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Spectroscopic Investigation of Interactions between Dipeptides and Vanadate(V) in Solution

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The reaction of vanadate(V) with a series of dipeptides (Val-Gln, Ala-Gln, Gly-Gln, Gly-Glu, and Ala-Gly) was investigated by UV–visible spectroscopy and multinuclear (^{51}V, ^{14}N, ^{13}C) NMR spectroscopy in solution. It was possible to evaluate the formation constants of the corresponding complexes for which a molecular structure was proposed. Complex formation is favored by the presence of a functionalized or a sterically demanding side chain. The Val-Gln dipeptide which combines both properties exhibits one of the highest formation constant reported so far for dipeptides.

Introduction

The biodistribution and biological and pharmacological activity of vanadium have attracted much attention, and a great deal of work has been devoted to different aspects of the coordination chemistry of vanadium relevant to its presence and activity in biological systems.[1−6] This trace element may be involved in biological processes in both its cationic form (mainly as $V(IV)O^{2+}$ and sometimes as V(III)) and its anionic form (mainly as vanadate(V)). The inhibitory, stimulatory, and regulatory functions of vanadates $[H_xVO_4]^{(3-x)-}$ toward phosphohydrolases and phosphotransferases should be related to their structural analogy with phosphates $[H_xPO_4]^{(3-x)-}$.[1−3] Vanadium behaves like a typical transition metal ion, and both vanadate(V) and vanadyl(IV) bind tightly to a number of proteins, particularly serum albumins[7,8] and transferrins.[9,10] Moreover, as vanadium has a good propensity to change its coordination environment and its oxidation state, vanadates may be easily reduced under physiological conditions. This biometal can replace other metal ions in enzymes and be involved in some complexation reactions with biological ligands.[1−6,11] Among the different biological functions of vanadium recognized so far, there is the catalytic activity of vanadium in the nitrogenase system of a special strain of *Azotobacter chroococcum*[12] and in the vanadate(V)-dependent halide peroxidase of several marine brown algae.[13] Since 1977, vanadium has been also known to inhibit the Na, K ATPase (sodium−potassium pump).[14] Although numerous biochemical and physiological functions of vanadium were evidenced, its biological role in some higher organisms still remains unclear. In particular, the nature of the vanadium interactions with proteins is not easy to determine because of the wide

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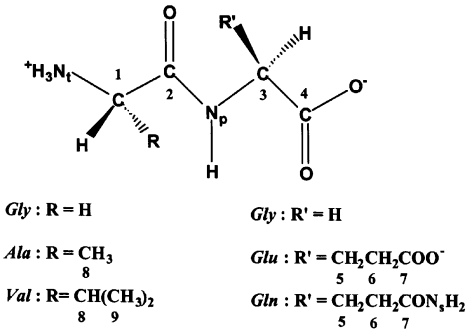
range of vanadium species in aqueous solutions. Depending on pH and vanadium concentration, aqueous solutions containing vanadium(V) will give rise to a large family of vanadate oligoanions including monomers ($H_2VO_4^-$, HVO_4^{2-}), dimers ($H_2V_2O_7^{2-}$, $HV_2O_7^{3-}$), tetramer ($V_4O_{12}^{4-}$), pentamer ($V_5O_{15}^{5-}$), and decamer ($V_{10}O_{28}^{6-}$). They are known to exhibit different affinities for proteins and consequently different inhibitory or stimulatory properties.[11] Therefore, the reaction of vanadate with selected amino acids, peptides, proteins, and different biomolecules was studied to determine the nature of these interactions.[3,15−25] The complexes formed between vanadate and several amino acids and small peptides were investigated mainly by ^{51}V NMR spectroscopy.[22−24] Dipeptides appear to react more strongly with vanadates than amino acids. Most of the dipeptides studied so far do not bear any functionalized side chain, and a large number of them bear a glycine N-terminal amino acid such as Gly-Gly, Gly-Ser, Gly-Val, and Gly-Asp. The major V−dipeptide complexes were evidenced by a ^{51}V NMR signal between −510 and −520 ppm.[19,20,22−25] The terminal amino, terminal carboxylate, and peptide nitrogen are presumably involved in these complexes. Some molecular structures were proposed, but except for the V−Gly-Tyr, V−Ala-His, V−Ala-Ser, and V−His-Ser complexes, they were mainly based on ^{51}V NMR analyses.[19,20,22,24,25]

The role of functionalized side chains in the formation of V−dipeptide complexes was investigated mainly for aromatic (His, Tyr, Trp) and seryl or threonyl side chains.[19,20,24,25] The results showed that, in histidine-containing compounds, the protonation state of the imidazole ring of the free ligand strongly influences product formation. No vanadium-to-imidazole bond was formed for any of the histidine-containing complexes studied.[19,24] In addition, no vanadium-to-tyrosyl bond was evidenced in the complex V−Gly-Tyr.[25] On the other hand, if a side chain contains a hydroxy group as in serine or threonine, an additional coordination of the hydroxyl group to vanadium was possibly present for some V−dipeptide complexes such as V−Gly-Ser[22] but not for V−Ala-Ser.[20] Apparently, dipeptides containing other aliphatic side chains, such as those of asparagine or glutamic acid, do not appear to react much differently with vanadate than do nonfunctionalized peptides.[24] However, no detailed spectroscopic study was performed on these V−dipeptide complexes. Moreover, the side chain carboxylate group of glutamate or aspartate is supposed to react with vanadium to form an anhydride similar to those formed with acetate and other carboxylic acids.[26]

Chart 1. Structure of the Dipeptides Val-Gln, Ala-Gln, Ala-Gly, Gly-Gln, and Gly-Glu



Therefore in an attempt to develop the understanding of the interactions between dipeptides bearing aliphatic or functionalized side chains and vanadate, we have studied the reaction of vanadate with a series of five dipeptides Val-Gln, Ala-Gln, Gly-Gln, Gly-Glu, and Ala-Gly. These dipeptides were chosen to estimate the role of the peptide sequence in the nature and strength of the interactions with vanadium. In addition to the presence of carbonyl functions on C-terminal amino acids (amide group for Gln and carboxylate group for Glu), most of the dipeptides studied exhibit an aliphatic side chain (Val and Ala) on the N-terminal amino acid, allowing us to evaluate the effect of the steric hindrance induced by this group in the stability of the complexes. All the vanadium(V)−peptide complexes were fully characterized in solution by UV−visible and multinuclear (^{51}V, ^{13}C, ^{14}N) NMR spectroscopy. UV−visible spectroscopy was complementary to NMR to evidence vanadium complexation. ^{14}N NMR spectroscopy was found to be particularly efficient to detect the nitrogen sites interacting with vanadium.

Experimental Section

Starting Compounds. The dipeptides L-H_2N-alanine-glycine-OH (L-Ala-Gly), H_2N-alanine-glutamine-OH (Ala-Gln), H_2N-valine-glutamine-OH (Val-Gln), H_2N-glycine-glutamine-OH (Gly-Gln), and H_2N-glycine-glutamic acid-OH (Gly-Glu) were purchased from Sigma. Their structure is presented in Chart 1. The vanadium solutions were prepared from sodium metavanadate $NaVO_3$ purchased from Prolabo. All the reagents were used without further purification.

