A Potentiometric and ^{51}V NMR Study of the Aqueous $H^+/H_2VO_4^-/H_2O_2$/L-α-Alanyl-L-histidine System**

Hauke Schmidt,[a] Ingegärd Andersson,[a] Dieter Rehder,[b] and Lage Pettersson*[a]

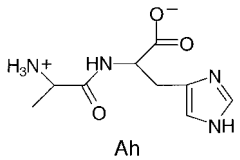
Abstract: The speciation in the quaternary aqueous $H^+/H_2VO_4^-/H_2O_2$/L-α-alanyl-L-histidine (Ah) system has been determined from quantitative ^{51}V NMR measurements and potentiometric data (glass electrode). The study was performed in 0.150 M Na(Cl) medium at 25 °C. Data were evaluated with the computer program LAKE, which is able to treat combined EMF and NMR data. The pK_a values for Ah were determined as 8.06, 6.72 and 2.64. In the ternary $H^+/H_2VO_4^-$/Ah system, two complexes, $(H^+)_p(H_2VO_4^-)_q(Ah)_r$, for which ($p$, q, r) values of (0, 1, 1) and (1, 1, 1) with log $\beta_{0,1,1} = 2.52 \pm 0.03$ and log $\beta_{1,1,1} = 9.40 \pm 0.05$ (p$K_a = 6.88$), respectively, explain all data. The errors given are 3σ. In the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah system, eight complexes were determined in addition to all binary and ternary complexes, four with a V/X/Ah ratio 1:1:1 and four with a ratio 1:2:1 (X = peroxo ligand). VX_2Ah^{2-} and VX_2Ah^- (p$K_a = 8.19$) are the main complexes and predominate in the pH range 5 to 9. Three additional minor species have also been found but their compositions could not be determined owing to their small amounts. Equilibria are slow, significant decomposition of peroxide occurs only in acidic solutions. Data in the pH range 5 to 10 have been used for the LAKE calculations. Chemical shifts, compositions, and formation constants for the eight quaternary complexes are given, and equilibrium conditions are illustrated in distribution diagrams. Structural proposals for VX_2Ah^{2-} and VX_2Ah^- are made from ^1H and ^{13}C NMR measurements.

Keywords: bioinorganic chemistry · NMR spectroscopy · peptides · peroxo complexes · vanadates

Introduction

Vanadium as a biometal in its +V and +IV/+III oxidation states is founded on, inter alia, the action (mostly inhibitory) of vanadate towards phosphate-metabolising enzymes.[1] This includes the insulin-mimetic behaviour of vanadate, peroxovanadate, vanadyl and several vanadium complexes,[2] which is most probably because of inhibition of a protein tyrosine phosphatase.[3] In addition vanadium is present in the cofactor for some nitrogenases and haloperoxidases. The structure of a chloroperoxidase from the mould *Curvularia inaequalis* (*C.i.*) has recently been determined by X-ray diffraction both in its native and its peroxo form.[4] This enzyme catalyses the two-electron oxidation of chloride by peroxide, and a peroxovanadium complex presumably is the active intermediate.[5] In the native form, vanadium is in a trigonal-bipyramidal array in a O_4N donor set with only one covalent link to the enzyme by N^ε of a histidine residue. In the peroxo form the geometry of the retained O_4N donor set changes to tetragonal pyramidal.

In order to model unspecific vanadium binding to proteins, the complexation behaviour of vanadate to protein fragments, for example dipeptides, is of interest. ^{51}V NMR data for vanadium complexes with different dipeptides have been published.[6, 7] The vanadium binding to L-α-alanyl-L-histidine (Ah) has been investigated in 0.600 M Na(Cl) medium at 25 °C to obtain information on the complexation of vanadium in biogenic compounds through ligands dominated by nitrogen functional groups.[8] The coordinating functions in those and in other vanadate dipeptide systems studied over the last decade have been determined to be the terminal amino group, the terminal carboxylate and the deprotonated peptide-N.[9] The imidazole residue of alanylhistidine does not coordinate to vanadium, possibly because of its involvement in protonation/deprotonation equilibria of the imine-N.[8] The speciation for the $H^+/H_2VO_4^-$/Ah system has now been rechecked in 0.150 M Na(Cl) medium, at 25 °C, as we intended



Ah

[a] Prof. Dr. L. Pettersson, Dr. H. Schmidt, Res. eng. I. Andersson
Department of Chemistry, Inorganic Chemistry
Umeå University, 901 87 Umeå (Sweden)
Fax: (+46) 90-786-9195
E-mail: lage.pettersson@chem.umu.se

[b] Prof. Dr. D. Rehder
Inorganic and Applied Chemistry, University of Hamburg
20146 Hamburg (Germany)

[**] Speciation in Vanadium Bioinorganic Systems; Part 7.

  0947-6539/01/0701-0251 $ 17.50+.50/0

to investigate the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah system at physiological medium and accurate knowledge of the ternary $H^+/H_2VO_4^-$/Ah system under these conditions is therefore necessary.

Systems with peroxo and/or bis-peroxo vanadates and peptides have been extensively studied by ^{51}V NMR spectroscopy.[10] The crystal structure of the complex (NEt_4)-$[VO(O_2)(GlyGly)]$ shows the same tridentade coordination mode of the dipeptide[11] which was presumed for the ternary vanadium dipeptide complexes in solution.[9] Investigations with histidine containing peptides have also revealed complexation of diperoxovanadate with the imidazole residue of histidine.[12] Interestingly, the imidazole binding to vanadium is favoured by the presence of peroxide, while vanadium–imidazole complexes without peroxide are very weak.[13] In addition to the unspecific interaction of vanadate and proteins, peroxovanadate complexes with histidine- or histidine-containing peptides can be considered as structural models for the peroxo form of *C.i.* chloroperoxidase owing to similarities in their structural properties.[4b]

Since no full speciation of the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah system has been reported so far, a detailed investigation of this system was performed, with quantitative ^{51}V NMR spectroscopic and potentiometric data. These data were treated with the computer program LAKE, which is designed to simultaneously treat multimethod data.[14] Furthermore, ^1H and ^{13}C NMR measurements were made to obtain information about structural properties of new quaternary complexes.

