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Speciation in Vanadium Bioinorganic Systems. 3. A Potentiometric and ^{51}V, ^{13}C and ^{1}H NMR Study of the Aqueous H^+-Vanadate(V)–L-Prolyl-L-alanine/L-Alanyl-glycine Systems

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The speciation as well as some structural and kinetic properties of the complexes that form between vanadate and prolylalanine(PAH), and vanadate and alanylglycine(AGH) respectively, have been characterised using potentiometry and ^{51}V, ^{13}C and ^{1}H NMR spectroscopy. Formation constants were determined in 0.600 M Na(Cl) at 25 °C. Data from the combined EMF and ^{51}V NMR study were evaluated with the computer program LAKE and cover the pH range $3.3 \leqslant pH \leqslant 9.8$ for the PAH system, and $2.5 \leqslant pH \leqslant 9.2$ for the AGH system. The pK_a values for prolylalanine were determined as 8.79 and 3.27, and for alanylglycine as 8.11 and 3.15. The ternary system H^+–$H_2VO_4^-$–PAH contains one −1 charged complex, $VPAH^-$, having $\log \beta_{0,1,1} = 2.44 \pm 0.02$. The corresponding AGH system also contains one −1 charged complex, $VAGH^-$, with $\log \beta_{0,1,1} = 1.715 \pm 0.009$. The errors given are 3σ. The structural characteristics were further explored by ^{13}C and ^{1}H NMR spectroscopy. Both ligands were found to bind to vanadium through the α-N, the deprotonated peptide N and the carboxylate O, thus acting as a tridentate ligand. Equilibrium conditions are illustrated in distribution diagrams, and proposed structures of the complexes are given. Furthermore, some kinetic measurements at neutral pH were performed. The formation of the vanadium–prolylalanine complex is complete after 60 h, whereas the vanadium–alanylglycine system needs only 5 h to reach equilibrium.

Vanadium is considered one of the most important metals used in the primitive earth.[1] However, little is known about the structure and function of vanadium compounds of biogenic ligands. The accumulation of vanadium in tunicates (e.g. *Ascidia nigra*), fan worms (*Pseudopotamilla*) and toadstools (e.g. *Amanita muscaria*), where the metal is stored as V^{III}/V^{IV} (tunicates and fan worms) and V^{IV} (*Amanita* toadstools), may provide an integral part of the organism's protection system.[1,2a] Vanadium, in the form of vanadium–iron–sulfide clusters, also occurs as the cofactor in the vanadium–nitrogenase of nitrogen-fixing bacteria (e.g. *Azotobacter*).[3] The vanadate-dependent haloperoxidases in marine brown algae, such as *Ascophyllum*, *Laminaria* and *Chorda*, and in marine red algae (*Ceramium* and *Corallina*) and in the fungus *Curvularia inaequalis* are also believed to be important for defensive purposes.[2b] Here, vanadium is in the oxidation state V and bound to oxygen and nitrogen histidine donors. In the oxidation states III, IV and V, vanadium binds to the protein transferrin. Vanadotransferrin, a complex with O/N coordination but with the oxygen functions dominating, is probably the form in which vanadium is transported in more advanced organisms.[2a] Moreover, vanadate is known to inhibit or stimulate a variety of phosphorylation enzymes,[4a] suggesting bonding interactions between vanadium and side-chains of the protein matrix.

In order to model vanadium binding to proteins, we have earlier investigated the complexation behaviour of vanadate to a dipeptide containing the *N*-heterocyclic amino acid histidine, viz. L-alanyl-L-histidine (AH).[5] In the H^+–$H_2VO_4^-$–AH system, a zero-charged 1 : 1 vanadate-to-ligand complex is formed, with the formation constant $\log \beta_{1,1,1} = 9.44$. This complex can be deprotonated and the pK_a value is 6.89, identical to that of alanylhistidine itself (6.85). This suggests that the protonation site is the aromatic imidazole N(1), which is further strongly supported by the fact that protonation

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occurs without any noticeable change in the ^{51}V NMR chemical-shift value.

In the present study, the complex formation between vanadate and L-prolyl-L-alanine (PAH; Fig. 1a) or L-alanyl-glycine (AGH; Fig. 1b) has been investigated. AGH was chosen in order to deduce any difference in complexation behaviour of a heterocyclic and a very simple amino acid. In PAH the α-N is the heteroatom in a five-membered ring and hence in a fixed position, while access to the α-N in AGH is more flexible. The study was performed in an aqueous 0.600 M Na(Cl) medium, since when the present study started the H^+–$H_2VO_4^-$ system was best known at this ionic strength.[6] A recent reinvestigation,[7] using a 500 MHz spectrometer and a more sophisticated evaluation of peak integrals, especially for overlapping peaks, has resulted in even more accurate, though similar, values of the vanadate formation constants. These new values were tabulated in the foregoing dipeptide study[5] and have been used in the present work. Since the equilibrium conditions and NMR characteristics of the vanadate system are now also determined in the physiologically relevant 0.150 M Na(Cl) medium,[7] we will use this medium in future work on vanadium bioinorganic systems. A study of the medically important insulin mimetic vanadium–maltol system has already been completed in this medium.[8]

The dependence of product formation on pH and concentration of vanadate and dipeptides has been determined, and the combined potentiometric and quantitative ^{51}V NMR data have been evaluated with the computer program LAKE.[9] As a result, the complete speciation in the systems is presented. In order to obtain more information about the rate of complexation, some kinetic measurements have been carried out.

