Speciation in Vanadium Bioinorganic Systems. 5. Interactions between Vanadate, Uridine, and Imidazole−An Aqueous Potentiometric, ^{51}V, ^{17}O, and ^{13}C NMR Study

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The full speciation has been determined in the aqueous vanadate−uridine (UrH_2) and vanadate−uridine−imidazole (ImH) systems. The studies performed allow direct comparison with the corresponding adenosine systems, and the role of the pyrimidine and purine bases on various complex properties could be evaluated. The studies also provide insight into possible biological mechanism of action. Formation constants have been determined in 0.600 M Na(Cl) medium at 25 °C in the pH range 2−11, using a combination of potentiometry and ^{51}V NMR spectroscopy. In the $H^+-H_2VO_4^-$−UrH_2 system, two dimeric complexes form: $V_2Ur_2^{2-}$ with $\log \beta = 7.66 \pm 0.02$ and $V_2Ur_2^{3-}$ with $\log \beta = -1.09 \pm 0.04$. The errors given are 3σ. Both species give rise to NMR resonances at −523 ppm. A minor ^{51}V NMR resonance at −507 ppm is probably originating from a VUr^{2-} species. No evidence was found for a 1:1 species at −523 ppm or a 1:1 species with ^{51}V NMR shift superimposed on the vanadate monomer. In the $H^+-H_2VO_4^-$−UrH_2−ImH system, two monomeric, mixed ligand species are formed: $VUrIm^-$ with $\log \beta = 3.12 \pm 0.04$ and $VUrIm^{2-}$ with $\log \beta = -6.26 \pm 0.20$. The p$K_a$ values for the $V_2Ur_2^{2-}$ and $VUrIm^-$ species, 8.75 and 9.38, are both close to that of uridine (9.02) and are consistent with the interpretation that the deprotonation site is located on the base part of the nucleoside. For both the $V_2Ur_2^{n-}$ and $VUrIm^{n-}$ species, isomers have been identified. Equilibrium conditions are illustrated in distribution diagrams. A quantitative comparison with the analogous vanadate−adenosine and vanadate−adenosine−imidazole systems is presented. Furthermore, the solution structure of $V_2Ur_2^{2-}$ was examined using ^{17}O NMR spectroscopy. The complex gives rise to a broad resonance at approximately −1010 ppm, which at higher temperature sharpened into two signals of equal intensities at −1007 and −1032 ppm. This spectrum is consistent only with a solution structure containing pentacoordinate vanadium in an arrangement in which one hydroxyl group of the uridine forms an ester with one vanadium atom, and the other hydroxyl group bridges the two vanadium atoms. In addition, ^{13}C NMR studies revealed that the dinuclear vanadate−uridine species exchange while no evidence for intermolecular exchange between the complexes and free uridine was observed. The similarity between the vanadate−uridine and vanadate−adenosine complexes suggests that the role of the base in these complexes is very limited with respect to structural features and thermodynamic and kinetic properties.

Introduction

The aqueous chemistry of vanadate has attracted a great deal of interest due to its ability to induce a large number of biological responses, including e.g. insulin-mimetic activity and cardiovascular effects as described in late compilations of its role in life.[1,2] Vanadium acts not only as a growth factor,[3] but is also the cofactor for a number of haloperoxidases and nitrogenases.[1,4−7] Some organic vanadates can act as enzyme substrates,[1,8−11] although vanadium is also widely recognized for its inhibitory effects on enzymes.[1,2] Both ribonucleases[1,12] and Na,K-ATPase[1] are potently inhibited by vanadium. The crystallographic characterizations of the interactions of a 1:1 complex of vanadate and uridine with ribonuclease A[13] contrast with the fact that in aqueous solution of vanadate and uridine the major species is a −2 charged 2:2 complex.[14−19] The

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evidence for the existence of the cyclic monomeric −1 charged 1:1 vanadate−uridine complex bound by ribonuclease A is indirect, since the ^{51}V NMR signal originating from this species is superimposed on the major 2:2 complex signal. Thus, additional evidence for the existence and, if so, exact formation constants of this 1:1 species are important for those interested in transition state inhibitors of enzyme reactions.