Results and Discussion

Subsystems: To establish the complete speciation in the ternary $H^+/H_2VO_4^-$/Ah and the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah systems, the equilibrium constants for the subsystems $H^+/H_2VO_4^-$, H$^+$/Ah and $H^+/H_2VO_4^-/H_2O_2$ have to be accurately known under the same experimental conditions. The ionic medium was chosen to represent physiological concentrations, 0.150 M Na(Cl) and a temperature of 25 °C.

The speciation in the vanadate system has already been published (Table 1).[15] For Ah no acidity constants have been reported in 0.150 M Na(Cl) medium. Four potentiometric titrations were carried out to establish the speciation in this binary system. To find the exact amount of Ah in the chemical from Bachem, the concentration of the ligand and the acidity constants were optimised together, using the computer program LAKE, and 91.2% of the chemical was found to be Ah. This is in contrast to the ethanol content quoted for the commercially available chemical and is attributed to attempts to remove the ethanol by evaporation. The acidity constants obtained from these titrations are presented in Table 2.

The ternary $H^+/H_2VO_4^-/H_2O_2$ system has recently been investigated in 0.150 M Na(Cl) medium.[13] Depending on both the pH and the peroxide to vanadate ratio, VX, VX_2, V_2X_4 and VX_3 were found as major species (X = peroxo ligand). In addition, some minor vanadium peroxide complexes also appear. Table 3 shows the full speciation of this ternary system.

Table 1. Species and formation constants for the inorganic vanadates (0.150 M Na(Cl), 25 °C) used in LAKE calculations on the VAh and VXAh systems.[15]

p, q	Notation	Formula	$\log\beta$	pK_a
−1, 1	V_1	HVO_4^{2-}	−8.17	
0, 1		$H_2VO_4^-$	0	8.17
2, 1		VO_2^+	7.00	
−2, 2	V_2	$V_2O_7^{4-}$	−16.19	
−1, 2		$HV_2O_7^{3-}$	−5.85	10.34
0, 2		$H_2V_2O_7^{2-}$	2.65	8.50
−2, 4	$1-V_4$	$V_4O_{13}^{6-}$	−9.98	
−1, 4		$HV_4O_{13}^{5-}$	−0.63	9.35
0, 4	V_4	$V_4O_{12}^{4-}$	9.24	
0, 5	V_5	$V_5O_{15}^{5-}$	11.17	
4, 10	V_{10}	$V_{10}O_{28}^{6-}$	50.28	
5, 10		$HV_{10}O_{28}^{5-}$	56.90	6.62
6, 10		$H_2V_{10}O_{28}^{4-}$	61.07	4.17
7, 10		$H_3V_{10}O_{28}^{3-}$	62.93	1.86

Table 2. Species and acidity constants for L-α-alanyl-L-histidine (0.150 M Na(Cl), 25 °C).

p, q	Notation	$\log\beta$ (3σ)	pK_a
−1, 1	Ah^-	−8.06 (1)	
0, 1	Ah	0	8.06
1, 1	Ah^+	6.72 (1)	6.72
2, 1	Ah^{2+}	9.36 (1)	2.64

Table 3. Species and formation constants for the $H^+/H_2VO_4^-/H_2O_2$ system (0.150 M Na(Cl), 25 °C) used in LAKE calculations on the VXAh system.[13]

p, q, r	Notation	$\log\beta$ (3σ)	pK_a	^{51}V NMR shift
−1, 1, 1	VX^{2-}	−2.27 (6)		−624.5
−1, 1, 2	VX_2^{2-}	3.61 (10)		−764.5
0, 1, 2	VX_2^-	11.28 (9)	7.67	−691.1
−1, 1, 3	VX_3^{2-}	5.14 (10)		−732.2
−1, 2, 4	$V_2X_4^{3-}$	16.48 (20)		−754.5
*2, 1, 1	$*VX^+$	11.51 (9)		−539.5
2, 2, 3	V_2X_3	23.73 (19)		−669
−1, 2, 1	V_2X^{3-}	−0.34 (10)		−621.8 [VX]
				−563.0 [V]
−1, 2, 2	$V_2X_2^{3-}$	5.33 (13)		−737.0 [VX_2]
				−554.9 [V]
*−1, 2, 2	$*V_2X_2^{3-}$	3.6		−633.6

The $H^+/H_2VO_4^-$/Ah system: The full speciation of this ternary system was determined from ^{51}V NMR data, which were recorded in the pH range 2.2 to 10.6. The spectra show a relatively broad signal at $\delta = -518$ over a wide pH range with an optimum integral intensity near pH 6 (Figure 1). This observation agrees with the results from the earlier study in 0.600 M Na(Cl) medium.[8a] In acidic solutions, the resonance signal of the VAh complex overlaps with the V_{10}'' peak, one of the three resonance signals from the decavanadate species (see Figure 1). Therefore, integral deconvolution is necessary and was performed with the program WIN-NMR, distributed by Bruker. Two series of ^{51}V NMR spectra have been recorded: constant c(V) with variable c(Ah) at neutral pH, and constant concentrations of the reactants with variable pH.

The experimental data were evaluated with the computer program LAKE as described in reference [8a] to find the set of complexes that gives the best fit to experimental data. Two