Syntheses of Vanadate Complexes with Dipeptides. A representative sample containing 50 mmol·L^{-1} vanadate and 50 mmol·L^{-1} Ala-Gln (vanadium/peptide, V/P = 1) at pH 7 is described. A 50 mmol·L^{-1} vanadate solution was prepared by dissolution of $NaVO_3$

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(122 mg) in 20 mL of distilled water. The solution was heated at 323 K to speed up the process. After dissolution, the pH of the solution was close to 7.6. The vanadate solution was cooled at room temperature, and Ala-Gln (217 mg, 1 mmol) was then added as a powder directly to the solution under magnetic stirring. After the addition of the dipeptide to the vanadate solution, the pH decreased. It was then measured and adjusted to a final value close to 7 with 0.1 or 1 mol·L^{-1} NaOH. For the samples whose final pH is higher than 7, the solutions were not acidified to avoid the formation of decavanadates. Indeed, decavanadates, once formed at pH <6.5, take several hours at room temperature to dissociate to vanadates of lower nuclearities as the pH is adjusted to 7 or above. For the different vanadate/dipeptide solutions, the final pH was adjusted to a constant value in the range 7.0−7.5. Equilibration of the solutions required a few hours. Solutions were normally stored overnight, and the pH was then checked before starting spectroscopic experiments. For all the vanadate/dipeptide solutions, the ionic strength was kept constant (50 mmol·L^{-1} Na^+ from the vanadium precursor $NaVO_3$).

To determine the complex stoichiometry, a series of solutions was prepared for each dipeptide by varying the dipeptide concentration from 10 to 200 mmol·L^{-1} (V/P from 5 to 0.25) and keeping the vanadium concentration constant (50 mmol·L^{-1}). The study of the vanadate/dipeptide solutions over a pH range (2−9) was performed with a peptide concentration of 25 mmol·L^{-1} and a vanadium concentration of 10 mmol·L^{-1} (V/P = 2/5). The complexes of vanadium(V) with the dipeptides were designed as follows: V−Val-Gln; V−Ala-Gln; V-Gly-Gln; V-Gly-Glu; V−Ala-Gly.

UV−Visible Spectroscopy. UV−visible spectra were recorded on a Uvikon XS spectrometer between 350 and 420 nm. The stability of the solutions was checked by recording the spectra 15 min after adding the peptide to the 50 mmol·L^{-1} vanadate solution with the desired V/P ratio. The solutions were transferred to 0.2 mm quartz cells.

^{51}V NMR Spectroscopy. ^{51}V NMR spectra were recorded at 78.9 MHz on a Bruker AC 300 MHz NMR spectrometer and at 105.2 MHz on a Bruker Avance 400 MHz NMR spectrometer. The chemical shifts were measured relative to the external standard $VOCl_3$ at 0 ppm. For the spectra recorded at 78.9 MHz, 6000 transients were accumulated at 298 K. A spectral width of 31250 Hz, a pulse width of 11 μs ($\theta \sim 90°$), an accumulation time of 0.13 s, and no relaxation delay were used. The 5 mm NMR tubes were filled with 500 μL of the vanadate/dipeptide solutions, prepared from 250 μL of D_2O and 250 μL of H_2O. For the spectra recorded at 105.2 MHz, 256 transients were accumulated at 298 or 323 K. We typically used a spectral width of 31 447 Hz, a pulse width of 17 μs ($\theta \sim 90°$), an accumulation time of 500 ms, and no relaxation delay. The 10 mm NMR tubes were filled with 3 mL of the vanadate/dipeptide solutions, prepared from 1.5 mL of D_2O and 1.5 mL of H_2O. The spectra were simulated with the WIN-FIT program.[27]

^{14}N NMR Spectroscopy. ^{14}N NMR spectra were recorded on the sole dipeptides and on the vanadate/dipeptide solutions at 28.9 MHz on a Bruker Avance 400 MHz NMR spectrometer with a spectral width of 100 kHz. The spectra were recorded at 298 and 323 K with a spin−echo sequence $\theta-\tau-2\theta-\tau$ ($\theta \sim 90°$, which corresponds to a pulse width of 30 μs) to avoid the baseline distortion due to the acoustic ringing. Chemical shifts were referenced to a 8 mol·L^{-1} $NaNO_3$ solution ($\delta(^{14}NO_3) = -3.7$ ppm compared to CH_3NO_2 ($\delta = 0$ ppm)).[28] The number of transients per spectrum varied from 6400 to 26 000 depending on the solution.

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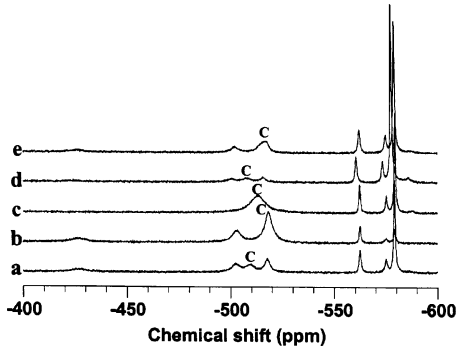

Figure 1. ^{51}V solution NMR spectra of vanadate/dipeptides solutions with V/P = 1, $[V]_{TOT}$ = 50 mmol·L^{-1}, pH 7, 298 K, ^{51}V Larmor frequency = 78.9 MHz, and NS (number of scans) = 6000: (a) Gly-Gln; (b) Ala-Gln; (c) Val-Gln; (d) Gly-Glu; (e) Ala-Gly. C: V−dipeptide complex.

The 10 mm NMR tubes were filled with 3 mL of the vanadate/dipeptide solutions, prepared from 1.5 mL of D_2O and 1.5 mL of H_2O. The spectra were simulated with the WIN-FIT program.[27]

^{13}C NMR Spectroscopy. ^{13}C NMR spectra were recorded at 298 K on the vanadate/dipeptide solutions and on the sole dipeptides at 100.6 MHz on a Bruker Avance 400 MHz NMR spectrometer. The number of transients per ^{13}C NMR spectrum varied from 16 000 to 84 000 depending on the solution. For the dipeptides Ala-Gln and Ala-Gly, 5 mm NMR tubes were filled with 500 μL of the vanadate/dipeptide solutions (V/P = 1 and $[V]_{TOT}$ = 50 mmol·L^{-1}) prepared from 250 μL of D_2O and 250 μL of H_2O. For Val-Gln, Gly-Glu, and Gly-Gln, 5 mm NMR tubes were filled with 500 μL of the vanadate/dipeptide solutions (V/P = 5 and $[V]_{TOT}$ = 50 mmol·L^{-1}) prepared from 250 μL of D_2O and 250 μL of H_2O. TMSP-2,2,3,3-d_4 (sodium 3-(trimethylsilyl)propionate) was used as external reference ($\delta = -2$ ppm). In addition proton-detected ^{1}H−^{13}C HMBC (heteronuclear multiple bond correlation) correlation[29] and proton-detected ^{1}H−^{13}C HMQC (heteronuclear multiple quantum coherence) correlation[30] spectra using a ^{13}C decoupling and a gradient-adapted pulse sequences were acquired on the vanadate/dipeptide solutions at 298 K. For HMBC correlations, typically 32−160 transients and 512−1024 experiments were necessary to achieve reasonable signal-to-noise ratios. These two sequences were used to assign the ^{13}C signals of the free dipeptides and the dipeptides bound to vanadium.

