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Oxovanadium(IV) complexes of *N*-D-gluconylamino acids

Béla Gyurcsik,*[ab] Tamás Jakusch[b] and Tamás Kiss*[ab]

[a] *Biocoordination Chemistry Research Group of the Hungarian Academy of Sciences, University of Szeged, H-6701 Szeged, P.O. Box 440, Hungary. E-mail: gyurcsik@chem.u-szeged.hu; tkiss@chem.u-szeged.hu*
[b] *Department of Inorganic and Analytical Chemistry, University of Szeged, H-6701 Szeged, P.O. Box 440, Hungary*

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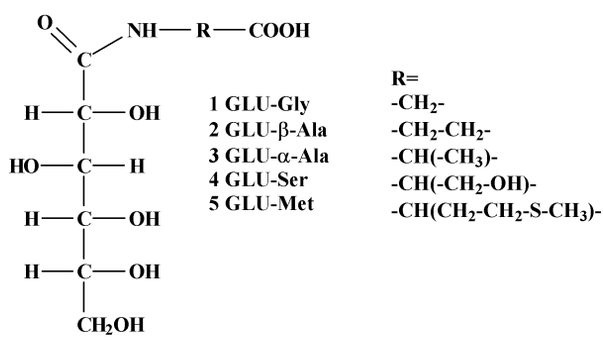
The complex formation between five *N*-D-gluconylamino acids (the derivatives of glycine, α-L-alanine, β-alanine, L-serine and L-methionine) and $V^{IV}O$ ion was studied in aqueous media by means of combined pH-metric, CD, UV-Vis and EPR spectroscopic methods. The equilibrium study (25 °C, $I = 0.2$ mol dm^{-3} (KCl)) revealed the formation of various mono- and di-nuclear complexes, deprotonated to extents depending on the pH. The EPR spectra confirmed the formation of EPR-inactive complexes involving strong antiferromagnetic coupling between the metal centres in the pH interval 5–10. A dinuclear species with the composition $[(VO)_2L_2H_{-4}]^{2-}$ predominates at pH ≈ 5 in each system; the changes in shape of the CD spectra strongly suggested the deprotonation and hence coordination of the amide nitrogen in this species. The CD results provided evidence of the metal ion-promoted deprotonation and coordination of the C(2)–OH group too under these conditions. On increase of the pH further alcoholic OH groups are deprotonated, but the amide group remains in the first coordination sphere both in the dinuclear species $[(VO)_2L_2H_{-5}]^{3-}$ and $[(VO)_2L_2H_{-6}]^{4-}$ and in the monomeric complex $[(VO)LH_{-4}]^{3-}$. No mononuclear bis complexes were observed, even at a high excess of ligand.

Introduction

Studies on the biological role of $V^{IV}O$ have shown that this ion binds to proteins such as carboxypeptidase, nucleases and phosphatases with enzyme regulatory functions; it also serves as an EPR and Vis probe for studies of the active centre of many other zinc(II) enzymes.[1] A number of investigations have been initiated to model potential binding sites of the $V^{IV}O$ ion, including amino acids[2–7] and dipeptides.[8,9] Although $V^{IV}O$ ion-promoted peptide nitrogen deprotonation has been suggested in certain cases, hydrolysis of the metal ion at physiological pH was not always prevented by these ligands. There is X-ray crystallographic evidence of the above process in the solid state for the ternary complexes with 1,10-phenanthroline and dipeptides or pseudopeptides.[10] A recent study on the $V^{IV}O$–SalGly system[11] revealed the coordination of the deprotonated amide group in aqueous solution, where the hydrolysis was shifted to pH > 10. Like other metal ions, $V^{IV}O$ needs a primary coordinating anchor group (such as the phenolate or/and the carboxylate group in salicylglycine, SalGly), which binds to the metal ion strongly enough to be able to promote deprotonation and subsequent coordination of the amide group.[12] As a continuation of this work we are searching for more efficient peptide deprotonation-promoting groups. The alcoholate groups of carbohydrates are similarly hard, but more basic donor groups than phenolate. Carbohydrate derivatives have been demonstrated to exhibit high affinity toward the $V^{IV}O$ ion.[13,14] We have published several papers on the coordination properties of modified sugar ligands, including sugar–amino acid derivatives, and established that parallel deprotonation of the alcoholic OH and the amide groups leads to these ligands being effective complexing agents for transition metal ions[15] and for organotin(IV).[16] The present paper reports the results of an investigation of five *N*-D-gluconylamino acids (Scheme 1) in which the very weakly acidic amide and alcoholic OH groups provide potential binding sites for the hard $V^{IV}O$ ion, which usually prefers to coordinate to oxygen donors. Potentiometry was used to determine the compositions and stabilities of the complexes, while UV/Vis, CD, and EPR spectroscopic measurements were performed to characterize the solution structures of these complexes.