Experimental

Chemicals and analyses. L-Prolyl-L-alanine, $C_8H_{14}N_2O_3 \cdot H_2O$ (Sigma, H_2O content 1.0 mol/mol), and L-alanyl-glycine, $C_5H_{10}N_2O_3$ (Sigma), must be stored at less than 0 °C but were tempered to 25 °C before use. Both dipeptides were used without purification. The amount of water in the PAH chemical was determined from titration analysis to be 10.3 ± 1.0% (1.06 ± 0.10 mol/mol). Sodium chloride (E. Merck *p.a.*) was dried at 180 °C and used without further purification. Boiled

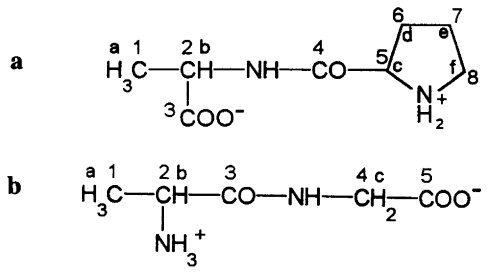

Fig. 1. Schematic structure of L-prolyl-L-alanine (a) and L-alanyl-glycine (b).

distilled water was used for preparation of all solutions. After boiling, the water was cooled to 25 °C and stored in the absence of air. Neutral and alkaline solutions were prepared and stored under argon to protect them from atmospheric $CO_2(g)$. Vanadate stock solutions and solutions of hydrochloric acid and sodium hydroxide were prepared and standardised as described in Ref. 5. All V-dipeptide solutions were kept for at least 4 days before measuring to ascertain equilibration.

Notation. The equilibria studied are written with the components H^+, $H_2VO_4^-$ and PAH/AGH. Thus, the complexes are formed according to

$$pH^+ + qH_2VO_4^- + r\text{PAH/AGH} \rightleftarrows (H^+)_p(H_2VO_4^-)_q(\text{PAH/AGH})_r^{p-q}$$

Formation constants are denoted $\beta_{p,q,r}$ and, for brevity, complexes are often given the notation (p,q,r). The total concentrations of vanadium, prolylalanine and alanylglycine are denoted $[V]_{tot}$, $[PA]_{tot}$ and $[AG]_{tot}$, respectively.

Potentiometric measurements. The EMF measurements in the binary H^+-PAH/AGH systems were carried out in 0.600 M Na(Cl) medium at 25 °C as described in Ref. 5. Owing to slow equilibration in parts of the pH range for the V-dipeptide systems, the conventional titration technique had to be complemented. Therefore, instead of titrating a solution over a period of several days, individual samples were prepared directly at different pH values, total concentrations and concentration ratios. After equilibration these samples were used for NMR measurements, directly after which pH was measured with an Ingold U402-M6-S7/100 combination electrode calibrated against buffer solutions of known $[H^+]$ in 0.600 M Na(Cl).

NMR spectroscopy. ^{51}V NMR spectra were recorded at 131.6 MHz using a Bruker AMX 500 MHz spectrometer as described in Ref. 5. The probe temperature was 25 ± 1 °C, and the chemical shifts are reported relative to the external reference $VOCl_3$ (0 ppm). ^1H NMR spectra were obtained with a Bruker AM 360 MHz spectrometer in rotating 5 mm diameter vials. The probe temperature was 20 ± 1 °C. All chemical shifts are given in ppm from external sodium 1,1,2,2-tetramethylpropionate in D_2O. ^{13}C NMR spectra were scanned at 20 ± 1 °C with the same instrument as ^1H NMR but at 90.6 MHz and referenced against DMSO. The samples were measured in 10 mm precision tubes.

Potentiometric data. The acidity constants for prolylalanine were determined from five titrations (88 experimental points). The pH range was $2.0 \leqslant pH \leqslant 9.3$ and the total concentration range was $5 \leqslant [PA]_{tot}/mM \leqslant 10$. Data used to evaluate the acidity constants for alanylglycine were from four titrations (69 experimental points). The pH and concentration ranges covered were $2.5 \leqslant pH \leqslant 9.0$ and $5 \leqslant [AG]_{tot}/mM \leqslant 10$.

In the three component systems, pH values were measured and EMF effects calculated for each solution that was prepared for the NMR measurements (see above). In the case of the H^+–$H_2VO_4^-$–PAH system, a total of 38 individual samples were measured, and for the H^+–$H_2VO_4^-$–AGH system the number of samples was 42.

