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Speciation in Vanadium Bioinorganic Systems. 1. A Potentiometric and ^{51}V NMR Study of Aqueous Equilibria in the H^+–Vanadate(V)–L-α-Alanyl-L-histidine System

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The full speciation in the system H^+–$H_2VO_4^-$–L-α-alanyl-L-histidine(Ah) has been determined from potentiometric (glass electrode) and ^{51}V NMR measurements. The study was performed in 0.6 M Na(Cl) medium at 25°C. Data cover the range $2 \leq pH \leq 9.5$ and were evaluated with the computer program LAKE, able to treat combined EMF and NMR data. The pK_a-values for Ah were determined as 8.08, 6.85 and 2.76. In the ternary system, two complexes, $(H^+)_p(H_2VO_4^-)_q(Ah)_r$, having the (p,q,r) values (0,1,1) and (1,1,1) with log $\beta_{0,1,1} = 2.55 \pm 0.04$ and log $\beta_{1,1,1} = 9.44 \pm 0.05$ ($pK_a = 6.89$) explain all data. The errors given are 3σ. The complexes formed are fairly strong. At a ratio Ah/V = 4:1 about 60% of V is bound in ternary complexes, with the optimum amount at around pH 6. Equilibrium conditions are illustrated in distribution diagrams and structures of the complexes formed are proposed. The system H^+–$H_2VO_4^-$–L-histidine(His) has been partly studied. The pK_a-values for His were determined as 9.103, 6.146 and 1.778. His has a maximum complexation to vanadate near pH 5, and the complexes formed are weaker than those for Ah. Spontaneous reduction was, however, found to occur, so accurate quantitative evaluation could not be performed on the $H_2VO_4^-$–His system.

Vanadium is, among the 29 elements having a biological role, the tenth most abundant in the earth's crust.[1] Because of the increasing amount of vanadium in the atmosphere, the toxicology of vanadium has become of great interest.[2a] Vanadium is used as a catalyst in industrial processes and as an alloy additive in various types of steel.[3] Furthermore, it is for instance used in dental implants and in pharmaceutical agents.[2b] Several vanadium compounds of therapeutic importance are related by the way they lower glucose and cholesterol levels, their diuretic and natriuretic effects, a contracting effect on blood-vessels and an ability to increase the oxygen-affinity of hemoglobin and myoglobin. Vanadium can act as a stimulatory, regulatory and inhibitory agent in various enzymatic phosphate-metabolizing reactions.[2d,4a] While vanadium in the oxidation state IV is the more stimulatory form of the metal, vanadium(V) is the more inhibitory form.[2c] Because of the physiological relevance of vanadium, there is much interest in the complexation behaviour of vanadate(V) with organic ligands. Histidine (His, Fig. 1A) present in the protein matrix is able to coordinate several metal ions. Studies have indicated the presence of histidine in the coordination sphere of VO^{2+} in transferrin and in reduced vanadate-dependent haloperoxidase.[4b–e] Although histidine seems to play an

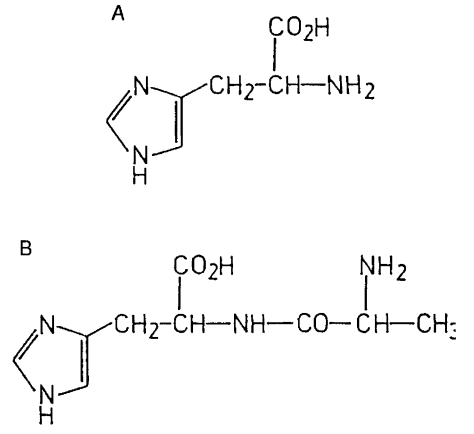

Fig. 1. Structure of L-histidine (A) and L-α-alanyl-L-histidine (B).

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important role in several biochemical processes, relatively weak complexes are formed with vanadate at physiological pH levels.[5]

The complexation of the His-containing dipeptide alanylhistidine (Ah, Fig. 1B) by vanadate in aqueous medium has earlier been studied by Fritzsche *et al.*, at an ionic strength of 0.2 M NaCl.[5] The study was performed in the pH range 7.2–8.8 but focused on the physiological pH value 7.2. Data were best explained by a dinuclear monoligate complex, V_2Ah. This species was, however, not unambigously determined. A complete equilibrium study analysing data with a sophisticated computer program was considered necessary to establish firmly the stoichiometry and formation constants of complexes formed. Therefore, a collaboration was started with the Umeå group. Since the equilibrium conditions in the H^+–$H_2VO_4^-$ system were best known in 0.6 M Na(Cl),[6] this medium was chosen for the present study. A recent reinvestigation[7] using a 500 MHz NMR spectrometer and more accurate evaluation of peak integrals, especially for overlapping peaks, have resulted in even more accurate vanadate formation constants which have been used in the present study (Table 1).