Scheme 1 The ligands investigated: 1, *N*-D-gluconylglycine (GLU-Gly); 2, *N*-D-gluconyl-β-alanine (GLU-β-Ala); 3, *N*-D-gluconyl-L-α-alanine (GLU-α-Ala); 4, *N*-D-gluconyl-L-serine (GLU-Ser) and 5, *N*-D-gluconyl-L-methionine (GLU-Met).

Experimental

Chemicals

The preparation and characterization of the ligands was described elsewhere.[17] The purity of the ligands was checked and the concentrations of their solutions were determined by the Gran method.[18] The $V^{IV}O$ stock solution was prepared as described earlier[19] and standardized for the metal ion concentration by permanganate titration and for hydrogen-ion concentration by potentiometry. The ionic strength in all solution studies was adjusted to 0.20 mol dm^{-3} KCl. In all cases the temperature was 25.0 ± 0.1 °C.

Potentiometric measurements

The stability constants of the proton and $V^{IV}O$ complexes of the ligands were determined by pH-potentiometric titration of 25.0 cm^3 samples. The metal-to-ligand ratio was varied from 1 : 1 to 1 : 6, with metal ion concentrations between 7×10^{-4} and 4×10^{-3} mol dm^{-3}. The titrations were performed over the pH range 2–11.5 with carbonate-free KOH solution of known concentration (*ca.* 0.2 mol dm^{-3}) under a purified argon atmosphere. The reproducibility of the titration points included in the evaluation was within 0.010 pH units throughout the whole pH range. The following stability constants (log β) were assumed for the hydroxo complex of $V^{IV}O$: for $[VO(OH)]^+$, log $\beta_{1-1} = -5.94$, for $[(VO)_2(OH)_2]^{2+}$, log $\beta_{2-2} = -6.95$, calculated from the data published by Henry *et al.*,[20] using the Davies equation to take into account the different ionic strengths, for $[VO(OH)_3]^-$, log $\beta_{1-3} = -18.0$ and for $[(VO)_2(OH)_5]^-$, log $\beta_{2-5} = -22.5$, taken from ref. 21.

For potentiometric titrations an automatic titration set including a Dosimat 665 (Metrohm) autoburette, an Orion 710A precision digital pH-meter and an IBM-compatible personal computer was used. A Metrohm 6.0234.1#00 semimicro combined pH electrode was calibrated for hydrogen ion concentration according to the method of Irving *et al.*[22] The pK_w calculated from strong acid–strong base titrations was 13.755 ± 0.010. The concentration stability constants, $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$, were calculated with the aid of the computer program PSEQUAD.[23]

Spectral measurements

UV-Vis spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer in the wavelength interval from 350 to 820 nm. In a cell of 3 cm optical path the metal ion concentration was 4×10^{-3} mol dm^{-3}.

CD spectra were recorded on a Jobin Yvon CD6 spectropolarimeter in the wavelength interval from 350 to 800 nm. The metal ion concentration was 5×10^{-3} mol dm^{-3} in a cell of 1 cm optical path. CD data are given as the differences in molar absorptivity between left and right circularly polarized light, normalized to the (total) metal ion concentration in dm^3 mol^{-1} cm^{-1} unit.

