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Interactions of VO(IV) with oligopeptides

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Contents

Abstract

The oxovanadium(IV)-binding features of various oligopeptides are reviewed in the paper. The decisive role of amide coordination and the presence of a suitable anchoring donor group in the molecules are discussed through numerous examples. It is found that the effectiveness of the anchoring donors in promoting peptide amide deprotonation and coordination follows the sequence: phenolate-O^- > alcoholate-O^-, thiolate-S^- > carboxylate-COO^- > NH_2. This basic sequence is finely tuned by the presence of additional donors in the molecule, and also by the presence of additional binder molecules in the biological fluids.

Keywords: VO(IV) complexes; Oligopeptides; Solution speciation; Structure

1. Introduction

Vanadium is an important nutrient for most higher animals and is known to be essential for certain organisms, including tunicates, some fungi and bacteria [1]. Although its essentiality for human life has not yet been clearly established, it does generate significant physiological responses; for example, vanadate inhibits a number of enzymes, such as ion transport ATP-ases, phosphotyrosine phosphatase, etc. [2]. The most important biological response of vanadium is its insulin-enhancing activity [3]. Our understanding of the mechanism of the insulin-mimetic action of vanadium is far from complete, but it is known that the original biologically active complexes undergo considerable transformations in the organism, including ligand-exchange processes and redox reactions [4]. A knowledge of the distribution and chemical speciation of vanadium in biological fluids and tissues is therefore of basic importance. Proteins, which are widespread biomolecules both extra- and intracellularly, may be intricately involved in vanadium binding in organisms. Peptides are not the best molecules with which to model the metal ion bindings of proteins, as the specially arranged side-chain donor groups of the amino acid units, determined by the tertiary structure of the proteins, are usually the primary

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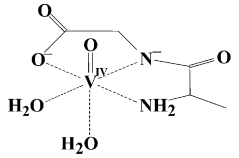
binding sites of proteins, while in the oligopeptides the N or C terminal donors are the basic sites for metal ion coordination. However, peptides are the most closely related models of proteins, and in some cases proteins may have specific metal-binding sites at one of the termini of the proteins, as in the case of albumin. The important cell constituent, glutathione, may play a role in the redox transformations of vanadium in the cells [5]. Detailed structural, thermodynamic, speciation and kinetic investigations on model complexes of vanadium with oligopeptides will certainly contribute greatly to our understanding of the biological roles and actions of vanadium.

A simple oligopeptide contains three kinds of potential donors: the terminal amino and carboxylate groups and the intermediate peptide groups. Chelation through the terminal-NH_2 group together with the carbonyl-O of the peptide group, or through the terminal COO^- alone or together with the carbonyl-O, is generally not a strong enough binding mode to keep metal ions in solution at the physiological pH. In order to achieve this, the amide-N^- should also be coordinated. However, it should first deprotonate, a process that can be promoted by metal ions. Such a binding mode means a strong interaction between a metal ion and the oligopeptide. It has been found for many metal ions that the presence of a suitable anchoring donor in the ligand molecule, which can bind metal ions strongly enough to be able to promote deprotonation of the amide, plays a crucial role in metal binding. Various metal ions have been found to be able to do this, e.g. Pt(II), Pd(II), Cu(II), Ni(II), and in some special cases Zn(II), Co(II), and some others [6]. These basic binding modes may be altered considerably by strongly coordinating side-chain donors, such as imidazole-N or thiolate-S.

In this paper the VO(IV)-binding capabilities of oligopeptides and derivatives are briefly surveyed, with special emphasis on the role of the presence of a suitable anchoring donor group in the molecule. The anchoring abilities of various donor groups (e.g. amino, phenolate, thiolate and alcoholate), and chelating functions (e.g. dialcoholates or aminophenolate) are discussed.

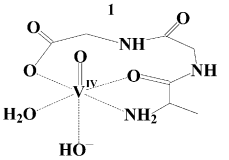
2. Glycine-type oligopeptides

The coordination of a few synthetic ligands to VO(IV) via amide has been observed in the solid state [7]. Kabanos et al. [8] isolated the first VO(IV) complexes of simple dipeptides containing deprotonated amide-N^-. Peptide-amide coordination has subsequently been proved in a series of VO(IV) complexes, such as $[VO(GlyGlyH_{-1})(phen)] \cdot 2CH_3OH$ [9], $[VO(GlyTyrH_{-1})(phen)]$ [10], $[VO(GlyAlaH_{-1})(phen)]$, $[VO(GlyPheH_{-1})(phen)]$ and $[VO(GlyValH_{-1})(phen)]$ [11] (phen = 1,10-phenantroline). In these complexes, the three donor groups of the dipeptides (NH_2, CON^-, COO^-) and an aromatic ring N in phen are coordinated equatorially, while the second aromatic ring N in phen is bound axially. However, no such unambiguous proof of the amide deprotonation has been obtained with such simple dipeptides in aqueous solution. It has been found, for example, that GlyGly and GlyAla are not strong enough binders to prevent the hydrolysis of VO(IV) [12,13]. In acidic solution, combined pH-metric and spectroscopic techniques have indicated the formation of various binding isomers with 1:1 and 1:2 stoichiometries involving coordination of the terminal NH_2, COO^- and the peptide carbonyl. After the dissolution of $VO(OH)_2$ at pH > 7 in the presence of a high excess of ligand (VO(IV): ligand > 1:80), spectral measurements suggested the existence of a species $[VOLH_{-1}]$ containing deprotonated amide-N^- (see Structure 1) [13]. Interestingly, no clear evidence of amide deprotonation/coordination was found for Gly and Ala-containing tripeptides; the same composition ($[VOLH_{-1}]$) could be ascribed to a hydroxo complex involving (NH_2, CONH, COO^-) coordination of the tripeptides (see Structure 2). The EPR signal for basic solutions are very weak as EPR-silent oligomers predominate in this pH range. For AlaGlyGly some resemblance of the CD spectra at pH > 11 with those obtained for AlaGly could suggest the deprotonation/coordination of the amide-N close to the N-terminus, which would correspond to a $pK_a > 11.5$ [13]. It may be concluded that neither the terminal NH_2 nor the terminal COO^- is a strong enough VO(IV) binder to behave as an efficient anchoring donor in the promotion of amide deprotonation. In order to achieve this, the simultaneous coordination of both donors adjacent to the amide group seems necessary. Accordingly, in the case of dipeptides VO(IV) can induce deprotonation of the peptide-NH, while with tripeptides, where the two terminal anchors are separated by two peptide groups, this does not occur.



