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Oxovanadium(IV) complexes of peptides with non-co-ordinating side chains and related ligands; a spectroscopic study†

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The systems VO^{2+} + L (L = Gly-Gly, Gly-Gly-Gly, L-Ala-Gly, Gly-L-Ala, L-Ala-L-Ala, Gly-Gly-L-Ala or L-Ala-Gly-Gly; Gly = glycine, Ala = alanine) have been studied in the range pH 1.5–13 by a combination of spectroscopic methods (ESR, circular dichroism and visible absorption). Very extensive hydrolysis and precipitation of $[VO(OH)_2]$ occurs at pH > 4–5, even when using a L : M ratio of 180 : 1. Plausible isomeric structures are discussed and for dipeptides N_{amide} deprotonation/co-ordination occurs at pH > ≈7. Several hydrolysis products (monomeric and oligomeric) are formed at higher pH values. Spectroscopic studies with Gly-Sar (glycyl-*N*-methylglycine), Gly-L-Pro (proline) and *N*-acetyl-L-alanine support the proposed co-ordination geometries. The results are compared with those for glycylglycine and glycylglycylglycine with VO^{2+}. For tripeptides, optically inactive oligomeric complexes are the major species; there is no evidence of amide deprotonation/co-ordination.

Interactions of oxovanadium(IV) and vanadate with proteins may provide[1,2] the key to our understanding of the biological role of vanadium. Oxovanadium(IV) binds specifically (tight binding) and non-specifically (weak binding) to various proteins including carboxypeptidase, nucleases and phosphatases.[3,4] We and others have investigated complexation of vanadyl by several α-amino acids[5–16] and by the glycine peptides Gly-Gly and Gly-Gly-Gly in the range pH 1.5–4 by potentiometric and spectroscopic methods (EPR and visible absorption).[17] With such peptides, at vanadyl concentrations like those in our α-amino acid systems,[5–12] vanadyl hydroxide precipitates at pH > 4–5 even when using high amino acid : metal ratios (L : M), *e.g.* 150–180 : 1. At pH > 7.5–8 the hydroxide slowly dissolves to give brown solutions, indicating that the oxovanadium(IV) is extensively hydrolysed. This rules out potentiometry at high pH. For pH < 4 potentiometry is also of limited value: with low L : M ratios much oxovanadium(IV) is free and to form the important complexes, *via* monodentate carboxylate (and eventually O_{amide}), involves no H^+.[17] At pH > 8 the very high absorbance values (especially for λ < 650 nm)[18] also precludes the use of visible spectra. Minor oxidation of V^{IV} may also affect these spectra. Visible spectroscopy is very useful at pH < 4–5, because high L : M ratios may be used. Such spectra in the Gly-Gly and Gly-Gly-Gly + VO^{2+} systems made it possible to calculate reliable formation constants. The final equilibrium model included[17] stoichiometries M(HL), $M(HL)_2$ and MLH_{-1}: for each, the visible and EPR spectra give some clues to the mode of co-ordination of these simple peptides.

Since circular dichroism (CD) spectra are more informative than the corresponding absorption spectra, optically active peptides have now been selected to clarify their mode of co-ordination. These include; Ala-Gly, Gly-Ala and Ala-Ala, Gly-Gly-Ala and Ala-Gly-Gly (Ala = alanine). Other optically active compounds were also studied: glycylsarcosine (Gly-Sar), glycyl-L-proline (Gly-Pro), which may chelate only through carboxylate, amine and carbonyl oxygen; alanine amide, *N*-acetyl-L-alanine, L- and (*S*)-(+)-2-methylbutyric acid. The stoichiometries and structures of species that form in the Gly-Ala, Ala-Gly and Ala-Ala systems with VO^{2+} are probably the same as in the Gly-Gly system, and the same should apply for Ala-Gly-Gly and Gly-Gly-Ala compared to Gly-Gly-Gly. Only the peptide–vanadium complexes contribute to Δ*A* values (differential absorption) in CD spectra, and the sign patterns of Cotton effects can be compared for bands I, II (and III) of oxovanadium(IV) between different peptides and/or amino acid systems as the pH is varied.

A fundamental objective here is to decide whether N_{amide} deprotonation/co-ordination occurs in the physiological pH range. This process requires a primary ligating group (or 'anchor'), *i.e.* carboxylate or amine, and has been suggested for vanadium-(IV)[16] and -(V)[19–27] peptide complexes. It does not occur with oxovanadium(IV) and Gly-Gly or Gly-Gly-Gly[17] at least till pH ≈ 5. Part of this work has been presented in a preliminary form.[28] Process (1), with M = Cu^{2+}, Ni^{2+} or Pd^{2+},

$$ML \overset{K_a}{\rightleftharpoons} MLH_{-1} + H^+ \qquad (1)$$

has pK_a 4–5, 9–10 and ≈2, respectively.[29–31] In the absence of metal ion, N_{amide} (peptidic) only deprotonates at high pH values (>14). As the amide group is planar and the C–N bond has some double-bond character, the nitrogen atom has a positive partial charge[29–31] and the oxygen atom a negative one. Some NMR measurements[32–34] have demonstrated that O_{amide} is the main protonation centre in the whole pH range.

The increase in double-bond character in the C–N bond on substitution of a metal ion for the proton reinforces the planarity of the peptide. Since the chelate rings are nearly planar even when there are side chains, the effect of chelate-ring puckering makes a negligible contribution to optical activity.[29] Analysis of Cu^{2+}–peptide complexes based on CD additivity calculations suggests that the trend may be accounted for if the ability for the metal ion to sense asymmetry on a nearby side chain is transmitted in the order:[29] $N_{amide} > (CO)N_{amide} > CO_2^- \geqslant NH_2$. This conclusion is probably valid for other metal ions.

As only pK_{a1} (terminal CO_2H) and pK_{a2} (terminal NH_3^+) can be determined for these peptides, the formation constants correspond to the general reaction (2). For the formulations

$$pM^{2+} + qL^- + rH^+ \overset{\beta_{pqr}}{\rightleftharpoons} M_pL_qH_r^{2p-q+r} \qquad (2)$$

$(VO)_p(ligand)_qH_r^{2p-q+r}$ we normally use the abbreviation $M_pL_qH_r$, the Δε values for which will be designated by $\Delta\varepsilon_{pqr}$.

† *Non-SI unit employed*: G = 10^{-4} T.

Experimental

All solutions were prepared in an inert atmosphere (high-purity dinitrogen passed through soda lime and glass-wool). All measurements were at 25 °C with solutions containing 2.25 mol dm^{-3} $NaNO_3$. Amino acids (from Sigma) were dried for several days (over silica gel *in vacuo*). The experimental difficulties in the present systems were discussed in ref. 5.

The stock solution of VO^{2+} was prepared and standardised as described in ref. 5. Solutions of NaOH and HNO_3 (both 4 mol dm^{-3}) were used to adjust the pH.

