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Coordination Chemistry Reviews 237 (2003) 77–87

COORDINATION CHEMISTRY REVIEWS

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Speciation in peroxovanadate systems☆

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Received 22 February 2002; accepted 23 August 2002

Contents

Abstract

Detailed and thorough potentiometric and ^{51}V-NMR spectroscopic investigations of H^+–$H_2VO_4^-$–H_2O_2–Ligand systems have been performed at 25 °C in 0.15 M Na(Cl) ionic medium. Extensive ranges of vanadate, hydrogen peroxide and ligand concentration and of pH have been covered. The medium was chosen to represent the physiological conditions in human blood. The computer program LAKE, designed to treat different types of data simultaneously, has been used to establish the entire speciation in the systems. Before studying systems containing the ligand (L), the complete speciation in the subsystem H^+–$H_2VO_4^-$–H_2O_2 must be known under the same experimental conditions. The formation constants in this subsystem have earlier been determined and it was found that hydrogen peroxide interacts with vanadate in the whole pH range studied (0.5–10.5). In all, 10 peroxovanadate species were identified and diperoxovanadate species were found to be exceptionally stable at physiological pH. The ligands studied so far include imidazole (Im), L-α-alanyl-L-histidine (Ah), L-α-alanyl-L-serine (As), picolinic acid (Pi), and L-(+)-lactic acid (La). In these five systems, as many as 3, 8, 6, 8, and 5 different peroxovanadate—L species (isomers included) were identified. A feature common to all these systems is that $V(H_2O_2)_2L$ species are formed at physiological pH. Notably, the ^{51}V chemical shift values of diperoxovanadate moieties are always found in the range −670 to −770 ppm, and those of monoperoxovanadate from −540 to −670 ppm. The equilibrium conditions are illustrated in distribution diagrams and the effectiveness of the different ligands as complexation agents are compared. In the case of diperoxovanadate complexes, ligands with aromatic nitrogen (Im, Pi, Ah) are the most effective, the one with both aliphatic nitrogen and oxygen (As) is less effective, and the one with oxygen only (La) is the least preferred.

Keywords: Potentiometry; ^{51}V-NMR; Speciation; Bioinorganic chemistry; Insulin-mimetic activity; Peroxovanadate complexes

☆ Speciation in vanadium bioinorganic systems; Part 8.

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0010-8545/02/$ - see front matter
PII: S0010-8545(02)00223-0

1. Introduction

Recently, vanadium and its compounds came to focus in many areas including the medical and biological sciences due to their discovered biochemical activity [1,2]. Many of these activities are thought to be related to the chemical similarity of vanadate to phosphate, which allows vanadate compounds to interact with numerous enzymes in living organisms by either inhibiting or activating them [3]. One particular case of these interactions is when vanadium interferes with the glucose uptake processes by mimicking insulin. This can occur via different pathways and the exact role of vanadium in the different processes is not completely known, but probably involves the inhibition of tyrosine phosphatase (PTPase) enzymes. Hydrogen peroxide has also been found to mimic insulin to some extent. Furthermore, synergism is found when vanadates and hydrogen peroxide are used together [4], presumably because the resulting peroxovanadates inhibit the PTPase enzymes irreversibly by oxidizing them, while vanadates inhibit them only reversibly [5]. This would make peroxovanadium compounds ideal candidates for a possible oral anti-diabetes drug in the future, provided that some current problems with toxicity and side effects are solved. These problems are probably attributable to the low adsorption and low selectivity of these compounds, which in theory can be helped by suitable complexing ligands. For both finding the most suitable ligand, that is the one that gives the most effect and least side effects, and for elucidating the role and interactions of peroxovanadates in cells, it is vital to know the complete speciation of vanadium in the presence of hydrogen peroxide and biologically important ligands. Furthermore, in order to model the human physiological environment, studies have been performed in 0.15 M Na(Cl) medium, which is isotonic to blood. The parenthesis around Cl means that the Na^+ ion concentration is kept constant, while the Cl^- concentration is allowed to vary somewhat. This kind of ionic medium is to be recommended when studying equilibria where negatively charged species are formed.

Before any study of peroxovanadate systems containing an organic ligand (L), the complete speciation in the inorganic subsystems H^+–$H_2VO_4^-$ and H^+–$H_2VO_4^-$–H_2O_2 must be known under the same experimental conditions (concentration ranges, ionic medium and temperature). The formation constants in the binary H^+–$H_2VO_4^-$ system in 0.15 M Na(Cl) medium have been published [6], and recently the ternary H^+–$H_2VO_4^-$–H_2O_2 system has been studied [7].

The organic ligands we have studied so far include imidazole (Im) [7], L-α-alanyl-L-histidine (Ah) [8], L-α-alanyl-L-serine (As), picolinic acid (Pi), and L-(+)-lactic acid (La). The As and Pi studies are experimentally complete while the La study remains in progress. The structures of the ligands are given in Table 1.

Study of the speciation in the complicated quaternary H^+–$H_2VO_4^-$–H_2O_2–L systems also requires a knowledge of the complete speciation in all the subsystems containing the ligand, H^+–L and H^+–$H_2VO_4^-$–L. Potentiometry combined with ^{51}V-NMR spectroscopy has proved to be a very powerful technique in this field. By using the computer program LAKE [9], designed to treat data from different methods simultaneously, it has been possible to establish the speciation in all the systems.

In this paper, some general comments on the inorganic subsystems H^+–$H_2VO_4^-$ and H^+–$H_2VO_4^-$–H_2O_2 are given, and the results from the speciation studies of the quaternary systems with the ligands Im, Ah, As, Pi and La are presented. The relative effectiveness of these five organic ligands as complexation agents is discussed and illustrated by distribution diagrams.