$[VO(GlyGly)H_{-1}]^-$

1



$[VO(GlyGlyGly)H_{-1}]^-$

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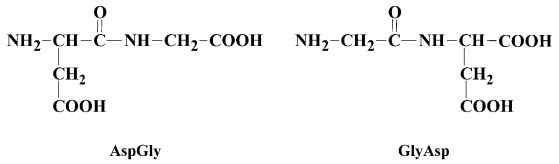


Scheme 1. The formulae of GlyAsp and AspGly.

3. Asp-containing dipeptides

As negatively charged O donors (such as COO^-, phenolate-O^-) are usually much more efficient than neutral ones (such as CONH, CO) as VO(IV) binders, the presence of an extra carboxylate group in the molecule might result in their stronger binding ability. Hence, the Asp-containing dipeptides GlyAsp and AspGly (see Scheme 1) were expected to be more efficient VO(IV) binders than simple Gly-type dipeptides. In fact a somewhat lower excess of ligand (VO(IV): ligand < 30) was sufficient to prevent the precipitation of $VO(OH)_2$, although slow equilibration indicated extensive hydrolysis at pH > 6 [14]. The speciation diagrams depicted in Fig. 1 indicate significant differences in the stoichiometries of the complexes formed. In both systems, complex formation starts through monodentate carboxylate coordination: $[VOLH_2]^{2+}$ (one proton in the terminal NH_3^+, and the other in the non-coordinated COOH group). Then, in the case of GlyAsp bis complexes predominate in the pH range 3.0–5.5, while with AspGly only 1:1 complexes are formed throughout the pH range (2 < pH < 6.5) studied pH-potentiometrically. Spectroscopic studies have suggested that only the carboxylate, the peptide-CO and the amino groups (this latter being somewhat less favoured) participate in the metal ion binding in the weakly acidic pH range. The position of the tridentate Asp residue in the peptide chain affects the coordination mode of the ligand to a slight extent: when Asp is C-terminal (GlyAsp), it rather behaves as a succinic acid derivative, favouring equatorial carboxylate chelation of the two neighbouring groups with some involvement of the peptide carbonyl-O in the metal ion binding. This results in the formation of bis complexes in different protonation states $[VOL_2H_3]^+$, $[VOL_2H_2]$ and $[VOL_2H]^-$ (the non-coordinating terminal NH_3^+ groups deprotonate in a stepwise manner). In the N-terminal Asp dipeptide AspGly, the involvement of either the peptide carbonyl or the terminal amino groups seems more essential, since the two carboxylates are much further apart [14].

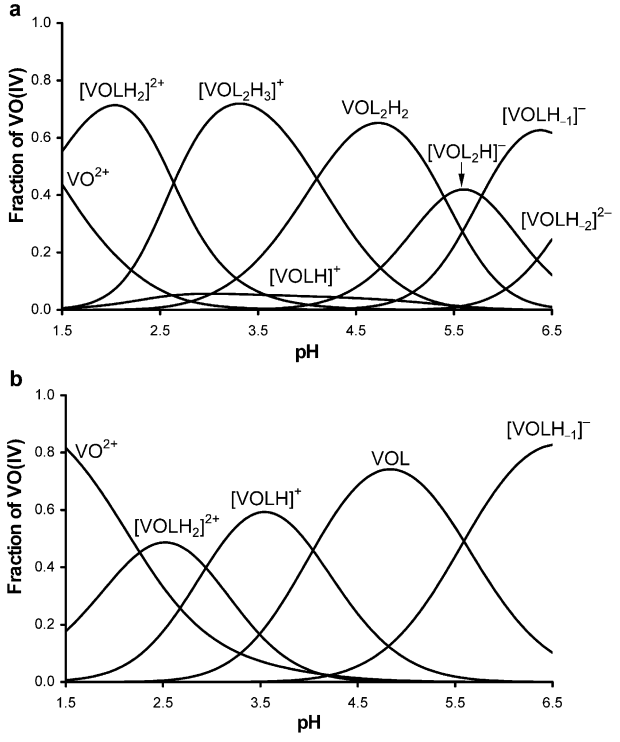


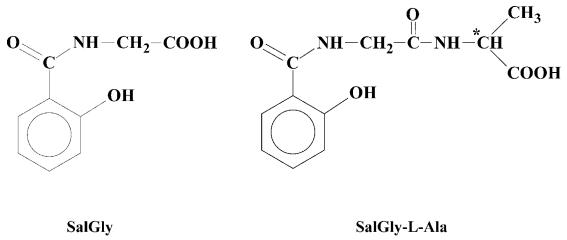
Fig. 1. Speciation curves for the complexes formed in the VO(IV)–GlyAsp (a) and VO(IV)–AspGly systems (b) at 1:30 metal ion to ligand ratio, $c_{VO(IV)} = 0.002$ M, (adapted from Ref [14]).

At pH > 6–7, the predominant stoichiometries for the EPR and CD-active complexes are $[VOLH_{-1}]^-$ and $[VOLH_{-2}]^{2-}$. The spectroscopic results allow the formation of a binding isomer with coordinated amide: $(COO^-, CON^-, NH_2, Y)_{equatorial}$ with $Y = H_2O$ or OH^- for both GlyAsp and AspGly. Besides this, the axial coordination of the β-COO^- is possible, but not proved. Deprotonation of the amide might be a little less favoured for GlyAsp then for AspGly. In the former case, the succinate-type chelation of the ligand may hinder the amide-type coordination.