NMR data. For the V-PAH system, 38 spectra were recorded in the ranges $3.3 \leqslant pH \leqslant 9.8$; $1.25 \leqslant [V]_{tot}/mM \leqslant 64$ and $2.0 \leqslant [PA]_{tot}/mM \leqslant 80$. For V-AGH, a total of 42 spectra were recorded in the ranges $2.5 \leqslant pH \leqslant 9.2$; $1.25 \leqslant [V]_{tot}/mM \leqslant 10$ and $4 \leqslant [AG]_{tot}/mM \leqslant 120$. Directly after recording the NMR spectra, the pH of each of the solutions was measured. Spectra were then quantitatively evaluated, using the Bruker software computer program UXNMR/P, to obtain precise integral values.

Computer calculations. The EMF and quantitative ^{51}V NMR data were evaluated with the least squares program LAKE,[9] as described in Ref. 5. Calculation and plotting of distribution diagrams were performed with the program SOLGASWATER.[10]

Results and discussion

The H^+–$H_2VO_4^-$–PAH system. To determine the speciation in the ternary H^+–$H_2VO_4^-$–PAH system correctly, the binary subsystems H^+–$H_2VO_4^-$ and H^+-PAH should be precisely known. The equilibrium conditions and NMR characteristics of the H^+–$H_2VO_4^-$ system are, as mentioned in the introduction, well known in 0.600 M Na(Cl).[5–7] The notation used for vanadate species in the present work is in accordance with Table 1 in Ref. 5, viz. V_1 = monomeric vanadate, V_2 = dimeric vanadate, V_4 = cyclic tetrameric vanadate V_5 = cyclic pentameric vanadate and V_{10} = decavanadate species. The acidity constants for prolylalanine have not been reported earlier in 0.600 M Na(Cl) and at 25 °C, and were thus determined under these conditions. The determination was made with the program LAKE, by evaluation of data from potentiometric titrations. Formation constants and pK_a values obtained are given in Table 1.

^{51}V NMR spectra for the ternary system show, in addition to the vanadate resonances, only one rather broad and symmetric resonance at a chemical shift value of −495 ppm, irrespective of pH. Since resolution enhancement of the resonance gives no indication of overlapping hidden peaks, it originates from a single V-PAH complex. A series of spectra from solutions at a 4/1 ligand-to-vanadate ratio ($[V]_{tot}$ = 4 mM), is shown in Fig. 2. As can be seen, complexation occurs in a wide pH range from around 3 to 10.

Table 1. Species and acidity constants for prolylalanine (PAH) and alanylglycine (AGH) [0.600 M Na(Cl), 25 °C]. The (*p,q,r*) notation is defined in the Experimental section.

(*p,q,r*)	Notation	log β ± 3σ	pK_a
1,0,1	PAH_2^+	3.27 ± 0.01	3.27
0,0,1	PAH	0	8.79
−1,0,1	PA^-	−8.79 ± 0.01	-
1,0,1	AGH_2^+	3.15 ± 0.02	3.15
0,0,1	AGH	0	8.11
−1,0,1	AG^-	−8.11 ± 0.02	-

To determine the speciation in the vanadate–prolylalanine system, both EMF and ^{51}V NMR data were used. However, since the V-PAH complexes formed were found to have a proton consumption very similar to the binary vanadate subsystem, the EMF effects are small. Thus, EMF data are not very decisive, and the study was focused on NMR measurements. By spreading data with respect to pH, total concentrations and concentration ratios, a relatively small number of data was enough to establish the speciation. Earlier results from the vanadate–alanylhistidine system showed a 1:1 vanadate to dipeptide complex to form.[5] From the similarity of coordination properties of the two dipeptides, the same stoichiometry was expected for the vanadate–prolylalanine complex. As expected, the data analysis shows the presence of only one vanadium–ligand complex, $VPAH^-$, with the formation constant $\log \beta_{0,1,1} = 2.44 \pm 0.02$. Selected results from LAKE calculations are summarised in Table 2a. Formation constants for systematically chosen complexes $(H^+)_p(H_2VO_4^-)_q(PAH)_r^{p-q}$ were varied so as to minimise the error squares sum, *U*, and that for the (0,1,1) complex is by far the lowest. Furthermore, there are no systematic deviations in the EMF and NMR data for this model, and the (0,1,1) species is thus firmly established.

The distribution of vanadium-containing species as a function of pH was calculated for $[V]_{tot}$ = 4 mM and $[PA]_{tot}$ = 16 mM, and is illustrated in Fig. 3a. For clarity, no species containing less than 5% of total vanadium is shown. For the concentrations given, the $VPAH^-$ complex has its maximum concentration at pH between 5.5 and 8, where it binds ca. 40% of the total vanadium. It does, however, exist in a wide pH range from 3 to 10. In Fig. 3b a simplified distribution diagram is shown with the experimental ^{51}V NMR data points indicated by symbols. The calculated model fits the experimental data well. The small deviations that occur at low pH are due to evaluation problems, originating from overlapping of one of the decavanadate peaks (V_{10}') and the $VPAH^-$ peak, cf. Fig. 2. The corresponding PAH distribution is shown in Fig. 4. Since the diagram represents a condition in which an excess of PAH ($[PA]_{tot}/[V]_{tot} = 4$) is employed, only at most 10% of the dipeptide is bound in the $VPAH^-$ complex.