0947-6539/01/0701-0252 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 1

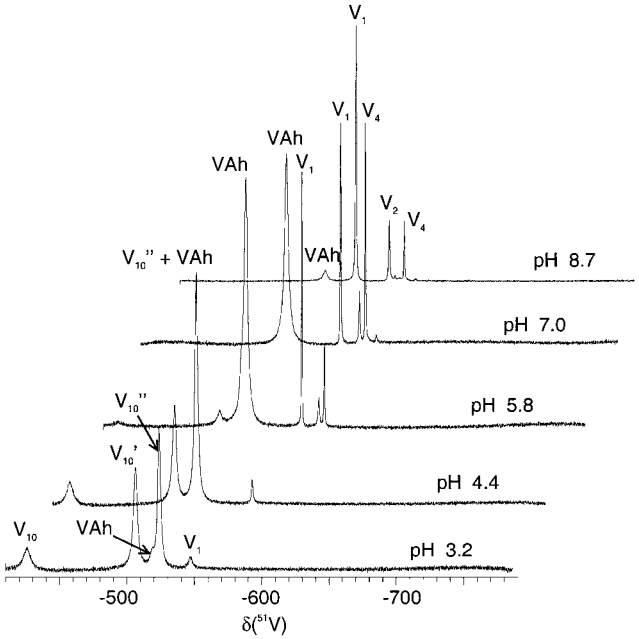
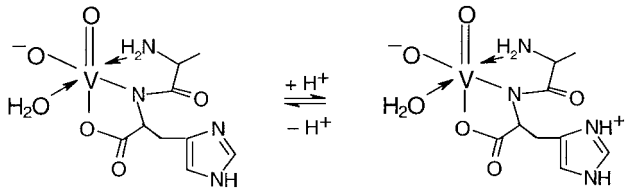


Figure 1. ^{51}V NMR spectra of aqueous solutions at $c(\mathrm{V}) = 3.7$ mM, $c(\mathrm{Ah}) = 16$ mM at different pH values.

complexes, $(H^+)_p(H_2VO_4^-)_q(Ah)_r$, with (p, q, r) values (0, 1, 1) and (1, 1, 1) with $\log \beta_{0,1,1} = 2.52 \pm 0.03$ and $\log \beta_{1,1,1} = 9.40 \pm 0.05$ ($pK_a = 6.88$) provide the best fit. The errors given are 3σ. The differences from the results of the earlier study in 0.600 M Na(Cl) are only marginal. The pK_a of the VAh complex (6.88) is very similar to the value for Ah^+ (6.72), and indicates that the proton of the histidine residue is responsible for this protonation step. In addition, changing the pH does not alter the position of the ^{51}V NMR shift, which indicates that the protonation site is located far away from the vanadium center. This is in agreement with the proposed structure for the complex (Scheme 1), obtained from ^{1}H, ^{13}C and ^{14}N NMR data in an earlier study.[9a]



Scheme 1. Proposed structures of VAh^- and VAh. It has not yet been clarified whether the vanadium is penta- or hexacoordinate.[8a]

The distribution for all vanadium-containing species was calculated in the pH range 2 to 11 ($c(\mathrm{V}) = 4$ mM, $c(\mathrm{Ah}) = 16$ mM) and is shown in Figure 2. The two VAh species exist from pH 2.5 to 9.5 and predominate in the pH range 5 to 8. A maximum of 70% of the vanadium is integrated into the ternary complexes at pH 6. An earlier study by Elvingson et al.[8a] under the same conditions in 0.600 M Na(Cl) medium (Figure 2b, marked #) shows a lower percentage of the VAh species at the maximum, this is explained by the more favoured formation of highly charged species like $V_4O_{12}^{4-}$ in the higher ionic strength medium used in this study. Figure 3

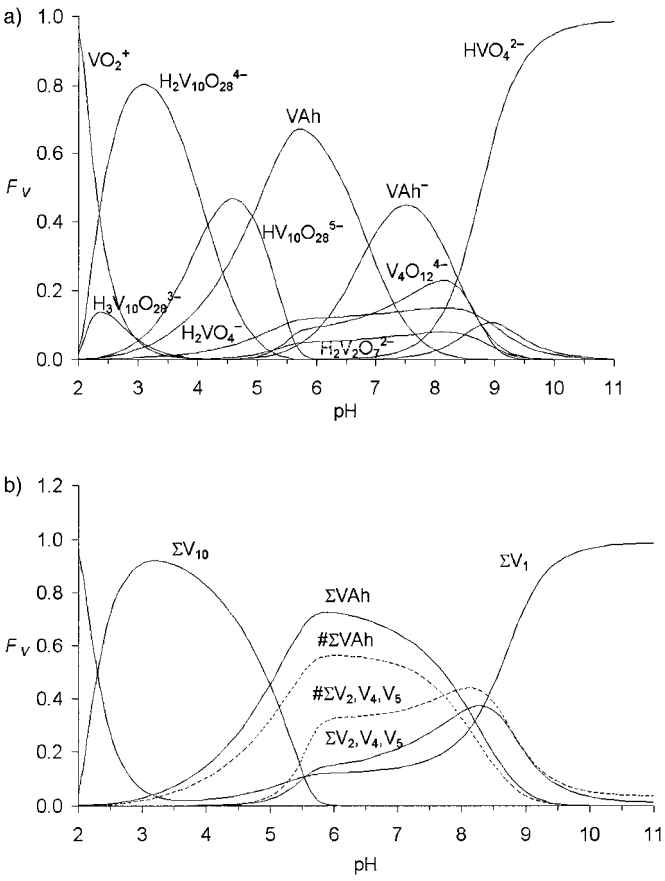


Figure 2. Distribution of vanadium (F_V) versus pH at $c(\mathrm{V}) = 4$ mM, $c(\mathrm{Ah}) = 16$ mM. F_V is defined as the ratio between c(V) in a species and total c(V). a) All vanadium-containing species are shown except those with $< 5\%$ of total c(V). b) The sum of the decavanadates, oligovanadates, monovanadates and VAh species are shown. The corresponding amounts of oligovanadates and VAh species in 0.600 M Na(Cl) medium[8a] (marked with #) are shown by dashed curves.

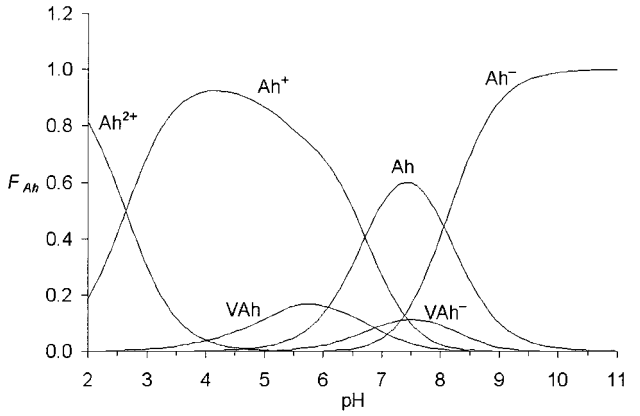


Figure 3. Distribution of alanylhistidine (F_{Ah}) versus pH at $c(\mathrm{V}) = 4$ mM, $c(\mathrm{Ah}) = 16$ mM.

shows the distribution of Ah (F_{Ah}) versus pH for the same concentrations used in Figure 2. Here, a maximum amount of 20% of the ligand is integrated into the ternary complexes.