EPR spectra were recorded on a JEOL-JES-FE 3X spectrometer in the X band at 25 °C with 100 kHz field modulation. Mn^{II}-doped MgO powder served as field standard. The $V^{IV}O$ ion concentration was 5×10^{-3} mol dm^{-3}. The EPR spectra were simulated and the parameters calculated by means of a recently developed computer program.[24]

Results and discussion

The protonation constants determined from the potentiometric titrations did not exhibit significant differences from those determined earlier in 0.1 mol dm^{-3} $NaClO_4$ medium.[15] In solutions containing $V^{IV}O$ and an excess of ligand no precipitation was observed in the investigated pH region 2–11.5. The only solution where opalescence was detected *via* the increased baseline in the spectrophotometric measurements was an equimolar solution of the metal ion and GLU-β-Ala. This sample was excluded from the evaluation. The best speciation model included a series of dinuclear species $[(VO)_2L_2H_{-x}]^{-(x-2)}$, where $x = 2$–6, besides the mononuclear complexes $[(VO)L]^+$ and $[(VO)LH_{-4}]^{3-}$, where L denotes the deprotonated ligand molecule with a charge of −1. The stability constants for the above species are listed in Table 1.

As revealed by the species distribution diagram for the $V^{IV}O$–GLU-Gly system (Fig. 1), a species with composition $[(VO)L]^+$ is formed below pH 4.5 in all cases. The basicity-corrected stability constants (Table 1) for this species show a slight increase, in the decreasing sequence GLU-Ser, GLU-Gly, GLU-β-Ala, as compared with the $V^{IV}O$ complexes of alkanoic acids (−2.75 for ethanoic, −2.78 for propanoic and −2.76 for butanoic acid).[25] This may be attributed to partial coordination of some other, more distant ligand site(s), such as the amide oxygen or the alcoholic OH groups of the sugar moiety. Indeed, these complexes display a very weak CD effect, in support of the above assumption. The weakness of the CD effect in the complexes of ligands with optically active amino acid residues (Fig. 2) can be attributed to the low ability of the monodentate carboxylate coordination mode to transfer the chiral perturbation from the chiral centre toward the d–d orbitals and the very weak coordination of non-deprotonated alcoholic OH groups. The EPR parameters in Table 2 differ slightly from those determined for $[VO(H_2O)_5]^{2+}$ at pH ≈ 2.0. As the lines are relatively broad, almost no spectral changes are observed at room temperature, and only small but continuous shifts of the lines at the temperature of liquid nitrogen between pH 2 and 4. These changes are consistent with transformation of the aqua ion into the complex $[(VO)L]^+$.[26] Carboxylate-coordinated dipeptide complexes display similar changes; their respective EPR parameters are also shown in Table 2.

With increasing pH, the difference between the titration curves of the systems without and with the metal ion revealed the extra consumption of two equivalents of base per metal ion by pH ≈ 5. The liberation of these two protons takes place

Table 1 Protonation constants (log K) and $V^{IV}O$ complex formation constants (log β) of GLU-Gly, -β-Ala and -Ser at $I = 0.20$ mol dm^{-3} (KCl) and 25 °C

Complex	GLU-Gly	GLU-β-Ala	GLU-Ser
log K_{HL}	3.32(1)	4.14(2)	3.10(2)
$[(VO)L]^+$	1.75(2)	1.91(6)	2.19(5)
$[(VO)_2L_2H_{-2}]$	−1.37(6)	−1.19(6)	−1.37(20)
$[(VO)_2L_2H_{-3}]^-$	−5.57(6)	−5.48(6)	−5.08(20)
$[(VO)_2L_2H_{-4}]^{2-}$	−10.17(6)	−10.17(4)	−9.21(5)
$[(VO)_2L_2H_{-5}]^{3-}$	−16.67(5)	−16.52(5)	−15.11(6)
$[(VO)_2L_2H_{-6}]^{4-}$	−25.01(5)	−24.43(4)	−22.84(11)
$[(VO)LH_{-4}]^{3-}$	−24.80(7)	−23.98(3)	−23.74(10)
$VO^{2+} + HL \rightleftharpoons [(VO)L]^+ + H^+$	−1.57	−2.23	−0.91
$pK([(VO)_2L_2H_{-2}])$	4.20	4.29	3.71
$pK([(VO)_2L_2H_{-3}]^-)$	4.60	4.69	4.13
$pK([(VO)_2L_2H_{-4}]^{2-})$	6.50	6.35	5.90
$pK([(VO)_2L_2H_{-5}]^{3-})$	8.34	7.91	7.73
No. of points	321	448	176
Fitting[a]	0.017	0.017	0.016

[a] Average differences in the calculated and experimental titration curves expressed in cm^3 of the titrant.