To summarise the effect of the presence of an extra carboxylate in the dipeptide, we can say that the VO(IV)-binding ability of the ligands is somewhat increased as compared to that of the Gly-type dipeptides, because of the presence of new chelating sites: (COO^-, COO^-) in GlyAsp and (NH_2, COO^-) in AspGly. However, these sites are more competitive than the monodentate groups in VO(IV) binding, and the possibility of amide deprotonation/coordination is hardly enhanced as compared to the Gly-type dipeptides.

4. Phenolate as anchoring donor

Phenolate, another negatively charged O donor group, is known to have a much higher affinity for VO(IV). Accordingly, replacement of the terminal NH_2 group by the hard phenolate-O^- may enhance the metal-binding ability of the molecule more significantly. In fact, the dipeptide analogue 2-OH-hippuric acid (salicylglycine, SalGly) and its tripeptide derivative SalGly-L-Ala (for their formulae, see Scheme 2) proved to be much stronger VO(IV) binders: they can keep



Scheme 2. The formulae of SalGly and SalGly-L-Ala.

VO(IV) in solution in the pH range 2–12, even in equimolar solution. No precipitation or slow equilibration indicative of hydrolysis of the metal ion or its complexes is observed in these systems. Detailed pH-metric and spectral studies [15,16] indicated that complex formation starts at the C-terminal of the peptides, with probable involvement of the peptide-CO in binding. This is different from what was observed for the metal complexes of normal dipeptides containing a terminal NH_2 group, as in these complexes the N-terminal site, and not the C-terminal group, is the primary binding site [6]. At pH ~ 3.5 rearrangement of the binding mode occurs through the formation of [VOL], in which the metal ion is 'transferred' to the other terminus of the molecules and phenolate also becomes involved in metal binding. The strong coordination to the VO(IV) can now induce deprotonation of the peptide-NH group(s), and the compound containing the usual binding mode with (O^-, CON^-, COO^-) for SalGly (Structure 3), and with (O^-, CON^-, CON^-, COO^-) for SalGly-L-Ala (Structure 4) becomes the predominant species by pH ~ 6 (see Fig. 2). Interestingly, deprotonation of the two adjacent amide-NH groups in VO(SalGly-L-Ala) takes place in a strongly overlapping way ($pK(VOA) = 5.37$ and $pK(VOAH_{-1}) = 5.39$); the intermediate species with one deprotonated amide-N^- being formed only in low concentration. Amide coordination is accompanied by a characteristic colour change from blue to pinkish-red at pH ~ 5.5, with hardly any subsequent change up to pH ~ 11. The changes in the EPR parameters (see Table 1) indicate more covalent bonding in the equatorial plane, which is in accordance with the above-mentioned rearrangement of the binding modes. A detailed analysis of the CD data (including deconvolution of the data for the individual species) obtained for the VO(IV)–SalGly-L-Ala system revealed that a significant positive Cotton effect accompanies only the formation of $[VOLH_{-2}]^{2-}$ with both peptide-N^- atoms coordinated. This strongly suggests that, although the deprotonation of the two amides take place in a cooperative way, it starts on the one closer to the phenolate terminal. This is in agreement with what has been observed for most metal ions, but is in contrast with the situation with $R_2Sn(IV)$, where the C-terminal COO^- proved to be the anchoring donor [17].

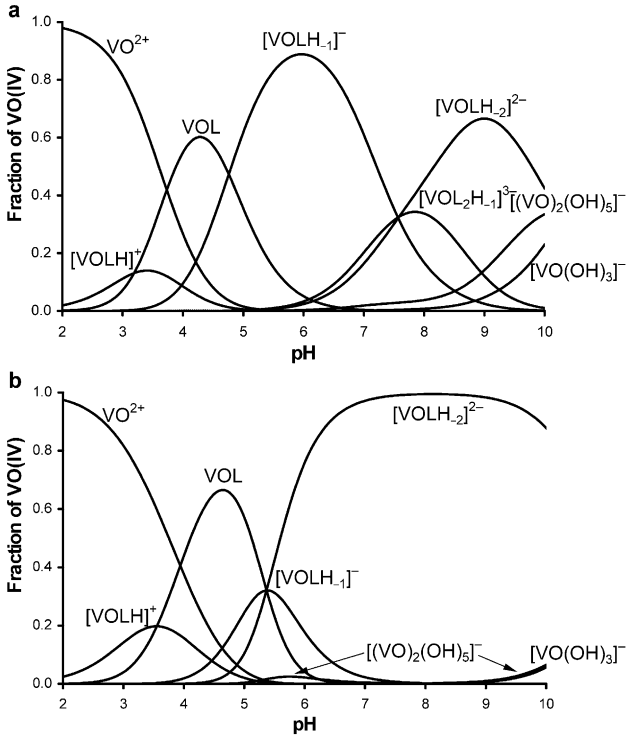
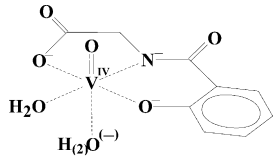


Fig. 2. Speciation curves for the complexes formed in the VO(IV)–SalGly (a) and VO(IV)–SalGly-L-Ala systems (b) at 1:4 metal ion to ligand ratio, $c_{VO(IV)} = 0.001$ M, (adapted from Refs. [15] and [16]).



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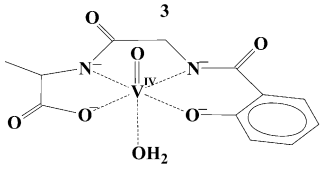


4

For SalGly, the deprotonation of $[VOLH_{-1}]^-$ should be ascribed to the liberation of a proton from a coordinated water molecule. This process is seen in the electron absorption spectra through the occurrence of a third absorption at ~400 nm, as is common in the visible spectra of VO(IV) complexes of strong donors. The negligible changes in the EPR parameters suggest, however, that the proton is liberated from axial and not equatorial water [15]. An alternative explanation could be that the equatorially coordinated OH^- forms such a strong bond that the individual contributions ($A_{||,i}$, see

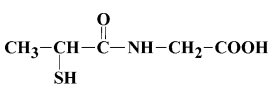
Table 1
Stabilty constants, EPR parameters and proposed binding modes of the complexes formed in the VO(IV)–SalGly and VO(IV)–SalGly-L-Ala systems [15,16]