TLC

These experiments were performed on Merck TLC plates (Art. 5626, 10 × 20 cm). The compounds and solutions used for spectroscopic measurements were monitored throughout the whole pH range studied to check their purity and test for reactions (*e.g.* hydrolysis): neither contamination nor decomposition was detected. Usually, 2 μl samples were applied to the plates 20 mm from the bottom. Elution was carried out in Camag twin chambers with walls covered with filter-paper impregnated with the eluent butanol–ethanol–propionic acid–water (10:10:2:5). When it reached ≈120 mm from the bottom the plates were removed and dried. The chromatogram was developed with a ninhydrin–collidine (2,4,6-trimethylpyridine)–copper solution according to Moffat and Lytle.[35] In some cases, after such development, the plate was placed in an enclosed chamber for development with iodine vapour. Typically, samples were taken for TLC after dissolution of the amino acid and addition of VO^{2+} at several pH values.

pH measurements

For preparation of the solutions and pH calibrations we used a special glass vessel with a double wall, with entries for the glass electrode (Orion Ross 81-01) and reference electrode (Orion Ross 80-05), thermometer, nitrogen and reagents (*e.g.* base). A computerised system developed locally (for an IBM-PCXT 286 computer) was used to control the titration conditions for pH calibrations. The electromotive force measurements were made with a Crison 517 pH meter.

Spectroscopic measurements

The CD spectra were recorded with a JASCO 720 spectropolarimeter and a red-sensitive photomultiplier (EXWL-308). Cell compartments were kept at 25 °C with circulating water. By CD spectra we normally mean a representation of $\Delta\varepsilon_m$ values *vs.* λ [$\Delta\varepsilon_m$ = differential absorption/(bc_{vo}) where b = optical path, c_{vo} = total oxovanadium(IV) concentration and differential absorption = $\Delta A = A_L - A_R$]. All measurement operations of the spectropolarimeter were computerised and controlled by a Compaq 386 computer. A rapid Fourier-transform noise-reduction routine (JASCO) was used without affecting peak shapes. At pH > 10–12, depending on the system, c_{vo} and L:M ratio, the absorption at λ < 600–650 nm is high and the signal-to-noise ratio is unfavourable, so these CD spectra are not entirely reliable. The ESR spectra were usually recorded at 77 K (on glasses made by freezing solutions in liquid nitrogen) with a Bruker ESR-ER 200D X-band spectrometer.

As vanadyl hydroxide precipitates in the range pH ≈ 4.5–7.5 even at high L:M, CD and EPR spectra were recorded in most cases for the following types of solutions: (*i*) at pH < 4–5, with low and high L:M ratios, varying the pH with approximately fixed total vanadium and ligand concentration, at L:M ratios of ≈3 and ≈15:1 for Ala-Gly, Gly-Ala and Ala-Ala, ≈15:1 for Gly-Gly-Ala, Ala-Gly-Gly and alanine amide, ≈17:1 for Gly-Pro, ≈30:1 for Gly-Sar, 5:1 for *N*-acetylalanine and 4:1 for (*S*)-(+)-2-methylbutyric acid; (*ii*) at approximately fixed pH (≈2, 3 and 4) and total ligand concentration, varying $2 \leqslant L:M \leqslant 20$ by addition of oxovanadium(IV) stock solution; (*iii*) at pH > 7.5–8, as in (*i*) using high L:M. To study basic solutions (*iii*) and avoid precipitation of vanadyl hydroxide, 4 mol dm^{-3} NaOH was rapidly added to solutions at pH ≈ 4.

The liquid (*S*)-(+)-2-methylbutyric acid with VO^{2+}(aqueous) does not give a homogeneous 'solution'. The CD spectra of such solutions (L:M = 4:1) are relatively weak at pH < 3 ($|\Delta\varepsilon_m| < 4 \times 10^{-4}$ dm^3 mol^{-1} cm^{-1}).

Results and Discussion

The X-band ESR spectra of frozen 'solutions' containing peptides may be simulated as axial spectra. The field region corresponding to $A_\parallel$ and $M_I = \frac{5}{2}$ and $\frac{7}{2}$ gives more information about the type and number of species present. Fig. 1 shows ESR spectra recorded in this range. When the pH is increased from ≈3 to ≈4 [*e.g.* Fig. 1(*a*) and 1(*b*)] each spectrum appears like that of only one contributor, but the peaks actually shift to lower field so more than one species contribute to each spectrum. Table 1 summarises the g and A (∥ and ⊥) parameters at low pH, calculated using equations[36] based on Chasteen's[37] iterative method. Throughout, superscripts 'exptl' and 'est' refer to 'experimental' and estimated parameters [equation (3)], where $A_{\parallel,i}$ are

$$A_\parallel^{est} = \tfrac{1}{4}\sum_{i=1}^{4} A_{\parallel,i} \qquad (3)$$

the contributions to $A_\parallel^{est}$ of each of the four equatorial groups (most presented by Chasteen[37] with accuracy estimated to be ±3 × 10^{-4} cm^{-1}). When reliable $A_{\parallel,i}$ are not available we do not include values of $A_\parallel^{est}$.

The spin-Hamiltonian parameters calculated for solutions at pH ≈ 3 are practically the same for all systems (Table 1). Spectra of neutral/basic solutions containing Ala-Ala, Gly-Ala or Ala-Gly are also similar [*e.g.* Fig. 1(*c*) and 1(*d*)]. As the pH is increased, the intensity decreases but the peaks do not change position, with $A_\parallel^{exptl}$ (161 ± 1) × 10^{-4} cm^{-1} (Table 2). Decreasing L:M from ≈15 to ≈5:1 (by adding VO^{2+}) caused no changes in positions of the lines. The ESR spectra of tripeptides and Gly-Sar are very weak but we can estimate $A_\parallel^{exptl} \approx 167 \times 10^{-4}$ cm^{-1} for Gly-Sar solutions (pH 8–10) (see Table 2).

At low pH (1.5–4.5) the visible spectra for solutions of VO^{2+} and the optically active di- and tri-peptides resemble those for

Table 1 Vanadium hyperfine coupling constants and g values calculated[36,37] from the first-derivative ESR spectra at 77 K of frozen acidic 'solutions' containing VO^{2+} and dipeptides using high L:M ratios (see text and Fig. 1)

Dipeptide	pH	$g_\parallel$*	$10^4A_\parallel$*/cm^{-1}	$g_\perp$*	$10^4A_\perp$*/cm^{-1}
Ala-Gly	2.97	1.946	177.9	1.978	63.5
	3.87	*1.946*	*176.4*	*1.977*	*62.5*
Gly-Ala	2.95	1.949	178.3	1.977	62.2
	3.87	*1.947*	*175.6*	*1.976*	*61.3*
Ala-Ala	2.97	1.944	177.9	1.975	63.5
	3.86	*1.947*	*175.6*	*1.976*	*61.3*
Gly-Gly	2.95	1.947	178.1	1.977	63.5
	3.97	*1.945*	*176.2*	*1.977*	*62.5*
Gly-Gly-Gly	3.02	1.947	177.6	1.970	59.5
	3.93	*1.947*	*176.4*	*1.976*	*61.1*
Ala-Gly-Gly	2.98	1.946	177.3	1.978	63.6
	3.93	*1.945*	*175.3*	*1.979*	*62.7*
Gly-Gly-Ala	2.99	1.946	177.1	1.978	63.7
	3.87	*1.947*	*176.2*	*1.978*	*62.5*
Gly-Sar	1.97	1.945	180.2	1.979	65.8
	2.58	1.949	178.7	1.976	62.1
	2.98	1.948	177.4	1.977	62.3
	3.88	*1.946*	*175.4*	*1.979*	*62.7*

* Absolute values of g and A^{exptl} (∥ and ⊥) are probably to ± 0.007 and ±1, respectively. We present the $A_\parallel$ value to one decimal place only for comparison.

