Henriques, P. M. Matias, R. D. Gillard, *J. Chem. Soc. Dalton Trans.* **1996**, 1989–1996; e) C. R. Cornman, K. M. Geisre-Bush, S. R. Rowley, P. D. Boyle, *Inorg. Chem.* **1997**, *36*, 6401–6408.
[36] A_z is 43.7 for [VO(*N*-salicylidene-L-alaninato)(H_2O)] and 42.4×10^{-4} cm^{-1} for [VO(*N*-salicylidene-L-alaninato)(bipy)];[34] 40.8 for [VO(salen)] and 43.4×10^{-4} cm^{-1} for [VO(acen)];[35a] 40.8×10^{-4} cm^{-1} for [VO(salen)];[35b] 42.1 for [VO(tsalen)], 38.1 for VO(tsaltn)], 40.6 for [VO(tsalphen)] and 40.6×10^{-4} cm^{-1} for VO(tsal-4,5-Me_2phen)];[35c] 42.7 for [VO(*N*-salicylidene-L-methioninato)(H_2O)], 40.2 for [VO(*N*-salicylidene-L-methioninato)(bipy)], 41.7 for [VO(*N*-salicylidene-L-valinato)(H_2O)], 41.8 for [VO(*N*-salicylidene-L-valinato)(bipy)], 41.6 for [VO(*N*-salicylidene-L-phenylalaninato)(H_2O)], 41.1 for [VO(*N*-salicylidene-L-phenylalaninato)(bipy)], 43.0 for [VO(*N*-salicylidene-L-isoleucinato)(H_2O)], 42.6 for [VO(*N*-(2-oxido-1-naphthylmethylene-L-valinato))(H_2O)] and 41.9×10^{-4} cm^{-1} for [(*N*-(2-oxido-

1-naphthylmethylene-L-valinato))(bipy)];[35d] 41.8 for [VO(*N*,*N*′-ethylenebis-*o*-(*tert*-butyl-*p*-methylsalicylaldiminato))$_2$], 42.0 for [VO(*N*,*N*′-propanediylbis-*o*-(*tert*-butyl-*p*-methylsalicylaldiminato))$_2$], 41.8 for [VO(N-methylsalicylaldiminato)$_2$], 41.6 for [VO(*N*-methyl-*o*-*tert*-butyl-*p*-methylsalicylaldiminato)$_2$], 41.6 for [VO(*N*-isopropyl-*o*-methylsalicylaldiminato)$_2$] and 39.8×10^{-4} cm^{-1} for [VO(*N*-methyl-*o*-(*tert*-butyl-*p*-methylsalicylaldiminato))$_2$].[35e]

[37] A. J. Tasiopoulos, A. N. Troganis, A. Evangelou, C. P. Raptopoulou, A. Terzis, Y. Deligiannakis, T. A. Kabanos, *Chem. Eur. J.* **1999**, *5*, 910–921.

[38] A. D. Keramidas, A. P. Papaioannou, A. Vlahos, T. A. Kabanos, G. Bonas, A. Makriyannis, C. P. Raptopoulou, A. Terzis, *Inorg. Chem.* **1996**, *35*, 357–367.

[39] I. Cavaco, J. Costa Pessoa, S. M. Luz,, M. T. Duarte, P. M. Matias, R. T. Henriques, R. D. Gillard, *Polyhedron* **1995**, *14*, 429–439.

[40] A. J. Tasiopoulos, E. J. Tolis, J. M. Tsangaris, A. Evangelou, J. D. Woollins, A. M. Z. Slawin, J. Costa Pessoa, I. Correia, T. A. Kabanos, *J. Biol. Inorg. Chem.* **2002**, *7*, 363–374.

[41] J. Costa Pessoa, T. Gajda, R. D. Gillard, T. Kiss, S. M. Luz, J. J. G. Moura, I. Tomaz, J. P. Telo, I. Török, *J. Chem. Soc. Dalton Trans.* **1998**, 3587–3600.

[42] A. J. Tasiopoulos, Y. G. Deligiannakis, J. D. Woollins, A. M. Z. Slawin, T. A. Kabanos, *J. Chem. Soc. Chem. Commun.* **1998**, 569–570.

[43] G. R. Hanson, T. A. Kabanos, A. D. Keramidas, D. Mentzafos, A. Terzis, *Inorg. Chem.* **1992**, *31*, 2587–2594.

[44] C. R. Cornman, E. P. Zovinka, Y. D. Boyajina, K. M. Geisre-Bush, P. D. Boyle, P. Singh, *Inorg. Chem.* **1995**, *34*, 4213–4219.

[45] F. H. Allen, O. Kennard, 3D Search and Research Using the Cambridge Structural Database, *Chemical Design Automation News* **1993**, *8*, pp. 1 and 31–37.

[46] E. J. Tolis, K. D. Soulti, C. P. Raptopoulou, A. Terzis, Y. Deligiannakis, T. A. Kabanos, *J. Chem. Soc. Chem. Commun.* **2000**, 601–602.

[47] B. R. McGarvey, *J. Phys. Chem.* **1967**, *71*, 51–67.

[48] T. S. Smith II, C. A. Roof, J. W. Kampf, P. G. Rasmussen, V. L. Pecoraro, *J. Am. Chem. Soc.* **2000**, *122*, 767–775.

[49] J. Selbin, *Coord. Chem. Rev.* **1966**, *1*, 293–314.

[50] G. Gran, *Acta Chem. Scand.* **1950**, *4*, 559–577.

[51] I. Nagypál, I. Fábián, *Inorg. Chim. Acta* **1982**, *61*, 109–113.

[52] H. Irving, M. G. Miles, L. D. Pettit, *Anal. Chim. Acta* **1967**, *38*, 475–481.

[53] P. Gans, A. Vacca, A. Sabatini, *J. Chem. Soc. Dalton Trans.* **1985**, 1195–1200.

[54] R. P. Henry, P. C. H. Mitchell, J. E. Prue, *J. Chem. Soc. Dalton Trans.* **1973**, 1156–1159.

[55] C. W. Davies, *J. Chem. Soc.* **1938**, 2093–2098.

[56] A. Komura, M. Hayashi, H. Imanaga, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2927–2931.

Received: July 2, 2004