The potentiometric and ^{51}V NMR spectroscopic study of the V-Ah system reported here has been performed over a wide pH range, and the combined EMF and quantitative NMR data have been evaluated with the computer program LAKE.[8] As a result, the complete speciation in the system is presented. The V-Ah species were found to have a 1:1 stoichiometry. The reason why Fritzsche *et al.*[5] in their preliminary study arrived at the 2:1 stoichiometry is caused by (i) a non-specified ethanol and water content present in the Ah chemical and (ii) an error in the graphical evaluation of the stoichiometry and the corresponding equilibrium constants. A re-evaluation on corrected data has shown that a 1:1 species also gives the best explanation of their data.

In this study, besides the histidine-containing dipeptide Ah, the amino acid histidine itself has been studied. Imidazole and uridine (Ur) are known to form strong mixed ligand complexes with vanadate.[9] Since histidine contains an imidazole residue, a complex between vanadate, histidine and uridine is likely to form. This has also been verified in the present work.

Table 1. Species and formation constants for vanadium [0.6 M Na(Cl), 25 °C] used in LAKE calculations on the V-Ah system.

(*p*,*q*,*r*)	Formula	NMR assignment	log β
−1,1,0	HVO_4^{2-}	V_1	−7.946
0,1,0	$H_2VO_4^-$		0
2,1,0	VO_2^+		6.919
−2,2,0	$V_2O_7^{4-}$	V_2	−15.23
−1,2,0	$HV_2O_7^{3-}$		−5.44
0,2,0	$H_2V_2O_7^{2-}$		2.79
−2,4,0	$V_4O_{13}^{6-}$	e-V_4	−8.60
−1,4,0	$HV_4O_{13}^{5-}$		0.13
0,4,0	$V_4O_{12}^{4-}$	V_4	9.89
0,5,0	$V_5O_{15}^{5-}$	V_5	12.16
0,6,0	$V_6O_{18}^{6-}$	V_6	13.9
4,10,0	$V_{10}O_{28}^{6-}$	V_{10} V'_{10} V''_{10}	51.76
5,10,0	$HV_{10}O_{28}^{5-}$		57.83
6,10,0	$H_2V_{10}O_{28}^{4-}$		61.43
7,10,0	$H_3V_{10}O_{28}^{3-}$		62.64

Experimental

Chemicals and analyses. L-Histidine, $C_6H_9O_2N_3$ (E. Merck > 99%) and L-α-alanyl-L-histidine, $C_9H_{14}O_3N_4$ (Sigma, ethanol content 18%, H_2O content 0.5 mol/mol), must be stored at less than 0 °C, but here they were tempered to 25 °C before use. To test possible interference caused by the ethanol content of the dipeptide chemical, the ethanol was completely evaporated. NMR measurements using the pure dipeptide showed that there is no difference in the complexation behavior between pure alanylhistidine and the alanylhistidine containing 18% ethanol. The constants of formation of vanadate–ethanol esters have in fact been shown to be negligible small.[10] The ethanol-containing chemical could therefore be used. Uridine, $C_9H_{12}O_6N_2$ (Janssen Chimica 99%), was first dried at 70 °C, but was found to contain no water. It was thus used without further purification. Vanadate stock solutions were prepared by dissolving sodium metavanadate (E. Merck *p.a.*) in hot water. The solution was cooled to room temperature and then filtrated through porous glass G4 and standardized by evaporation to the solid. Sodium chloride (E. Merck *p.a.*) was dried at 180 °C and used without further purification. Diluted solutions of hydrochloric acid (E. Merck *p.a.*) were standardized against tris(hydroxomethyl)aminomethane (TRISMA-base). Diluted sodium hydroxide was prepared from 'oljelut' (50% NaOH and 50% H_2O) and standardized against the hydrochloric acids. In all preparation of solutions boiled distilled water was used. Alkaline solutions were protected from CO_2(g) by an argon gas stream. All solutions were kept for 4 days before measuring to ascertain equilibria.

Potentiometric measurements. The EMF measurements in the binary H^+-Ah/His systems were carried out as a series of potentiometric titrations in 0.6 M Na(Cl) medium at 25 °C with an automated potentiometric titrator. The glass electrodes used were of the general purpose type, Ingold 201-NS. The free hydrogen ion concentration was determined by measuring the EMF of the cell:

− Ag,AgCl | 0.6 M Na(Cl) || equilibrium solution | glass electrode +

The measured EMF (in mV) may be written as $E = E_0 + 59.157 \log[H^+] + E_j$, where $E_j/mV = -76[H^+]$

$+42.5K_w[H^+]$. E_j is the liquid junction potential at the 0.6 M Na(Cl) || equilibrium solution interface. K_w (1.875×10^{-14}) is the ionic product of water in 0.6 M Na(Cl) and at 25 °C. The constant E_o was determined separately in a solution with known $[H^+]$, before and after each titration. Owing to slow equilibria in parts of the pH range, and in the case of histidine also because of reduction, it was not suitable to study the ternary systems H^+–V(V)–Ah/His by the conventional titration technique. Therefore, point solutions were prepared and the pH was measured separately with an Ingold U402-M6-S7/100 combination electrode. The electrode was calibrated against buffer solutions of known $[H^+]$ in 0.6 M Na(Cl). All solutions were protected from atmospheric carbon dioxide by using an argon gas stream.