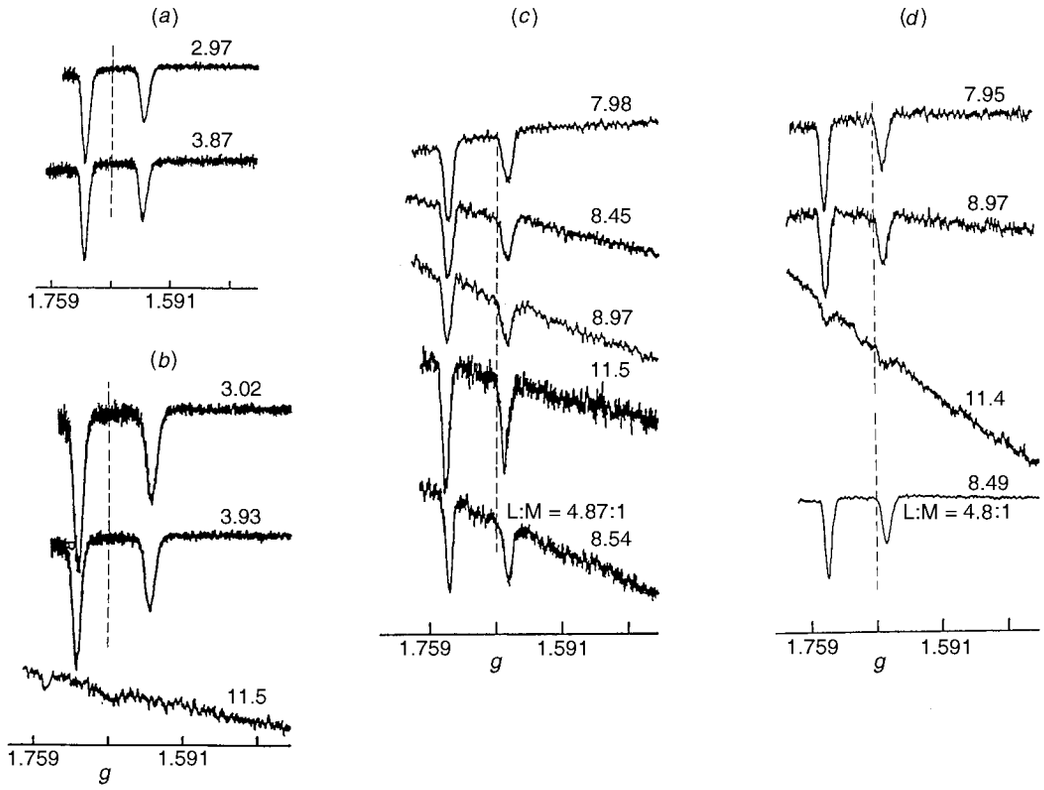


Fig. 1 High-field range (3800–4400 G) of the first-derivative ESR spectra at 77 K of frozen 'solutions' containing (*a*) Ala-Gly + VO^{2+}, L : M = 15.2 : 1 c_{vo} = 0.025–0.027 mol dm^{-3}, (*b*) Gly-Gly-Gly + VO^{2+}, L : M = 15.5 : 1, c_{vo} = 0.025–0.027 mol dm^{-3}, (*c*) Ala-Ala + VO^{2+}, L : M = 14.3 : 1, c_{vo} = 0.011–0.019 mol dm^{-3} and (*d*) Gly-Ala + VO^{2+}, L : M = 15.1 : 1, c_{vo} = 0.018–0.020 mol dm^{-3}. The pH values are indicated. The last spectrum of (*c*) and (*d*) (pH ≈ 8.5, L : M ≈ 5 : 1) was obtained by addition of stock VO^{2+} solution to the solution at pH ≈ 11.5

Table 2 Vanadium hyperfine coupling constants and *g* values calculated[36,37] from the first-derivative ESR spectra at 77 K of frozen basic 'solutions' containing VO^{2+} and dipeptides using high L : M ratios (see text and Fig. 3)

Dipeptide	pH	$10^4 A_{\parallel}$[a]/ cm^{-1}	$g_{\parallel}$[a]	$10^4 A_{\perp}$[a]/ cm^{-1}	$g_{\perp}$[a]
Gly-Ala	7.95	161.7	1.955	52.5	1.981
	8.97	161.5	1.955	54.5	1.982
	11.4	159.8	1.955	54.7	1.983
	8.49 (L : M ≈ 5 : 1)	161.5	1.955	54.5	1.982
Ala-Gly	8.00	160.1	1.953	53.4	1.981
	8.96	161.5	1.955	54.2	1.983
	11.5	161.9	1.960	49.2	1.976
	8.51 (L : M ≈ 5 : 1)	161.3	1.953	54.5	1.982
Ala-Ala	8–9	161.5	1.955	54.5	1.982
	11.5	159.4	1.956	49.5	1.977
	8.54 (L : M ≈ 5 : 1)	163.0	1.956	54.3	1.981
Gly-Gly	7.99	161.8	1.958	53.1	1.981
	9.00	161.5	1.955	54.5	1.982
	11.5	161.8	1.958	54.4	1.980
	8.46 (L : M ≈ 5 : 1)	161.7	1.957	54.5	1.982
Gly-Sar[b]	6.12	≈169.8	≈1.949	≈59.7	≈1.980
		≈162.3[c]	≈1.951[c]	≈60.7[c]	≈1.983[c]
	7.97	≈167.0	≈1.950	≈56.2	≈1.978
	9.03	≈167.0	≈1.950	≈56.2	≈1.978
	9.9	≈166.9	≈1.949	≈56.2	≈1.978

[a] Absolute values of *g* and A^{expt} (|| and ⊥) are probably to ±0.007 and ±1, respectively. We present the $A_{\parallel}$ value to one decimal place only for comparison. [b] Weak signal. [c] Minor component: impurity of Gly-Gly?

Gly-Gly or Gly-Gly-Gly.[17] At pH > 7–8 the absorption in the range 400–600 nm increases markedly, and the bands for acidic solutions at λ_{max} ≈ 600 and ≈770 nm are substituted by a weak broad band. The visible and ESR spectra indicate that VO^{2+} is extensively hydrolysed, and mainly present as oligomers with no measurable CD.

Low pH

Our previous study[5] of L-Ala + VO^{2+} did not include CD at pH <1.5. Such solutions have negative CD in the range 400–850 nm, with λ_{max} ≈ 700 (band I) and ≈ 560 nm (band II).