The interaction between vanadate and the nucleoside adenosine has recently been studied in a wide pH range.[14] Uridine is the pyrimidine base analogue of adenosine and would be expected to form similar derivatives with vanadate with respect to the ribose ring, but to show different protonation behavior with respect to the base. Since a new species was identified for the vanadate−adenosine system when using the combined potentiometry and ^{51}V NMR spectroscopy method,[14] it is very likely that a corresponding new species exists in the vanadate−uridine system. Some evidence for acyclic mono- and diesters of monomeric vanadium complexes with different ligands, e.g. ethanol, ethylene glycol, and 1,2-propanediol, has been presented.[20−22] Also it has been proposed that nucleosides form these kind of esters.[15−17]

The crystal structure of $[N(C_2H_5)_4]_2[\{VO_2Ad\}_2]\cdot 4.74H_2O$ has been determined.[23] In this complex, each vanadium is coordinated to the ribose oxygens in an irregular pentacoordinate arrangement. Considering the similarity between uridine and adenosine, the structure of the $V_2Ur_2^{n-}$ complexes should be in accordance with this V_2Ad_2 structure. Although extensive studies have been carried out on various systems, ^{17}O NMR spectroscopy has only been used in the case of the simple ethylene glycol system.[24] The proposed solid state and aqueous structure for the vanadate−uridine 2:2 complex contains two five-coordinate vanadium atoms, and this possibility can conveniently be scrutinized by ^{17}O NMR spectroscopic studies.

Imidazole is often an essential functionality for enzymatic catalysis, since the amino acid histidine contains an imidazole group.[1] Furthermore, imidazole is often used as a buffer given its pK_a value of 7 and its ability to prevent undesirable metal ion based reactions.[1] In addition, in the vanadate−adenosine−imidazole system a 1:1:1 complex of surprisingly high stability is formed.[14] This 1:1:1 complex is significantly more stable than other ternary complexes.[21,25] Documentation of a second complex of this type and the elucidation of the source of the stability of this type of complex would be important for future complex design.

The work presented in this paper describes the complete speciation in the vanadate−uridine and the vanadate−uridine−imidazole systems. To achieve this, potentiometric and ^{51}V NMR spectroscopic data have been recorded. Accurate potentiometric data are needed to obtain precise pH-independent formation constants. With such constants it is possible to model the system and to illustrate equilibrium conditions by means of distribution diagrams.

The quantitative study of the vanadate−uridine system was principally conducted in order to study the vanadate−uridine−imidazole system, but also to identify a possible deprotonated major V_2Ur_2 species and any 1:1 V−Ur species, which so far have only been shown by indirect methods. ^{17}O and ^{13}C NMR spectroscopic studies were carried out to provide information of the solution structure and lability of the major aqueous species. The vanadate−uridine−imidazole system was studied in order to examine the effect of a second ligand on complex formation.

Experimental Section

Chemicals and Analyses. Uridine, $C_9H_{12}N_2O_6$ (Janssen Chimica 99%), was dried at 80 °C but found to contain no water. The concentration was further verified by titrations and the uridine was found to be >99% pure. It was thus used as provided. However, for selected experiments the uridine was recrystallized before use. Saturated uridine solutions in boiling ethanol were cooled to room temperature. After a few hours the crystals were recovered by filtration, washed with cold ethanol and dried in air. The crystals were then ground in a mortar and dried under vacuum over $CaCl_2$(s). The solubility of uridine in 0.600 M Na(Cl) medium at 25 °C is quite high. A solution of at least 800 mM is easily obtained. Imidazole ($C_3H_4N_2$), sodium chloride, stock solutions of sodium metavanadate, and diluted solutions of hydrochloric acid and sodium hydroxide (all E. Merck *p.a.*) were prepared and standardized as described elsewhere.[14] The samples for ^{17}O NMR were prepared by mixing a stock solution of sodium metavanadate with the appropriate amounts of water, $^{17}O-H_2O$ and/or D_2O, to produce a range of concentrations from 400 to 800 mM. Uridine was added either in the form of a solid or as a stock solution in V−Ur ratios from 1:1 to 1:2. Preliminary studies were carried out using 10 atom % ^{17}O-labeled water, and later a series of studies using 50 atom % ^{17}O water was performed.

Equilibration. In the vanadate−uridine and vanadate−uridine−imidazole systems equilibration is fast at neutral and alkaline pH. The complexes form within minutes after mixing the ligands with a vanadate solution. In acid solutions, equilibration can take up to 24 h due to slow decomposition of initially formed decavanadates.