Species	Log β	$g_{\|\|}$	$A_{\|\|}$	$g_\perp$	$A_\perp$	Binding mode
SalGly						
VOLH	10.24					(COO^-,CONH)
VOL	7.05	1.938	175			(O^-, CONH, COO^-)
$VOLH_{-1}$	2.29	1.949	165	1.980	57	(O^-,CON^-,COO^-)
$VOLH_{-2}$	−5.28	1.951	163	1.980	55	O^-,CON^-,COO^-,OH^-)
VOL_2H_{-1}	5.55					(O^-,CON^-,COO^-)(O^-,CONH)
SalGlyAla						
VOLH	9.99					(COO^-,CONH)
VOL	6.43	1.937	176	1.977	64	(O^-,CONH,COO^-)
$VOLH_{-1}$	1.06	1.945	165	–	–	(O^-,CON^-,CONH,COO^-)
$VOLH_{-2}$	−4.32	1.958	159	1.984	52	(O^-,CON^-,CON^-,COO^-)

below) of the other equatorial donors to the $A_{||}$ change in such a way that the EPR parameters do not change much.

5. Thiolate as anchoring donor

Vanadium in oxidation states +4 and +5 is considered a rather hard metal ion, which prefers coordination to O donors, and especially to negatively charged O donors, such as carboxylate or phenolate. According to the hard–soft theory, its coordination to soft sulphur donors is expected to be fairly weak, although sulphur coordination occurs with macromolecules in biological systems [18], or even with small molecules such as dimercaptosuccinic acid, which forms a complex with ($4 \times S^-$) coordination at high pH [19]. Mercaptopropionylglycine (Mpg; for structure, see Scheme 3), one of the synthetic structural analogues of glutathione [20,21], has been found to form a complex $[VO(MpgH_{-1})(phen)]^-$ [20]. X-ray crystallography revealed that Mpg is (S^-, CON^-, COO^-)-coordinated and thiolate serves as the anchoring donor. In solution, a tenfold excess of Mpg proved sufficient to keep the metal ion in solution and prevent hydrolysis throughout the pH range studied. Strong complex formation with Mpg was indicated by the colour of the solution, which was brownish-yellow at the neutral pH, but became light-red at pH > 9 [22]. The species distribution curves of the complexes depicted in Fig. 3 reveal that, in the pH range between 2 and 4, the protonated species are formed in strongly overlapping processes. EPR suggests monodentate carboxylate coordination of the ligand in



Mpg

Scheme 3. The formula of Mpg.

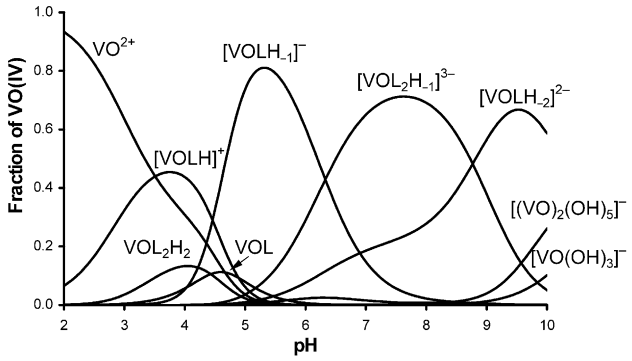
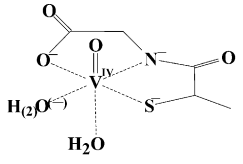


Fig. 3. Speciation curves for the complexes formed in the VO(IV)–Mpg system at 1:10 metal ion to ligand ratio, $c_{VO(IV)} = 0.004$ M, (adapted from Ref. [22]).

these complexes. The significant and sudden changes in the EPR parameters as the pH is increased from 4 to 5.5 indicate deprotonation and coordination of the thiolate and the amide in practically a single step, resulting in the complex with Structure 5.



$[VO(Mpg)H_{-1}]^-$ $[VO(Mpg)H_{-2}]^{2-}$

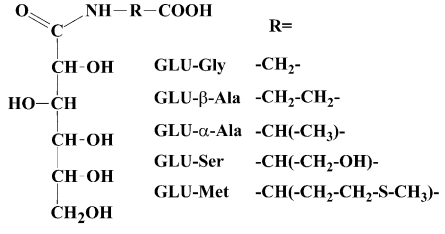
5

The speciation in the VO(IV)–Mpg system is similar to that in the VO(IV)–SalGly system (vide supra), the species formed have the same stoichiometries (c.f. Figs. 2a and 3), and the amide deprotonation occurs at pH ~ 4.5 in both systems. However, the basicity-adjusted stability constants characterising the equilibrium $VO^{2+} + HL^- = [VOLH_{-1}]^- + 2H^+$ reveal that the $[VOLH_{-1}]^-$-type complex of SalGly (log $K^* = -5.87$) [15] is about one and a half orders of magnitude more

stable than the corresponding species formed with Mpg (log $K^* = -7.23$) [22]. This indicates that both phenolate and thiolate coordinate to the VO(IV) ion strongly to promote amide deprotonation and coordination in such arrangements, but the thiolate group appears to be a less effective anchoring donor than phenolate for VO(IV). Interestingly, it was also found that ligands, such as bpy, containing N donors with empty π orbitals, and thus capable of back-coordination, promote deprotonation of the amide-NH through enhanced ternary complex formation. At the same time, ligands such as tiron, maltol or oxalate, with negatively charged O donors, increase the electron density on the metal ion and inhibit deprotonation of the amide-NH, thereby making ternary complex formation unfavoured [22].