Results

^{51}V NMR and UV−Visible Spectroscopic Studies: Evidence of the Vanadium Complexation by Dipeptides. The reaction of vanadate with the different dipeptides at a pH close to 7 was investigated by ^{51}V NMR spectroscopy. The ^{51}V NMR spectra of the vanadate/dipeptide solutions with $[V]_{TOT}$ = 50 mmol·L^{-1} and V/P = 1 display the signals typical of vanadates present at pH 7 (Figure 1). The signal close to −560 ppm can be assigned to the diprotonated monovanadate $[H_2VO_4]^-$ (H_2V_1), and the signal near −573 ppm, to the diprotonated divanadate $[H_2V_2O_7]^{2-}$ (H_2V_2).[31] Two signals at −577 and −585 ppm can be assigned respectively to the cyclic metavanadates $[V_4O_{12}]^{4-}$ (V_4) and

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Table 1. ^{51}V NMR Data, Formation Constants, and Isosbestic Points at pH 7 of the V−Peptide Complexes

complexes	δ (ppm)	$\Delta\nu_{1/2}$ (Hz)	$10^{-1}\times$ formation consts	isosbestic pts (nm)
V−Val-Gln	−511	600	21 ± 2	
V−Ala-Gln	−517	460	16 ± 2	386 ± 3
V−Gly-Gln	−509	390	6 ± 2	373 ± 3
V−Gly-Glu	−508	460	5	
V−Ala-Gly	−513	340	7 ± 2	383 ± 3

$[V_5O_{15}]^{5-}$ (V_5).[31] For some dipeptides, the spectrum also displays the resonances at −427, −502, and −518 ppm due to the decavanadate polyanion $[H_nV_{10}O_{28}]^{(6-n)-}$.[32] An additional NMR signal near −510 ppm can be assigned to a vanadate−dipeptide complex. The chemical shifts of the signals corresponding to the different V−peptide complexes are given in Table 1. Such chemical shifts are consistent with those reported for V−dipeptide complexes.[19,20,22−25,33,34] In particular, the ^{51}V NMR chemical shift of the V−Ala-Gly complex is quite close to the one reported in the literature for this complex in an aqueous 0.6 mmol·L^{-1} NaCl solution.[33] The ^{51}V NMR chemical shift provides information about the local environment around the metal center as it depends on the electronegativity of the atoms bound to vanadium.[35,36] The five V−dipeptide complexes are characterized by a ^{51}V NMR signal between −508 and −517 ppm. This chemical shift range is consistent with a six-coordinate or a five-coordinate vanadium atom. Indeed, it is known that VO_6 and VO_5 environments of polyoxovanadate species are typically observed in the range −400 and −500 ppm.[37] However, as the electronegativities of oxygen and nitrogen are close, the vanadium atom may also be coordinated by nitrogen ligands. Therefore, coordination spheres such as VO_5N or VO_4N_2 are also possible. Actually, the chemical shift of the V−dipeptide complexes are quite close to the one of the $[VO_2(EDTA)_2]^{3-}$ complex (−519 ppm), which exhibits a VO_4N_2 environment.[36] For each complex, the resonance is rather broad: half-height widths ($\Delta\nu_{1/2}$) are typically in the 340−600 Hz range (see Table 1). This is not unexpected for a vanadium center with a low-symmetry site. Indeed, when the vanadium coordination is tetrahedral, then the room temperature line widths are typically 50−200 Hz. They are generally larger when the coordination number is higher.[37]

The complexation of vanadium with the different dipeptides at a pH close to 7 was also evidenced by UV−visible spectroscopy. Vanadium(V), lacking d-electrons, does not exhibit any d−d transition in the visible spectrum.[38] The yellow color observed with some vanadium(V) solutions (pH < 7) is due to a strong oxygen-to-metal charge-transfer absorption of decavanadates tailing in from the ultraviolet. Deconvolution techniques were used to obtain the UV absorption spectra of the various vanadate species.[38] Figure 2 displays a series of spectra corresponding to the solutions $NaVO_3$/Ala-Gln for $[V]_{TOT}$ = 50 mmol·L^{-1} and different V/P molar ratios. This study was not possible for the peptide Val-Gln due to its poor solubility. No significant results were obtained with the peptide Gly-Glu as its dissolution in water leads to a solution with a pH < 7, which therefore contains a large quantity of decavanadates. The spectra obtained for the peptides Ala-Gly and Gly-Gln show a similar behavior. These spectra are available as Supporting Information.

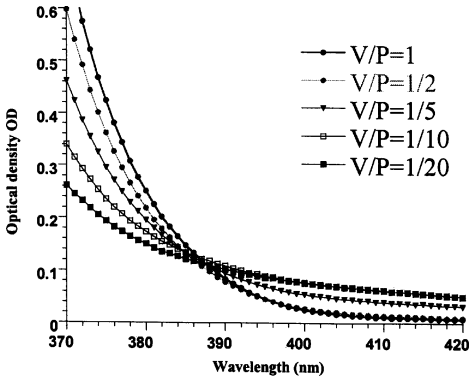

Figure 2. UV−visible absorption spectra of vanadate/Ala-Gln solutions for different V/P ratios: $[V]_{TOT}$ = 50 mmol·L^{-1}; pH 7; 298 K.

The UV−visible spectra of Figure 2 exhibit an isosbestic point close to 386 nm. By addition of dipeptide to the vanadate solution (decrease of the V/P ratio), the absorbance of the band at λ < 386 nm decreases while the one of the band at λ > 386 nm increases. Therefore, the band at λ < 386 nm can be assigned to the different vanadates H_2V_1, H_2V_2, V_4, and V_5 present at pH 7 and the band at λ > 386 nm to a V−Ala-Gln complex. The presence of the isosbestic point is consistent with an equilibrium between the V−Ala-Gln complex and the vanadates. The isosbestic point of the $NaVO_3$−dipeptide solutions (dipeptide = Gly-Gln, Ala-Gln, Ala-Gly) are reported in Table 1.

^{51}V NMR Spectroscopy: Determination of the Complex Stoichiometry and Effect of Temperature and pH. By the variation of peptide concentrations (from 10 to 200 mmol·L^{-1} corresponding to V/P ratios from 5 to 0.25) and maintenance of a constant vanadium concentration ($[V]_{TOT}$ = 50 mmol·L^{-1}), ^{51}V NMR spectra were recorded on equilibrated solutions. Therefore, the stoichiometry of the V−peptide complexes can be obtained from reactions 1−4 as described previously for some vanadium (V)−peptide complexes:[22,24,25]

$$V_1 + p \leftrightarrow V_1{-}p \quad K = [V_1{-}p]/[V_1][p] \tag{1}$$

$$2V_1 + p \leftrightarrow (V_1)_2{-}p \quad K = [(V_1)_2{-}p]/[V_1]^2[p] \tag{2}$$

$$V_1 + 2p \leftrightarrow V_1{-}(p)_2 \quad K = [V_1{-}(p)_2]/[V_1][p]^2 \tag{3}$$

$$2V_1 + 2p \leftrightarrow (V_1)_2{-}(p)_2 \quad K = [(V_1)_2{-}(p)_2]/[V_1]^2[p]^2 \tag{4}$$

$[V_1]$ represents the concentration of monovanadate, and [p], the concentration of the peptide in solution. Equations 1−4

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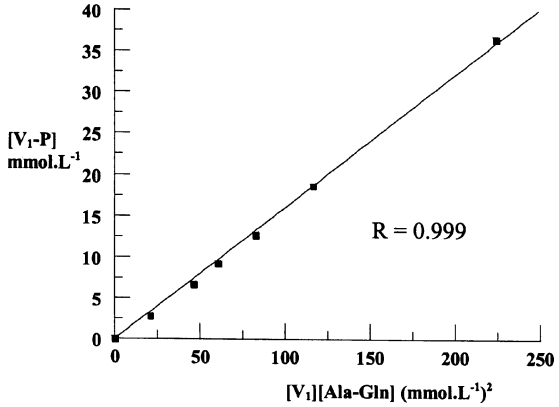


Figure 3. Plot of the concentration of V−Ala-Gln complex as a function of V_1 concentration multiplied with Ala-Gln concentration. This series was conducted using constant vanadium concentration [V] = 50 mmol·L^{-1}, pH 7, and 298 K.