Peptides. The CD spectra with Ala-Ala, Gly-Ala and Ala-Gly modify as the pH is varied from ≈1.5 to 4–4.5, but there are some similarities. In general, the $\Delta\varepsilon_m$ in the range 400–850 nm are negative for pH < 2, as for similar solutions containing L-Ala. Band II is well defined and corresponds to λ_{max} ≈ 580 nm (Fig. 2), except for Ala-Gly where λ_{max}(band II) ≈ 600–610 nm. At pH > ≈ 2 two positive bands (IA and IB), corresponding to λ_{max} ≈ 680 and ≈ 780 nm, respectively, become important, especially at high L : M ratios (*e.g.* Fig. 2). Till pH ≈ 3.5 the CD spectra gradually change from the predominant pattern for pH < 2 to resemble spectrum 5 of Fig. 2(*b*). Alanylglycine is an exception: band II grows with pH but the pattern does not change much till pH ≈ 3.6, even for solutions with L : M ≈ 15. The $|\Delta\varepsilon_m|$ values are also much lower than those for Xaa-Ala peptides (Xaa = Gly or Ala). For Ala-Ala and Gly-Ala systems, $|\Delta\varepsilon_m|$ gradually increases with pH up to ≈3.5. At pH > 4 the CD spectra change: three bands may be detected [band II (λ_{max} ≈ 500, $\Delta\varepsilon_m$ < 0), IB (≈660–680, >0) and IA (≈780 nm, >0 dm^3 mol^{-1} cm^{-1})], but the relative intensities of bands IA and IB change.

For all dipeptides at pH ≈ 2 and variable L : M ratio (from 20 to 5 : 1), the $|\Delta\varepsilon_m|$ values are low and decrease with L : M. The CD spectra have the same pattern indicating a single species. At pH ≈ 3 and variable L : M the spectra present three bands (two of them weak). The pattern of the CD spectra at pH ≈ 4 (varying the L : M ratio) is fairly similar for the Ala-Ala and Gly-Ala systems [*e.g.* spectra 3–5 of Fig. 2(*b*)]. However, for Ala-Gly under similar conditions, the pattern of the CD spectra is quite different: only bands IB (λ_{max} ≈ 600, $\Delta\varepsilon_m$ < 0) and IA (λ_{max} ≈ 770 nm, $\Delta\varepsilon_m$ > 0 dm^3 mol^{-1} cm^{-1}) are clear; II is probably

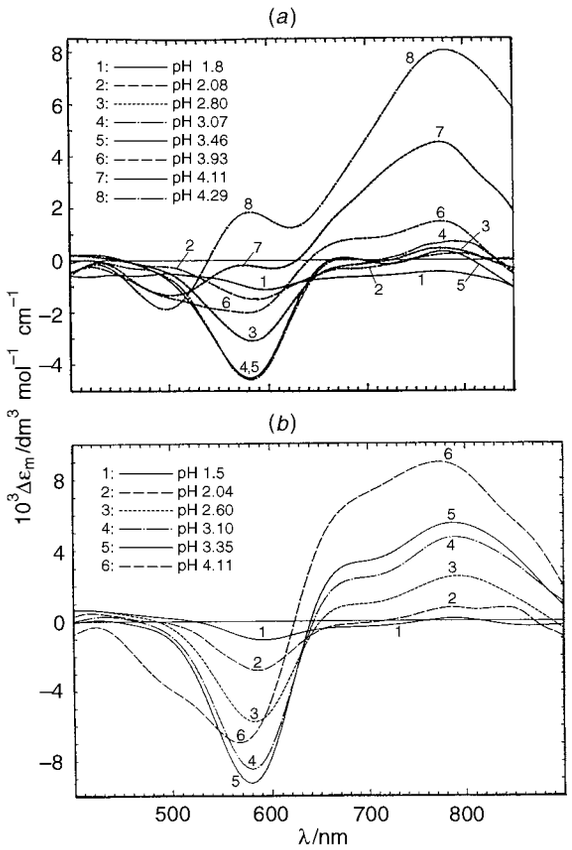
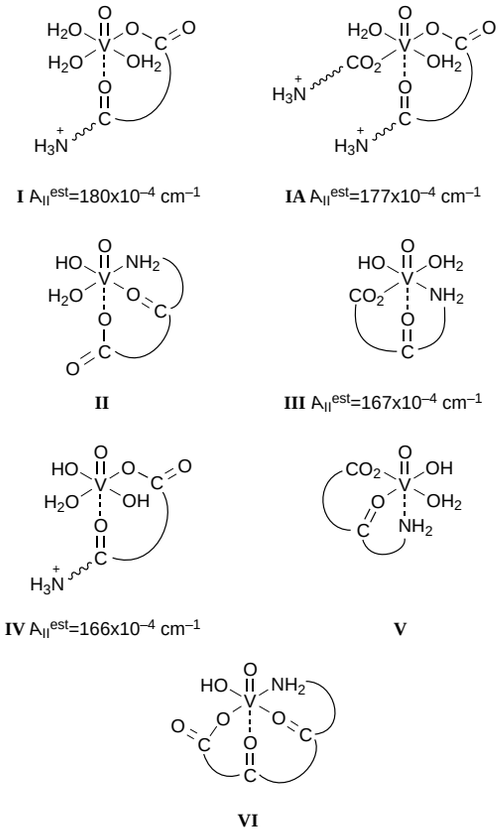


Fig. 2 The CD spectra of acidic solutions containing L-Ala-L-Ala + VO^{2+} at (*a*) L : M = 3.0 : 1 and c_{vo} = 0.031–0.039 mol dm^{-3} and (*b*) L : M = 15 : 1 and c_{vo} = 0.014–0.021 mol dm^{-3}. The pH corresponding to each spectrum is indicated



under IB and possibly corresponds to lower $|\Delta\varepsilon_m|$ values. At pH ≈ 4, the spectra at L : M ≈ 2 : 1 differ from those at higher L : M, possibly due to more extensive hydrolysis.

In the range pH 1–4 the CD spectra of solutions containing Gly-Gly-Ala and VO^{2+} (L : M = 15 : 1) are very similar to those for Ala-Ala. An isodichroic point is observed in the range pH ≈ 2.5–3.5 at ≈615 nm ($\Delta\varepsilon_m$ < 0 dm^3 mol^{-1} cm^{-1}). At pH > 4 vanadyl hydroxide precipitates. At pH 1–4.5 the CD spectra for Ala-Gly-Gly and VO^{2+} (L : M = 15 : 1) are weak. However, at pH > 2.4 a positive band is seen with λ_{max} ≈ 775 nm; its intensity increases up to pH 4.3. Values of $\Delta\varepsilon$ < 0 dm^3 mol^{-1} cm^{-1} we recorded in the range 500–620 nm. At pH > 4 the CD spectra are positive in the wavelength range studied; another band arises at λ_{max} ≈ 595 nm and grows up to pH 4.9. At higher pH vanadyl hydroxide precipitates.

These results are consistent and corroborate the equilibrium model proposed[17] for the Gly-Gly and Gly-Gly-Gly systems with VO^{2+} based on spectroscopic and potentiometric studies, which includes the stoichiometries M(HL), $M(HL)_2$ and MLH_{-1} and several hydrolysis products.