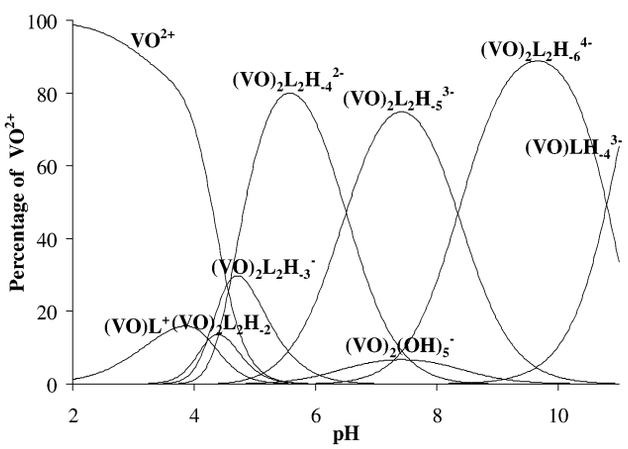


Fig. 1 Concentration distribution curves for the complexes formed in the $V^{IV}O$–*N*-D-GLU-Gly system as a function of pH; $c_{VO^{2+}} = 0.001$ mol dm^{-3} and $c_{ligand} = 0.005$ mol dm^{-3}.

Table 2 EPR parameters for $V^{IV}O$–GLU-Gly 1 : 5 solutions compared to those of related systems. The A values are assumed to be precise within $\pm 0.5 \times 10^{-4}$ cm^{-1}, the g values within ±0.002

	$g_\perp$	$g_\parallel$	$A_\perp$[a]	$A_\parallel$[a]	Coordinated groups	Ref.
pH 2.0	1.978	1.936	69.0	181.3	$\{H_2O\}$	This work
pH 3.5	1.982	1.944	63.7	174.6	$\{CO_2^-\}$	This work
pH 4.6	1.981	1.944	62.4	174.0	$\{CO_2^-\}$	This work
pH 11.5	1.988	1.961	48.3	144.9	$\{amide\ N^-, OH^-, 2 \times RO^-\}$	This work
Gly-Asp/$[(VO)LH_2]^{2+}$	1.978	1.936	65	177	$\{CO_2^-\}$	9
Gly-Asp/$[(VO)LH_{-1}]^-$	1.981	1.953	53.5	160.7	$\{amide\ N^-, NH_2, CO_2^-\}$	9
Sal-Gly/(VO)L		1.938		175	$\{CO_2^-\}$	11
Sal-Gly/$[(VO)LH_{-1}]^-$	1.980	1.949	57	165	$\{amide\ N^-, PhO^-, CO_2^-\}$	11
D-Ribose		1.958		149	$\{4 \times RO^-\}$	13
D-Galacturonic acid	1.98	1.96	49	150	$\{4 \times RO^-\}$	13
$[(VO)(H_2O)_5]^{2+}$	1.978	1.935	67	183	$\{H_2O\}$	21
$[(VO)(OH)_3]^-$	1.977	1.957	52	161	$\{3 \times OH^-\}$	21

[a] Units of the hyperfine coupling constants, $\times 10^{-4}$ cm^{-1}.

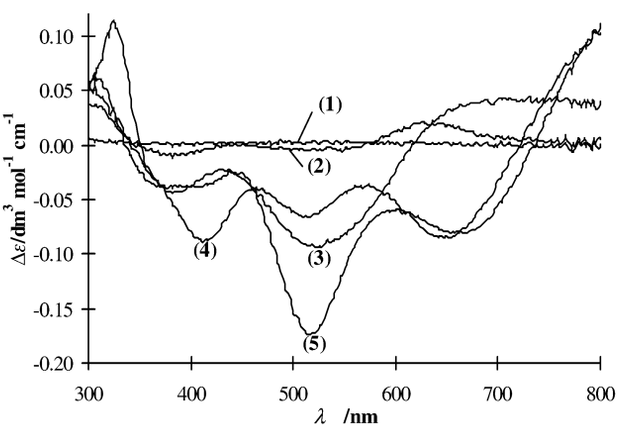


Fig. 2 CD spectra recorded in the $V^{IV}O$–GLU-β-Ala system at different pH values: 2.2 (1), 3.9 (2), 5.2 (3), 8.3 (4) and 11.5 (5).