2. Experimental

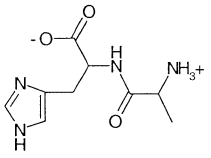
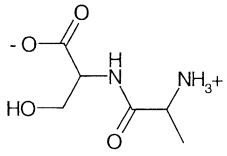
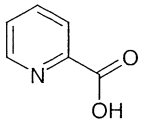
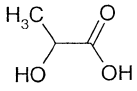
All studies were carried out in 0.15 M Na(Cl) physiological medium at 25 °C. The potentiometric (glass electrode) and ^{51}V-NMR (Bruker AMX-500 MHz) measurements were performed as described in Refs. [6–8]. The chemicals used and the analyses of HCl, NaOH, sodium metavanadate, H_2O_2, imidazole, L-α-alanyl-L-histidine and NaCl are given in Refs. [7,8]. L-α-Alanyl-L-serine (As), $C_6H_{12}N_2O_4$ (Bachem), must be stored below 0 °C, but here it was tempered to 25 °C before use. Picolinic acid, $C_6H_5NO_2$ (Acros), was used without further purification. Sodium-L-lactate, $CH_3CH(OH)CO_2Na$ (Aldrich), was used instead of L-lactic acid to avoid possible oligomerization of the ligand. The chemical was used without further purification.

The potentiometric and quantitative ^{51}V-NMR data were evaluated using the least-squares program LAKE [9], as described previously [10]. The LAKE program is able to calculate formation constants from a combination of different kinds of data. In the present work, quantitative ^{51}V integral and shift data, and potentiometric data (obtained in titrations, or mostly, individual solutions), have been used. Calculation and plotting of distribution diagrams were performed using the program SOLGASWATER [11].

2.1. Equilibria and notation

The equilibria studied are written with the components H^+, $H_2VO_4^-$, H_2O_2 and the organic ligand L (Im, Ah, As, Pi and La). Thus, complexes are formed according to

Table 1
Summary of peroxospecies formed in the H^+–$H_2VO_4^-$–H_2O_2–Ligand systems. X represents the peroxo ligand

Structure	Ligand (Notation)	Composition	No. of species	pH range used for logβ determ.
	Imidazole (Im)	$VXIm^-$ VX_2Im^- $V_2X_4Im^{3-}$	1 1 1	2 - 10
	L-α-Alanyl-L-histidine (Ah)	$VXAh^-$ / VXAh VX_2Ah^{2-} / VX_2Ah^-	4 4	5 - 10
	L-α-Alanyl-L-serine (As)	$VXAs^{2-}$ / $VXAs^-$ VX_2As^{2-} / VX_2As^-	4 2	5 - 10
	Picolinic acid (Pi)	$VXPi^{2-}$ / $VXPi^-$ / VXPi $VXPi_2^-$ VX_2Pi^{2-}	3 3 2	1 – 10
	L-(+)-Lactic acid (La)	VX_2La^{2-} $V_2XLa_2^{2-}$ $V_2X_2La_2^{2-}$ ($V_5X_4La_4^{4-}$)	1 2 1 1	4 - 10

$$pH^+ + qH_2VO_4^- + rH_2O_2 + sL^{x-} \rightleftharpoons (H^+)_p(H_2VO_4^-)_q(H_2O_2)_r(L)_s^{p-q-sx}$$

Complexes are given the notation $V_qX_rL_s^{n-}$, where X is used instead of the peroxo ligand to shorten the formulae. The total concentrations of vanadate, H_2O_2 and organic ligand are denoted V, H_2O_2 and L. When in a system more than one resonance arise from species having the same nuclearity ($V_qX_rL_s$), the dominant species is given the notation $V_qX_rL_s$. The others are denoted $*V_qX_rL_s$ and $**V_qX_rL_s$ in decreasing order of dominance.

3. H^+–$H_2VO_4^-$ system

The hydrolysis of pentavalent vanadium, V(V), is quite complex. Besides monomeric species, a variety of polyoxovanadate species with the nuclearities 2, 3, 4, 5, 6 and 10 are known to form in equilibrated solutions. The charges vary from +1 to −6. As a consequence, the speciation is strongly dependent both on the total concentration of vanadium, V, and on the ionic medium concentration. Equilibria are fast except for the pH range 4–7. Here, the slow equilibrium is caused by the slow decomposition of initially formed deca- to so-called metavanadate species having a charge per vanadium equal to −1. It was not until the early 80's that the full speciation was established for the whole pH range in the same ionic medium (0.6 M Na(Cl)) [12]. This was accomplished by combining potentiometric and ^{51}V-NMR data. The chemical shift values for the different species are shown in Fig. 1 as a function of pH in two different media. Except for the cyclic tetramer, c-V_4, and pentamer, V_5, the shifts are pH dependent due to protonation/deprotonation. The proposed structures are shown in the figure as well. Since the decavanadate structure contains three structurally different vanadium atoms, two central (V_{10}), four corner (V'_{10}) and four capping (V''_{10}) ones, three resonances with the integral ratio 1:2:2 are obtained. The ionic medium dependence of the chemical shifts is considerable for highly charged species, e.g. the −4 charged c-V_4 and −5 charged V_5, as shown in the figure.