For M(HL) and $M(HL)_2$ stoichiometries the carboxylate and probably O_{amide} group (in axial position) co-ordinate as in structure **I** or **IA** respectively. We assume that a seven-membered ring involving equatorial/axial co-ordination may be relatively stable because the distance from vanadium to an axial donor is 0.3–0.4 Å longer than for equatorially co-ordinated atoms (*e.g.* ref. 18). For tripeptides a second O_{amide} group may co-ordinate equatorially replacing water. As for the co-ordination of the first amide, this reaction results in no consumption of H^+. For the dipeptides, structures such as **II** and/or **III** are most probable for the MLH_{-1} stoichiometry, but we cannot exclude **IV** and **V**. For tripeptides structures such as **VI** (and others) can also be envisaged. For most co-ordination geometries discussed other isomers (*e.g.* those resulting from *cis–trans* isomerism) could be envisaged. Usually we only include sketches for one of the possible isomers.

For Gly-Gly and Gly-Gly-Gly at pH < ≈ 2, M(HL) is the predominant stoichiometry at low pH.[17] The $|\Delta\varepsilon_m|$ changes for Ala-Gly, Gly-Ala and Ala-Ala at fixed pH (≈2) with varying L : M ratio suggest that M(HL) is the only optically active complex present. The similarities observed for Xaa-Ala ligands (Xaa = Gly, Ala; *N*-acetyl or H), and the lack of CD signal for L-alanine amide indicate that the carboxylate co-ordinates. The CD spectrum for the M(HL) stoichiometry is probably similar to spectrum 1 of Fig. 2(*a*).

For the present peptides the observations suggesting O_{amide} co-ordination for M(HL) stoichiometry may be summarised: (*i*) K_1 values [equation (4)] for Gly-Gly and Gly-Gly-Gly systems

$$VO(HL)_{n-1} + HL \overset{K_n}{\rightleftharpoons} VO(HL)_n \qquad (4)$$

(≈71 and ≈69 dm^3 mol^{-1}, respectively) are higher than for Gly (14.8),[13] L-Ala (15.6),[5] L-Ser (serine) (12.5),[6] L-Thr (threonine) (12.9),[6] 3-sulfanyl-D-valine (20.0),[7] L-Cys (cysteine) (15.0)[7] and L-Glu (glutamic acid) (9.3),[10] (*ii*) $\Delta\varepsilon_m$ values for Ala-Gly at low pH are finite; (*iii*) the CD spectra for Ala-Gly-Gly at low pH are weak but finite; (*iv*) CD spectra for *N*-acetyl-L-alanine are very weak at low pH, but are quite like those for Ala-Ala, Gly-Ala and Ala-Gly under similar conditions. There is no evidence for O_{amide} co-ordination in copper(II)–*N*-acetylamino acid complexes,[31] but apparently this can occur axially to oxovanadium(IV).

Relatively strong carboxylate co-ordination (and weaker O_{amide}) and/or more efficient transmission of the vicinal effect through the CO_2^- than through C=O_{amide} are evident in the range pH 2–3.5 from: (*i*) the near absence of CD for L-alanine amide; (*ii*) the CD for Ala-Gly and particularly for Ala-Gly-Gly is significantly weaker than for Xaa-Ala (Xaa = Ala, Gly, Gly-Gly or *N*-acetyl); (*iii*) CD spectra for Ala-Ala, Gly-Ala, Gly-Gly-Ala or *N*-acetylalanine present many similarities, including an isodichroic point at λ ≈ 615–630 nm corresponding to M(HL) ⇌ ML_2H_2; (*iv*) the CD spectra for 2-methyl-

butyric acid are relatively similar to those for Xaa-Ala (Xaa = Ala, Gly, Gly-Gly or *N*-acetyl).

In the model proposed for Gly-Gly and Gly-Gly-Gly systems with VO^{2+},[17] species with M(HL) and ML_2H_2 stoichiometries predominate for $2 < pH < 3.5$. On analysing the CD spectra and those corresponding to pH ≈ 3 on varying the L : M ratio, the CD spectrum of ML_2H_2 species is seen to be similar to spectra 3–5 of Fig. 2(*b*). Depending on the system, the intensities of individual bands can differ, influenced by differences in the ratio between *cis* and *trans* isomers, in $pK_a^{CO_2H}$ and formation constants.

The K_2 values [equation (4)] for Gly-Gly (≈6.6)[17] and Gly-Gly-Gly (≈8.9)[17] are like those for amino acids [*e.g.* L-Ala (1.9),[5] L-Ser (1.9),[6] 3-sulfanyl-D-valine (3.5),[7] L-Cys (3.0)[7] and L-Glu (1.6)[10]]. This suggests that in ML_2H_2 the second peptide is monodentate. We cannot however exclude such other interactions as hydrogen bonds between a co-ordinated water and *e.g.* O_{amide} or between the oxo group and NH_3^+ or NH_{amide}.

The CD spectra at pH > 3.5 indicate the presence of a third optically active species, MLH_{-1}, according to the model for Gly-Gly.[17] At low L : M ratios (2–3 : 1) species with low L : M are favoured, and for pH > ≈ 4 the CD spectra give important information about the pattern of the individual spectra of MLH_{-1} for the several systems studied. Using high L : M it is possible to reach higher pH values without hydroxide precipitation, favouring the contribution of MLH_{-1} to the CD. The formation of ML_2H_2 is also favoured but its relative concentration decreases at pH > ≈ 3.8. The CD spectrum for the MLH_{-1} stoichiometry is probably like spectrum 8 of Fig. 2(*a*).

The following suggest that the N_{amine} group is co-ordinated in MLH_{-1}: (*i*) the CD pattern drastically changes at pH > ≈ 3.5 (when MLH_{-1} forms); (*ii*) the CD for *N*-acetylalanine differs completely from those involving the peptides; (*iii*) at pH ≈ 4 the similarities between (*a*) ESR for Gly-Sar, (*b*) CD for Gly-Pro, and the counterparts for the simple peptides rule out deprotonation/co-ordination of N_{amide}; (*iv*) at pH ≈ 4–4.5, overall the CD spectra of Ala-Xaa present more similarities; (*v*) the ESR spectra in the range 3800–4300 G shift significantly to lower field when the pH is increased from 3 to 4 ($A_{\parallel}^{exptl}$ become lower), in agreement with equatorial co-ordination of N_{amine}.

The $A_{\parallel}^{exptl} \approx (175–176) \times 10^{-4}$ cm^{-1} at pH ≈ 4 are consistent with the sum of the weighted contributions of species corresponding to stoichiometries M(HL), ML_2H_2 and MLH_{-1}, assuming structures such as **II** or **III** for MLH_{-1}, O_{amide} contributing to $A_{\parallel}^{est}$ like carboxylate, and a concentration distribution similar to those of Gly-Gly and Gly-Gly-Gly systems.[17]

The CD for Gly-Pro at L : M = 17.2 : 1 and pH 1.1 is negative for λ = 400–1000 nm: band II corresponds to λ_{max} ≈ 580–590 nm. On increasing the pH, besides this (negative) band (II), two additional (positive) bands appear at λ_{max} ≈ 820 (IA) and ≈670 nm (IB). All three strengthen as the pH is increased to ≈4. At higher pH the spectra change slightly and at pH ≈ 5.2 hydroxide precipitates.