NMR measurements. ^{51}V NMR spectra were recorded at 131.5 MHz (11.7 T) using a Bruker AMX-500 MHz spectrometer. The probe temperature was 25 ± 1 °C. The chemical shifts are reported relative to the external reference standard $VOCl_3$. The field frequency stabilization was locked to deuterium by placing the 8 mm sample tubes into 10 mm tubes containing D_2O. Usually, spectral widths of 200 ppm (26.9 kHz) were used and data for the FID were accumulated in 4K blocks. A 90° pulse angle was used, and owing to short relaxation times no relaxation delay was used.

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

$$p\mathrm{H}^+ + q\mathrm{H_2VO_4^-} + r\mathrm{Ah/His} \rightleftarrows (\mathrm{H}^+)_p(\mathrm{H_2VO_4^-})_q(\mathrm{Ah/His})_r^{p-q}$$

Formation constants are denoted $\beta_{p,q,r}$ and complexes are often given in the notation (p,q,r).

Computer calculations. The EMF and quantitative ^{51}V NMR data were evaluated with the least-squares program LAKE.[8] LAKE is able to calculate formation constants with standard deviations out of, for instance, EMF data from titrations/point solutions, quantitative NMR data or combined EMF–NMR data. Formation constants for arbitrary but systematically chosen complexes $(H^+)_p(H_2VO_4^-)_q(Ah)^{p-q}_r$ are varied, so that the error squares sum, $U = \Sigma(W_i\Delta A_i)^2$, is minimized. The complex, or set of complexes, having the lowest U-value forms the model which best explains the experimental data. A_i can be either the total concentrations of components, free species concentrations, NMR peak integrals, chemical shifts or combinations of these. W_i is a weighting factor that must be set to give the different types of data their proper weights. Here we have used a weighting factor that gives NMR peak integrals a predominant contribution to the sum of residuals. In addition, a quality weight is used, giving even low vanadium concentrations a considerable contribution to the error squares sum. Calculation and plotting of distribution diagrams were performed with the program SOLGASWATER.[11]

Potentiometric data. The acidity constants for Ah were determined from three titrations with a total of 85 points. The pH range was 1.5–8.5 and the total concentration range $5 \leq [Ah]/mM \leq 14$. Because of an equilibrium time of at least 4 days and small EMF effects, only one titration ($2.4 \leq pH \leq 9.1$; [V] = 4 mM, [Ah] = 16 mM) was made for the three-component system H^+–$H_2VO_4^-$ – Ah.

Data used to evaluate the acidity constants for histidine comprised two titrations with totally 46 points. The pH range covered was 2.2–9.8 and [His] = 80 mM. For the three-component system H^+–$H_2VO_4^-$ –His only small effects were obtained, and also reduction occurred. Therefore, no usable titrations could be made. Reduction has not been reported earlier, but was revealed by this combined potentiometric/NMR study performed over a period of time of 2 months.

In both ternary systems most of the pH values were measured and the EMF effects calculated from the point solutions that were prepared for the NMR measurements. In the case of H^+–$H_2VO_4^-$ –Ah, a total of 26 point solutions were prepared, and for the system H^+–$H_2VO_4^-$ –His the number of solutions was 27.

NMR data. In the V-Ah system, 26 spectra were recorded in the ranges $2.4 \leq pH \leq 9.5$, $1.25 \leq [V]/mM \leq 40$ and $2.0 \leq [Ah]/mM \leq 64$; for V-His a total of 27 spectra were recorded in the ranges $1.9 \leq pH \leq 9.6$, $1.25 \leq [V]/mM \leq 10$ and $10 \leq [His]/mM \leq 80$. The pH of each solution was measured directly after recording the NMR spectrum, with the carefully calibrated combination electrode mentioned above. Spectra were then quantitatively evaluated using the Bruker software computer program UXNMR/P, or the NMRi program,[12] to obtain precise integral values.