6. *N*-D-Gluconylamino acids

As shown above, negatively charged phenolate and carboxylate are efficient anchoring donors for the promotion of amide deprotonation and coordination. The alcoholate groups of carbohydrates are similarly hard, but more basic donors than phenolate. It has been demonstrated that carbohydrate derivatives exhibit high affinity toward the VO(IV) ion [23,24]. The pseudopeptides formed between gluconic acid and various α- and β-amino acids (see Scheme 4) provide a donor set of carboxylate, amide and vicinal alcoholic-OH groups for metal ion binding. As illustrated in the speciation diagram of the complexes formed with one of the *N*-D-gluconylamino acids studied, GLU-Gly (Fig. 4), this donor set is able to bind VO(IV) very efficiently, but not in mononuclear complexes, as for the other ligands discussed above, but in dinuclear species in which alcoholate-O^- groups behave as bridging units [25]. A similar speciation feature has been observed for all VO(IV)–ligand systems studied (see Scheme 4). After the formation of a monodentate carboxylate coordinated complex $[VOL]^+$, two protons are liberated from the complex in practically one step, resulting in a species $[VOLH_{-2}]_n^{n-}$, with $n = 1$ in the case of a mononuclear, and $n > 1$ for an oligonuclear species. In the VO(IV)–dipeptide systems, the amino group is considered not to be an effective coordinating site for the hard VO(IV) ion, while the initially coordinated carboxylate could be



Scheme 4. The formulae of the variuos *N*-D-gluconylamino acids.

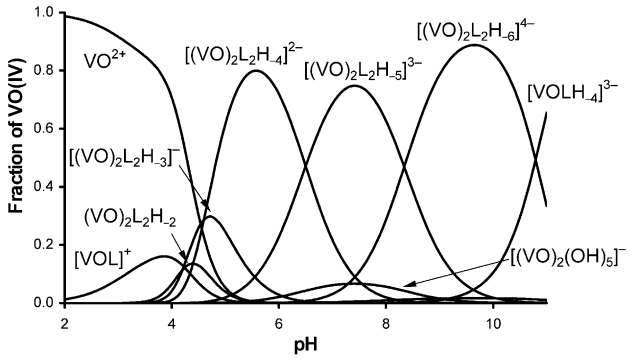


Fig. 4. Speciation curves for the complexes formed in the VO(IV)–GLU-Gly system at 1:5 metal ion to ligand ratio, $c_{VO(IV)} = 0.001$ M, (adapted from Ref. [25]).

such an anchor (vide supra). However, this group alone can not promote amide deprotonation in the weakly acidic pH range. The alcoholic-OH groups of D-gluconic acid have been suggested to coordinate in non-deprotonated form to VO(IV) at pH ~ 6, forming a mixed dihydroxo complex [26]. Alcoholic-OH groups adjacent to a carboxylate function have been shown to deprotonate with pK ~ 4 (as in these systems), e.g. in the VO(IV) complexes formed with glycolic acid and lactic acids [19]. In *N*-D-gluconylamino acids, however, the carboxylate group is not in such a favourable steric arrangement with the alcoholic-OH groups (see Scheme 4), and deprotonation of only these alcoholic-OH groups therefore cannot be expected at such a low pH. Further, there is no significant difference in pH range for the liberation of the first two protons between the complexes formed with the α-amino acid and the β-amino acid derivatives. These points all indicate once more that carboxylate cannot be the only anchoring donor.

The CD spectra of the complexes $[(VOLH_{-2})_n]^{n-}$ formed with GLU-Gly and GLU-β-Ala (ligands which do not contain an optically active amino acid residue) exhibit relatively low, but significant CD intensity (see Fig. 5). The complexes of ligands with an optically active amino acid residue furnish more intense CD spectra. The most reasonable explanation for this

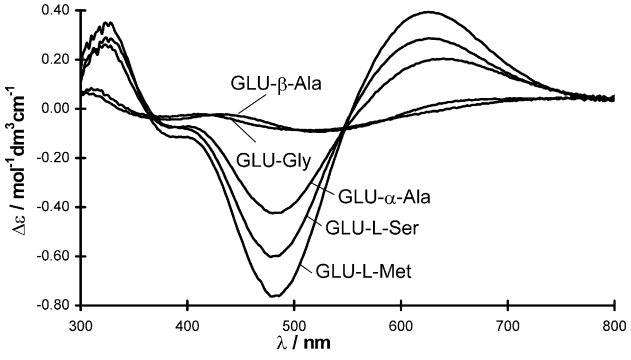
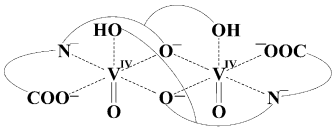


Fig. 5. CD spectra of the VO(IV)–N-D-gluconylamino acid systems at pH 5, (adapted from Ref [25]).

behaviour is that the ligands are coordinated through both the amino acid and the carbohydrate functional groups, involving the carboxylate-O^-, amide-N^- and alcoholate-O^- donors, forming a joined (5+5)-membered (5+6 for GLU-β-Ala) chelate ring system. This binding mode would mean that the role of the carboxylate in the metal ion binding is significant (it serves as a primary binding site), and the almost cooperative deprotonation of the above two slightly acidic groups is a consequence of their very favoured steric arrangement. The coordination of the not yet deprotonated OH groups presumably promotes this process. As both the isotropic and anisotropic EPR spectra of these systems reveal a significant decrease in intensity from pH ~ 5, a strong antiferromagnetic interaction between the VO(IV) centres can be assumed, indicating the formation of dinuclear species. The above-mentioned joined chelate system can be formed in a double-bridged dimer through the alcoholate-O^- groups as bridging donors (see Structure 6).



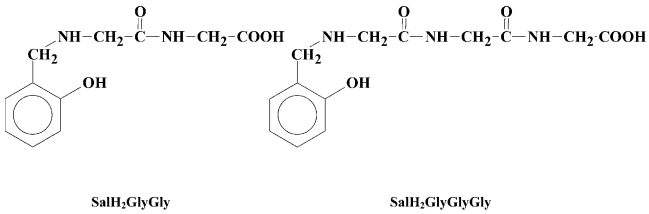
$[(VO)_2(GLU\text{-}Gly)_2H_{-4}]^{2-}$

6

Thus, the formulae of the complexes formed in this pH range can be written as $[(VO)_2L_2H_{-4}]^{2-}$. The molecular models indicate that this species may be stabilised through coordination of the non-deprotonated alcoholic-OH groups in the axial positions, forming new ligand bridges between the two metal centres. As the coordination spheres of both metal ions are saturated, any further coordination process would result in replacement of one of the already coordinated groups. This may happen during the next two deprotonation steps which take place up to pH ~ 10, where the lack of EPR signals reveals that the species should remain as dimers. The most probable explanation to explain these processes is that the OH^- and the deprotonating alcoholic-OH groups of the ligand molecule displace the carboxylate group from the equatorial plane of the coordination sphere. It is noteworthy that the usual bis complexes with two deprotonated vicinal alcoholic functions from each ligand, characteristic of metal ion–carbohydrate complexes at high pH [23,24] are not formed, but the deprotonated amide-N^- remains in the coordination sphere even at pH ~ 12 [25].