practically in one step; the concentration of the intermediate deprotonated species must therefore be low, and the predominant species should have the formula $(MLH_{-2})_n$, with $n = 1$ in the case of a monomer and $n > 1$ for oligomers. There are several possibilities to explain this behaviour. Since hydrolysis of the metal ion would result in precipitation under the given conditions, we assume that one or both protons are released from the ligand molecule. Both the amide and the alcoholic OH groups may undergo deprotonation at pH > 13 in the absence of metal ions. In the presence of a suitable metal ion and an effective anchoring donor in the ligand, the pK of these groups may decrease by several orders of magnitude. In the $V^{IV}O$–dipeptide systems the amino group is considered not to be an effective coordinating site for the hard $V^{IV}O$ ion, while the initially coordinated carboxylate could be such an anchor. However, this group alone could not promote amide deprotonation in the weakly acidic pH range.[8,9] The alcoholic OH groups of D-gluconic acid (GLU) have been suggested to coordinate in non-deprotonated form to $V^{IV}O$ at pH ≈ 6, forming a mixed ligand dihydroxo complex.[27] Alcoholic OH groups adjacent to a carboxylate function have been shown to deprotonate with p$K \approx 4$ (as in our systems), *e.g.* in the $V^{IV}O$ complex formed with glycolic acid and lactic acid.[28] In *N*-D-gluconylamino acids, however, the carboxylate group is not in such a favourable steric arrangement with the alcoholic OH groups (see Scheme 1), and therefore deprotonation of only these alcoholic OH groups cannot be expected at such a low pH. Further, there is no significant difference in pH range for liberation of the first two protons between the complexes of the α-amino acid and β-amino acid derivatives, indicating again that the carboxylate cannot be the only anchoring donor. In the $V^{IV}O$–Sal-Gly system, where the phenolic OH (p$K = 8.16$) is in a position similar to that of the C(2)–OH group in our ligands, two successive deprotonation processes were observed, with p$K_1 = 3.19$ and p$K_2 = 4.76$, assigned to the phenolic OH and amide NH, respectively.[11]

The broad band with increased intensity at lower wavelengths in the Vis spectra indicates a ligand field energy increase and a large contribution from charge transfer transitions. Since all the ligands have the same carbohydrate chain, very similar CD spectra would be expected for the complexes of all the ligands if only the sugar residue were coordinated to the metal ion. On the other hand, only a very small change in CD intensity is to be expected as compared with the $[(VO)L]^+$ complexes, at least for the GLU-Gly and GLU-β-Ala complexes, in the event of coordination of only the amino acid residue in the mixed ligand hydroxo complexes. However, the CD spectra recorded in this pH region (Fig. 3) can be divided into two distinct groups on the basis of their shape and intensity. The spectra of $V^{IV}O$–GLU-Gly and $V^{IV}O$–GLU-β-Ala (the ligands do not contain an optically active amino acid residue) belong in the first group, with relatively small, but significant intensity. The complexes of the ligands with an optically active amino acid residue (belonging in the second group) exhibit more intense CD spectra. The most reasonable explanation for this 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 fused 5 + 5-membered (5 + 6 for GLU-β-Ala) chelate ring system. Thus, the difference in the CD spectra between the two groups points rather to the similarity than to the difference in the local donor atom arrangement around the metal ion in these complexes.

This binding mode would mean that the role of the carboxylate in the metal ion binding is significant (it serves as a primary anchor), and the almost co-operative deprotonation of the above two slightly acidic groups is a consequence of their very favoured arrangement. Besides, the supplementary coordination of the not yet deprotonated OH groups presumably also promotes this process.

Both the isotropic EPR spectra and those recorded at 77 K revealed a significant decrease in intensity from pH ≈ 5, indicating a strong antiferromagnetic interaction between the $V^{IV}O$ centres, due to the formation of di- or oligo-nuclear complexes. If the coordination of the ligand occurs through a fused chelate ring system, the formation of double-bridged dimers is possible only through the deprotonated alcoholic OH groups as bridging donors. Thus, the formula of the complexes formed at pH 5 can be written as $[(VO)_2L_2H_{-4}]^{2-}$. According to the molecular models, this species (Scheme 2) may also be stabilized through coordination of the non-deprotonated alcoholic OH groups in the axial positions, forming new ligand bridges between the two metal centres. The ligand configuration allows only the binding