^1H and ^{13}C NMR studies of the V-PAH system have led to the identification of the binding sites in the ligand. As is the case for other dipeptides when coordinated to vanadium, PAH forms two five-membered rings.[11] The ligating atoms are the α-N, the carboxylate O and the

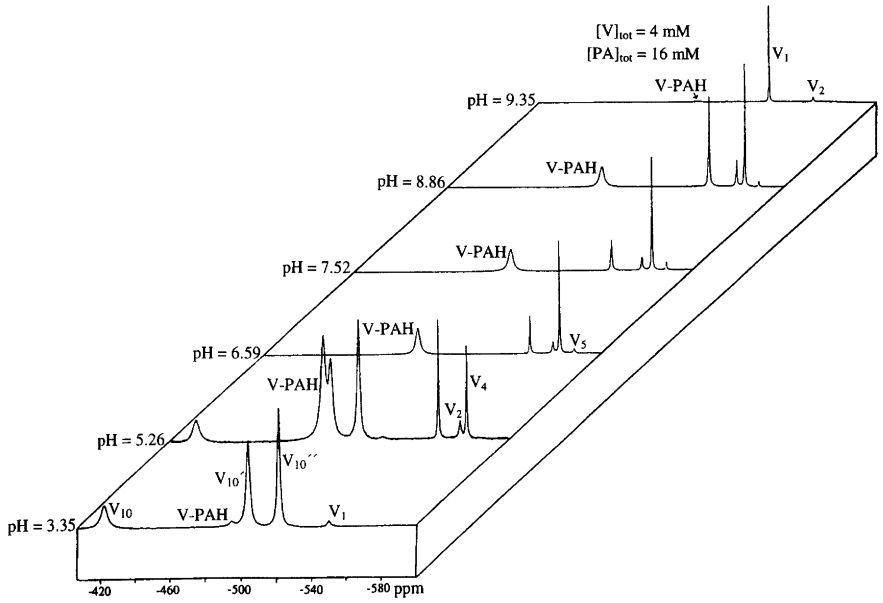

Fig. 2. ^{51}V NMR spectra of aqueous solutions containing vanadate and prolylalanine ($[V]_{tot}/[PA]_{tot}=4/16$ mM) at different pH values. V_1, V_2, V_4, V_5 and V_{10} refer to monomeric, dimeric, tetrameric, pentameric and decavanadate species, respectively.

Table 2. Results from the LAKE calculations. The binary H^+–$H_2VO_4^-$ constants are published in Ref. 5 and the binary constants for the peptide systems are compiled in Table 1. The (*p,q,r*) notation is defined in the experimental section.

(a) The vanadate-prolylalanine system

(*p,q,r*)	log β±3σ	10^3 *U*
0,1,1	2.44±0.02	1.95
0,2,2	6.98±0.08	13.36
0,1,2	4.08±0.07	11.75
0,2,1	5.23±0.04	7.45

(b) The vanadate-alanylglycine system

(*p,q,r*)	log β±3σ	10^5 *U*
0,1,1	1.715±0.009	3.6
0,2,2	5.76±0.07	136.3
0,1,2	2.86±0.06	101.8
0,2,1	4.66±0.02	16.3

deprotonated amide N. This has also been reported for the dipeptide alanylhistidine,[12] and is strongly supported by the coordination shifts $\Delta\delta(^{13}C)=\delta(V\text{-}PAH)-\delta(PAH)$ (Table 3 and Fig. 1a). The carbons adjacent to the nitrogen of the peptide linkage, C(2) and C(4), are shifted to low field by 9.63 and 11.84 ppm in the complex with respect to the free ligand. It is also evident from the ^{13}C NMR results that the NH_2 and the CO_2^- groups are involved in the coordination. C(8) and C(5) show Δδ values of 7.84 and 4.58 ppm, while the carboxylate C is shifted by 6.19 ppm. The carbons 1, 6 and 7 are shifted only marginally. The binding mode has been further studied by ^1H NMR spectroscopy. The coordination shifts, $\Delta\delta(^1H)$, on complexation are between −0.38 and 0.55 ppm (Table 3). The protons close to a coordinating nitrogen atom are shifted upfield, whereas those close to a coordinating oxygen are shifted downfield.

From the results above it is evident that the carboxylate group, the amide nitrogen and the α-amino group are involved in complexation. Two five-membered rings are thus formed, and vanadate will be hexacoordinated or pentacoordinated, coordination geometries that are preferred in vanadium(V) complexes containing an NON donor set.[13] In Fig. 5a penta-coordination is shown. The involvement of the deprotonated peptide N in coordination is further supported by the observation that alanylproline, with a secondary amide in the peptide linkage, does not coordinate to vanadate.