The $H^+/H_2VO_4^-/H_2O_2$/Ah system: Upon addition of H_2O_2 to solutions containing vanadate and Ah, a large number of new resonance signals appear at higher field than those from

binary vanadates and ternary vanadate alanylhistidine species. This indicates the formation of peroxovanadate species and quaternary peroxovanadate complexes with Ah. The chemical shifts and assignments for the peaks are presented in Table 4 and Figure 4. The preliminary assignments for the quaternary species were made by comparing the results with those of earlier investigations in peroxovanadate ligand systems, for example, the peroxovanadate–imidazole system.[13] Only one of the new quaternary species (*VXAh) shows a change in the ^{51}V NMR shift due to protonation/deprotonation. The asterisk in Table 4 indicates the formation of an isomeric species. For all other complexes, no changes in the chemical shift occur.

Table 4. Species and formation constants for the $H^+/H_2VO_4^-$/Ah and the $H^+/H_2VO_4^-/H_2O_2$/Ah system (0.150 M Na(Cl), 25 °C).

p, q, r, s	Notation	$\log \beta$ (3σ)	pK_a	^{51}V NMR shift
0, 1, 0, 1	VAh^-	2.52(3)		
1, 1, 0, 1	VAh	9.40 (5)	6.88	−518.0
0, 1, 1, 1	$VXAh^-$	7.68 (21)		
1, 1, 1, 1	VXAh	14.80 (5)	7.12	−659.9
0, 1, 1, 1	$*VXAh^-$	8.01 (8)		−683.3
1, 1, 1, 1	*VXAh	13.93 (8)	5.92	−626.7
−1, 1, 2, 1	$*VX_2Ah^{2-}$	5.56 (18)		
0, 1, 2, 1	$*VX_2Ah^-$	13.58 (9)	8.02	−738.9
−1, 1, 2, 1	VX_2Ah^{2-}	6.29 (6)		
0, 1, 2, 1	VX_2Ah^-	14.48 (4)	8.19	−749.7

Equilibria in this quaternary system are slow due to slow formation of the peroxovanadate–dipeptide complexes and the slow decomposition of decavanadate initially formed in weakly acidic solutions (pH range 3 to 6). Serial ^{51}V NMR measurements of vanadate- and Ah-containing solutions were carried out after addition of peroxide to determine the equilibration time and the influence of decavanadate and peroxide decomposition. A total of seven serial measurements was recorded with different V/H_2O_2/Ah ratios in the pH range 3.6 to 7.4. At neutral pH, the bis(peroxo)species (VX_2Ah, $*VX_2Ah$) are already fully formed after 2 h, while an equilibrium for the monoperoxo (VXAh) species is not established until 12 to 15 h have elapsed. It has not been clarified whether the monoperoxo species are formed originally from the components (V, Ah or the complex VAh) or whether their formation is due to decomposition of diperoxo species. In acidic solutions (pH 3 to 4), where only small amounts of quaternary complexes are formed, the formation of all peroxovanadium species (mainly VX_2) is controlled by the slow decomposition of decavanadate which takes up to 6 h under these conditions. In addition, reformation of decavanadate species occurs after this time because of decomposition of peroxide in acidic solutions. Figure 5 shows the results of a serial ^{51}V NMR measurement of a solution with the V/H_2O_2/Ah ratio 1/1.7/4.4 at pH 5.2. The formation of the bis(peroxo)vanadium species is relatively fast, while decomposition of decavanadate and the formation of the monoperoxovanadium species takes about 10 to 15 h. Therefore, the equilibration time was estimated as 15 h and all solutions for quantitative measurements were allowed to equilibrate for at least this period of time after addition of hydrogen peroxide.

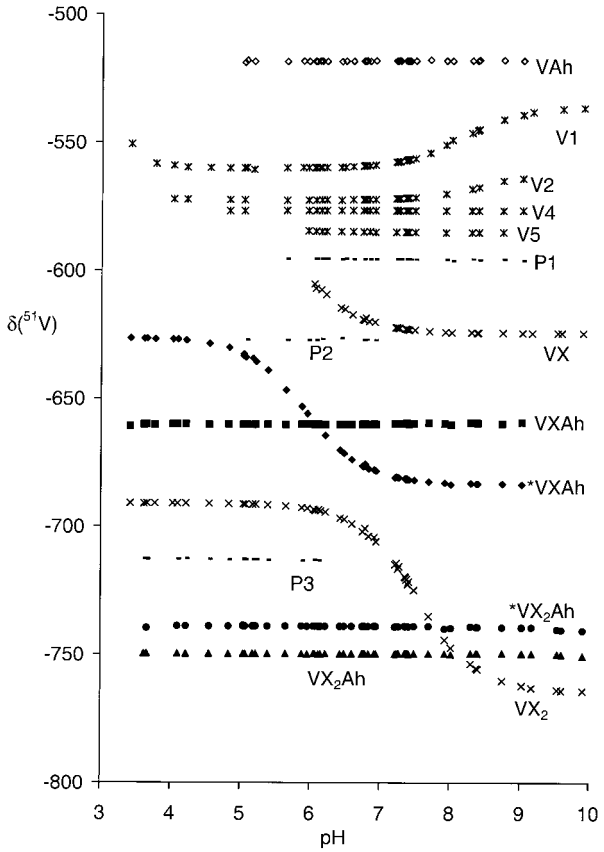


Figure 4. ^{51}V NMR shifts in the $H^+/H_2VO_4^-/H_2O_2$/Ah system versus pH. The three additional minor species at $\delta = -595$ (P1), −627 (P2), and −712 (P3) have not been determined.