Results and discussion

The H^+–$H_2VO_4^-$ –Ah system. From potentiometric and ^{51}V NMR data, the full speciation in the H^+–$H_2VO_4^-$ –Ah system has been determined. To perform a complete equilibrium analysis of a ternary system, the binary subsystems must each be accurately known in order to characterize properly the speciation. The equilibrium conditions in the vanadate system are well known from a recent study in 0.6 M Na(Cl)[7] (Table 1). For the peptide system no acidity constants have been reported earlier in 0.6 M Na(Cl). In the former study by Fritzsche *et al.*[5] Ah from Serva was used, but is no longer commercially available. To find the exact amount of ethanol plus water in the Sigma chemical used in this study, the concentration of the ligand and the acidity constants were optimized together, using the program LAKE. 80.3% of the chemical was found to be Ah. A titration on a small

Table 2. Species and acidity constants for histidine and alanylhistidine [0.6 M Na(Cl), 25 °C].

(*p,q,r*)	Notation	log β±3σ	pK_a
−1,0,1	His^-	−9.103±0.003	–
0,0,1	His	0	9.103
1,0,1	His^+	6.146±0.003	6.146
2,0,1	His^{2+}	7.924±0.008	1.778
−1,0,1	Ah^-	−8.076±0.019	–
0,0,1	Ah	0	8.08
1,0,1	Ah^+	6.845±0.011	6.85
2,0,1	Ah^{2+}	9.609±0.020	2.76

residue of the Serva chemical gave identical results. The Serva chemical thus contains undeclared ethanol. The calculations in this study are based on two binary titrations. The constants obtained are presented in Table 2.

For the ternary system H^+–$H_2VO_4^-$–Ah, ^{51}V NMR data and EMF point solution data were used to determine the speciation in a pH range from 2.4 to 9.5. The spectra indicate the occurrence of a rather broad symmetric resonance at −518 ppm in addition to the vanadate resonances (Fig. 2). Even after extreme solution enhancement of the peak there is no indication of a second hidden species. This V-Ah complex exists in a wide pH range, having a maximum near pH 6. Severe overlapping with the V''_{10} peak occurs (Fig. 2). Therefore, a program for integral evaluation is necessary. In an effort to gain as much information as possible about the equilibrium species, the following factors have been considered: constant [Ah] with variable [V], constant [V] with variable [Ah], constant concentrations of the reactants with variable pH and [V]/[Ah] = 1/4 with increasing total concentrations.

In the wide pH range covered in this work several species are formed in vanadate solutions,[6] and the resulting equilibrium mixture contains mono- and oligovanadates. When a complexing ligand is added to a vanadate solution the original equilibrium mixture will change. The concentration of mono-/oligovanadates will be decreased owing to formation of vanadate–ligand complexes. The higher the nuclearity of the oligovanadate species the more pronounced is the decrease.

To find the complex or set of complexes that best fitted the experimental data, different models were tested. By use of the computer program LAKE, formation constants with corresponding errors were calculated for different species. The model having the lowest error squares sum, *U*, is the one that best explains the experimental data. The results are presented in Table 3. As can be seen, two 1:1 vanadate to ligand complexes having the compositions (0,1,1) and (1,1,1) explain the data best. The *U*-value is more than 3 times lower than that for the binuclear monoligate species model, and the errors are also low. The values of log $\beta_{0,1,1} = 2.55 \pm 0.04$ and log $\beta_{1,1,1} = 9.44 \pm 0.05$ give a pK_a of 6.89. The pK_a for Ah^+ is 6.85. These very similar values indicate that it is the proton on the histidine residue that accounts also for the pK_a of the V-Ah complex. Moreover, the protonation of the (0,1,1) complex occurs without any noticeable change in the ^{51}V NMR chemical shift. We therefore propose that the protonation takes place at the aromatic