To characterise further the product that is formed, some kinetic experiments were carried out. A solution providing a maximum of complex concentration ($[V]_{tot}=20$ mM and $[PA]_{tot}=80$ mM) was prepared at physiological pH (6.54), and development of the complex with time was investigated by ^{51}V NMR spectroscopy. The quantitatively evaluated spectra show that the $VPAH^-$ complex concentration increases continuously until it reaches equilibrium after 60 h. The result from the kinetic study does not give an unambiguous answer as to whether the reaction is of first or second order.

The H^+–$H_2VO_4^-$–AGH system. A study similar to that outlined above for prolylalanine was performed for the vanadate–alanylglycine system. As for prolylalanine, no acidity constants have previously been published in 0.600 M Na(Cl). From potentiometric titrations, the pK_a values were determined as 3.15±0.02 and 8.11±0.02 (Table 1).

The complexation between vanadate and the simple dipeptide alanylglycine at different pH values is illus-

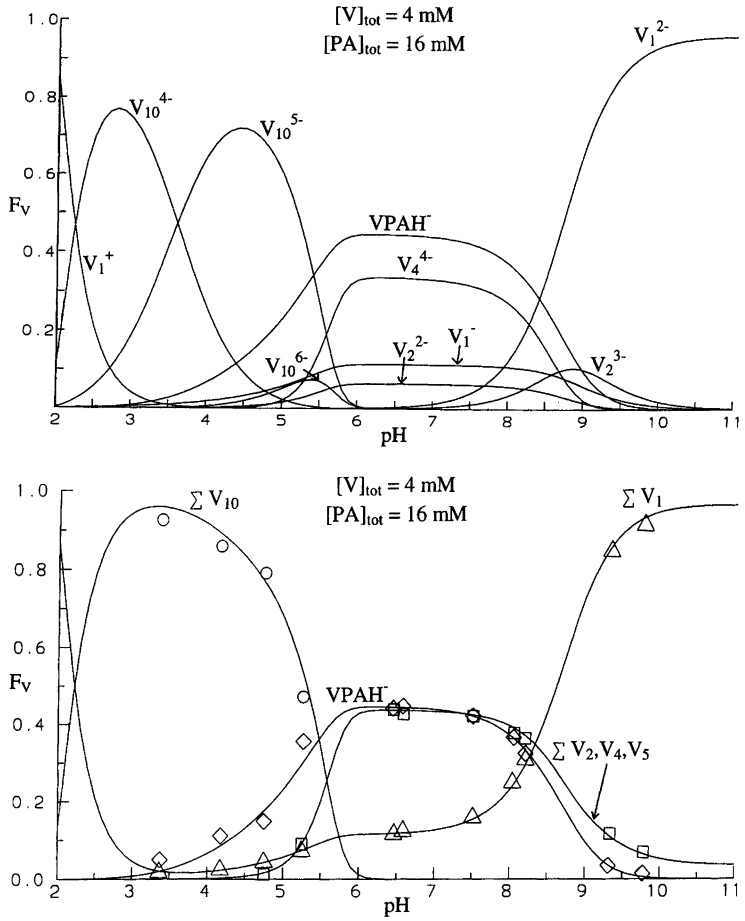

Fig. 3. Diagrams showing the distribution of vanadium, F_V, vs. pH at $[PA]_{tot}/[V]_{tot}=4$. F_V is defined as the ratio between [V] in a species and $[V]_{tot}$. (a) All vanadium-containing species are shown except those containing <5% of $[V]_{tot}$. (b) The $VPAH^-$ complex and the sums for the decavanadate, oligovanadate and monovanadate species are shown. The symbols represent experimental NMR data points.

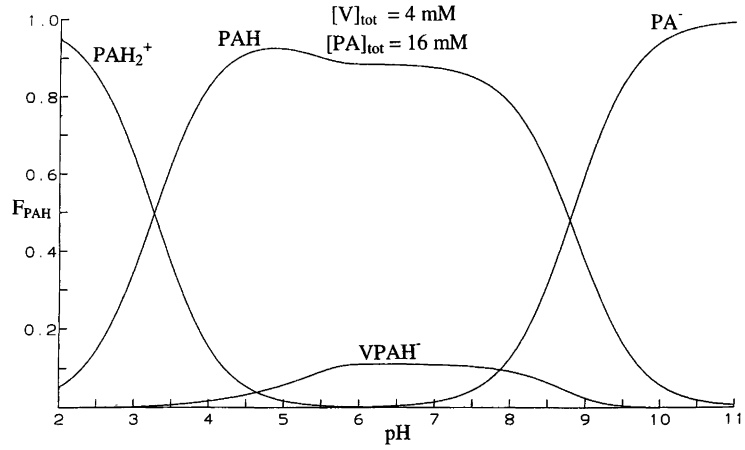

Fig. 4. Distribution of PAH, F_{PAH}, vs. pH at $[PA]_{tot}/[V]_{tot}=4$. F_{PAH} is defined as the ratio between [PAH] in a species and $[PA]_{tot}$.

trated in Fig. 6. Since the complexation is weaker than for PAH, a 12/1 ligand-to-vanadate ratio has been chosen for the illustration. Vanadate, in the presence of AGH, gives rise to an NMR signal at −512 ppm in addition to the resonances from free vanadates coexistent under these conditions. As in the PAH system, complexation occurs over a wide pH range, and no change in the chemical shift is observed. Complexation is weak throughout. To obtain quantitative data of high accuracy, a large excess of the ligand is necessary. Using only