Using the formation constants obtained in our studies, distribution diagrams can be calculated. In Fig. 2 the speciation is shown for three different Na(Cl) media, the physiological medium included, at

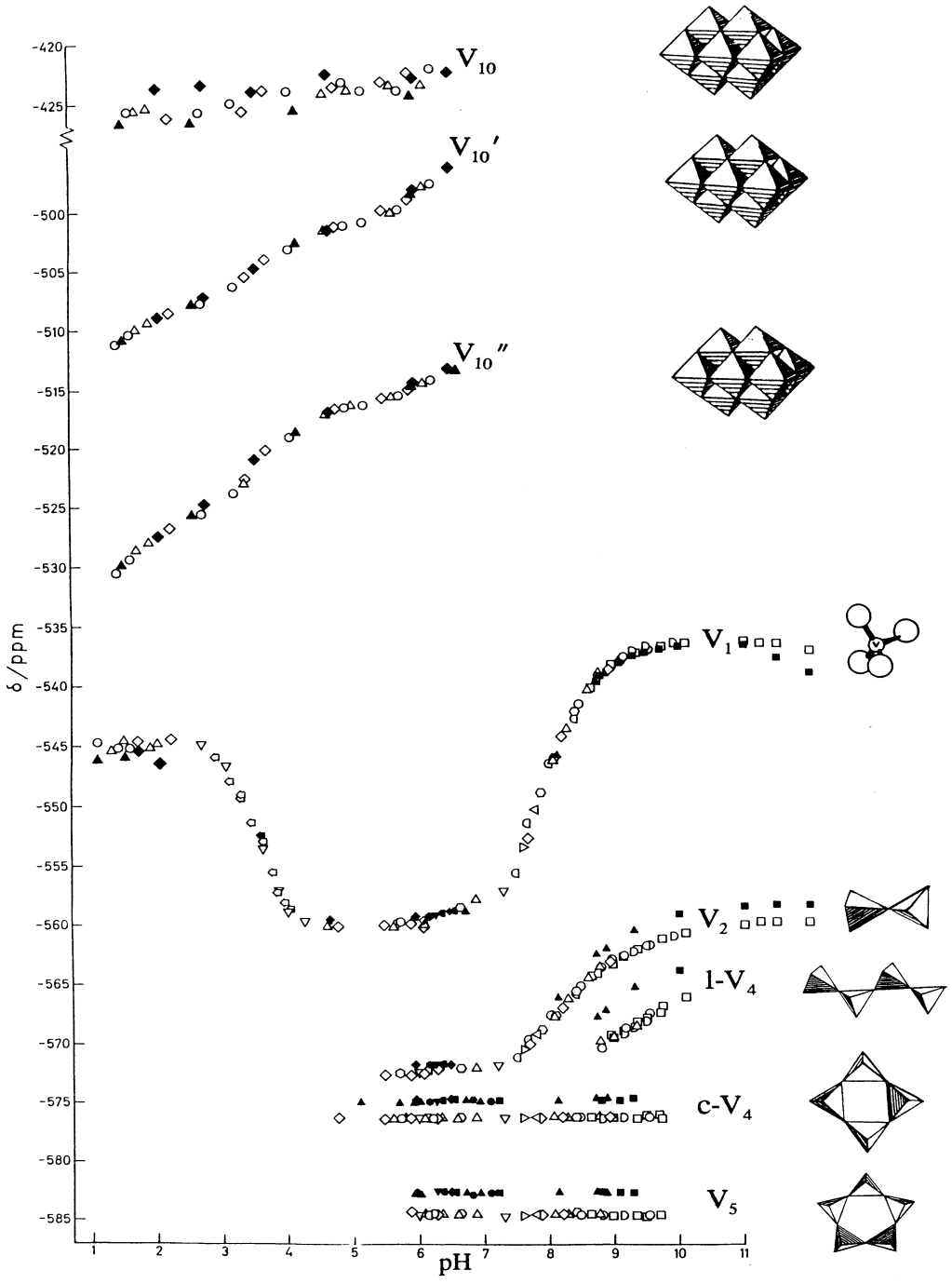


Fig. 1. ^{51}V chemical shifts as a function of pH for the H^+–$H_2VO_4^-$ system in the range $0.3 < V < 160$ mM. The open and filled symbols represent experimental NMR points in 0.60 M Na(Cl) and 3.0 M Na(ClO_4), respectively.

$V = 1.25$ mM. The relative abundance of the different species is, as expected, dependent not only on pH but also on the concentration of the ionic medium. For example, at physiological pH, the tetramer is the dominant species in the medium with the highest concentration, and the monomer in the medium with lowest concentration. A plausible explanation is that the Na^+ cations in the medium form complexes with vanadate ions, especially with those having a high negative charge. This has been verified for $V_2O_7^{4-}$ and HVO_4^{2-} and their sodium ion formation constants have been determined [13].

4. H^+–$H_2VO_4^-$–H_2O_2 system

This system has recently been studied [7]. It was found that hydrogen peroxide interacts with vanadate over the