Related compounds. The CD of solutions containing *N*-acetyl-L-alanine (L : M ≈ 5) is relatively low at pH < 2 ($|\Delta\varepsilon_m| < 4 \times 10^{-4}$ dm^3 mol^{-1} cm^{-1}). The degree of complexation is low and 1 : 1 species certainly predominate. For pH ≈ 2 the CD spectrum is relatively intense and like those of the dipeptides Xaa-Ala, indicating that the 2 : 1 species are important. On increasing the pH to 3.5 two positive bands [λ_{max} ≈ 790 (IA) and ≈660 nm (IB)] and one negative [λ_{max} ≈ 575 nm (II)] are detected and gradually grow. The basicity of the amide group is low and it is not clear whether O_{amide} co-ordinates axially to vanadium. For other metals (*e.g.* Ni^{2+} and Cu^{2+}) there is no literature reference to amide group co-ordination in *N*-acetylamino acids with non-co-ordinating side chains.[29,31] At pH > 3.5 band II strengthens and λ_{max} is red-shifted (to 605 nm). At pH > 4.2 the solution is dark greyish blue, and at pH > 4.5 hydroxide precipitates. The changes observed in the CD spectra, particularly the increase in intensity and red shift of band II, indicate a gradual increase in the extent of complexation, *i.e.* formation of ML_2H_2 and MLH_{-1}, the latter possibly involving substitution of an H_2O molecule by OH^-. The ESR and VIS studies of Dessì *et al.*[15] with glycine amide led to similar conclusions.

At pH < 3.3 the CD for solutions containing (*S*)-(+)-2-methylbutyric acid is very weak. As carboxylic acids have relatively high pK_a values compared with those of peptides, the extent of complexation is low in the present system, at least at pH < 3.3. At higher pH the CD shows three bands: $\lambda_{max} > 830$ (IA) (+), ≈670–680 (IB) (+) and ≈580–590 nm (II) (−). The intensity increases with pH up to ≈4.3. At pH > 4.3 hydroxide precipitates. The spectra have similarities with those for peptides indicating that for the latter systems the carboxylate has an essential role in co-ordination and in the transmission of the vicinal effect to the metal centre. The isodichroic point at λ ≈ 690 nm is probably due to an equilibrium between 2 : 1 and 1 : 1 complexes.

Glycine amide chelates Cu^{2+} and Ni^{2+} *via* the amino nitrogen and carbonyl oxygen, but stronger chelation occurs in neutral (Cu^{2+}) or basic (Ni^{2+}) solutions upon deprotonation/co-ordination of the amide nitrogen.[29–31] Solutions containing L-alanine amide (L : M = 15 : 1) have almost no CD at pH < 3.5. This suggests that the carboxylate group has a role in co-ordination, and that for peptides N_{amine} is not co-ordinated in this pH range. The CD spectrum at pH ≈ 3.7 has some similarities with those for Ala-Xaa peptides at pH > ≈ 4. This probably arises from the contribution of 1 : 1 (and 2 : 1) species having at least one ligand co-ordinated equatorially through N_{amine} and O_{amide}, forming a five-membered chelate ring. In basic solutions a precipitate formed and the CD signal at pH ≈ 11 and ≈13 is extremely weak.

High pH

Dipeptides. For solutions containing Ala-Ala (L : M ≈ 15 : 1) [Fig. 3(*a*)] two bands are detected in the range pH 7.5–9.5

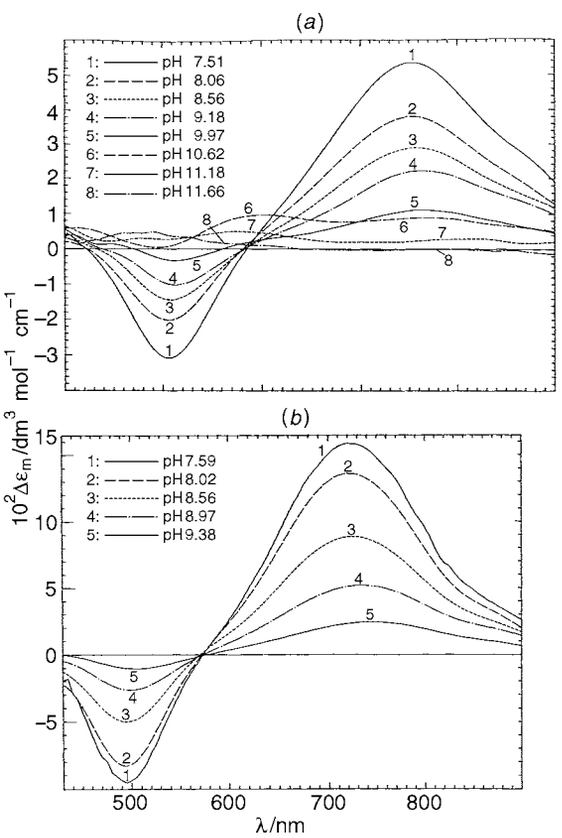


Fig. 3 The CD spectra of solutions containing (*a*) L-Ala-L-Ala + VO^{2+} at L : M = 15 : 1 and c_{vo} = 0.009–0.011 mol dm^{-3} and (*b*) Gly-L-Ala at L : M = 15 : 1 and c_{vo} = 0.013–0.017 mol dm^{-3}. The pH corresponding to each spectrum is indicated

corresponding to $\lambda_{max} \approx 750$–$760$ ($\Delta\varepsilon_m > 0$ dm^3 mol^{-1} cm^{-1}) and ≈505–510 nm (<0). The $|\Delta\varepsilon_m|$ values decrease as the pH is increased with a slight red shift and an isodichroic point at 585 nm ($\Delta\varepsilon_m \approx 0$ dm^3 mol^{-1} cm^{-1}). At pH > ≈10 the spectral pattern changes and the intensity decreases: an isodichroic point exists at ≈530 nm; a band with $\Delta\varepsilon_m > 0$ dm^3 mol^{-1} cm^{-1} ($\lambda_{max} \approx 600$ nm) is detected, with maximum intensity at pH ≈ 10.6.

For Gly-Ala in the range pH 7.5–9.4 the CD pattern [Fig. 3(*b*)] is similar to those for Ala-Ala but the λ_{max} differ: band I ($\lambda_{max} \approx 720$, $\Delta\varepsilon_m > 0$ dm^3 mol^{-1} cm^{-1}) and II (≈495–500 nm, <0). An isodichroic point is detected at 570 nm ($\Delta\varepsilon_m \approx 0$ dm^3 mol^{-1} cm^{-1}). At higher pH the CD intensity continues to decrease and the λ_{max} values shift gradually to the red.

At pH > 7.5 the CD of solutions containing Ala-Gly (L : M ≈ 15 : 1) differ completely, being much weaker than for Ala-Ala and Gly-Ala. At pH > 10.4 the spectra clearly differ from those for Ala-Ala and Gly-Ala.