Notation. The equilibria studied are written with the components H^+, $H_2VO_4^-$, UrH_2, and ImH. Thus, the complexes are formed according to the equation

$$p\mathrm{H}^+ + q\mathrm{H_2VO_4}^- + r\mathrm{UrH_2} + s\mathrm{ImH} \rightleftharpoons (\mathrm{H}^+)_p(\mathrm{H_2VO_4}^-)_q(\mathrm{UrH_2})_r(\mathrm{ImH})_s^{p-q}$$

Formation constants are denoted $\beta_{p,q,r,s}$, and complexes are given the notation (p, q, r, s) or $V_xUr_yIm_z^{n-}$. The total concentrations of vanadium, uridine, and imidazole are denoted $[V]_{tot}$, $[Ur]_{tot}$, and $[Im]_{tot}$, respectively.

Potentiometric Measurements. The measurements were carried out as potentiometric titrations in 0.600 M Na(Cl) medium at 25 °C or as separate pH measurements, as described in ref 14.

Potentiometric Data. The first acidity constant for uridine (9.02) was determined from six automated titrations (73 experimental points). The pH and concentration ranges studied were $1.7 \le pH \le 9.5$, and $5 \le [Ur]_{tot}/mM \le 80$. The second acidity constant (12.6) was determined from manual titrations using a combination electrode specially designed for measurements at high pH values. The electrode was calibrated as described in ref 14. The total concentration of uridine was 80 mM, and the pH range covered was $7.5 \le pH \le 12.7$. Four titrations were performed representing 66 experimental points.

For the ternary $H^+-H_2VO_4^--UrH_2$ system, data were obtained from nine automated titrations with a total of 77 experimental points ($2.3 \le pH \le 9.4$, $[V]_{tot} = 10$ or 80 mM, $[Ur]_{tot} = 3-160$ mM). In the quaternary $H^+-H_2VO_4^--UrH_2-ImH$ system, six titrations (79 experimental points) were performed ($7.1 \le pH \le 10.1$, $[V]_{tot} = 10$ mM, $[Ur]_{tot} = 10$, 20, and 80 mM, $[Im]_{tot} = 80$ and 160 mM). Moreover, for the vanadate−uridine and vanadate−uridine−imidazole systems, the potentiometric titrations were supplemented by additional measurements where pH values were measured for each solution that was prepared for the NMR measurements (see below).

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NMR Spectroscopy. Using a Bruker AM-500 MHz spectrometer, ^{51}V NMR spectra were recorded at 131.5 MHz (11.7 T) and 25 ± 1 °C as described earlier.[14] ^{17}O NMR spectra were recorded at 40.7 MHz using a 5 mm tunable probe on a modified Bruker AC-300P spectrometer (7.0 T) in unlocked mode. Spectral windows in the ^{17}O NMR spectra ranged from 20 000 to 150 000 Hz. A 90° pulse width and a relaxation delay of 0.07 s were employed. The spectra were recorded at various temperatures as indicated in the text and were referenced to water (0 ppm). Exponential line broadening of 20 Hz and zero-filling of the time domain data to 2 K was applied to the FID prior to Fourier transformation. Baseline correction was made by selecting a 300−400 ppm window of the spectrum, using the EP subroutines in the Bruker DISNMR software. The K subroutine was used to make manual interactive corrections on the spectra. The spectra were simulated with the WinNuts software, using Lorentzian line shape for a two-peak spectrum with variable Lorentzian fractions (from 0.5 to 1.0) depending on the specific peak line shape.

^{51}V NMR Data. For the V−UrH_2 system, 37 spectra were recorded in the ranges $3.6 \le pH \le 10.0$, $0.5 \le [V]_{tot}/mM \le 80$, and $10 \le [Ur]_{tot}/mM \le 320$. For V−$UrH_2$−ImH, a total of 44 spectra were recorded in the ranges $5.8 \le pH \le 10.1$, with concentration ranges as follows: $2.5 \le [V]_{tot}/mM \le 20$, $20 \le [Ur]_{tot}/mM \le 80$, and $0 \le [Im]_{tot}/mM \le 640$. Immediately after we recorded the NMR spectra, the pH of each of the solutions was measured with a combination electrode. Spectra were quantitatively evaluated using the NMRi[26] or the Bruker UX-NMR/P program to obtain precise integral values.

Computer Calculations. The potentiometric and quantitative ^{51}V NMR data were evaluated with the least-squares program LAKE,[27] as described in ref 14. The LAKE program is able to calculate formation constants from multimethod data. In the present work potentiometric data, obtained from titrations or individual solutions, and quantitative integral NMR data have been used simultaneously to identify species formed and to determine their formation constants. Calculation and plotting of distribution diagrams were performed with the program SOLGASWATER.[28]

Results and Discussion

Subsystems. To establish the complete speciation in the ternary $H^+-H_2VO_4^-$−UrH_2 and quaternary $H^+-H_2VO_4^-$−UrH_2−ImH systems, the equilibria in the subsystems H^+−vanadate, H^+−uridine, H^+−imidazole, H^+−vanadate−imidazole, and H^+−uridine−imidazole have to be known under the same experimental conditions (0.600 M Na(Cl), 25 °C). The speciation in the vanadate system[29,30] and the acidity constant for imidazole[14] were presented earlier. The formation constants and pK_a values of uridine were determined from pH titrations (Table 1).