The complexation of vanadium with peroxide is quite strong; at, for example, a ratio $H_2O_2/V = 2$, almost all of the vanadium is present in the form of peroxovanadates and quaternary complexes. In addition, the complexation of Ah to vanadium is enhanced by the presence of peroxide; in the ternary $H^+/H_2VO_4^-$/Ah system, a large excess of Ah is necessary to get appreciable amounts of vanadium bound in ternary complexes (see above and reference [8a]). The same observation has been made in a study of the quaternary $H^+/H_2VO_4^-/H_2O_2$/imidazole system where the complexation of imidazole to vanadium is extremely favoured by the presence of peroxide.[13]

The potentiometric data and the ^{51}V NMR integral and shift data for VXAh, *VXAh, $*VX_2Ah$ and VX_2Ah (Figure 4) were evaluated with the computer program LAKE to find compositions and formation constants for the set of complexes which best explain the experimental data. The results of these calculations are presented in Table 4. Eight complexes, four with a V/H_2O_2/Ah ratio 1:1:1 and four with a ratio 1:2:1, gave the best explanation. In a preliminary communication, we reported calculations for only three complexes with the ratio 1:2:1.[16] A recent evaluation of the experimental data revealed the presence of an additional complex. No attempts were made to evaluate the composition of the very minor species which gave rise to resonances at $\delta = -595$, −627 and −712 (marked P1, P2 and P3 in Figure 4) owing to very small integral values for these signals. The minor species

0947-6539/01/0701-0254 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 1

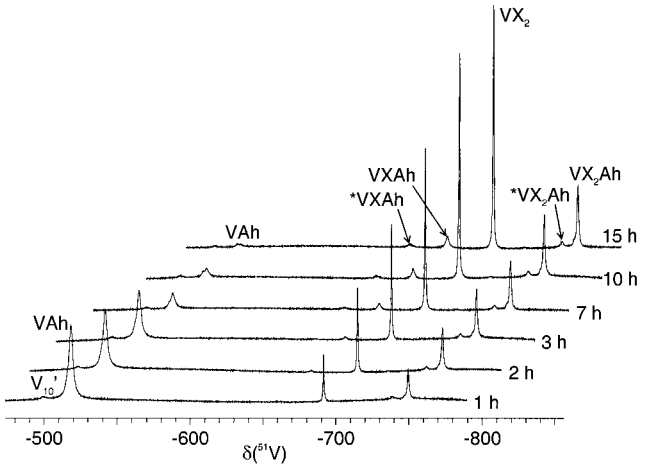


Figure 5. ^{51}V NMR spectra of an aqueous solution at $c(V) = 3.3$ mM, $c(Ah) = 14.4$ mM, $c(H_2O_2) = 5.7$ mM, pH = 5.2 after different reaction times.

at $\delta = -627$ appears at pH values higher than 5 where the shift of *VXAh, $\delta = -626$ at pH 3, already has moved to higher field, indicating that these are different complexes.

Two complexes, VX_2Ah^{2-} and VX_2Ah^-, with (p, q, r, s) values (−1, 1, 2, 1) and (0, 1, 2, 1) (see Equation (1) in Experimental Section), give the best fit for the data of the main resonance signal at $\delta = -750$. At a H_2O_2/V ratio of 2, this signal predominates in the pH range 5 to 8. The resonance signal at $\delta = -739$ was found to arise from isomeric species denoted $*VX_2Ah^{2-}$ and $*VX_2Ah^-$. The two signals for monoperoxovanadium species ($\delta = -660$ and $-683/-626$) exist over a wide pH range (3 to 8) with maximum amounts at pH 5. At a H_2O_2/V ratio of 1, these signals predominate in weakly acidic solutions. Two complexes for each signal, $VXAh^-$ and VXAh for $\delta = -660$, and $*VXAh^-$ and *VXAh for $\delta = -683/-626$, give the best fit for the data. $VXAh^-$ and $*VXAh^-$, and VXAh and *VXAh are isomers. As *VXAh shows a change in the ^{51}V NMR shift due to its deprotonation, the shift data for this peak could be used in the calculations.

Figure 6 shows the distribution of vanadium (F_V) versus pH at different conditions. At a H_2O_2/V ratio 2 (Figure 6a; $c(V) = 4$ mM, $c(H_2O_2) = 8$ mM, $c(Ah) = 8$ mM), bis(peroxo)vanadium species predominate over the whole pH range. The two complexes VX_2Ah^{2-} and VX_2Ah^-, calculated for the main ^{51}V NMR signal at $\delta = -750$ predominate in the pH range 6 to 8 with a maximum amount of about 70% at pH 7, while the isomer $*VX_2Ah^-$ makes up about 10% of the overall vanadium amount. In more acidic or alkaline solutions, ternary bis(peroxo)vanadates are the predominant species. This distribution confirms that the complexation of vanadate with peroxide is quite strong. Furthermore, the complexation of alanylhistidine to vanadate is favoured by the presence of peroxide. In contrast to the ternary $H^+/H_2VO_4^-$/Ah system, no large excess of the dipeptide is necessary to get appreciable amounts of quaternary complexes.

At low vanadium concentrations ($c(V) = 0.1$ mM), with a ten-fold excess of H_2O_2 and a five-fold excess of Ah (Figure 6b), all of the vanadium is bound in diperoxovanadate and the two VX_2Ah species of the main ^{51}V NMR signal. The diperoxovanadates predominate over the whole range. At