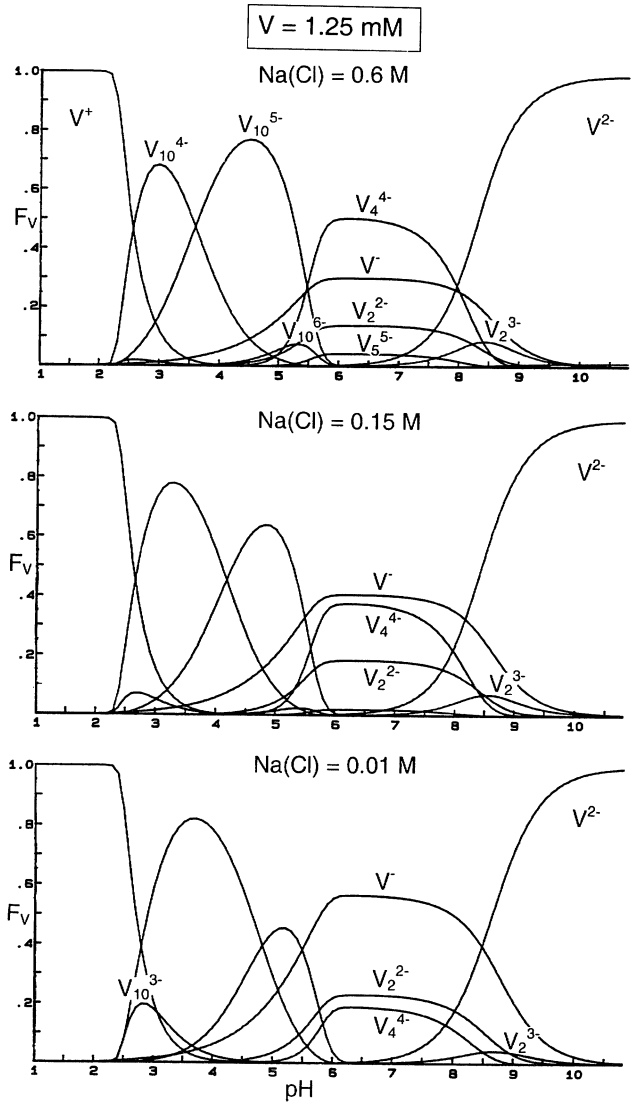


Fig. 2. Diagrams showing the distribution of vanadium, F_V, as a function of pH at V = 1.25 mM in three different ionic media. F_V is defined as the ratio between V in a given species and total V in the solution.

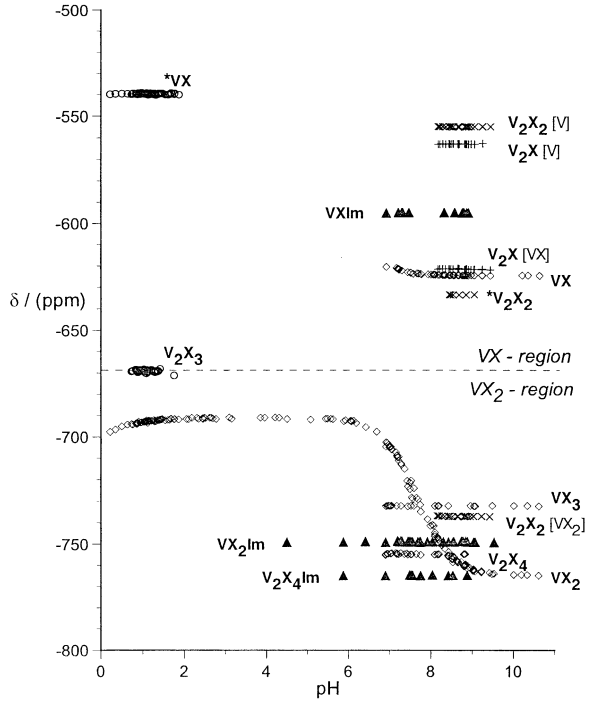


Fig. 3. ^{51}V chemical shifts as a function of pH for the H^+–$H_2VO_4^-$–H_2O_2–Im system. Symbols represent experimental NMR points.

whole pH range studied (0.5–10.5). Equilibria are fast, except for the slow decavanadate equilibration. The decomposition of hydrogen peroxide is fast in acidic but slow in neutral and akaline solutions. The peroxovanadate species are either monomeric or dimeric in vanadium, and four resonances of the dimeric species have been unambiguously assigned by 2D ^{51}V-NMR. Seven major complexes were found, VX^{2-}, VX_2^-, VX_2^{2-}, VX_3^{2-}, $V_2X_4^{3-}$, V_2X_3 and VX^+ (X = peroxo ligand) and three minor complexes, V_2X^{3-} and two isomeric $V_2X_2^{3-}$ species, which are only observed in the pH range 7–10 at high V.

The species are strongly dependent on the H_2O_2/V ratio. The VX^{2-} species is only present at low ratios, but under such conditions, a substantial amount of vanadium has not reacted with peroxide. The VX_2^{n-} species are the favoured species at pH 2–10, when sufficient peroxide is present and are exceptionally stable at physiological pH. The VX_3^{2-} species becomes predominant with excess of peroxide at high pH. At low pH the VX^+ species occurs as a predominant species.

The chemical shifts for each peroxovanadate species are shown in Fig. 3. For comparison, the shifts from the quaternary H^+–$H_2VO_4^-$–H_2O_2–Im system (Section 5.1) are included as well. Besides showing the pH range of existence the plot also gives information about which species undergo protonation. Another important information obtained from the figure is that the shift values of the upfield resonances arise from species containing VX_2 moieties and the downfield from species containing VX. The resonance from the species V_2X_3 is very broad and minor. It probably consists of two overlapping peaks, one arising from a VX moiety and the other from a VX_2 moiety, placing the chemical shift of the resulting broad resonance close to the border of these regions.

5. H^+–$H_2VO_4^-$–H_2O_2–Ligand systems

To be able to solve the speciation in the complicated quaternary H^+–$H_2VO_4^-$–H_2O_2–L systems, the complete speciation in all the subsystems containing the ligand, H^+–L and H^+–$H_2VO_4^-$–L, must be known under the same experimental conditions as for the inorganic subsystems (0.15 M Na(Cl), 25 °C). Complete descriptions of these systems with Im, Ah, As, Pi and La

were not available in the physiological medium prior to our studies. Therefore, the pK_a values for each ligand had to be determined and complete speciation studies of the ternary H^+–$H_2VO_4^-$–L systems had to be performed before studying the quaternary systems. Moreover, direct interactions between hydrogen peroxide and the organic ligand need to be ruled out, preferably by ^{1}H- and ^{13}C-NMR. No such interactions were observed for any of the ligands Im, Ah, As, Pi and La.

5.1. Imidazole (Im) as ligand [7]

The pK_a value for Im^+ was determined as 7.016±0.004. In the H^+–$H_2VO_4^{2-}$–Im system, no vanadate–imidazole complexes could be detected at the concentrations used, not even with very high excess of Im (V = 1 mM, Im = 640 mM) [14].