In the range pH 8–10 the CD spectra of solutions containing Ala-Ala or Gly-Ala are therefore much stronger than for Ala-Gly under similar conditions. The brown colour, high absorption at λ < 500 nm, and weak ESR signal suggest oligomeric hydroxy complexes. The isodichroic points at λ ≈ 570–585 nm ($\Delta\varepsilon \approx 0$) and the similar behaviour of $|\Delta\varepsilon_m|$ values corresponding to both bands (negative and positive) suggest that only one optically active species contributes to the CD spectra. The red shift at pH > ≈ 9.5 indicates the formation of another optically active species with a higher extent of hydrolysis. The weaker CD and ESR spectra at higher pH are mainly due to oligomers like $[\{(VO)_2(OH)_5\}_n]^{n-}$ and/or $[\{(VO)_2(OH)_6\}_m]^{2m-}$.[5,18]

As ESR spectra of dipeptides (Gly-Ala, Ala-Gly and Ala-Ala) do not depend on the system and apparently correspond to a single species [Fig. 1(*a*)], the co-ordination geometry of all monomeric species is similar. The relatively strong CD indicates N_{amide} co-ordination. This demands the previous co-ordination of an anchor group. The CD spectra of Gly-Ala are more intense than those of Ala-Gly (*e.g.* for pH ≈ 7.5: $\Delta\varepsilon_m^{Gly\text{-}Ala} \approx 20\Delta\varepsilon_m^{Ala\text{-}Gly}$), so carboxylate co-ordinates equatorially. The simultaneous co-ordination of N_{amine} is also possible. Overall for Ala-Ala, Gly-Ala and Ala-Gly the deprotonation/co-ordination of N_{amide} is indicated by: (*i*) in the range pH ≈7.5–9.5 the ESR spectra are similar and reasonably intense, the calculated parameters being consistent with tridentate co-ordination *via* CO_2^-, N^-_{amide} and NH_2 and an equatorial water molecule (*e.g.* **VII**), assuming a contribution by N^-_{amide} to $A_\parallel$ of (130–131) × 10^{-4} cm^{-1} (see below); (*ii*) the ESR spectra of solutions containing Gly-Sar are much weaker than for Gly-Gly, Gly-Ala, Ala-Gly and Ala-Ala even at L : M as high as 30 : 1, and correspond to higher $A_\parallel^{exptl}$ values (Table 2); (*iii*) the CD spectra of basic solutions containing the three dipeptides are much stronger than at pH ≈ 4.5, despite a significant content of optically inactive hydroxo complexes; (*iv*) in the range pH 7–10 the CD spectra of Gly-Pro are weak and differ from those for Ala-Ala, Ala-Gly and Gly-Ala.

This work presents the first clear evidence of peptidic N_{amide} co-ordination to oxovanadium(IV). A previous suggestion[15] of such co-ordination in glycyl-*N*-acetylcysteamine, glycyl-*N*-acetyl-L-cysteine and γ-glutamylcysteinylglycine at pH > 7–8 assumed that the amino and peptide nitrogens co-ordinated equatorially contribute equally to the ^{51}V hyperfine constant. That is not so. Several reports[38–40] clearly indicate much lower contributions of N_{amide} (see below), particularly[40,42] when not bonded to a phenyl group.

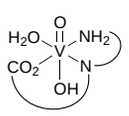
The position of the optically active amino acid residue in the peptidic chain is important in understanding the CD spectra. Ligand asymmetry is induced to the metal ion (vicinal effect) through the co-ordinated functional groups, and the nature and positions of the substituents are also important. As there are no well characterised VO^{2+}–peptide complexes, it is not possible to predict CD band intensities by the additivity calculations used for Cu^{2+}–peptide complexes.[29] The O_{amide} and N_{amide} may be donor atoms in VO^{2+}–peptide complexes so it is important to predict their relative effectiveness in transmitting asymmetry from a nearby side chain. To rationalise our CD results at low and high pH we extend the sequence of Sigel and Martin[29] including such transmission: $N_{amide} > (CO)N_{amide} > CO_2^- > C{=}O_{amide} > (NH)C{=}O_{amide} > NH_2$. We assume here and elsewhere[5–12] that optical activity transmits better from the chiral centres of the ligand into the optical transitions through equatorial rather than axial donor atoms.

According to the CD and ESR spectra and by analogy with systems involving Cu^{2+} (or Ni^{2+}), structures like **VII** for MLH_{-2} stoichiometry are plausible. It involves two five-membered fused rings without significant steric constraints. Although we expect the OH^- ligand to co-ordinate equatorially, here that would be *trans* to N_{amide}. Such complexes involving equatorial OH^- would show much lower values of $A_\parallel^{exptl}$ (see below). Overall our CD results can be rationalised assuming structure **VII** and the order of vicinal transmission above.

Since N_{amide} of Gly-Sar does not co-ordinate, structures such as **II**, **III**, **VIII** or **IX** could be envisaged for moderately basic solutions. Assuming $A_\parallel^{exptl}$ for Gly-Sar (≈167 × 10^{-4} cm^{-1}) corresponds to one of these, we can estimate the contribution of O_{amide} for $A_\parallel$ [$A_{\parallel,i}(O_{amide})$] for additive calculations. Kabanos and co-workers[40] characterised, by X-ray diffraction and ESR spectroscopy (at 77 K), two complexes (with rhombic symmetry) corresponding to the co-ordination geometry N (pyridine), N (amine), N (amide), O (ketone), and calculated A_z values of 151.4 × 10^{-4} and 152.4 × 10^{-4} cm^{-1}. The non-bonding nature of the spin-containing d_{xy} orbital decreases dependence on the geometry of the compound and so one may take $A_z \approx A_\parallel$. For structures **II**, **VIII** or **IX** one can then back-calculate the contribution of O_{amide} to $A_\parallel$, assuming the global $A_\parallel^{exptl}$ is 167 × 10^{-4} cm^{-1}. Taking the average A_z (=$A_\parallel$) from Kabanos' complexes[40] gives the back-calculated contribution of $A_\parallel(N_{amide})$. We estimate $A_{\parallel,i}(O_{amide})$ of 170 × 10^{-4}, 154 × 10^{-4} and 182 × 10^{-4} cm^{-1} if the correct structures are **II**, **VIII** or **IX**, respectively, and $A_{\parallel,i}(N_{amide})$ of 114 × 10^{-4}, 131 × 10^{-4} and 103 × 10^{-4} cm^{-1}, respectively.

According to these estimates for $A_{\parallel,i}(O_{amide})$ and $A_{\parallel,i}(N_{amide})$, structure **IX** is ruled out and **II** less probable; **VIII** leads to acceptable $A_{\parallel,i}$ values but has an improbable seven-membered equatorial chelate ring. Therefore, of **II**, **III**, **VIII** and **IX**, only **III** appears acceptable and we expect it to correspond to the monomeric species in moderately basic solutions containing Gly-Sar. A similar geometry was proposed for simple dipeptides at pH > ≈ 4.