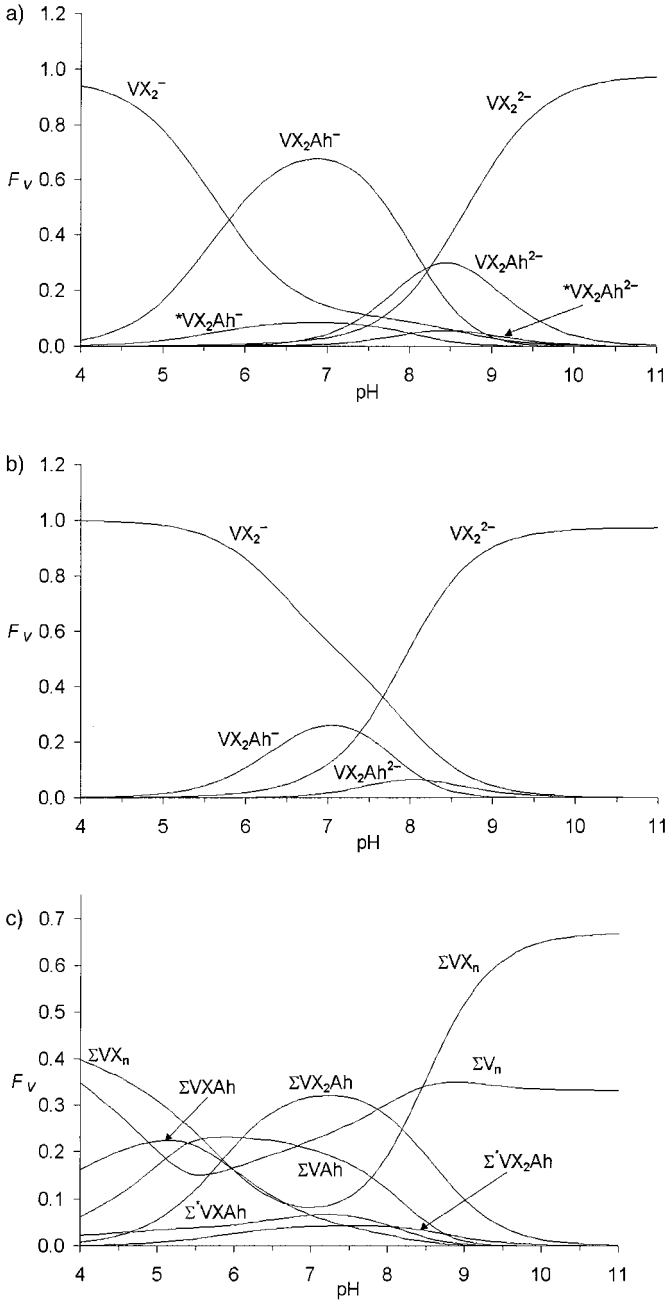


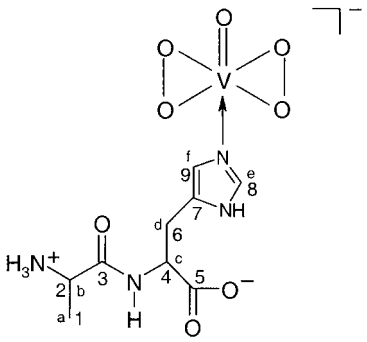
Figure 6. Distribution of vanadium (F_V) versus pH. All vanadium-containing species are shown except those with <5% of total $c(V)$. a) Distribution at $c(V) = 4$ mM, $c(H_2O_2) = 8$ mM, $c(Ah) = 8$ mM. b) Distribution at $c(V) = 0.1$ mM, $c(H_2O_2) = 1$ mM, $c(Ah) = 0.5$ mM. c) Distribution at $c(V) = 4$ mM, $c(H_2O_2) = 4$ mM, $c(Ah) = 8$ mM. The sum of binary vanadates, ternary peroxovanadates and VAh species and the different quaternary VXAh species are shown.

physiological pH, approximately 30% of the vanadium are bound in VX_2Ah complexes.

The distribution of vanadium becomes more complicated if less than two equivalents of peroxide are present. Addition of only one equivalent of peroxide to a solution containing vanadate and Ah gives rise to the formation of several monoperoxovanadium complexes particularly in weakly acidic solutions. The distribution diagram calculated for a H_2O_2/V ratio of 1 (Figure 6c; $c(V) = 4$ mM, $c(H_2O_2) = 4$ mM,

$c(\mathrm{Ah}) = 8\,\mathrm{mM}$) exhibits VXAh and *VXAh species with maximum formation at pH 5. At physiological pH, VX_2Ah^- is still the predominant complex, and comprises more than 30% of the vanadium. Furthermore, at this H_2O_2/V ratio, ternary VAh complexes are present with an optimum at pH 5 to 7.

Comparison of the ^{51}V NMR shift value for the predominant complex VX_2Ah^- with the data for the recently investigated $H^+/H_2VO_4^-/H_2O_2$/imidazole (Im) system (in which the resonance signal for the complex VX_2Im^- appears at $\delta = -750$)[10b, 13] indicates nitrogen coordination to vanadium through the imidazole residue of Ah (Scheme 2). ^1H NMR measurements on a solution with a maximum



Scheme 2. Coordination of nitrogen to vanadium through the imidazole residue of Ah.

amount of the complex VX_2Ah^- ($c(\mathrm{V}) = 40\,\mathrm{mM}$, $c(H_2O_2) = 80\,\mathrm{mM}$, $c(\mathrm{Ah}) = 60\,\mathrm{mM}$, pH = 7.45) show significant differences for the shifts of the imidazole residue protons between the free and the bound ligand, while no noticeable changes in the shifts for the other protons occur (Table 5). These changes towards lower field are caused by the deshielding effect of the oxovanadium(v) unit and indicate the interaction of this unit with the imidazole residue of Ah. Since the pK_a for VX_2Ah^- (8.19) is very similar to that for Ah (8.06), it is likely that the proton on the terminal ammonium group accounts for the protonation/deprotonation. In addition, the deprotonation of

Table 5. $^1H/^{13}C$ NMR shifts in the $H^+/H_2VO_4^-/H_2O_2$/Ah system ($c(\mathrm{V}) = 40\,\mathrm{mM}$, $c(H_2O_2) = 80\,\mathrm{mM}$, $c(\mathrm{Ah}) = 60\,\mathrm{mM}$, pH = 7.45).