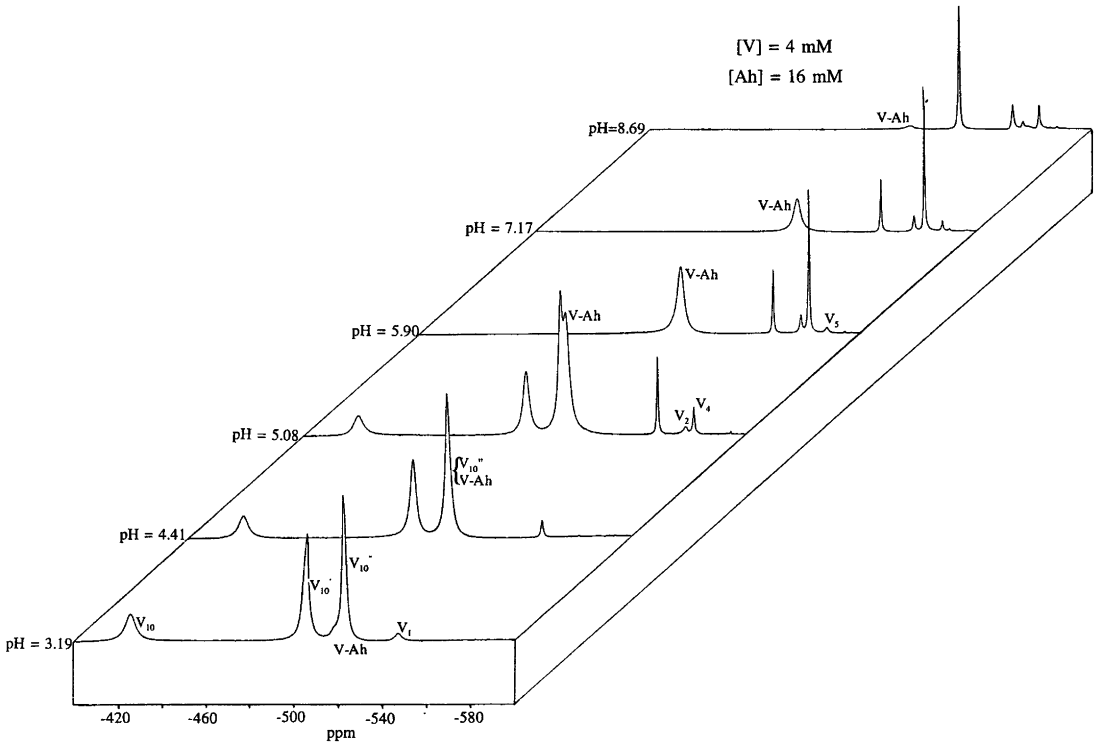

Fig. 2. ^{51}V NMR spectra of aqueous solutions of vanadate and alanylhistidine ([V]/[Ah]=4/16 mM) at different pH values.

Table 3. Results from the LAKE calculations. The binary constants used are compiled in Tables 1 and 2.

p,q,r	$\log\beta\pm3\sigma$	10^6 *U*
0,1,1	2.55±0.04	209
1,1,1	9.44±0.05	
0,1,2	3.96±0.70	1052
1,1,2	11.82±0.19	
2,1,2	18.05±0.30	
0,2,1	5.41±0.07	709
1,2,1	2.17±0.15	
2,2,1	17.41±0.26	
0,2,2	7.16±0.11	971
1,2,2	–	
2,2,2	21.21±0.13	
0,2,2	7.18±0.10	920
1,2,2	–	
2,2,2	21.12±0.16	
3,2,2	26.02±0.42	
1,2,2	14.50±0.11	1068
2,2,2	20.95±0.28	
3,2,2	26.14±0.36	

imidazole N(1), which is located far away from the vanadium atom (Fig. 3). The proposed structure of the complex is based on considerations in previous work.[13, 14]

The distribution of vanadium-containing species from pH 2 to 11 in a solution containing 4 mM V and 16 mM Ah was calculated and is shown in Fig. 4, while the corresponding Ah distribution is shown in Fig. 5.The constants used for the calculations are from Tables 1 and 2 and the best ternary model in Table 3. In Fig. 4a it is seen that at pH 6 predominantly an uncharged complex (1,1,1) and at pH 7.5 a −1 charged complex (0,1,1) exist. The complexation occurs over a wide pH range, from about 2 to 9.5. Also, at neutral and slightly acidic pH, the V-Ah species is dominant over the uncomplexed vanadate species. The points in Fig. 4b are the experimental values from the NMR integral evaluation. They fit well to the calculated model. The deviations that sometimes occur are due to evaluation problems, especially for small and overlapping peaks, and are not systematical when all data are taken into account. In Fig. 5 it is seen that at the most 15% of the Ah is bound in the V-Ah complexes at this V/Ah ratio.

In a former study[5] the V-Ah system was investigated using ^{51}V NMR as well as ^{13}C NMR and ^{14}N NMR Data were consistent with the formation of two five-membered rings with vanadate, with the binding sites at the carboxylate O, the deprotonated peptide N and the α-amino N (Fig. 3). To obtain definitive structural information, crystallisation experiments are in progress.

The H^+–$H_2VO_4^-$–His system. Before studying the ternary system, the acidity constants of histidine had first to be determined, since no such determination has been reported earlier in 0.6 M Na(Cl). From our potentiometric titration data it was found, in contrast to the Ah chemical, that no correction of the concentration was needed. From calculations on the collected EMF data the values given in Table 2 were obtained. To examine the complex formation to vanadate, ^{51}V NMR spectra were recorded. In an earlier study was shown that histidine (Fig. 1A) forms rather weak complexes with vanadate at neutral pH.[5] In the present study a wide pH range from 2 to 9.8 was covered. One set of spectra at the ratio [V]/[His] = 1/8, with V = 10 mM, His = 80 mM and at pH 1.95–6.54, is shown in Fig. 6. Besides the "free" vanadate peaks two sharp resonances can be seen: $\delta(^{51}V) = -544$ ppm (1) and −571 ppm (3). The large change in relative resonance intensities of the complexes with changing pH was clearly depicted. In this system complexation occurs at pH 3, reaches a maximum at around pH 5.4 and ends at pH approximately 8. The shift values of the signals arising from complexation are independent of pH. In the earlier study by Fritzsche *et al.*[5] an additional resonance at −561 ppm (2) is present at neutral pH. It has been suggested that the signal at $\delta = -544$ ppm indicates complexes in which histidine is coordinated in a monofunctional fashion through the NH_2 group.[5] The signal at −561 ppm is, according to Jaswal and Tracey,[13] probably due to a tetrahedral complex which is formed by reaction of vanadate and the carboxylic group. Investigation of the V-His system cov-