Uridine contains a pyrimidine base and resultantly stack to a much smaller extent in aqueous solution than adenosine which contains a purine base.[31] Potential stacking between molecules of uridine was examined using ^{1}H NMR measurements of solutions containing 2.5−540 mM uridine. Only above 130 mM concentrations were chemical shift changes observed that could be consistent with stacking interactions. In the quantitative part of the present studies, the concentration of uridine was kept below 160 mM to minimize the effects of intermolecular stacking on complex speciation.

The evidence for a weak vanadate−imidazole complex has been inferred from changes in the vanadate equilibria and from the kinetics of vanadate exchange with simple alcohols in the presence of imidazole.[21] Quantitative data are difficult to extract from such experiments, since an increase in ^{51}V NMR resonance line widths can also arise from the increased viscosity of the solution at high ligand concentration.[32] However, to further examine the possibility of complexation in the $H^+-H_2VO_4^-$−ImH system, ^{51}V NMR spectra of solutions with $[V]_{tot} = 1$ mM and a 640 times excess of imidazole at pH 7.1 and 8.6 were recorded. No resonances apart from the monomeric, dimeric, tetrameric, and pentameric vanadate species were present, and no changes in the chemical shifts occurred compared to those from a similar solution containing no imidazole. The lack of chemical shift changes suggests that if a complex forms it has the same chemical shift or is in rapid equilibrium with vanadate. Since there is a considerable increase in the line widths of the V_1 and V_2 resonances, it is likely that imidazole interacts with V_1 and/or V_2.[21] The increase in line widths for V_4 and V_5 is much smaller, suggesting little or no interaction with these species. Another possible explanation is that imidazole is interacting with an intermediate, perhaps a trivanadate, which facilitates V_1/V_2 exchange. Imidazole may in fact exchange by decreasing the electrostatic repulsion of negatively charged species. It should be noted that the pK_a of imidazole is such that a significant fraction will be protonated in these solutions. Increased reactivity of vanadate is thus observed when imidazole is present in high excess, but for the concentrations and ratios used in this work no vanadate−imidazole complexes could be quantitatively determined. In the three-component system H^+−UrH_2−ImH, no uridine−imidazole complex forms.

Table 1. Composition, Notation, Formation Constants (β), and Acidity Constants (pK_a) for the H^+-UrH_2, $H^+-H_2VO_4^-$−UrH_2, and $H^+-H_2VO_4^-$−UrH_2−ImH Systems [0.600 M Na(Cl), 25 °C]

(p, q, r, s)[a]	notation	$\log \beta \pm (3\sigma)$	pK_a
0, 0, 1, 0	UrH_2	0	9.02
−1, 0, 1, 0	UrH^-	−9.02 (1)	12.6
−2, 0, 1, 0	Ur^{2-}	−21.61 (13)	−
0, 2, 2, 0	$V_2Ur_2^{2-}$	7.66 (2)	8.75
−1, 2, 2, 0	$V_2Ur_2^{3-}$	−1.09 (4)	−
−1, 1, 1, 0[b]	VUr^{2-}	−7.43 (33)	−
0, 1, 1, 1	$VUrIm^-$	3.12 (4)	9.4
−1, 1, 1, 1	$VUrIm^{2-}$	−6.26 (20)	−

[a] The (p, q, r, s) notation is defined in the Experimental Section and means, *e.g.*, that (−1, 2, 2, 0) represents the $[(H^+)_{-1}(H_2VO_4^-)_2(UrH_2^0)_2(ImH^0)_0]^{3-}$ complex which forms via a condensation reaction. [b] The minor −507 ppm ^{51}V NMR resonance.