7. Schiff bases and reduced Schiff bases of dipeptides

A range of VO(IV)–Schiff-base complexes derived from the condensation of salicylaldehyde and dipeptides



$SalH_2GlyGly$ $SalH_2GlyGlyGly$

Scheme 5. The formulae of $SalH_2GlyGly$ and $SalH_2GlyGlyGly$.

(GlyGly, GlySarcosine, AlaGly, AlaAla, SerGly), have been prepared and characterised [27]. The binding mode involves (O^-, N_{imine}, CONH, COO^-) (6+5+7)-membered joined chelate system. The solubility of the complexes in water or methanol is low and increasing the pH the Schiff bases hydrolyse easily. The reduced derivatives containing GlyGly and GlyGlyGly (see Scheme 5) have also been prepared and characterised [28]. In these ligands an aminophenolate chelate may behave as the anchoring donor site. The speciation diagrams depicted in Fig. 6 reveal significant differences in their binding abilities. In both systems, coordination starts at the terminal aminophenolate moiety through (O^-, NH) chelation. Amide deprotonation has been proved by EPR in both systems, but its extent differs in the two systems. With $SalH_2GlyGly$, formation of the (O^-, NH, CON^-, COO^-) (6+5+5)-membered joined chelate system (see Structure 7), which saturates the equatorial coordination sphere of VO(IV), favours

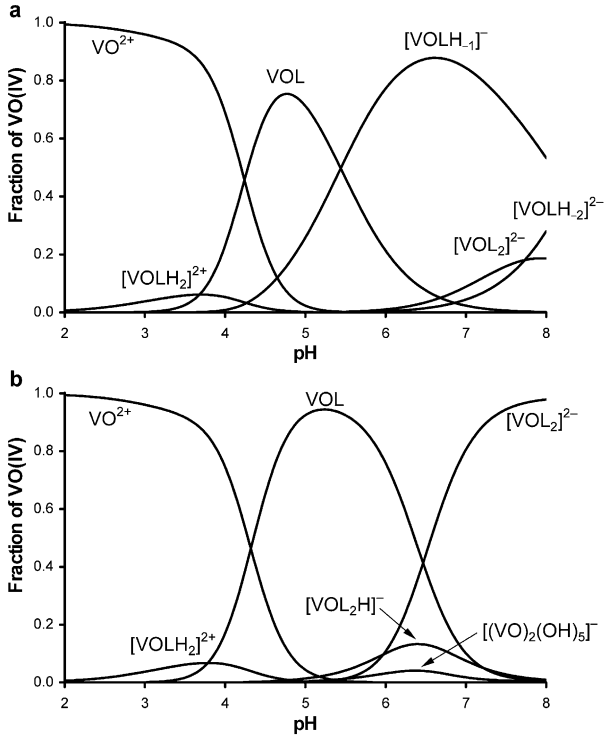
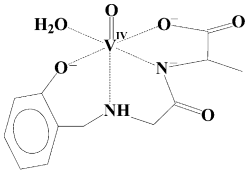


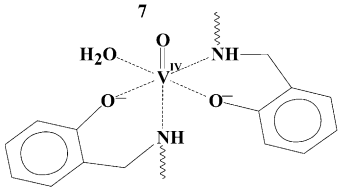
Fig. 6. Speciation curves for the complexes formed in the VO(IV)–$SalH_2GlyGly$ (a) and VO(IV)–$SalH_2GlyGlyGly$ systems (b) at 1:4 metal ion to ligand ratio, $c_{VO(IV)} = 0.001$ M, (adapted from Ref. [28]).

deprotonation of the amide-NH. At the same time, in the case of $SalH_2GlyGlyGly$ amide deprotonation is hindered and occurs only in a significantly higher pH range (pH > 8). As may be seen in Fig. 6b, at an excess of ligand the 2 × (O^-, NH) chelation (see Structure 8) is the predominant binding mode. At pH > 9, in complexes $[VOLH_{-1}]^-$ and $[VOLH_{-2}]^{2-}$, the ligand deprotonates through four or all five donor groups, resulting in (O^-, NH, CON^-, CONH, COO^-) and (O^-, NH, CON^-, CON^-, COO^-) coordination, respectively. In these binding modes, not only the four equatorial binding sites but also the fifth (weaker) site is occupied, and at physiological pH this binding mode seems to be less favoured than the bis chelation of the ligands in the equatorial plane of the metal ion, particularly in the case of $SalH_2GlyGlyGly$.



$[VO(SalH_2GlyGly)H_{-1}]^-$

7

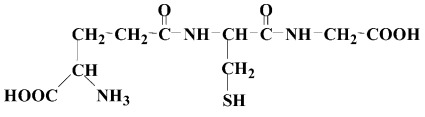


$[VO(SalH_2GlyGlyGly)_2]^{2-}$

8

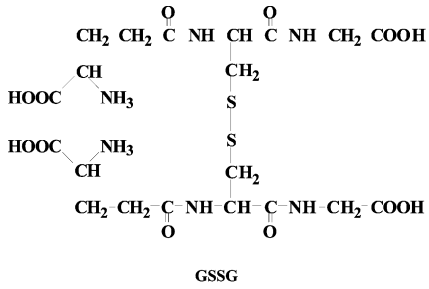
8. Glutathione and oxidised glutathione

Glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is one of the most important naturally occurring tripeptides (see Scheme 6); it is a normal and essential constituent of all living cells, and the most abundant intracellular non-protein thiol. As an example, in human erythrocytes, GSH is present typically at the 2–3 mM level [29]. Numerous studies have shown that it plays an important role in the biochemistry of vanadium [30–32]. Vanadium(V) is reduced to VO(IV) by GSH and this may also occur inside cells [32,33]. GSH may also act as



GSH

Scheme 6. The formula of GSH.