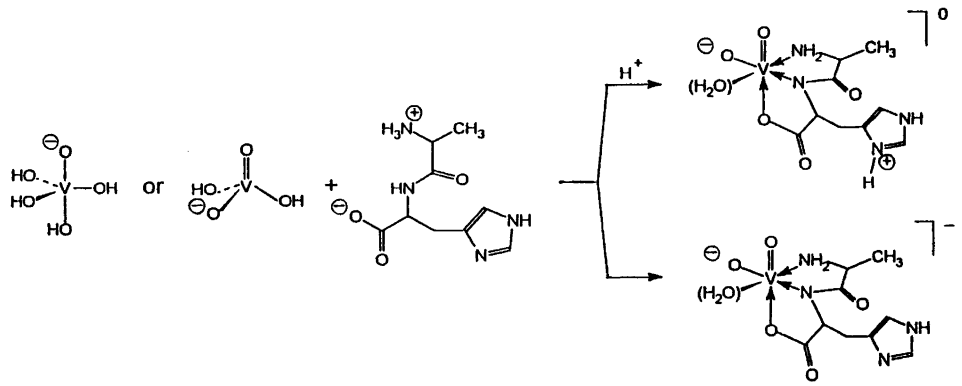

Fig. 3. Formation and schematic structure of the proposed (1,1,1) and (0,1,1) species. It has not yet been clarified whether the vanadium is penta- or hexacoordinated.

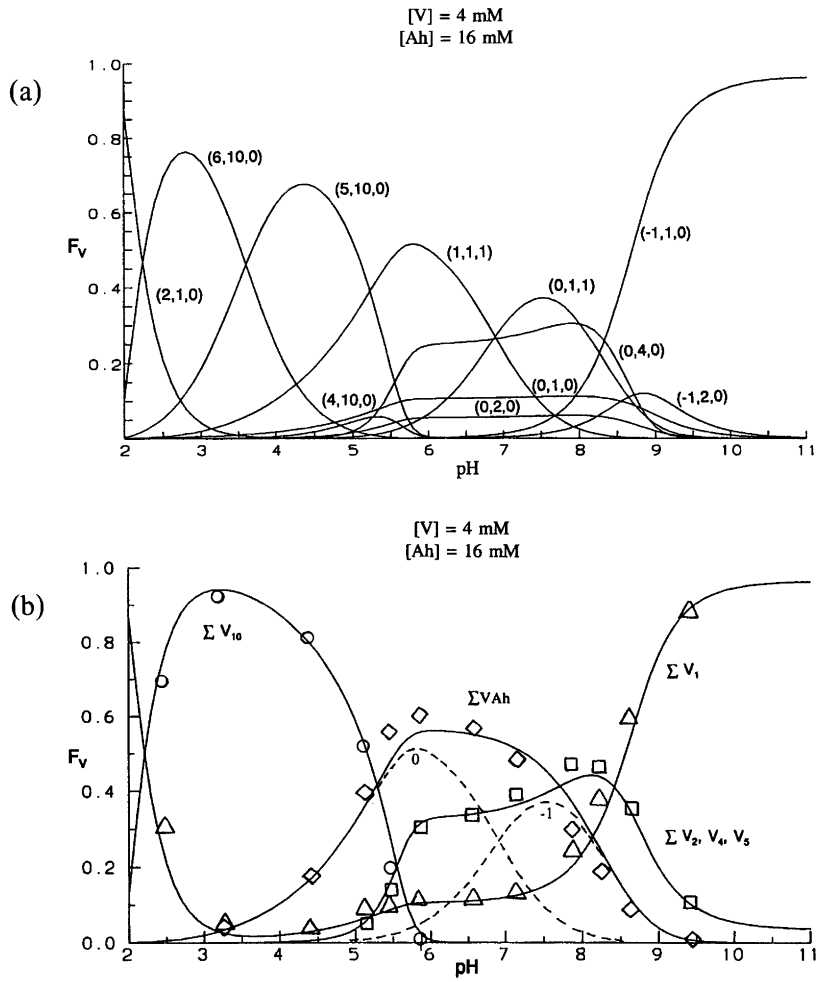

Fig. 4. Diagrams showing the distribution of vanadium, F_v, vs. pH at [Ah]/[V]=4. F_v is defined as the ratio between [V] in a species and total [V]. (a) All vanadium-containing species are shown except those with $<5\%$ of total [V]. (b) The sum of the decavanadates, oligovanadates, monovanadates and V-Ah species are shown. The symbols represent experimental NMR data points. The amount of the VAh^0 and the VAh^- complex is each shown by dashed curves.