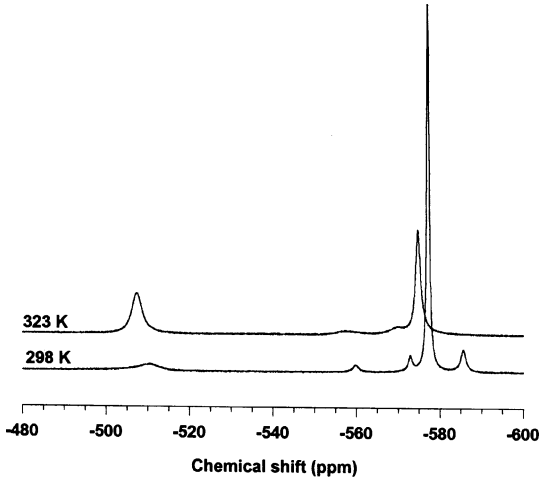


Figure 4. ^{51}V solution NMR spectra of vanadate/Val-Gln solutions at 298 and 323 K, V/P = 1, $[V]_{TOT}$ = 50 mmol·L^{-1}, pH 7, ^{51}V Larmor frequency = 105.2 MHz, and NS =256.

only provide the number of peptides and the number of vanadium atoms per complex. No information is given about the loss of water or protons. Figure 3 shows the relationship of complex concentration to the product of V_1 concentration and Ala-Gln concentrations. The stoichiometry of the other V−peptides complexes (except V−Gly-Glu) was also determined in a similar approach, and the corresponding graphs are available as Supporting Information. Figure 3 shows a good linear relationship which is also obtained for the other dipeptides studied in this work in agreement with literature data[22,24,25] and consistent with a 1:1 peptide:vanadate stoichiometry for the complexes. Formation constants K_f for the complexes could be deduced from the slope of the curves. They are listed in Table 1 for the different V−peptide complexes. The formation constant for V−Ala-Gly (K_f = 70) is slightly larger than the one reported in the literature (K_f = 52).[33] Nevertheless, this discrepancy between both formation constants may be due to the experimental conditions. Measurements reported in the literature were performed in an aqueous 0.6 mol·L^{-1} NaCl medium and therefore at a larger ionic strength when compared to the aqueous medium of the present study (a 50 mmol·L^{-1} Na^+ medium). It was previously shown that the equilibria of such vanadate/dipeptides solutions depend on the ionic strength and that the formation of some V−dipeptides complexes is favored at low ionic strength.[22] For the same reasons, the formation constant for V−Gly-Glu (K_f = 50) is also larger than the one reported in the literature (K_f = 23 in a 1.0 mol·L^{-1} KCl medium).[24] Moreover, the value reported in the present study is not accurate as it could only be determined from two solutions with the largest peptide concentration.

The spectra of the vanadate/Val-Gln solution for V/P = 1 at pH 7 were recorded at 298 and 323 K (Figure 4). The spectra of the vanadate/Ala-Gly solution for V/P = 1 at pH 7 were also recorded at those temperatures. It was previously shown that the vanadate oligomers observed at a pH 7−8 (V_1, V_2, V_4, and V_5) exchange with each other.[31,39] The major exchange paths responsible for the formation of various oligomers were identified and were quantified by 2D EXSY ^{51}V NMR.[39] In particular, V_1, V_2, V_4, and V_5 are in rapid and complex equilibrium.[39] In the presence of Val-Gln at 323 K (Figure 4), the ^{51}V NMR spectrum shows a broadening of the resonances associated with V_1 and V_2, as well as the coalescence of the V_4 and V_5 resonances which merge into a single peak as already described.[39] Deconvolution and integration of the signals show a modification of the V−Val-Gln complex and vanadate (V_1, V_2, V_4, and V_5) populations: from 20−80% at 298 K to 50−50% at 323 K, respectively. This feature indicates that the increase in temperature favors the formation of the complex. Similar results were obtained for the vanadate/Ala-Gly solution. The V−Ala-Gly complex and vanadate (V_1, V_2, V_4, and V_5) populations are changed from 8−92% at 298 K to 20−80% at 323 K.

The effect of temperature on the equilibria between vanadate and V−dipeptide complexes was confirmed by a series of ^{51}V NMR spectra recorded on vanadate/Ala-Gln solutions for V/P = 1 and $[V]_{TOT}$ = 0.2 mol·L^{-1} and at different temperatures (296, 306, 316, and 326 K). This series, available as Supporting Information, shows a significant increase of the concentration of V−Ala-Gln complex from 19% at 296 K to 33% at 326 K.

The vanadate/dipeptide solutions were studied by ^{51}V NMR spectroscopy over a wide pH range. For each dipeptide, one set of spectra was recorded at a ratio V/P = 2/5 and at $[V]_{TOT}$ = 10 mmol·L^{-1}. The distribution of vanadium-containing species from pH 2 to 9 in a solution containing Ala-Gln is shown in Figure 5. The corresponding distributions for the other dipeptides and the formation constants for V−Val-Gln and V−Ala-Gln complexes in the range 4 < pH < 9 are available as Supporting Information. For the other V−P complexes, it was not possible to estimate these constants as the V−P complexes are obtained in a very small amount. The large change in relative resonance integration of the V−dipeptide complexes with changing pH was clearly depicted. The V−Ala-Gln complex has its maximum concentration at a pH between 7 and 8, where it binds ca. 30%

(38) Borgen, O.; Mahmoud, M. R.; Skauvik, I. *Acta Chem. Scand.* **1977**, *A31*, 329−339.
(39) Crans, D. C.; Rithner, C. D.; Theisen, L. A. *J. Am. Chem. Soc.* **1990**, *112*, 2901−2908.