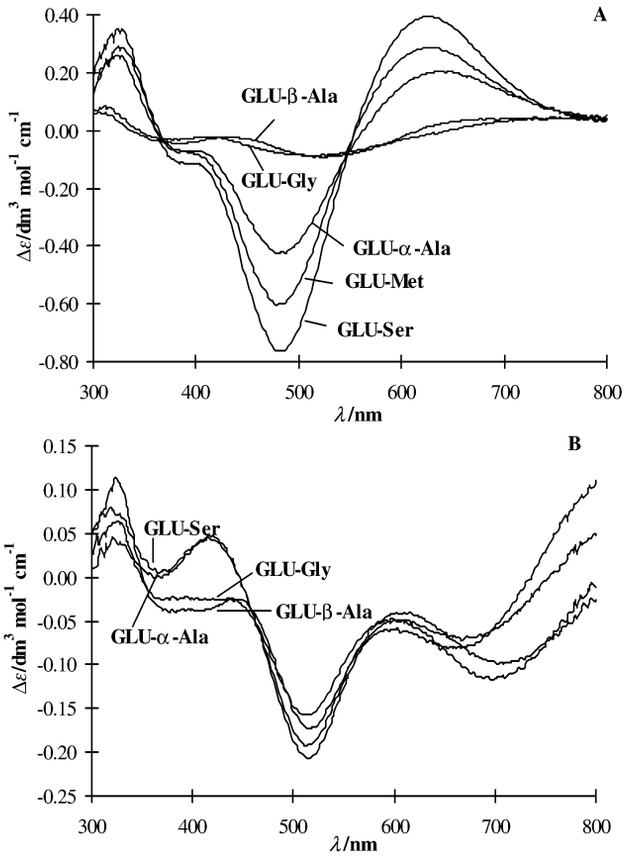
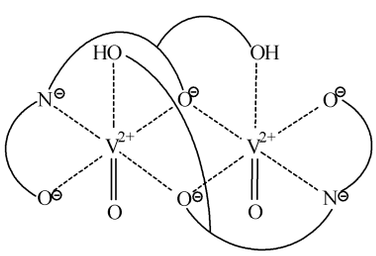


Fig. 3 CD spectra of the $V^{IV}O$–*N*-D-gluconylamino acid systems at: pH 5 (A) and >11.5 (B).



Scheme 2 The structure proposed for complex $[(VO)_2L_2H_{-4}]^{2-}$.

arrangement when both oxo groups of the $V^{IV}O$ centres are on the same side of the equatorial plane. In this case, as the coordination spheres of both metal ions are now saturated, any further coordination process would result in replacement of one of the already coordinated groups. This may happen in the next two, very slow deprotonation steps, which take place up to pH ≈ 10, where the lack of an EPR signal revealed that the species should remain dimers, with the compositions $[(VO)_2L_2H_{-5}]^{3-}$ and $[(VO)_2L_2H_{-6}]^{4-}$. The considerable changes in the Vis absorption and in the CD spectra indicate that the coordination sphere of the metal ion undergoes considerable changes as compared with that of $[(VO)_2L_2H_{-4}]^{2-}$. While these spectra for $[(VO)_2L_2H_{-5}]^{3-}$ and $[(VO)_2L_2H_{-6}]^{4-}$ display the same shape and have the same wavelength values for the extrema, a continuous change in absorption intensity accompanies the reaction $[(VO)_2L_2H_{-5}]^{3-} \rightleftharpoons [(VO)_2L_2H_{-6}]^{4-} + H^+$. It is difficult to suggest a single structure for these two species, since there are various possibilities for deprotonation. The most reasonable assumption is that the hydroxide ions and/or the deprotonating alcoholic OH groups of the ligand molecule displace the carboxylate group from the equatorial plane of the coordination sphere, the latter process being supported by the above mentioned changes in the CD spectra.