After adding imidazole to peroxovanadate solutions three new ^{51}V-NMR resonances were detected in the pH range 4–10, one major and one minor resonance in the upfield VX_2 moiety region, and one minor in the downfield VX region (see Fig. 3). The predominant species has the composition VX_2Im^- (−749 ppm). Such a complex has earlier been crystallised as an imidazolium salt and its structure has been determined [15]. The two minor species were found to have the compositions $VXIm^-$ (−595 ppm) and $V_2X_4Im^{3-}$ (−765 ppm).

5.2. L-α-Alanyl-L-histidine (Ah) as ligand [8]

The pK_a-values for Ah were found to be 8.06±0.01, 6.72±0.01 and 2.64±0.01. In the ternary H^+–$H_2VO_4^-$–Ah system, the ^{51}V-NMR spectra show a relatively broad resonance at −518 ppm over a wide pH range with an optimum integral intensity near pH 6. Two complexes, VAh^- and VAh give the best fit to the experimental data. Solutions were stored overnight for equilibration. The complexation behaviour of vanadate to Ah has also been investigated in a medium of four times higher sodium chloride concentration, 0.600 M Na(Cl) [10]. In this medium, the species found were the same but the V–Ah complexes are somewhat weaker. The reason is that in the more concentrated medium, the highly charged inorganic vanadate species are favoured over the low charged V–Ah species.

In the quaternary H^+–$H_2VO_4^-$–H_2O_2–Ah system, the time required to reach complete equilibration was investigated by a time course of ^{51}V-NMR spectra. At pH 3–6, the process of complex formation is slowed by decomposition of decavanadates initially formed in this pH range. Diperoxo species are formed within 2 h, while equilibrium between monoperoxo and diperoxo species is only established after 12–15 h. Therefore, solutions were allowed to stand overnight to equilibrate. Due to decomposition of hydrogen peroxide in acidic solutions, ^{51}V-NMR data were used for calculations only in the pH range 5–10.

Four major/medium resonances were found. Only one of these shows a change in its chemical shift with pH, thus indicating protonation behaviour. Two of the four resonances are in the downfield VX region and arise from isomers, which can both undergo protonation, although only one shows a chemical shift change upon protonation. The species are $VXAh^-$/VXAh (−660 ppm), $*VXAh^-$ (−683 ppm), and *VXAh (−627 ppm). The two resonances in the upfield VX_2 moiety region are explained by two isomers, which can both protonate, although they do not show any resulting changes of vanadium chemical shift. The species are VX_2Ah^{2-}/VX_2Ah^- (−750 ppm) and $*VX_2Ah^{2-}$/$*VX_2Ah^-$ (−739 ppm). Three additional extremely minor species giving rise to resonances at −595, −627 and −712 ppm were also detected. The composition of these species, however, could not be determined.

Notably, the complexation of Ah to vanadium is much enhanced by the presence of peroxide. This is also even more so the case with imidazole, which forms a very strong VX_2Im^- complex but no V–Im species under conditions used in our experiments.

5.3. L-α-Alanyl-L-serine (As) as ligand [16]

The pK_a values obtained for As in the physiological medium at 25 °C are 8.04±0.01 and 3.07±0.01. In the ternary H^+–$H_2VO_4^-$–As system, two new peaks were observed in the pH region 2.5–9.5. These peaks arise from the complexes VAs^- (−516 ppm) and $*VAs^{2-}$ (−503 ppm). Although the stoichiometry would indicate a simple protonation step between the two, no chemical shift change was observed for either resonances. This could mean either a different coordination mode of the ligand in the two complexes, or a change in the geometry around the vanadium center upon protonation/deprotonation, which is slow on the NMR timescale. Since ^{13}C-NMR investigations indicated that As utilizes the same dentation sites in both complexes, the latter explanation seems to be more valid. In addition, the protonation/deprotonation seems to take place on the amino group of the ligand. The broad peak of VAs^- exists in almost the whole pH range studied with an optimum integral intensity at pH about 6. The resonance of $*VAs^{2-}$ is also relatively broad and observed at pH values higher than 7, with a maximum intensity at about pH 9. As in the H^+–$H_2VO_4^-$–Ah system, solutions had to be stored overnight for equlibration.

In the H^+–$H_2VO_4^-$–H_2O_2–As system, a total of five new resonances were observed in the pH region 3–10. All of them showed constant chemical shift values. The complexes which give rise to the five peaks are $VXAs^-$/$VXAs^{2-}$ (−659 ppm), $*VXAs^-$ (−656 ppm), $**VXAs^{2-}$ (−677 ppm), VX_2As^{2-} (−743 ppm) and

*VX_2As^- (−714 ppm). In the case of the VXAs species, the protonation step does not result in a change of the chemical shift, indicating that the electronic environment of the central vanadium atom remains unchanged, i.e. the protonation occurs remotely. Asterisks here again refer to minor species of the same nuclearity. At pH values higher than 8, no quaternary monoperoxo species are formed, while in weakly acidic solutions VX_2As^{2-} does not appear. Complexation is strong but some species, e.g. the VXAs complexes, form very slowly. Solutions had to be stored for a minimum of 4 days before equilibrium was reached to the extent that calculations were feasible. Some solutions were stored up to 16 days, and surprisingly no substantial loss of peroxide was observed during this very long time. Tracey and Jaswal have also described slow equilibria and inhibited hydrogen peroxide decomposition upon complexation with numerous dipeptide ligands [17,18]. In those cases, however, equilibria usually required hours not days and 5–10% peroxide loss was observed already after 10 h even with a 100-fold excess of the ligand present [17].