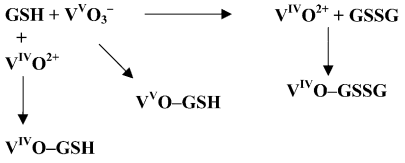


GSSG

Scheme 7. The formula of GSSG.

a ligand for the VO(IV) produced [34]. Oxidised glutathione (GSSG, see Scheme 7) is formed during the biological reduction of vanadium(V) to VO(IV). GSSG too is present in the erythrocytes, typically at a concentration some 1–2% of that of GSH [34]. The possible complex formation and redox reactions taking place between vanadium(V) and vanadium(IV) and GSH and GSSG are outlined in Scheme 8. A clear knowledge of the interactions of VO(IV) with GSH and GSSG is therefore important for an understanding of the biological role of vanadium both in vivo and in vitro. As their structural formulae show (Schemes 6 and 7) GSH and GSSG contain large numbers of potential donor atoms, which gives rise to the possibility of various types of coordination. For example, a metal ion can coordinate to GSH (i) at the N-terminal part of the molecule in an amino acid-like binding mode, (ii) via the thiolate group alone, or through (S^-, CONH) coordination or with the incorporation of deprotonated amide-N^-, and (iii) in a tripeptide manner, the coordination of both amide-N^- atom and the COO^- anchor also being possible [34]. In the case of GSSG, excluding the bridging sulphur atoms, the remaining above donors are all capable of metal ion binding. Unfortunately, no X-ray diffraction data are available for any VO(IV)–GSH or –GSSG complexes. A few solution studies have been made concerning these interactions [21,22,35,36], but there is controversy as regards the modes of binding of GSH and GSSG to VO(IV).

A recent combined pH-potentiometric and spectroscopic (EPR, UV–vis and CD) study led us to conclude the existence of a rather complicated speciation scheme,



Scheme 8. The possible complexation and redox reactions between vanadium(IV,V), GSH and GSSG.

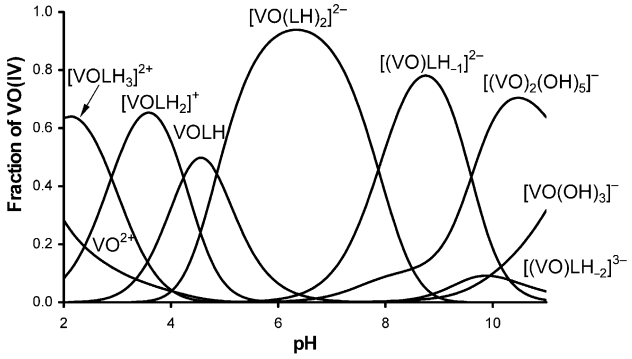


Fig. 7. Speciation curves for the complexes formed in the VO(IV)–GSH system at 1:25 metal ion to ligand ratio, $c_{VO(IV)} = 0.001$ M, (adapted from Ref. [37]).

including various protonated mono and bis complexes and several hydrolysed mixed ligand complexes. Interestingly, no unambiguous evidence was found for the coordination of deprotonated amide-N^- in any of these complexes [37]. GSH proved to be a moderately strong VO(IV) binder: an ~20-fold ligand excess was necessary to keep the metal ion in solution at and above the physiological pH. pH-metric titrations have revealed that a protonated bis complex $[VO(LH)_2]^{2-}$ is the predominant species in the neutral pH range (see Fig. 7). On increase of the pH, this complex is transformed to the deprotonated mono complexes $[VOLH_{-1}]^{2-}$ and $[VOLH_{-2}]^{3-}$. As these processes occur in parallel with extensive hydrolysis of the metal ion (formation of the dihydroxo bridged binary VO(IV) hydroxo complex $[(VO)_2(OH)_5]^-$), OH^- presumably displaces one of the ligand molecules, resulting in the formation of mixed hydroxo complexes. The EPR spectra shown in Fig. 8 clearly indicate the presence of these three species in the pH range 7–10. The additivity rule introduced by Chasteen [38] to estimate the hyperfine coupling constant $A_{\|}$ on the basis of the contributions $A_{\|}$,i of each of the four equatorial donor groups, suggests that it is very likely that both ligands coordinate to the metal ion through the α-aminocarboxylate moiety of the γ-glutamyl residue in the complex $[VO(HL)_2]^{2-}$ (see Structure 9). In contrast with Mpg, in GSH the thiolate-S^- does not seem to be in a favourable position to be able to induce amide deprotonation in the weakly acidic pH range. Although the deprotonated species $[VOLH_{-1}]^{2-}$ and $[VOLH_{-2}]^{3-}$ may contain a deprotonated amide-N^-, both EPR and the CD spectral results indicate that they are rather mixed hydroxo species with protonated and uncoordinated amide-NH groups (see Structures 10–13, respectively). These results are in contrast with those of Tasipoulous et al. [21], who suggested the coordination of GSH through the (S^-, CON^-, CON^- COO^-) donor set.