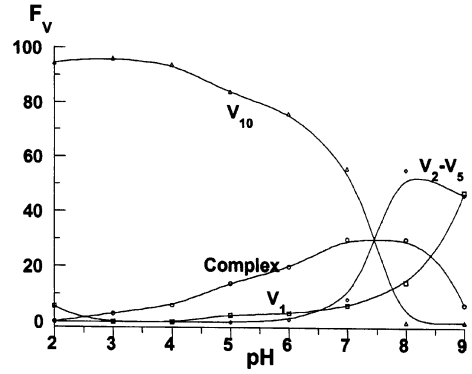


Figure 5. Diagram showing the distribution of vanadates species in the vanadate/Ala-Gln solution, F_v, versus pH at V/P = 2/5, $[V]_{TOT}$ = 10 mmol·L^{-1}. F_v is defined as the percentage of [V] in a species and total [V]. The symbols represent experimental NMR data points.

of the total vanadium. It does however exist in a wide pH range from 3 to 9. Similar distributions were obtained for the other dipeptides. The amount of V−dipeptide complexes at pH 7 is however particularly low for Gly-Glu, Gly-Gln, and Ala-Gly (almost 10%) and larger for Val-Gln (almost 55%). Increasing pH leads to a decreasing amount of the V−dipeptide complexes. Such an observation is in good agreement with the reported pK_a of the V−dipeptide complexes. They are between 8 and 10 for dipeptides with aliphatic side chains such as Gly-Gly, Gly-Ser, and Ala-Ser.[20,22]

For all V−dipeptide complexes, the ^{51}V NMR chemical shift is pH invariant. Since the resonance resolution gives no indication of overlapping peaks, this signal arises probably from one single V−dipeptide complex whose chemical environment (nature of oxo and OH ligands) and charge is about the same in the pH range 2−9.

Characterization of the V-Peptide Complexes by ^{14}N NMR Spectroscopy. ^{14}N NMR spectra were recorded in solution to get more information about the molecular structures of the V−dipeptide complexes. The nitrogen nucleus possesses two magnetic nuclei, ^{15}N and ^{14}N. Both have a rather low magnetogyric ratio (γ) and therefore a poor NMR sensitivity, about 1/10 that of ^{13}C. ^{15}N has a spin I = 1/2 but a low natural abundance (0.365%) and negative NOE factors (γ being negative). Moreover it may be slow to relax.[40] Therefore, observation of ^{15}N sites requires ^{15}N-enriched molecules. In contrast, the ^{14}N nucleus (I = 1) is quite attractive as it is nearly 100% abundant. Its quadrupole moment is relatively small ($Q = 0.017 \times 10^{-28}$ m^2), and the line broadening not too large.[40] However, an interference phenomenon (acoustic ringing) occurs between the resonance probe and the low resonance frequency of ^{14}N, giving rise to an important distortion of the baseline that strongly modifies the signals. This drawback was overcome by recording a spin−echo sequence (see Experimental Section).

The ^{14}N NMR spectra of the vanadate/Val-Gln and vanadate/Ala-Gly (V/P = 1) solutions were recorded at pH 7 and at 298 K. As a comparison, the ^{14}N NMR spectra of the sole dipeptides in solution were recorded in the same

(40) Mason, J. In *Multinuclear NMR*; Mason, J., Ed.; Plenum Press: New York, 1987; pp 335−367.

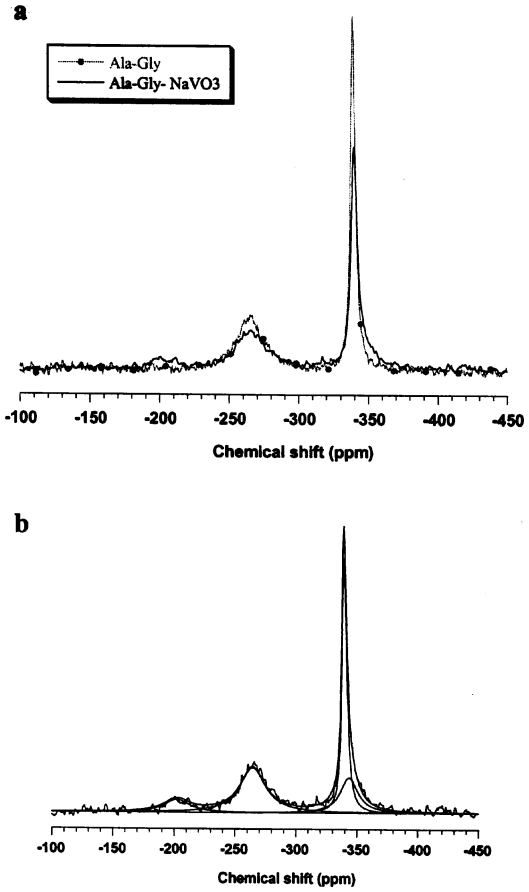


Figure 6. (a) ^{14}N solution echo NMR spectra of Ala-Gly and vanadate/Ala-Gly with V/P = 1, pH 7, $[V]_{TOT}$ = 50 mmol·L^{-1}, 323 K, NS = 6400, ^{14}N Larmor frequency = 28.9 MHz, and LB (line broadening) = 30 Hz. (b) Experimental and simulated ^{14}N solution echo NMR spectra of vanadate/Ala-Gly.

conditions. In the presence of vanadate, no additional signal is present but a decrease in intensity is observed which may be due to the complexation of vanadium by dipeptides. This effect is more pronounced for the terminal $N_tH_3^+$ site. However, the resolution of the spectra is not good enough to determine which nitrogen site interacts with vanadium. Therefore, ^{14}N NMR spectra of the vanadate/dipeptide solutions (V/P = 1, pH 7) were also recorded at 323 K to favor complexation as shown by ^{51}V NMR spectroscopy (vide supra). Moreover for quadrupolar nucleus such as ^{14}N, increasing the temperature leads to sharper lines and a better resolution. Figures 6 and 7 show the ^{14}N NMR spectra of vanadate/Ala-Gly and vanadate/Val-Gln solutions at 323 K. The ^{14}N NMR spectra corresponding to the other dipeptides and other vanadate/dipeptide solutions are available as Supporting Information. The chemical shifts and the line width of the signals observed for the five vanadate/dipeptide solutions are listed in Table 2. They were deduced from the simulation of the spectra made with the Winfit program.[27]

Figure 6 displays the ^{14}N NMR spectra of the vanadate/Ala-Gly solution and the sole Ala-Gly dipeptide at 323 K. The spectrum of the sole dipeptide Ala-Gly displays two signals at −265 ppm and at −340 ppm that can be assigned to the peptide nitrogen N_pH and to the terminal $N_tH_3^+$ site (Chart 1).[41] In the presence of vanadium, both signals are still present but their intensity decreases. Moreover, the

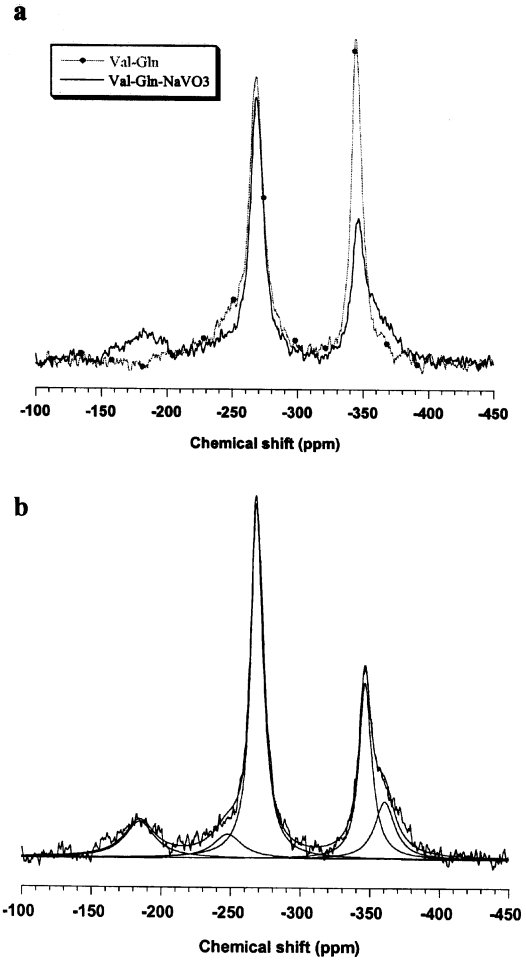


Figure 7. (a) ^{14}N solution echo NMR spectra of Val-Gln and vanadate/Val-Gln with V/P = 1, pH 7, $[V]_{TOT}$ = 50 mmol·L^{-1}, 323 K, NS = 6400, ^{14}N Larmor frequency = 28.9 MHz, and LB = 30 Hz. (b) Experimental and simulated ^{14}N solution echo NMR spectra of vanadate/Val-Gln.