Atom number[a]	$\delta(\mathrm{Ah})$[b] [ppm]	$\delta(VX_2Ah)$ [ppm]	$\Delta\delta$[c] [ppm]
H_a (d)	1.46	1.46	0.00
H_b (q)	3.96	3.95	−0.01
H_c (dd)	4.45	4.43	−0.02
H_d (ABX)	3.08/3.27	3.09/3.31	0.01/0.04
H_e (s)	7.92	8.22	0.30
H_f (s)	7.05	7.29	0.24
C_1	19.21	19.51	0.30
C_2	51.68	51.68	0.00
C_3	173.81	174.10	0.29
C_4	57.60	59.85	2.25
C_5	179.11	179.64	0.53
C_6	29.65	31.00	1.35
C_7	130.91	137.10	6.19
C_8	135.06	138.55	3.49
C_9	120.48	127.22	6.74

[a] See Scheme 2. [b] Free ligand in the presence of VX_nAh species. [c] $\Delta\delta = \delta(VX_2Ah) - \delta(\mathrm{Ah})$.

VX_2Ah^- occurs without any noticeable change in the ^{51}V NMR shift, which indicates that the protonation site is located far away from the vanadium centre. Also, ^{13}C NMR measurements on the same solution do not significantly differ for the shifts of the carbon atoms of the dipeptide backbone between the free and the bound ligand, either (Table 5).

In conclusion, we have shown that vanadate forms several mononuclear complexes with alanylhistidine in the presence of peroxide and we present stoichiometries and formation constants for them. In particular, the bis(peroxo) VX_2Ah^- complex is quite strong and exhibits coordination through the imidazole residue in contrast to ternary vanadate–dipeptide complexes with alanylhistidine, where the functional side chain does not participate in the coordination. Thus, VX_2Ah^- can be considered as a structural model for the peroxo form of *C.i.* chloroperoxidase.[4b] Furthermore, from insulin mimetic considerations and possible medicinal application of vanadium complexes, it is of interest that even at low vanadium concentrations high percentages of the total vanadium can be bound in VX_2Ah^- at physiological pH. Possible insulin mimetic effects of VX_2Ah^- will be tested in the near future using cell cultures.

Experimental Section

Chemicals and analyses: Sodium chloride (E. Merck p. a.) was dried at 180 °C and used without further purification. In the NaCl medium, the concentration of the cation was kept constant indicated by Na(Cl). A vanadate stock solution (1M) was prepared by the dissolution of sodium metavanadate (E. Merck p. a.) in hot water. After cooling, the solution was filtered and standardised by evaporation to the solid. Diluted solutions of hydrogen peroxide (E. Merck, 30%) were standardised against potassium permanganate and used without further purification. L-α-alanyl-L-histidine, $C_9H_{14}O_3N_4$ (Bachem, ethanol content 18%, H_2O content 0.5 mol mol^{-1}), was stored at less than 0 °C, but was heated to 25 °C before use. Possible interference by the ethanol content of the dipeptide has been checked by NMR spectroscopy. There is no difference in the complexation behaviour of pure and ethanol-containing Ah.[8] Furthermore, formation constants for vanadate ethanol esters have been shown to be negligible.[17] The ethanol-containing chemical could therefore be used. Possible interactions between Ah and peroxide have been checked by ^1H and ^{13}C NMR measurements, which show no changes after addition of peroxide. Diluted solutions of hydrochloric acid (E. Merck p. a.) were standardised against tris(hydroxymethyl)aminomethane (TRIZMA base, Sigma). Sodium hydroxide solutions were prepared from a saturated NaOH solution (50% NaOH and 50% H_2O) and standardised against hydrochloric acid. Boiled distilled water, stored under a CO_2 trap, was used for the preparation of all solutions. Alkaline solutions were prepared and stored under argon.

Notation: The equilibria studied are written with the components H^+, $H_2VO_4^-$, H_2O_2 and Ah. Thus, complexes are formed according to Equation (1).

$$p\mathrm{H}^+ + q\mathrm{H_2VO_4^-} + r\mathrm{H_2O_2} + s\mathrm{Ah} \rightleftharpoons (\mathrm{H}^+)_p(\mathrm{H_2VO_4^-})_q(\mathrm{H_2O_2})_r(\mathrm{Ah})_s^{p-q} \quad (1)$$

Formation constants are denoted $\beta_{p,q,r,s}$, and complexes are given the notation (p,q,r,s) or $V_qX_rAh_s^{n-}$. X is used instead of the peroxo ligand to shorten the formulae. The total concentrations of vanadate, hydrogen peroxide and L-α-alanyl-L-histidine are denoted $c(\mathrm{V})$, $c(H_2O_2)$ and $c(\mathrm{Ah})$ and are given in mM in the figures.

Equilibration of the solutions: Before the measurements in the ternary $H^+/H_2VO_4^-$/Ah system, the solutions were stored overnight for equilibration. In the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah system, the samples were in general prepared by addition of hydrogen peroxide to equilibrated solutions containing vanadate and dipeptide. However, cross-checks were

0947-6539/01/0701-0256 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 1

performed by addition of hydrogen peroxide before the dipeptide or the acid/base. The time for complete equilibration was determined by serial ^{51}V NMR measurements as 12 to 15 h. In particular, the formation of the monoperoxo species is slow. In addition, the process of complex formation is slowed down by decomposition of decavanadates initially formed in the pH range 3 to 6. Therefore, the solutions were allowed to stand overnight for equilibration. Owing to substantial decomposition of peroxide in acidic solutions, only ^{51}V NMR integral data in the pH range 5 to 10 were used for calculations. In this pH range, decomposition of peroxide is only marginal, but was taken into account. The peroxide concentrations used in the calculations were determined based on the presumption that all peroxide is bound to vanadium and no free peroxide exists. ^{51}V NMR shift data, however, were used without any restrictions.

Potentiometric measurements: The EMF measurements in the binary H^+/Ah system were carried out as potentiometric titrations in 0.150 M Na(Cl) medium at 25 °C with an automated potentiometric titrator as described in reference [8a]. For the ternary $H^+/H_2VO_4^-$/Ah and the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah systems, pH was measured on separately prepared \(point\) solutions using an Ingold U402-M6-S7/100 combination electrode which was calibrated against buffer solutions of known pH in 0.150[a] M Na(Cl).