5.4. Picolinic acid (Pi) as ligand [19]

The pK_a values obtained for picolinic acid (Pi) in physiological medium at 25 °C are 5.17±0.01 and 0.87±0.06. In the ternary H^+–$H_2VO_4^-$–Pi system, ^{51}V-NMR spectra were recorded from ca. 20 solutions to obtain an initial overview. At least seven resonances arise from the system. Some of them are very broad and overlap each other to such an extent that complete speciation study of the system is not finished yet.

Instead, in the study of the quaternary H^+–$H_2VO_4^-$–H_2O_2–Pi system, excess of peroxide was used to avoid the formation of complexes from the ternary H^+–$H_2VO_4^-$–Pi subsystem. Seven new resonances, belonging to eight species, were observed in the pH region 1–10. None of them showed changes in their chemical shifts, but LAKE calculations revealed protonation/deprotonation in the case of the minor *$VXPi^{2-}$/*$VXPi^-$ species. Five of the resonances are in the downfield VX region and the corresponding complexes are VXPi (−600 ppm), *$VXPi^{2-}$/*$VXPi^-$ (−658 ppm), $VXPi_2^-$ (−632 ppm), *$VXPi_2^-$ (−611 ppm) and **$VXPi_2^-$ (−616 ppm). The other two resonances are in the upfield VX_2 moiety region and originate from the complexes VX_2Pi^{2-} (−745 ppm) and *VX_2Pi^{2-} (−741 ppm). Asterisks in this system denote very minor species. The complexation is very strong and equilibrium is attained within 15 min. The decomposition of hydrogen peroxide is not substantial even in acidic solutions, which made it possible to investigate the system over the whole pH range 1–10. In a solution with twofold excess of peroxide and a Pi/V ratio of 1.5 (V = 80, H_2O_2 = 164, Pi = 120 mM), three species dominate over the wide pH range 1–9.5. The main species at pH < 2 is VXPi. Between pH 2–5, the dominating species is $VXPi_2^-$ and in the physiological and alkaline pH range VX_2Pi^{2-} is the predominant species. These complexes have been identified in an earlier study by Conte et al. [20]. The VX_2Pi^{2-} complex has also been crystallised and its structure has been determined [21].

5.5. L-(+)-Lactic acid (La) as ligand [22]

This system has already been investigated both with [23] and without peroxide [24], but not to date in the physiological medium. The pK_a value for La was determined to be 3.653±0.002 in 0.15 M Na(Cl) medium at 25 °C, and lactate ion (La^-) was used as a component in all the calculations. In the H^+–$H_2VO_4^-$–La system, a total of seven new resonances appear due to complex formation in the pH region 1–10. Only two of these show changes in the vanadium chemical shift with changing pH. Both are very minor peaks and belonging to complexes with a V:La ratio 1:1, but only one of them undergoes protonation. The change in the chemical shift values of the other one is attributable to an exchange process between the complex and the VO_2^+ cation, fast on the NMR timescale. The major ternary species in this system is a symmetrical $V_2La_2^{2-}$ dimer with a constant chemical shift of −533 ppm. The crystal structure of a species with such a composition is known from an X-ray study [25]. The other complexes in this system are: a $V_3La_2^{3-}$ trimer, giving rise to two peaks at −551 and −532 ppm with the integral ratio 1:2, and a $V_4La_2^{4-}$ tetramer showing two peaks at −525 and −540 ppm with the ratio 1:1.

Complexation in this system is relatively fast and is favoured in the acidic region, weak at neutral and physiological pH, and only marginal in alkaline solutions. Equilibrium is usually reached within 3 h, except for the decavanadate region, where it can take more than 16 h, and therefore all solutions were stored overnight before measurements. Substantial reduction occurs at pH below 2 and is more marked at higher ligand concentrations. Equilibrium analysis was therefore restricted to pH > 3. Interestingly, all complexes have a V:La ratio equal or close to 1, and species with high nuclearity are favoured in the acidic region, while the only complex present in alkaline solutions is the monomer.

In the H^+–$H_2VO_4^-$–H_2O_2–La system, nine new resonances were found in the pH region 2–10, belonging to five species. The major ones in the acidic region are $V_2X_2La_2^{2-}$ (−596 ppm), the crystal structure of which has recently been published [26], $V_2XLa_2^{2-}$ (−592 and −521 ppm) and *$V_2XLa_2^{2-}$ (−590 and −519 ppm). The latter two are different species with the same composition, each giving rise to two resonances of 1:1 integral ratio. One of these is in the VX moiety region of the

spectra, together with the single resonance from the $V_2X_2La_2^{2-}$ complex, while the other resonance falls into the region of ternary H^+–$H_2VO_4^-$–La species. The other four peaks observed are of less intensity and are belonging to two complexes: one peak to VX_2La^{2-} (−721 ppm), and three with the ratio 2:1:2 to presumably $V_5X_4La_4^{4-}$ (−565, −569 and −578 ppm). Equilibria are fast (reached within 30 min) and the formation of quaternary species is favoured in the acidic region, as with that of the ternary ones. Complexation with peroxide is not very strong, ternary species are present even when more than a twofold excess of peroxide is applied. The loss of hydrogen peroxide is substantial after 3 h at pH about 4, and even faster at more acidic pH. Due to the decomposition of hydrogen peroxide in acidic solutions, ^{51}V-NMR data were used for calculations only in the pH range 4–10. Also as in the ternary system, the only monomeric species is present in almost the whole pH range studied, but becomes preferred only in the neutral and alkaline region, while the higher nuclearity species dominate in acidic solutions. It is worth noticing that, with the exception of the tentative $V_5X_4La_4^{4-}$ species, all the quaternary species can be described as formed from the ternary ones by adding one or more peroxo ligands.