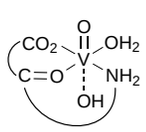
According to the value $A_\parallel^{exptl} \approx (161 \pm 1) \times 10^{-4}$ cm^{-1} for Gly-Ala, Ala-Gly and Ala-Ala systems in the range pH 7.5–10, and taking **VII**, which corresponds to MLH_{-2} stoichiometry, as the major species, one can back-calculate the contribution of $A_\parallel(N_{amide})$ to $A_\parallel^{est}$ as ≈(130 ± 4) × 10^{-4} cm^{-1}. Costa Pessoa *et al.*[38] proposed that $A_{\parallel,i}(N_{amide})$ is (130–146) × 10^{-4} cm^{-1} and Cornman *et al.*[39] determined values for $A_{\parallel,i}(N_{amide})$ of



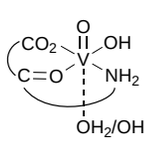
VII

A_{II}^{est}=(161-165)x10^{-4} cm^{-1}

[Assuming $A_{II,i}$ (N_{amide})=(130-146)x10^{-4} cm^{-1}]



VIII



(MLH$_{-1}$/MLH$_{-2}$)

IX

Xa **Xb**

A_{II}^{est}=(160-164)x10^{-4} cm^{-1} A_{II}^{est}=(157-161)x10^{-4} cm^{-1}

[Assuming $A_{II,i}$ (N_{amide})=(130-146)x10^{-4} cm^{-1}]

(128–148) × 10^{-4} cm^{-1} (depending on the ligand) and 150.4 × 10^{-4} cm^{-1} for $A_{\|,i}(O_{amide})$. All these values agree with our present results. Similar calculations for $g_{\|}$ taking $g_{\|}^{exptl}$ = 1.953–1.956 give estimates of $g_{\|,i}(N_{amide}) \approx$ 1.983–1.995, which appear to be high. The recent crystal-structure characterisation of $[NEt_4][VO(O_2)(Gly\text{-}Gly\text{-}O)]\cdot 1.58H_2O$,[41] where NH_2, N_{amide} and CO_2^- are equatorial, also supports the assignment of **VII** as the likely structure for this stoichiometry.

For N_{imine} the $A_{\|,i}(N_{imine})$ contributions in equation (3) were estimated to vary between 162 × 10^{-4} and 171 × 10^{-4} cm^{-1} in oxo(*N*-salicylideneamino acidato)vanadium(IV) complexes,[42] and 177.6 × 10^{-4} cm^{-1}.[39] The N_{amide} contributions also reflect the balance of electron donation from the ligands. Given inaccuracies in the determination of ESR parameters, we believe that the contribution of $A_{\|,i}(N_{amide})$ lies in the range (130–146) × 10^{-4} cm^{-1}, the lower limit being highly probable. This is consistent with the results of Cornman *et al.*[39] The values are lower than for N_{amine} or N (pyridine): N_{amide} induces higher electron density at the metal decreasing the interaction between the unpaired electron and ^{51}V nuclear spin.

The major species detected at pH ≈ 7–10 could also correspond to structures such as **Xa** or **Xb**. The former implies $A_{\|,i}(N_{amide}) \approx$ 136 × 10^{-4} cm^{-1}, which is reasonable, but the equatorial ring presents some steric constraint affecting the amide group planarity. The latter resembles that often proposed for vanadium(V)–dipeptide complexes.[20,25–27] Assuming that the vicinal effect is the main factor determining the CD intensity, structure **Xb** would not be in accord with experimental spectra. However, if inherent asymmetry is dominant, this structure is plausible and an acceptable contribution of $A_{\|,i}(N_{amide})$ can also be estimated assuming this structure: 146 × 10^{-4} cm^{-1}. However, the absence of CD for solutions containing L-alanine amide and VO^{2+} at high pH indicates that the carboxylate group is important in peptide co-ordination and probably occupies an equatorial position. Species with the ligand co-ordinated as in **VII** but with OH^- equatorial and H_2O axial are improbable; that would correspond to $A_{\|}^{est} \approx$ (154–158) × 10^{-4} cm^{-1} [assuming $A_{\|,i}(N_{amide}) \approx$ (130–146) × 10^{-4} cm^{-1}], too low compared with $A_{\|}^{exptl}$.

For dipeptides the stabilities of $CuLH_{-1}$ with CO_2^-, N^-_{amide}, NH_2 and H_2O equatorial follow the order Ala-Gly < Ala-Ala < Gly-Ala.[29,31] The VOL_{-2} complexes probably have identical equatorial co-ordination and the same order, partly explaining the weaker CD for Ala-Ala than for Gly-Ala. The lowest CD values for Ala-Gly solutions are expected for structure **VII**. Overall, the gradual decrease in CD at pH > 7.5 may be assigned to a decrease in MLH_{-2} concentration as the pH is increased.

At pH > ≈ 9.5 the weak ESR and CD spectra indicate low concentrations of monomeric and optically active species. The red shift and changes of CD at pH > 9.5–10.5 [*e.g.* Fig. 3(*a*)] suggest a new more hydrolysed optically active species. The major species are hydroxo complexes such as $[\{(VO)_2(OH)_5\}_n]^{n-}$ and/or $[\{(VO)_2(OH)_6\}_m]^{2m-}$. Species corresponding to MLH_{-3} and $M_2L_2H_{-5/-6}$, probably ESR silent, could also be considered.

At pH ≈ 10.6 the CD of Ala-Ala [Fig. 3(*a*)] differs probably due to small amounts of species such as $M_2L_2H_{-5}$. The isodichroic point at pH ⩾ 10.6 and the reasonably intense ESR spectrum pH ≈ 11.5 suggest an equilibrium between $M_2L_2H_{-5}$ and an optically and ESR-active species with stoichiometry MLH_{-3} (and $M_2L_2H_{-6}$?). At pH > 11.5–12 the solutions are reddish brown, *i.e.* $[VO(OH)_3]^-$. At pH ≈ 13, the ESR spectrum is indeed reasonably intense.

Tripeptides. The ESR spectra for basic solutions containing tripeptides and VO^{2+} are very weak; ESR-silent oligomers predominate. At pH > 7 solutions containing Gly-Gly-Ala are brown indicating extensive hydrolysis. The CD spectrum is weak especially at λ < 550 nm. A band corresponding to Δε > 0 dm^3 mol^{-1} cm^{-1} may (apparently) be detected in the range 530–700 nm. These spectra resemble those for Gly-Pro, so the N_{amide} close to CO_2^- does not deprotonate/co-ordinate. However, as the contribution of N_{amide} (near the amine group) to the vicinal effect will be small, we cannot exclude its co-ordination.

At pH > 8 solutions containing Ala-Gly-Gly are also brown, VO^{2+} being extensively hydrolysed. As for Gly-Gly-Ala, the $|\Delta\varepsilon_m|$ values are much lower than for dipeptides especially at λ < 550 nm. The $\Delta\varepsilon_m$ values are positive and at pH > 10.8 $\lambda_{max} \approx$ 600 nm as at pH 4.3–4.9; this may perhaps result from similar co-ordination geometries. Spectra at pH > 10.8 are akin to those for Ala-Gly (pH 7.5–8), particularly λ_{max} (I) ≈ 800 nm. Possibly, at least the N_{amide} group close to the amine group deprotonates and co-ordinates, which would correspond to a pK_a > 11.5.

In contrast with the analogous systems involving Cu^{II}, where tripeptides may form complexes even more stable than those with dipeptides,[29,31] we conclude that tripeptides without donor atoms in the side chains hardly co-ordinate to VO^{2+} in basic solutions, the non-optically active oligomeric complexes being the major species present. The tripeptide complexes are in very low concentrations and it is not possible to discuss their structures.

Acknowledgements

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