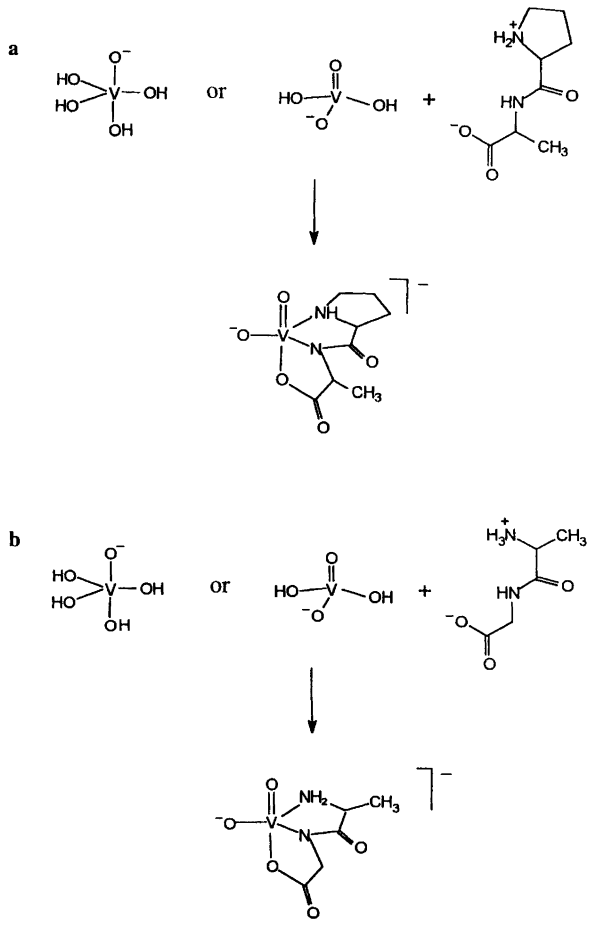

Fig. 5. Proposed formation and schematic structure of (a) the $VPAH^-$ and (b) the $VAGH^-$ complex.

a four-fold excess, as in the case of the prolylalanine–vanadate system, at most 1.6% of the vanadium is bound in the V-AGH complex. Therefore, most measurements were performed with a twelve-fold excess of AGH. However, in order to elucidate if the complex formed contains more than one vanadium per AGH, some measurements with vanadium in excess were also made.

The results from the equilibrium analysis of the V-AGH complexation are shown in Table 2b. A mononuclear, monoligand, -1 charged species, $VAGH^-$, gives the lowest residual error squares sum and explains all data. Despite the weak complexation, the formation constant has been determined with high precision. The symmetric resonance in the ^{51}V NMR spectra shows no hidden peaks even after resolution enhancement.

In Fig. 7 the distribution of vanadium-containing species in a solution with the same conditions as in Fig. 6 is shown. Even with this 12-fold excess of ligand, only ca. 25% of total vanadium is bound in the $VAGH^-$ complex ($5.5 \lesssim pH \lesssim 7.5$). The stability range, however, is from pH 3 to 9.5. The proposed model shows an excellent fit to the experimental ^{51}V NMR data points.

Comparing the structural formula of AGH with that of PAH leads to the assumption that the binding sites of AGH should be analogous to those of PAH. This is also strongly supported by the ^1H and ^{13}C NMR measurements. The coordination shifts in the ^1H and the ^{13}C spectra upon complexation are given in Table 3. The ^{13}C NMR results show that the methyl carbon atom has a coordination shift of only 1.22 ppm, whereas the carbons close to the coordinating atoms are shifted by 5.66 to 10.43 ppm. This indicates that AGH is a chelating ligand forming two five-membered rings with vanadate, as does