In all systems, crosschecks were performed with different order of addition of the components, but it has proved to be very important in the case of lactic acid, especially at acidic pH. If solutions were prepared in the way that first vanadate and lactate was allowed to equilibrate in acidic solution, and then hydrogen peroxide was added, immediate loss of peroxide was observed. On the other hand, if lactate and hydrochloric acid was added to already equilibrated peroxovanadate solutions, the loss of peroxide was much slower and the equilibrium analysis of the quaternary system was possible. A plausible explanation is that even small amounts of vanadium(IV) produced by lactic acid can catalytically decompose substantial amount of hydrogen peroxide, as experienced in the first case.

6. Comparison and concluding remarks

The complete speciation in peroxovanadate systems with different organic ligands has been established. One interesting question remains to be answered. Can mixed-ligand peroxovanadate species form? This is an important issue in several aspects. If there is more than one ligand per vanadium in a complex and different ligands are present, competition for the binding occurs, which can result in mixed-ligand complexes. This can very well be the case when vanadium(complexes) enter the bloodstream. It is therefore of fundamental interest to understand the role and actions of vanadium(complexes) in humans. Such mixed-ligand complexes were found earlier with vanadate–imidazole and either adenosine [27] or uridine [14]. This finding was particularly interesting, since imidazole alone did not bind to vanadate, pointing out again the complexity of factors that determine the fate of vanadium. Furthermore, mixed-ligand complexes have also been observed in the case of vanadium(IV) complexes of picolinic acid or 6-methylpicolinic acid and small blood serum ligands such as oxalate, lactate, citrate and phosphate [28]. It also points towards another important feature of vanadium complexes, namely their ability of changing the oxidation state of vanadium, with a possibility of retaining the ligand in the complex. This could mean a change in the charge of the complex, enabling it to enter the cell via either cationic or anionic transporters, which opens new possibilities in drug design and further emphasizes the complexity of vanadium chemistry in living organisms.

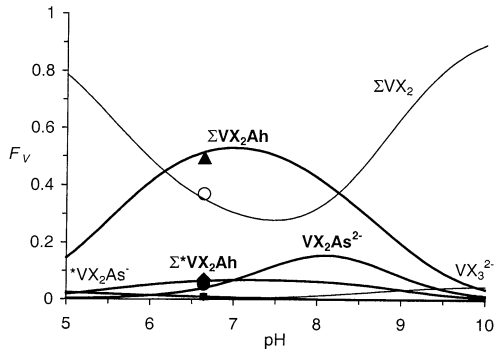


Fig. 4. Diagram showing the distribution of vanadium, F_V, as a function of pH in a solution with V = 10, H_2O_2 = 22, Ah = 8 and As = 40 mM. Symbols represent experimental values and X represents the peroxo ligand.

On the other hand, if there are no such mixed-ligand complexes present, the system can be modelled from the subsystems. This has been tested for the ligands Ah–As. A ^{51}V-NMR spectrum was recorded from a V = 10 mM solution containing a small excess of H_2O_2 (H_2O_2/V = 2.2) and the two ligands. Since As is a much weaker complexation agent than Ah, the former was in excess. As expected, no other resonances other than those already known from the two quaternary systems were detected, indicating that no mixed-ligand species were formed. To be sure that a resonance from a mixed-ligand species is not in perfect overlap with any of the known resonances, the spectrum was evaluated and the experimental points are shown in Fig. 4. The fit to the calculated distribution curves is very good. This is a firm proof that no mixed-ligand Ah–As species are formed at the prevailing pH value (6.7) and illustrates how well the model from the subsystems can describe speciation even with different ligands present. Some preliminary tests with Im–Pi, and Im–La also gave no indications of any formation of mixed-ligand species. This has, however, to be checked more carefully at different pH, concentra-

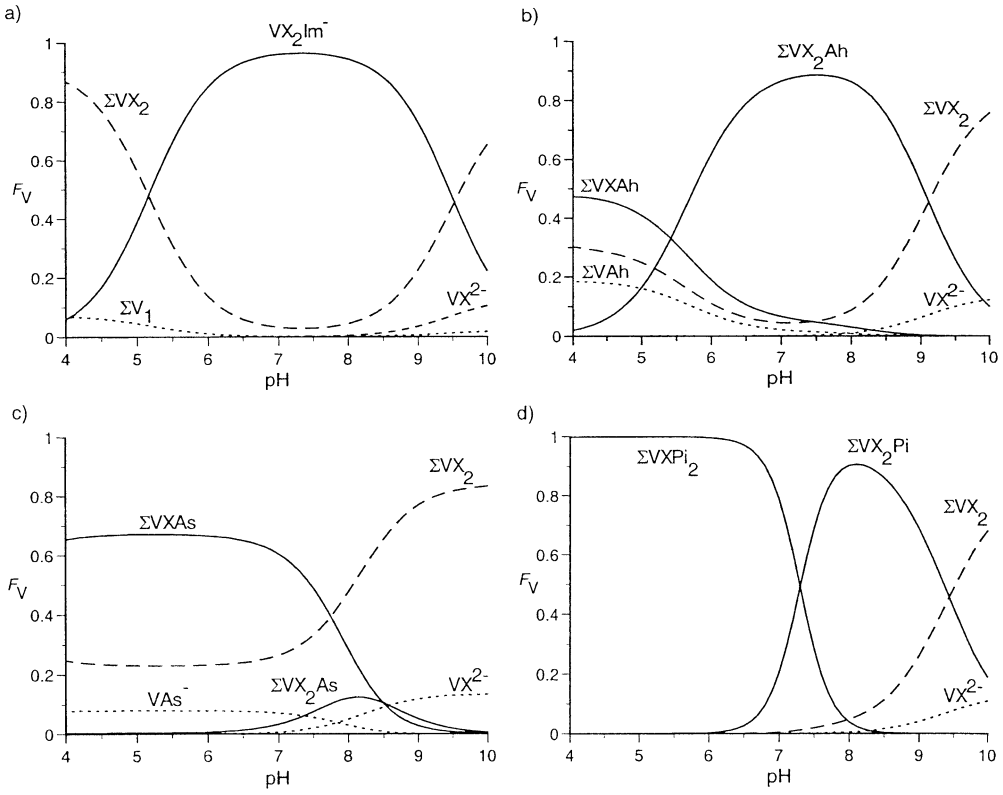


Fig. 5. Diagrams showing the distribution of vanadium, F_V, versus pH at V = 0.001, H_2O_2 = 0.01 and L = 20 mM. (a) Imidazole (Im) as ligand. (b) L-α-Alanyl-L-histidine (Ah) as ligand. (c) L-α-Alanyl-L-serine (As) as ligand. (d) Picolinic acid (Pi) as ligand. When for a specific nuclearity more than one species exist, the sum is shown. X represents the peroxo ligand.