spectrum displays two additional broad signals (Figure 6b). A signal whose line width is close to that of the N_pH sole peptide is observed at −202 ppm. It can be assigned to the N_p^- nitrogen bound to vanadium in the V−Ala-Gly complex. The OCN_p^- group is formed upon the reaction of vanadium with the OCN_pH peptide function. Actually, the replacement of peptide hydrogen by an alkyl group prevents complex formation.[22] As a consequence, a deprotonated secondary amide OCN_p^- is probably the coordination site. A broad signal at −344 ppm is slightly shifted toward high field compared to the sole peptide $N_tH_3^+$ nitrogen (Figure 6b). The line width of the $N_tH_3^+$nitrogen signal is larger than the one of the sole dipeptide (see Table 2). This may result from the interaction of this nitrogen site with vanadium, which leads to a change in the local electronic symmetry of the nitrogen nucleus. Therefore, this signal can be assigned to a terminal N_tH_2 site bound to vanadium resulting from the coordination reaction of the vanadium by the terminal $N_tH_3^+$ group of the peptide.

The ^{14}N NMR spectra of the vanadate/Val-Gln solution and the sole Val-Gln peptide at 323 K are presented in Figure 7. Three signals are evidenced for the sole peptide while only two signals could be distinguished at 298 K. The sharp peak at −346 ppm corresponding to the terminal $N_tH_3^+$ site is still present. However, two components at −247 and −269 ppm are clearly evidenced in the broad peak at −260 ppm. These resonances correspond respectively to the N_pH nitrogen and to the amide N_sH_2 of the glutamine side chain. In the presence of vanadate, five signals are observed (see Figure 7b). The signals of the sole peptide are present. The integration of the $N_tH_3^+$ and N_pH signals decreases strongly in the presence of vanadium while the integration of the amide N_sH_2 signal does not change. Moreover, the spectrum displays two broad signals at −185 and −362 ppm, which can be assigned respectively to the peptide nitrogen N_p^- and to the terminal N_tH_2 group that are both bound to vanadium. Similar spectra were obtained for the other V−peptides complexes. These experiments indicate a possible coordination of the vanadium center by the terminal $N_tH_3^+$ group and the peptide nitrogen N_pH. Such a coordination mode was previously evidenced by ^{15}N NMR spectroscopy for the V−Gly-Tyr complex.[25] For the peptides with glutamine, it seems that the amide NH_2 nitrogen of the side chain is not directly involved in complexation as no difference was observed for this nitrogen site between the ^{14}N NMR signal of the complex and the one of the sole dipeptide. A ^{13}C NMR study was undertaken to confirm these results and to evidence additional complexing functions.

Characterization of the V−Dipeptide Complexes by ^{13}C NMR Spectroscopy. The ^{13}C NMR spectra of the vanadate/dipeptide solutions (V/P = 1, $[V]_{TOT}$ = 50 mmol·L^{-1}) were recorded at pH 7 and at 298 K. They are available as Supporting Information. Chemical shifts of all carbon atoms in the complexes as well as the sole ligands are reported in Table 3. ^{13}C chemical shifts of the dipeptides bound to vanadium were assigned according to ^{1}H−^{13}C HMBC and ^{1}H−^{13}C HMQC experiments. Four carbon resonances in the complexes show significant shifts from the free ligand: the first carbon C(1) linked to the terminal $N_tH_3^+$ (between 4.2 and 6.4 ppm), the carbonyl carbon C(2) in the peptide group (between 10.8 and 13.3 ppm), the carbon C(3) linked to the peptide group (between 9.5 and 11.7 ppm), and the terminal carboxylate carbon C(4) (between 6.6 and 9.5 ppm). On the basis of these data, we can suggest that the terminal $N_tH_3^+$, the peptide nitrogen CON_p^- and the terminal carboxylate are all directly linked to the vanadium center, in agreement with the previous ^{14}N NMR experiments. Moreover, it has been reported that for peptides that do not contain any functionalized side chains, protection of either the N-terminal or C-terminal position or replacement of the peptide hydrogen by an alkyl group prevents complex formation.[22,25] On the other hand, the ^{13}C shift of the terminal carboxylate C(4) is quite small compared to the ^{13}C shift of the peptide carbon C(2). As a consequence, the interaction between the terminal carboxylate and vanadium should be fairly weak and we may assume that they could be induced by the trans effect of the oxo ligand. Such a coordination mode was previously proposed for the V−Gly-Tyr and V−Ala-Ser (isomer $[V-Ala-Ser]^{2-}$) complexes whose ^{13}C NMR shifts are also

(41) Richards, R. E.; Thomas, N. A. *J. Chem. Soc., Perkin Trans. 2* **1973**, 368−374.

Table 2. ^{14}N NMR Chemical Shifts, δ (ppm), and Line Widths, $\Delta\nu_{1/2}$ (Hz) (in Parentheses), of the V−Peptide Complexes at 323 K (V/P = 1, [V] = 50 mmol·L^{-1}, pH 7)

	Val-Gln		Ala-Gln		Ala-Gly	
N	peptide	complex	peptide	complex	peptide	complex
N_p[a]	−247 (730)	−247 (730)	−252 (880)	−252 (890)	−265 (730)	−265 (730)
N_p−V[b]		−185 (731)		−191 (880)		−202 (730)
N_s[c]	−269 (343)	−270 (348)	−269 (350)	−270 (320)		
N_t[d]	−346 (263)	−346 (340)	−340 (207)	−340 (203)	−340 (114)	−341 (130)
N_t−V[e]		−362 (558)		−343 (620)		−344 (500)

	Gly-Gln		Gly-Glu	
	peptide	complex	peptide	complex
N_p	−254 (810)	−254 (810)	−254 (997)	−254 (1044)
N_p−V		−188 (810)		−189 (512)
N_s	−270 (320)	−270 (320)		
N_t	−354 (160)	−357 (250)	−356 (238)	−353 (148)
N_t−V		−365 (520)		−361 (456)

[a] Peptide nitrogen. [b] Peptide nitrogen bound to vanadium. [c] Nitrogen of the side chain. [d] Terminal nitrogen. [e] Terminal nitrogen bound to vanadium.