As a consequence of the new deprotonation process, isosbestic points near 560 and 640 nm may be observed in the Vis absorption spectra in the basic pH range. This process results in decomposition of the dinuclear structure into mononuclear complexes as indicated by the reappearance of the EPR spectra. The formation of a mixed ligand hydroxo species and/or deprotonation and coordination of a further alcoholic OH group of the sugar moiety may again occur, resulting in a mononuclear complex with the composition $[(VO)LH_{-4}]^{3-}$. The formation of a bis complex $[(VO)L_2H_{-4}]^{x-}$ (x is dependent on the ligand molecule) has been described [13] for several carbohydrate ligands with $V^{IV}O$, with two deprotonated alcoholic OH goups from both ligands in the equatorial plane of the coordination sphere. The hyperfine coupling constants and g values for this type of coordination are around $A_{\parallel} \approx 150 \times 10^{-4}$ cm^{-1} and $g_{\parallel} \approx 1.96$.[13] The EPR parameters reported for the same donor group arrangement in ref. 29 are $A_{\parallel} = 141.3 \times 10^{-4}$ cm^{-1}, $g_{\parallel} = 1.967$, which are significantly different from those of ref. 13. The former values were obtained from the spectra of a number of carbohydrate complexes, but no real structural evidence was presented for the binding modes of these species. Therefore, we used the latter values to characterize coordination of the alcoholate O^- in the equatorial plane. Such contributions for the carboxylate ($A_{\parallel} = 170.9 \times 10^{-4}$ cm^{-1}, $g_{\parallel} = 1.941$),[29] the deprotonated amide nitrogen ($A_{\parallel} = 140.0 \times 10^{-4}$ cm^{-1}, $g_{\parallel} = 1.983$),[10] and the OH^- groups ($A_{\parallel} = 154.7 \times 10^{-4}$ cm^{-1}, $g_{\parallel} = 1.962$),[29] the probable coordinating donors in $[(VO)LH_{-4}]^{3-}$, have been suggested on the basis of EPR measurements on selected $V^{IV}O$ complexes.[29] With the assumption of the additivity of these contributions (p_i) in the equatorial plane,[29] the EPR parameters (p) can be estimated *via* the equation $p^{est} = \sum_{i=1}^{4} p_i/4$. The hyperfine coupling constants estimated in this way for the various potential binding modes in this species are as follows: 144.3×10^{-4} cm^{-1} for {2(RO^-), N^-, OH^-}, 148.4×10^{-4} cm^{-1} for {2(RO^-), N^-, CO_2^-}, 151.7×10^{-4} cm^{-1} for {RO^-, N^-, CO_2^-, OH^-}, 141.3×10^{-4} cm^{-1} for {4(RO^-)}, 141.0×10^{-4} cm^{-1} for {3(RO^-), N^-}, and 140.7×10^{-4} cm^{-1} for {2(RO^-), 2(N^-)}. Although the uncertainties in these calculated values are rather large (≈ $\pm 3 \times 10^{-4}$ cm^{-1}), their comparison with the experimental value of $A_{\parallel} = 144.9 \times 10^{-4}$ cm^{-1} obtained for $[(VO)LH_{-4}]^{3-}$ reveals that the binding mode (2RO^-, N^-, OH^-) with the possible additional coordination of the carboxylate in the axial position is the most probable one in this species. Such an arrangement of donor groups is supported by the CD spectra measured in basic (pH > 11) solutions of the complexes. Although these spectra are all very similar in intensity, Fig. 3B shows that, on the basis of their shape, they can still be divided into the same two groups as discussed for the spectra obtained at pH ≈ 5. This suggests that, although more deprotonated alcoholic OH groups are in the coordination sphere, which results in similarity of the spectra, the deprotonated amide nitrogen is still coordinated, leading to a small but significant difference in the respective CD spectra.

Conclusion

Combined pH-metric and Vis, CD and EPR spectroscopic measurements demonstrated that the *N*-D-gluconylamino acid ligands are efficient complex forming agents for the hard $V^{IV}O$ ion in the pH range from 4 to 12. The carboxylate coordination in acid solution and the favourable steric arrangement of the amide nitrogen and alcoholic OH groups allow parallel deprotonation of these groups, resulting in stable dialkoxo-bridged dimeric species even at pH ≈ 5. At physiological pH, EPR-inactive dimeric species are predominant, their stepwise deprotonation in slow equilibria leading finally to monomeric complexes in highly basic solution. However, these are not the usual bis complexes with two deprotonated vicinal alcoholic

OH groups from each ligand, characteristic of carbohydrate molecules at high pH; surprisingly, the deprotonated amide nitrogen remains in the coordination sphere ion even at pH ≈ 12.

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