tions, ratios, etc. Combinations of the other ligands will be tested in the near future.

A big advantage with the so-called equilibrium analysis method used in our studies, is that pH-independent formation constants are determined. Therefore, these constants can be used to calculate distribution diagrams for each system and over the whole pH range under study. Such diagrams show the effectiveness of the different organic ligands as complexing agents. Fig. 5 shows a set of diagrams for solutions with low V (1 μM), a 10-fold excess of hydrogen peroxide and very large excesses of the organic ligand Im, Ah, As and Pi, respectively. This modelling attempts to represent in vivo conditions. At physiological pH (pH ~7.4), Pi is the 'strongest' ligand, closely followed by Im and Ah, which are almost equally good, and As is the 'weakest' ligand. In weakly acidic solutions (pH ~4), Pi is still the strongest ligand, but the others show the reverse order As > Ah > Im. The reason for this order is that VXL species are formed at this pH for As and Ah but not for Im, and that the As complex is somewhat stronger than the Ah complex. In the Pi system, contrary to the other systems, a very strong bisligandmonoperoxo ($VXPi_2^-$) complex is formed, and all vanadium is bound in weakly acidic conditions. This complex predominates down to pH around 2 but at still lower pH an uncharged VXPi complex becomes dominant. Of the ligands studied so far, Pi is the only one that binds all the vanadate at excess of hydrogen peroxide over the wide pH range 1–7.5. This means that the complexes formed are stable both at the very acidic conditions of the stomach and at the neutral pH of blood, making Pi a very promising ligand for oral intake and so likely to be a potent antidiabetic drug.

The corresponding distribution diagram with the La ligand has not been included in the figure since the formation constants still are preliminary. The modelling with these preliminary constants shows, however, that La has the far weakest complexation ability in the pH range 4–10 at the chosen concentration conditions. Ongoing studies with citric acid [29] show similar features with respect to acidic complex formation, dominance of higher nuclearity complexes and severe reduction at acidic pH especially at high ligand concentrations.

Naturally, this attempt to predict speciations at much lower concentrations than can be experimentally studied has a limitation: it can be considered reliable only if it has been possible to determine formation constants of monomeric species. Otherwise, one cannot be sure that the model still holds at the very low vanadium concentrations present in cells. Moreover, one should be aware

of the possibility of the formation of complexes with higher ligand to vanadium ratio when the ligand is in extreme excess, even if no such complex is present under experimental conditions used in our studies.

The peroxovanadate complexes obtained with the different organic ligands are listed in Table 1. The Im and Pi systems are the only ones that have been possible to study over a wide pH range. In the others (Ah, As and La), equilibration is so slow that decomposition of hydrogen peroxide in acidic solutions made it impossible to perform complete speciation analyses i.e. to determine formation constants. Each system is unique, especially in the number of species formed, isomers and equilibration times. A common feature is that diperoxovanadate (VX_2L) and monoperoxovanadate (VXL) species are formed in all the systems, given that 'VXL' is a dimeric species when lactic acid is the ligand.

The ^{51}V chemical shift value of a particular complex is very informative. It can predict whether a complex contains no peroxide group, a VX or a VX_2 moiety. This is also valid for V_nX_n and V_nX_{2n} species. The resonances at downfield values up to -570 ppm arise from V or VL moieties, those in the range -540–-670 ppm from VX, and the high-field resonances at -670–-770 ppm from VX_2 moieties. Note that these boundaries are approximate and are to some extent dependent on the ligand.

One interesting and rather common feature is that protonation of a species can occur without any observable change in the chemical shift value. This can be attributed to remote protonation sites on the ligands. Except for the Im system, isomeric species are formed and occur frequently in the case of multidentate ligands.

Conclusions regarding the complexing abilities of the five different ligands we have studied can be drawn. In the case of diperoxovanadate complexes, ligands with aromatic nitrogen (Im, Pi, Ah) are the most, the one with both aliphatic nitrogen and oxygen (As) is less, and the one with oxygen only (La) is the least preferred. This contrasts with the vanadium–ligand systems, where Im forms no complexes at all. It suggests an electronic configuration for vanadium in peroxovanadates that favours the coordination of nitrogen atoms (preferably aromatic) over that of oxygen.

The insulin mimetic activity of peroxovanadate complexes is impossible to predict from speciation studies only. Therefore, complexes obtained by several research groups in the field are being tested on cell cultures (simian virus modified mice fibroblasts and human fibroblasts) within the European framework of COST D21-009 on 'Insulin-mimetic vanadium compounds' with promising preliminary results [30]. By means of such tests, connections between the speciation and the insulin mimetic effect can further be investigated. In addition to the aqueous speciation and biological studies, crystallographic data and theoretical calculations, considering the stability factors within the complexes, could also give valuable information to elucidate the role of vanadium in the glucose uptake processes.

Acknowledgements

This work was financially supported by the Swedish Natural Science Research Council. We thank Dr Oliver Howarth for valuable comments and linguistic corrections.

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