Table 3. ^{13}C NMR Chemical Shifts (ppm) of V−Dipeptide Complexes and Sole Dipeptides with $\Delta\delta(^{13}C)$ upon Complexation for the V−Dipeptide Complexes ([V] = 50 mmol·L^{-1}, pH 7)

	Val-Gln			Ala-Gln			Ala-Gly		
atom[a]	peptide	complex	$\Delta\delta$[b]	peptide	complex	$\Delta\delta$	peptide	complex	$\Delta\delta$
C_1	59.8	64.0	4.2	50.1	55.1	5.0	50.2	54.8	4.6
C_2	170.7	181.5	10.8	171.3	182.4	11.1	171.8	183.7	11.9
C_3	55.9	65.4	9.5	55.9	65.5	9.6	44.2	55.9	11.7
C_4	178.4	185.0	6.6	178.5	185.1	6.6	177.2	186.7	9.5
C_5	28.5	29.2	0.7	28.4	29.1	0.7			
C_6	32.5	30.9	−1.6	32.5	31.0	−1.5			
C_7	179.5	179.5	≈0	179.5	179.7	0.2			
C_8	31.1	30.9	−0.2	17.4	19.3	1.9	17.4	18.4	1.0
C_9	17.7/18.7	15.3/19.0							

	Ala-Ser[c]					
atom[a]	peptide	complex 1[d]	$\Delta\delta$	peptide	complex 2[d]	$\Delta\delta$
C_1	59.6	64.6	5.0	59.2	71.8	12.6
C_2	173.2	183.8	10.6	178.4	182.0	3.6
C_3	64.1	70.2	6.1	64.2	77.7	13.5
C_4	178.1	185.3	7.2	180.0	185.2	5.2
C_5						
C_6[e]	51.5	56.3	4.8	52.4	57.0	4.6
C_7						
C_8	18.9	20.6	1.7	21.8	19.0	−2.8
C_9						

	Gly-Gln			Gly-Glu			Gly-Tyr[f]		
atom[a]	peptide	complex	$\Delta\delta$	peptide	complex	$\Delta\delta$	peptide	complex	$\Delta\delta$
C_1	41.5	47.9	6.4	41.9	48.0	6.1	43.3	49.5	6.2
C_2	167.5	180.8	13.3	168.6	180.5	11.9	169.2	181.1	11.9
C_3	55.7	65.5	9.8	56.2	65.8	9.6	59.5	69.4	9.9
C_4	178.8	185.2	6.4	179.5	185.5	6.0	180.6	183.6	3.0
C_5	28.5	29.1	0.6	29.2	30.2	1.0			
C_6	32.5	31.0	−1.5	34.9	33.1	−1.8			
C_7	179.5	179.5	≈0	182.9	183.3	0.4			

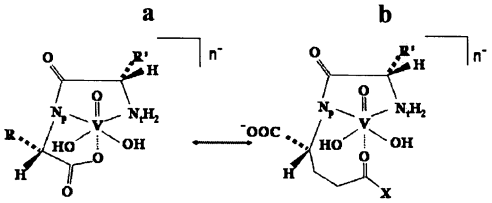
[a] See numbering in Chart 1. [b] Coordination shift $\Delta\delta = \delta$(complex) − δ(peptide). [c] NMR data according to Gorzsas et al.[20]. [d] Complex 1 corresponds to [V−Ala-Ser]$^-$, and complex 2, to [V−Ala-Ser]$^{2-}$. The maximum amounts are obtained respectively at pH 6.5 and 8.5.[20] [e] The C_6 carbon corresponds to the methylene carbon linked to the hydroxyl group. [f] NMR data according to Crans et al.[25]

reported in Table 3.[20,25] It was also observed for oxovanadium(V) triethanolamine, oxovanadium(V) (*S*,*S*,*S*)-tri-2-propanolamine, and oxotris(pivalato)vanadium that were characterized in solution and in solid state.[42] According to the above arguments, we can propose a possible molecular structure of the V−dipeptide complexes, which is presented in Scheme 1a.

Dipeptides Ala-Gln, Gly-Gln, Gly-Glu, and Val-Gln also exhibit a functionalized side chain. However, it is not obvious that the carbonyl group (amide or carboxylate) of the side chain is involved in coordination with vanadium as all the carbons of the side chain show small carbon shifts (<1 ppm for carbon C(7)). The assignment of the C(7) carbon in the complexes is mainly based on the HMBC experiment which reveals, for all the V−dipeptides complexes, a cross-peak

(42) (a) Crans, D. C.; Chen, H.; Anderson, O. P.; Miller, M. M. *J. Am. Chem. Soc.* **1993**, *115*, 6769−6776. (b) Rehder, D.; Priebsch, W.; von Oeynhausen, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1221−1222.

Scheme 1. Proposed Chemical Structure of the V−Dipeptide Complexes



Gln **: R = CH_2-CH_2-CO-NH_2** ***Gln*** **: X = NH_2**

Glu **: R = CH_2-CH_2-COO^-** ***Glu*** **: X = O^-**

Val **: R' = $CH(CH_3)_2$**
Ala **: R' = CH_3**
Gly **: R' = H**

n = 1 for the five V-dipeptide complexes
except n = 2 for the V-Gly-Glu complex

between the $CH_2(5)$ and $CH_2(6)$ protons and a carbon whose chemical shift is quite close to the one of the C(7) carbon of the free dipeptide. This observation is in agreement with the dipeptides Gly-Tyr, Ala-Ser, and Ala-His as no evidence for complexation of vanadium by the side chain could be established by ^{13}C NMR spectroscopy.[19,20,25,43] In contrast, for the His-Ser dipeptide, a large ^{13}C shift observed for the hydroxymethyl carbon supports a coordination of vanadium by CH_2O^- while no large coordination shifts are observed for the imidazole carbons, reinforcing the view that no vanadium−imidazole bond is formed upon complexation.[24] The $^1H-^{13}C$ HMBC experiment was crucial for the assignment of the carbonyl carbons in the complexes as their chemical shifts are all in the same range 179−185 ppm. Detection of cross-peaks for the carbonyl carbons (C(2), C(4), and C(7)) permits unambiguously the assignment of ^{13}C signals of the dipeptides bound to vanadium. However in some vanadate/dipeptide (dipeptide: Gly-Gln and Gly-Glu) solutions, an additional signal close to 186 ppm is present in the 1D ^{13}C spectrum, while no cross-peak was detected in the HMBC experiment. Moreover as its presence is randomly observed in samples which were prepared in the same way, this signal can be assigned to an impurity.

Nevertheless, the interaction of the side chains with vanadium cannot be ruled out on the basis of differences in chemical shifts. Indeed the ^{13}C chemical shift of ligands in metal complexes depends on a wide range of parameters such as metal−ligand bond lengths, metal−ligand bond energies, and the geometry of the complex. A lack of chemical shift change may be the result of opposite contributions to the ^{13}C chemical shift.

Therefore, isomer b (see Scheme 1) may also be present in solution in a small amount. The presence of a single ^{51}V NMR peak can be explained since the coordination sphere (geometry of the metal center and the nature of the ligands) at the vanadium is almost the same in both complexes a and b. The ^{51}V chemical shift difference is expected to be small and would contribute to the line width of several ppm. The structure of isomer b is consistent with the one of an oxovanadium(V) complex $[VOCl_3(Hpycan)]$ {Hpycan = *N*-(2-nitrophenyl)pyridine-2-carboxamide} which exhibits a distorted octahedral coordination with a $V-O_{amide}$ bond oriented trans to the oxo ligand.[44] However, isomer b exhibits a six-membered ring while a five-membered one is definitely more stable for vanadium. Interactions of both terminal carboxylate and the carbonyl group (amide or carboxylate) of the side chain with vanadium is only possible in the case of a fast exchange between two isomers a and b (Scheme 1) on the time scale of the ^{13}C NMR experiment. Therefore, as the carbon shift is the average over two positions, it is expected to be small especially as the terminal carboxylate and the carbonyl group of the side chain are both trans ligands in the isomers.