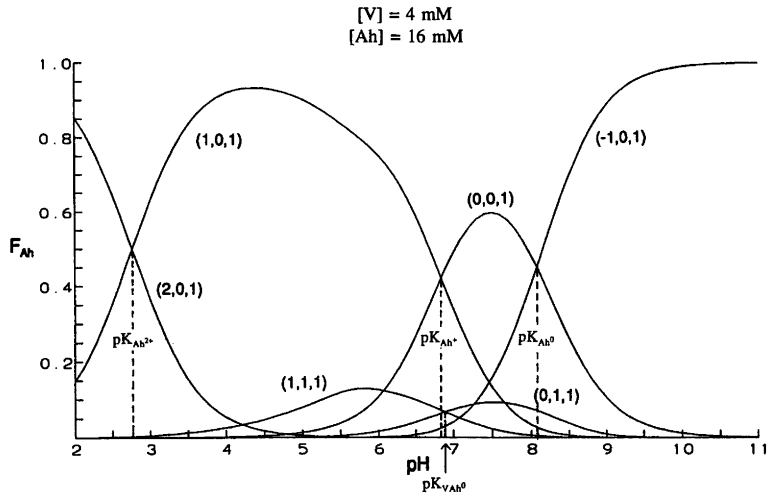

Fig. 5. Distribution of Ah, F_{Ah}, vs. pH at [Ah]/[V]=4. F_{Ah} is defined as the ratio between [Ah] in a species and total [Ah].

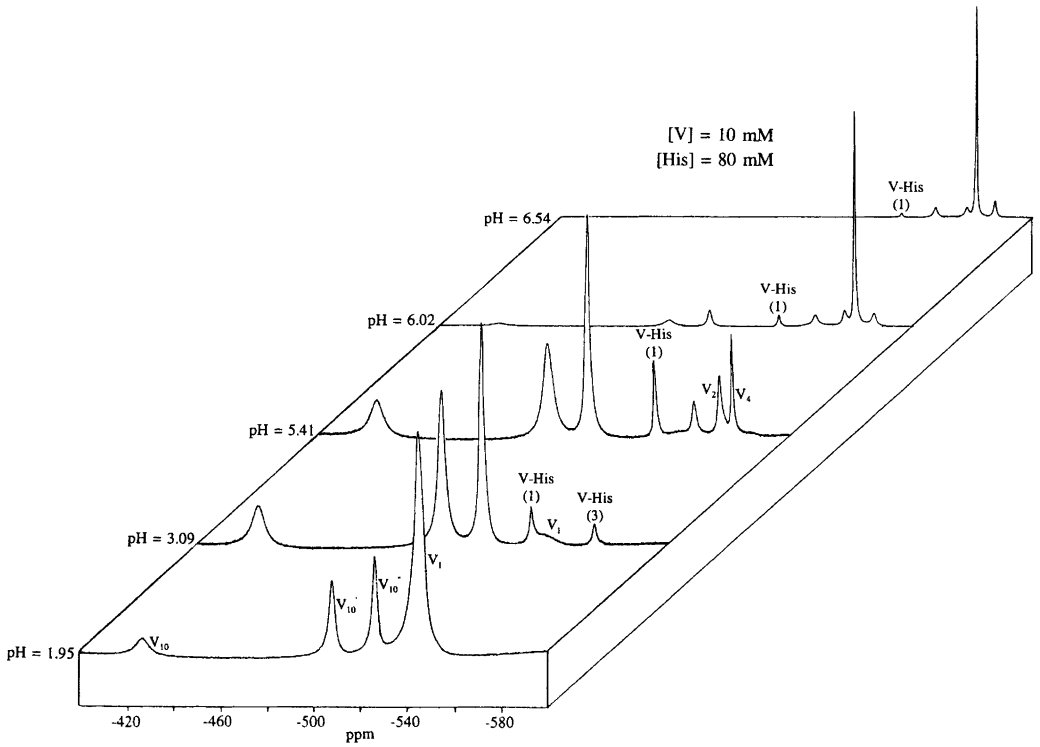

Fig. 6. ^{51}V NMR spectra of aqueous solutions of vanadate and histidine ([V]/[His]= 10/80 mM) at different pH.

ering a period of 4 weeks showed gradual reduction of vanadium, which occurred faster in acid than in neutral solution. Fifty percent of the vanadium became reduced at pH 3, and therefore further quantitative evaluation was not performed. Future plans include a combined EMF–NMR–ESR study of the V^{iv}/V^{v}–histidine system.