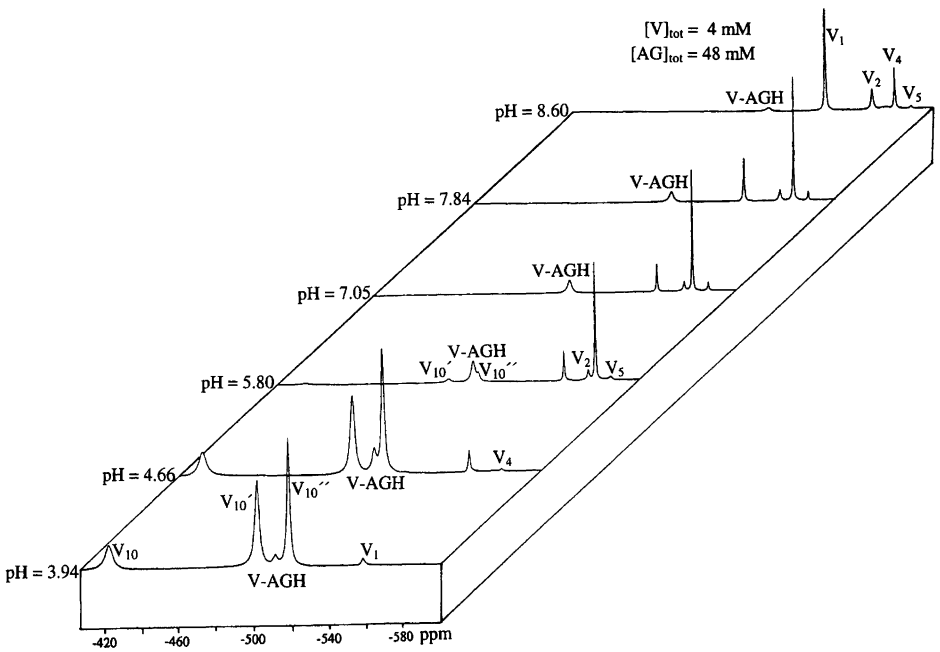

Fig. 6. ^{51}V NMR spectra of aqueous solutions containing vanadate and alanylglycine ($[V]_{tot}/[AG]_{tot} = 4/48$ mM) at different pH values.

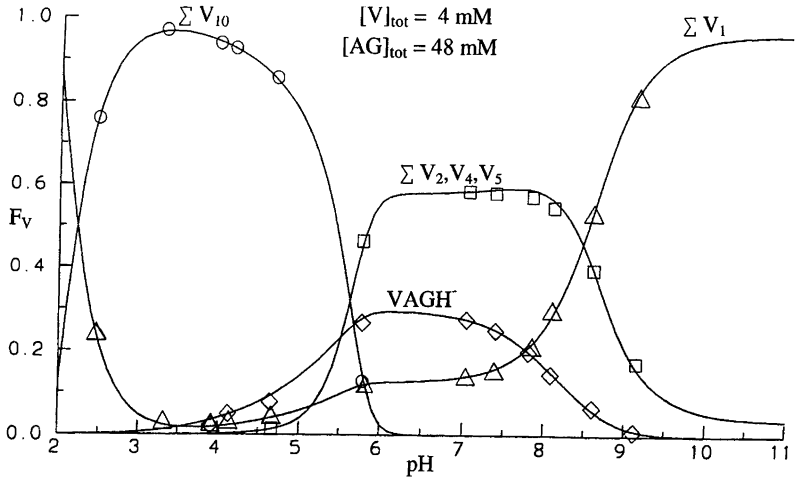

Fig. 7. Distribution of vanadium, F_V, vs. pH at $[AG]_{tot}/[V]_{tot} = 12$. The $VAGH^-$ complex and the sums for the decavanadate, oligovanadate and monovanadate species are shown.

Table 3. ^{13}C and ^1H NMR results for the V-PAH (20/80 mM) and V-AGH (10/120 mM) systems.

Atom no.[a]	PAH[b] (ppm)	V-PAH[c] (ppm)	$\Delta\delta^{c,d}$ (ppm)	AGH[b] (ppm)	V-AGH[c] (ppm)	$\Delta\delta^{c,d}$ (ppm)
C(1)	14.58	16.16	1.58	13.93	15.15	1.22
C(2)	49.03	58.66	9.63	46.84	52.51	5.66
C(3)	177.03	183.22	6.19	168.21	[e]	[e]
C(4)	166.26	178.10	11.84	40.94	51.37	10.43
C(5)	57.39	61.97	4.58	173.74	180.27	6.53
C(6)	26.97	26.27	−0.70			
C(7)	21.30	22.52	1.22			
C(8)	44.17	52.01	7.84			
H(a)	1.41	1.52	0.11	1.57	1.45	−0.12
H(b)	4.18	4.73	0.55	4.17	[e]	[e]
H(c)	4.44	[e]	[e]	3.84	4.42	0.58
H(d)	2.18/2.50	1.96/2.26	−0.22/−0.24			
H(e)	2.12	1.78	−0.34			
H(f)	3.47	3.09/3.28	−0.38/−0.19			

[a] See numbering in Fig. 1. [b] Uncomplexed ligand in the presence of the V-PAH complex. [c] C.f. Fig. 5. [d] Coordination shift $\Delta\delta$ = δ(complex) − δ(peptide); a positive sign indicates deshielding on coordination; minus denotes a shift to higher field. [e] Not visible.

PAH. The ^1H NMR measurements confirm these results. A five-coordinated structure is proposed but six-coordination, by addition of a water molecule, is also plausible. The proposed structure is presented in Fig. 5b.

The complex formation was evaluated in a kinetic study, using a solution with $[V]_{tot} = 20$ mM and $[AG]_{tot} = 240$ mM at pH 6.39. ^{51}V NMR spectra evaluated at defined time intervals show that the V-AGH system reaches equilibrium within 5 h. As for V-PAH, the result does not give an unambiguous answer as to whether the reaction is of first or second order.