NMR measurements: ^{51}V NMR spectra were recorded as described in reference [13]. Chemical shifts are quoted relative to $VOCl_3$. $^1H/^{13}C$ NMR spectra were measured in 5 mm/10 mm tubes at 500.13 MHz/125.77 MHz on a Bruker AMX-500 MHz spectrometer at 25 ± 1 °C. The spectra were externally referenced against the sodium salt of [D_4-2,2,3,3]3-(trimethylsilyl)propionic acid in D_2O, and dioxane in [D_6]benzene, respectively. All chemical shifts are given in ppm relative to TMS.

Potentiometric data: The acidity constants for Ah were determined from four titrations with a total of 84 points. The pH range was 1.5–8.1 and the total concentration range $5 < c(\mathrm{Ah})/\mathrm{mM} < 18$. Due to slow equilibration in the ternary $H^+/H_2VO_4^-$/Ah and the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah systems and to the decomposition of hydrogen peroxide, particularly in acidic solutions, no titrations were performed for these systems. Potentiometric data for these systems were obtained by measurement of "point" solutions with a combination electrode.

NMR data: In the ternary $H^+/H_2VO_4^-$/Ah system eighteen spectra were recorded in the ranges $2.2 < \mathrm{pH} < 10.6$, $3.4 < c(\mathrm{V})/\mathrm{mM} < 4$ and $2 < c(\mathrm{Ah})/\mathrm{mM} < 32$. In the quaternary $H^+/H_2VO_4^-/H_2O_2$/Ah system, a total of 60 spectra and seven serial measurements were recorded in the ranges $3.4 < \mathrm{pH} < 11.3$, $0.5 < c(\mathrm{V})/\mathrm{mM} < 15.8$, $1.0 < c(H_2O_2)/\mathrm{mM} < 26.0$ and $0.4 < c(\mathrm{Ah})/\mathrm{mM} < 28.3$. The pH of each solution was measured directly after recording the NMR spectrum.

Calculations: The EMF and quantitative ^{51}V NMR data were evaluated using the least squares program LAKE[14] as described previously in reference [8a]. The LAKE program is able to calculate formation constants from a combination of different kinds of data. In the present work, potentiometric data and quantitative ^{51}V integral and shift data have been used. Calculation and plotting of distribution diagrams were performed using the program SOLGASWATER.[18]

Acknowledgement

This work was supported by the Swedish Natural Science Research Council, the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie.

[1] a) D. Rehder, *Angew. Chem.* **1991**, *103*, 152–172; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 148–167; D. Rehder, *Coord. Chem. Rev.* **1999**, *182*, 297–322; b) A. Butler, C. J. Carrano, *Coord. Chem. Rev.* **1991**, *109*, 61–105; c) R. Wever, K. Kustin, *Adv. Inorg. Chem.* **1990**, *35*, 81–115.
[2] C. Orvig, K. H. Thompson, M. Battel, J. H. McNeil in *Metal Ions in Biological Systems*, *Vol. 31* (Eds.: H. Sigel, A. Sigel), Marcel Dekker, New York, **1995**, pp. 575–594.
[3] I. Goldwaser, D. Gefel, E. Gershonov, M. Fridkin, Y. Shechter, *J. Inorg. Biochem.* **2000**, *80*, 21–26.
[4] a) A. Messerschmidt, R. Wever, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 392–396; b) A. Messerschmidt, L. Prade, R. Wever, *Biol. Chem.* **1997**, *378*, 309–316.
[5] G. J. Colpas, B. J. Hamstra, J. W. Kampf, V. L. Pecoraro, *J. Am. Chem. Soc.* **1996**, *118*, 3469–3478.
[6] D. Rehder, *Inorg. Chem.* **1988**, *27*, 4312–4316.
[7] J. S. Jaswal, A. S. Tracey, *Can. J. Chem.* **1991**, *69*, 1600–1607.
[8] a) K. Elvingson, M. Fritzsche, D. Rehder, L. Pettersson, *Acta Chem. Scand.* **1994**, *48*, 878–885; b) V. Vergopoulos, W. Priebsch, M. Fritzsche, D. Rehder, *Inorg. Chem.* **1993**, *32*, 1844–1849.
[9] a) M. Fritzsche, V. Vergopoulos, D. Rehder, *Inorg. Chim. Acta* **1993**, *211*, 11; b) D. C. Crans, H. Holst, A. D. Keramidas, D. Rehder, *Inorg. Chem.* **1995**, *34*, 2524–2534; c) M. Fritzsche, K. Elvingson, D. Rehder, L. Pettersson, *Acta Chem. Scand.* **1997**, *51*, 483–491.
[10] a) A. S. Tracey, J. S. Jaswal, *J. Am. Chem. Soc.* **1992**, *114*, 3835–3840; b) A. S. Tracey, J. S. Jaswal, *Inorg. Chem.* **1993**, *32*, 4235–4243.
[11] F. W. B. Einstein, R. J. Batchelor, S. J. Angus-Dunne, A. S. Tracey, *Inorg. Chem.* **1996**, *35*, 1680–1684.
[12] J. A. Guevara-García, N. Barba-Behrens, R. Contreras, G. Mendoza-Díaz in *Vanadium Compounds*, *ACS Symposium Series Vol. 711* (Eds.: A. S. Tracey, D. C. Crans), **1998**, pp. 126–135.
[13] I. Andersson, S. Angus-Dunne, O. W. Howarth, L. Pettersson, *J. Inorg. Biochem.* **2000**, *80*, 51–58.
[14] N. Ingri, I. Andersson, L. Pettersson, A. Yagasaki, L. Andersson, K. Holmström, *Acta Chem. Scand.* **1996**, *50*, 717–734.
[15] K. Elvingson, A. Gonzáles-Baró, L. Pettersson, *Inorg. Chem.* **1996**, *35*, 3388–3393.
[16] H. Schmidt, I. Andersson, D. Rehder, L. Pettersson, *J. Inorg. Biochem.* **2000**, *80*, 149–151.
[17] A. S. Tracey, M. J. Gresser, *Can. J. Chem.* **1988**, *66*, 2570–2574.
[18] G. Eriksson, *Anal. Chim. Acta* **1970**, *112*, 375–383.

Received: April 14, 2000 [F2424]