Discussion

Vanadate tends to react with dipeptides Ala-Gln, Ala-Gly, Val-Gln, Gly-Gln, and Gly-Glu to form complexes with characteristic ^{51}V NMR chemical shifts of about −510 ppm. Such a chemical shift was already observed for V−dipeptides complexes such as V−Gly-Tyr and V−Ala-Ser.[19,20,22−25] Actually, vanadium(V) has a versatile coordination geometry and coordination numbers ranging between 4 and 7. For the V−dipeptide complexes, it is reasonable that the vanadium center is 6- or 5-fold-coordinated with oxygen or nitrogen ligands.

According to ^{14}N and ^{13}C NMR experiments and in agreement with the previous works reported in litterature,[19,20,22,25,33] a molecular structure can be proposed for the V−dipeptide complexes (see Scheme 1a). Dipeptides behave as multidentate ligands as three functionalities are required for complex formation. ^{14}N and ^{13}C NMR spectra clearly pointed out the involvement of the terminal amino N_tH_2 group, the terminal carboxylate group, and the peptide nitrogen CON_p^- in the complex. A similar structure was proposed for the complexes V−Gly-Ser, V-Gly-Tyr, V−Ala-His, and V−Ala-Ser (isomer $[V-Ala-Ser]^{2-}$).[19,20,22,25] This structure has both a deprotonated peptide (OCN_p^-) nitrogen and a deprotonated amine (N_tH_2) nitrogen. Indeed, solid-state and solution studies have shown the loss of the peptide proton in several other metal complexes.[45,46] It was also reported that protonation of the peptide nitrogen can lead to the dissociation of some metal−peptide complexes.[45] Furthermore, we can guess that the deprotonation of these nitrogen atoms increases their nucleophilic power and promotes complexation with vanadium. However, the presence of a terminal NH nitrogen cannot be ruled out. Actually, the protonation state of nitrogen depends on the nature of the V−N bond. One can expect a NH group in the case of an electrostatic interaction while a NH_2 group should be more appropriate in the case of a coordinative bond between the lone pair of the terminal amino group and the vanadium

(43) Fritzsche, M.; Vergopoulos, V.; Rehder, D. *Inorg. Chim. Acta* **1993**, *211*, 11−16.

(44) Kabanos, T. A.; Keramidas, A. D.; Papaioannou, A.; Terzis, A. *Inorg. Chem.* **1994**, *33*, 845−846.

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atom. To complete the vanadium coordination, three oxygen atoms (2 OH and 1 =O) not bound to the ligand could appear in any configurational arrangement, but only one form was illustrated in Scheme 1. Moreover the terminal carboxylate is located in the axial position as can be assumed by ^{13}C NMR analysis.

The experiments performed in this work allow us to estimate the role in complex formation of the complexing or sterically demanding side chain. The formation constants increase respectively from Gly-Gln (60), Ala-Gln (160), and Val-Gln (210), and these dipeptides can only be distinguished by the nature of the side chains. For valine and alanine, this effect should be related either to the increasing steric hindrance induced by the alkyl group or to its donor effect. We may assume that the donor effect of the alkyl groups is not significant. Indeed among the three dipeptides, no significant difference in ^{13}C chemical shift is evidenced for the coordinative sites that are the peptide group and the terminal carboxylate (see Table 3). Therefore, complexation should be promoted by the steric hindrance of the alkyl group. A sterically demanding alkyl group may favor a peptide conformation that more readily complexes vanadate. The arrangement around the carbon C_1 for valine is supposed to be quite bent compared to that of glycine. Such an assumption was previously proposed for the bulky dipeptides Val-Asp and Leu-Leu since there is no decrease in the formation of the corresponding complexes compared to small dipeptides.[22]

For glutamine, the complexing side chain also promotes V−dipeptide complex formation since the formation constants for Ala-Gly and Ala-Gln are respectively 70 and 160. The formation constant of Val-Gln ($K_f = 210$) is also higher than the one of Val-Gly ($K_f = 60$) already reported in the literature.[22] It is in agreement with the increased constant formation observed for the V−Gly-Gln complex ($K_f = 60$) compared to the one of V−Gly-Gly ($K_f = 19$).[22] However it was not possible to evidence the interaction of the carboxylate (Glu) or the amide (Gln) group of the side chain with vanadium by ^{13}C and ^{14}N NMR spectroscopy. Therefore, the role of the glutamine side chain could also be related to steric effects. Actually, the complexation of vanadium by the side chain substituents was only reported for dipeptide-containing seryl or threonyl amino acids.[22,24] Indeed, two products are obtained with Gly-Ser and they are retained when Gly-Ser is replaced by Gly-Ser ethyl ester showing that there is no necessity for the carboxylate functionality to be present if the peptide bears a complexing side chain like $-CH_2OH$ in glycylserine ethyl ester.[22] Nevertheless, the absence of any complexation of vanadium by dipeptides containing carboxylate or amide groups in the side chains remains quite surprising as different carbonyl groups are known to interact strongly with vanadium(V) and (IV) as established for acetate, dicarboxylic acids,[26] oxalate,[47] picolinate, dipicolinate,[18,48] EDTA ligand,[49] and amide ligands.[50,51] This shows that an ambiguity remains regarding the complexation of vanadium by the Gln and Glu side chains substituents. It is also difficult to explain how the functionalized side chains play a role in the formation of the complexes, and it has to be determined.

It appears that the presence of a side chain on each amino acid favors the formation of a stable complex. Indeed Val-Gln which exhibits a sterically demanding side chain on valine as well as a bulky functionalized side chain on glutamine binds more efficiently to vanadate ($K_f = 210$).

Conclusion

We have studied the complexation of vanadium(V) by five dipeptides differing by the nature of the side chains. These V−dipeptide complexes were characterized in solution by UV−visible spectroscopy and multinuclear (^{51}V, ^{14}N, ^{13}C) NMR spectroscopy. These experiments show that all complexes exhibit a 1:1 stoichiometry and that the terminal amino N_tH_2, the terminal carboxylate, and the peptide nitrogen $OC-N_p^-$ are involved in the coordination with vanadium. It seems that the presence of a sterically demanding side chain (Val or Ala) as well as a bulky functionalized side chain (Gln) are two important requirements for a fairly stable complex (V−Val-Gln and V−Ala-Gln).

This work shows that peptides or proteins cannot interact with vanadium by random binding at any peptide linkages as terminal amino and terminal carboxylate may be both required for a vanadium complexation. Moreover, as shown in this study, it seems that the nature of the side chains strongly modifies the stability of the vanadium−dipeptide complexes. As a consequence, side chains of amino acids differing by their complexing functions, their hydrophobicity, their charge, or their steric hindrance may impose different constraints on complex formation. This is a topic of continuing interest, and this work could be extended to more complex peptides to provide a better understanding of the mechanism by which vanadate interacts with polypeptides or proteins.

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Supporting Information Available: Figures showing UV−vis and NMR spectra, formation constant data, and distribution of species data. This material is available free of charge via the Internet at http://pubs.acs.org.

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