Comments

A comparison of the complexation between vanadate and the two dipeptides prolylalanine and alanylglycine shows that both form a single −1 charged 1 : 1 vanadate-to-dipeptide complex. While PAH is a fairly strong complexator for vanadium, the amount of complexation with AGH reaches only a fifth of the former at similar concentration and pH conditions. The 1:1 stoichiometry is often seen in similar systems.[5,11] In our recent study of the vanadate–alanylhistidine (AH) system, it was found that along with the −1 charged 1 : 1 complex, an additional 0 charged complex was formed, with the aromatic nitrogen of the imidazole ring providing the protonation site.[5] In Fig. 8, ^{51}V NMR spectra from the three systems are shown for the same vanadate and ligand concentrations. From the distribution diagram in Fig. 9 it is evident that the amount of vanadium bound to AGH is much lower than that bound to PAH or AH. The reason for this could be that hydrogen bonding/stacking interactions between the N-heterocycles in solution provide an additional stabilisation in the cases of PAH and AH. The pH range of existence is similar for the three V-dipeptide complexes, but with V-AGH having a slightly narrower range. We infer that the difference in complexation time between PAH (60 h) on the one hand

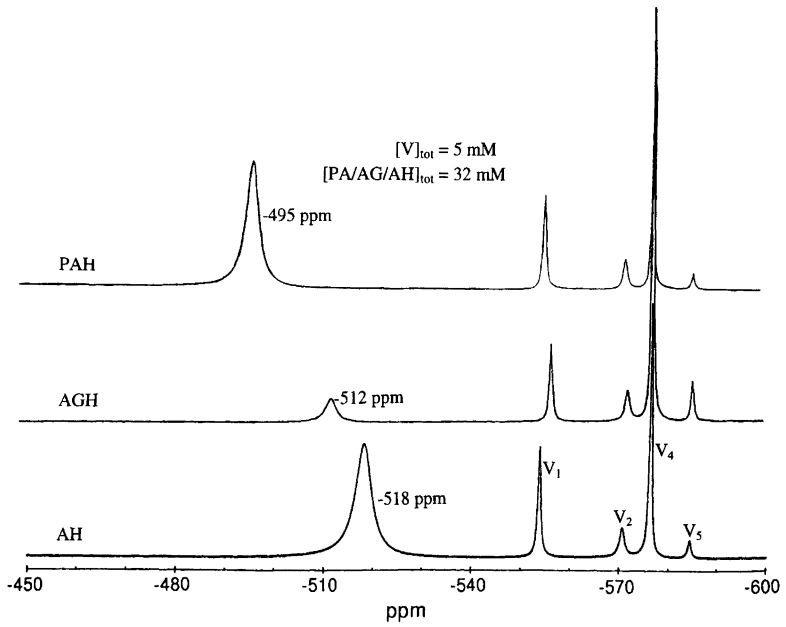

Fig. 8. ^{51}V NMR spectra of V-PAH, V-AGH and V-AH solutions at pH 7.3–7.5. $[V]_{tot}=5$ mM and $[peptide]_{tot}=32$ mM.

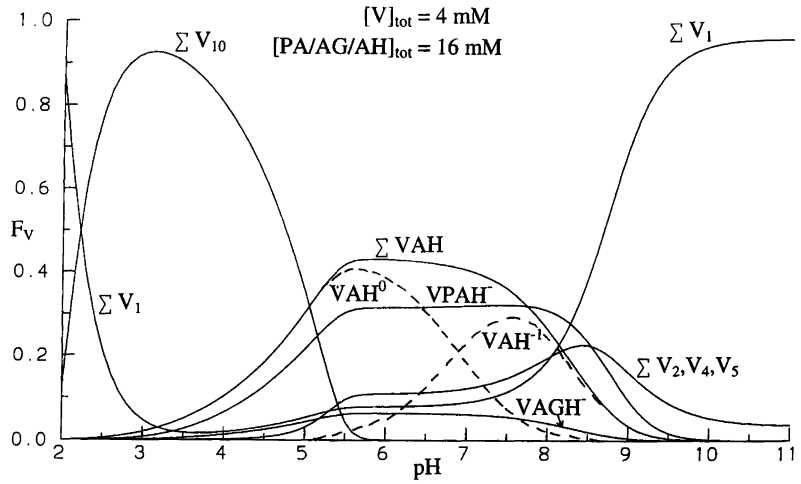

Fig. 9. Distribution of vanadium, F_V, vs. pH at $[V]_{tot}=4$ mM and $[PA]_{tot}$,$[AG]_{tot}$ and $[AH]_{tot}=16$ mM.

and AGH and AH (5 h) on the other, arises from the different accessibility for V to the α-N of the dipeptides. In PAH the α-N is in a five-membered ring and hence in a fixed position, while access to the α-N in AGH and AH is more flexible.

The equilibrium constants presented in the present study are determined from the components H^+–$H_2VO_4^-$–PAH/AGH. They are thus valid for all pH values. In other studies, e.g. Ref. 12, the equilibrium constants are often determined from the sum of the monomeric vanadate/ligand species concentrations and are therefore valid only at a certain pH value. In contrast to several other systems (V-His[5], V-Gly-Tyr[13]), the V-PAH and V-AGH systems are redox-stable.

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