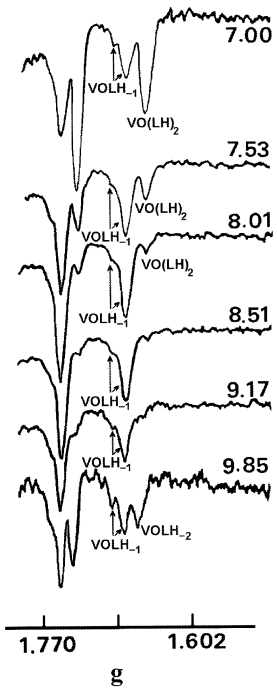
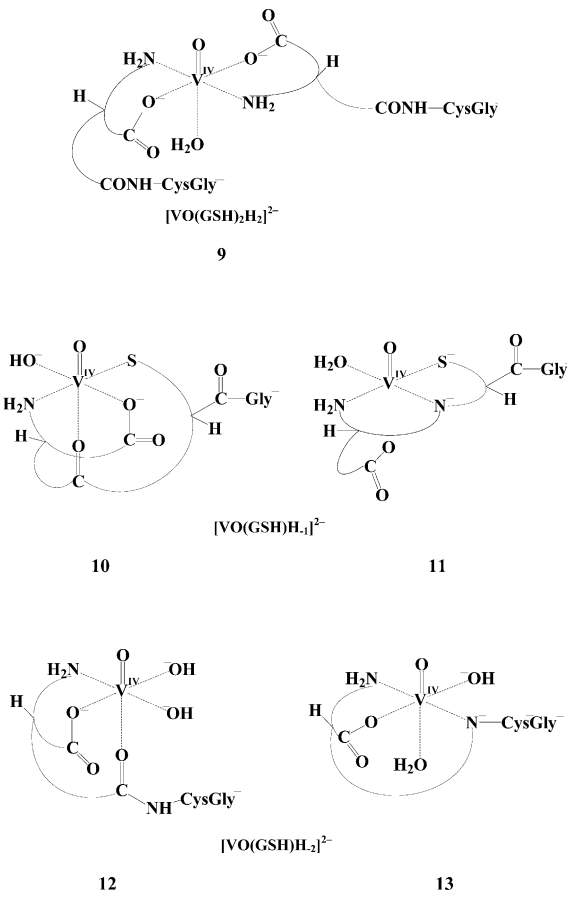


Fig. 8. High-field range (3800–4400 G) of the EPR spectra of the VO(IV)–GSH 1:25 system at 77 K at different pH values, $c_{VO(IV)} =$ 0.010 M, (adapted from Ref. [37]).



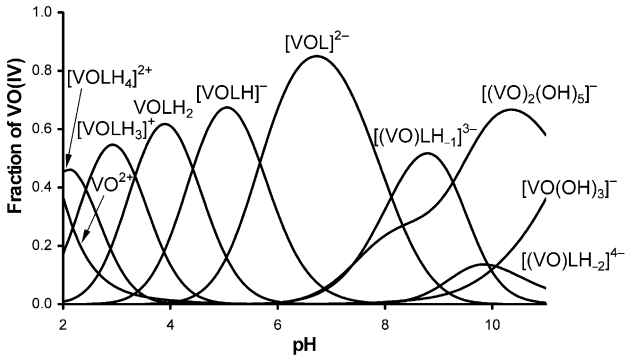
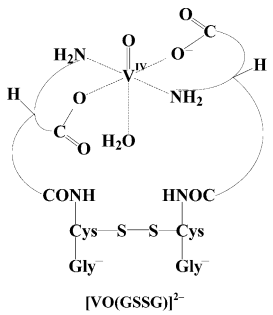


Fig. 9. Speciation curves for the complexes formed in the VO(IV)–GSSG system at 1:10 metal ion to ligand ratio, $c_{VO(IV)} = 0.007$ M. (adapted from Ref. [39]).

As shown in Fig. 9, oxidised glutathione forms a single 1:1 species $[VOL]^{2-}$ in the pH range between 6 and 8, this being a bis amino acid-type chelate [11]. However, because of the 'doubled' structure of the ligand, it has 1:1 stoichiometry (see Structure 14). As the pH is decreased this complex undergoes stepwise protonation ($[VOL]^{2-} \rightarrow [VOLH]^{-} \rightarrow [VOLH_2] \rightarrow [VOLH_3]^{+} \rightarrow [VOLH_4]^{2+}$), at the amino functions of the γ-glutamyl residue and the two non-coordinated Gly-COO^- groups. Detailed spectral measurements [39] demonstrated that amide deprotonation cannot be ruled out unambiguously in the deprotonated species $[VOLH_{-1}]^{3-}$ and $[VOLH_{-2}]^{4-}$ formed in the basic pH range, but if it occurs at all, this takes place only at pH > 9. It is interesting to note that, in spite of the same bis amino acid-type binding mode, GSSG is a somewhat more efficient binder of VO(IV) than GSH, in consequence of the larger entropy effect caused by some macrocyclic effect accompanying the formation of the complex $[VO(GSSG)]^{2-}$. When these speciation results are applied to cell conditions, it is clear that neither of these ligands is a strong enough binder of vanadium(IV), and hence some other bioligands must be considered (most probably biophosphates, such as ATP) to suppress vanadium hydrolysis, and to explain the actual chemical form(s) of VO(IV) in the cell [4,39].



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9. Conclusions

Detailed solution speciation and structural studies of numerous VO(IV)–oligopeptide systems and a critical survey of the relevant literature have revealed that a number of factors influence the VO(IV)-binding properties of peptide molecules. The strength of the coordination has been found to be largely determined by the possibility of participation of the amide-N in metal ion binding. This is affected primarily by the nature of the anchoring donor, according to the following sequence: phenolate-O^- > alcoholate-O^-, thiolate-S^- > carboxylate-COO^- > NH_2. This sequence is finely tuned by the presence of additional donors in the molecule, such as an extra COO^- in AspGly or GlyAsp, or an amino acid chelate in GSH and GSSG, or an aminophenolate chelate in $SalH_2GlyGly$ and $SalH_2GlyGlyGly$. The competitive chelation process involving these additional functional groups hinders the deprotonation and subsequent coordination of the amide group. As shown in the ternary systems formed with Mpg, and also in case of the isolation of several $V^{IV}O$(dipeptide)(phen) complexes, the presence of various endogenous and exogenous ligands can further influence the VO(IV)-binding properties of oligopeptides.

Besides the presence of a suitable anchoring donor in the peptide molecule and that of some other VO(IV) binding ligand, the third factor influencing the metal binding properties of oligopeptides is the role of any side chain donors and their position. Particularly, for longer oligopeptides this has hardly been studied. The interactions of VO(IV) and oligopeptides with special side chain donor arrangements through some extent of preorganisation of the molecule should also be investigated in order to obtain information on the binding ability of the side chain donors, which are the primary binding sites of proteins.

Acknowledgements

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