The H^+–$H_2VO_4^-$–His–Ur system. The vanadate–adenosine/uridine–imidazole systems have very recently been extensively studied.[9, 15] Since the complexation of imidazole to vanadium was found to be extremely favoured by the presence of the nucleoside, an analogous study using histidine instead of imidazole has been initiated. A ^{51}V NMR spectrum from a solution with Ur and His in excess (Fig. 7) shows resonances belonging to the free vanadate and the vanadate–histidine complex signals, as well as resonances at $\delta(^{51}V) = -481$ ppm (4) and -523 ppm (5). Peak (5) arises from a binuclear vanadate-uridine complex[9] and peak (4) arises from a species containing vanadate, histidine and uridine. The mixed ligand species in the previous studies containing vanadate, adenosine/uridine and imidazole were shown to have (1,1,1) stoichiometry and gave rise to a signal at -483 ppm.[9] Non-chelating metal–imidazole interactions usually exist through the imidazole N(1) atom.[16] Most probably, histidine is also complexed via the imidazole N(1) atom in a species with a (1,1,1) stoichiometry.

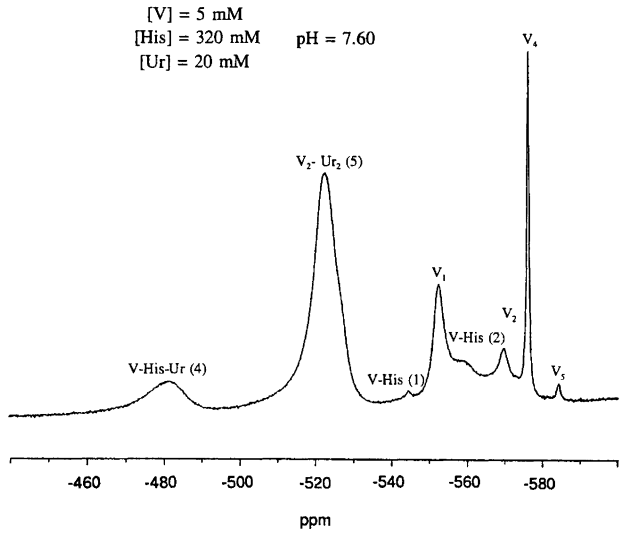

Fig. 7. ^{51}V NMR spectrum of an aqueous solution containing vanadate, histidine and uridine ([V]/[His]/[Ur]=5/320/20 mM) at pH 7.6.

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References

1. Chasteen, N. D. *Struct. Bond. 53* (1983) 107.
2. Chasteen, N. D. *Vanadium in Biological Systems*, Kluwer, Dordrecht, The Netherlands 1990, (a) p. 2, (b) p. 5, (c) p. 19, (d) 51–79.

3. World Health Organization, *Environmental Health Criteria 81: Vanadium*, 1988, p. 12.
4. (a) Crans, D. C., Bunch, R. L. and Theisen, L. A. *Am. Chem. Soc. 111* (1989) 7597; (b) Rehder, D. *Angew. Chem., Int. Ed. Engl. 30* (1991) 148; (c) Butler, A. and Carrano, C. J. *Coord. Chem. Rev. 109* (1991) 61; (d) Rehder, D. *Bio-Metals 5* (1992) 3; (e) Wever, R. and Kustin, K. *Adv. Inorg. Chem. 35* (1990) 81.
5. Fritzsche, M., Vergopoulos, V. and Rehder, D. *Inorg. Chim. Acta 211* (1993) 11.
6. Pettersson, L., Hedman, B., Andersson, I. and Ingri, N. *Chem. Scr. 22* (1983) 254.
7. Pettersson, L. and Andersson, I. *Unpublished results.*
8. Holmström, K. *Ph. D. Thesis*, University of Umeå, Sweden 1988.
9. Elvingson, K., Pettersson, L. and Crans, D. C. *Unpublished results.*
10. Tracey, A. S. and Gresser, M. J. *Can. J. Chem. 66* (1988) 2570.
11. Eriksson, G. *Anal. Chim. Acta 112* (1979) 375.
12. Duomulin, C. L. and Levy, G. C. *J. Mol. Struct. 113* (1984) 299.
13. Jaswal, J. S. and Tracey, A. S. *Can. J. Chem. 69* (1991) 1600.
14. Rehder, D. *Inorg. Chem. 27* (1988) 4312.
15. Crans, D. C., Harnung, S. E., Larsen, E., Shin, P. K., Theisen, L. A. and Trabjerg, I. *Acta Chem. Scand. 45* (1991) 456.
16. Eichhorn, G. *Ed. Inorganic Biochemistry*, Vol. 1, Elsevier, Amsterdam, The Netherlands 1973, p. 143.

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