Speciation in the Vanadate−Uridine System. Although this and related systems have been examined previously by others,[15−19] detailed knowledge of the speciation in a wide pH range and under the conditions used in the present work were required in order to accurately evaluate the interaction with imidazole. ^{51}V NMR spectra recorded on V−UrH_2 solutions show, in addition to the vanadate resonances, a broad unsymmetrical resonance at −523 ppm. Varying pH, concentrations, or $[Ur]_{tot}/[V]_{tot}$ ratios affected neither the chemical shift nor the shape of the resonance. A series of representative spectra at $[Ur]_{tot}/[V]_{tot} = 8$ is shown in Figure 1. As illustrated, complexation occurs in a wide pH range with an optimum around neutral pH. At alkaline pH (>8) additional minor resonances were observed at −507 (shown in Figure 1) and at −470 ppm (only at larger excess of uridine). These two minor resonances

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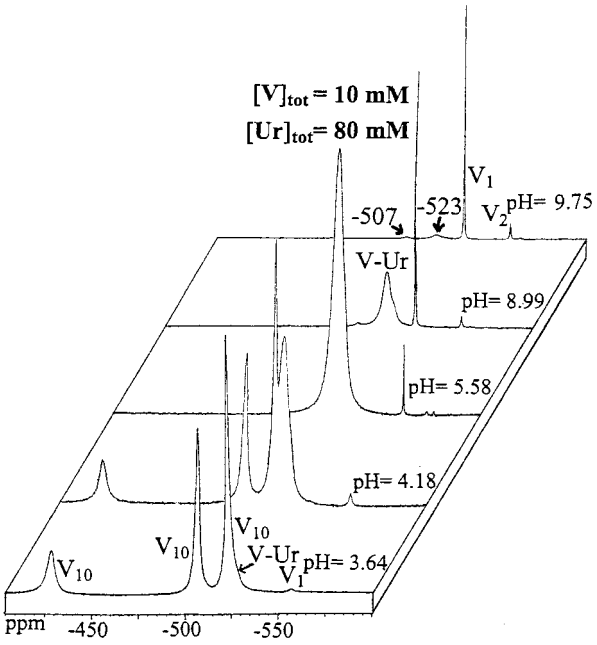

Figure 1. ^{51}V NMR spectra of aqueous solutions of vanadate and uridine ($[V]_{tot}/[Ur]_{tot}$ = 10/80 mM) at different pH values. The spectra are plotted with the highest peak at a preset value.

Table 2. Composition, Notation, Formation Constants, and Goodness of Fit (Expressed in Terms of *U*) for Different Species Models in (a) the V−UrH_2 and (b) the V−UrH_2−ImH Systems; The (*p*, *q*, *r*, *s*) Notation Is Explained in Table 1

(*p*, *q*, *r*, *s*)	notation	log $\beta \pm (3\sigma)$	10^5 *U*
	(a) V−UrH_2 System		
0, 2, 2, 0	$V_2Ur_2^{2-}$	7.88 (4)	730
0, 1, 1, 0	VUr^-	3.35 (6)	5621
0, 2, 1, 0	V_2Ur^{2-}	5.98 (5)	6627
0, 1, 2, 0	VUr_2^-	6.32 (5)	10506
0, 2, 2, 0	$V_2Ur_2^{2-}$	7.66 (2)	90
−1, 2, 2, 0	$V_2Ur_2^{3-}$	−1.11 (4)	
0, 2, 2, 0	$V_2Ur_2^{2-}$	7.66 (2)	86
−1, 2, 2, 0	$V_2Ur_2^{3-}$	−1.09 (4)	
−1, 1, 1, 0[a]	VUr^{2-}	−7.43 (33)	
	(b) V−UrH_2−ImH System		
0, 1, 1, 1	$VUrIm^-$	3.19 (3)	217
0, 1, 2, 1	VUr_2Im^-	4.79 (5)	591
0, 1, 1, 2	$VUrIm_2^-$	3.52 (5)	565
0, 1, 2, 2	$VUr_2Im_2^-$	5.30 (5)	425
0, 2, 2, 2	$V_2Ur_2Im_2^{2-}$	8.38 (6)	582
0, 2, 2, 1	$V_2Ur_2Im^{2-}$	8.02 (4)	318
0, 2, 1, 2	$V_2UrIm_2^{2-}$	6.68 (7)	714
0, 2, 1, 1	V_2UrIm^{2-}	6.34 (5)	471
0, 1, 1, 1	$VUrIm^-$	3.12 (4)	187
−1, 1, 1, 1	$VUrIm^{2-}$	−6.26 (20)	

[a] The minor −507 ppm ^{51}V NMR resonance.

were present in solutions prepared from recrystallized uridine as well, suggesting that they do not arise from impurities.

Different stoichiometric models were tested by using the LAKE program. In Table 2a selected results from the calculations are summarized. As seen, a 2:2 stoichiometry of the major −523 ppm resonance gives the lowest *U* value. This stoichiometry for a diol system has previously been reported by others,[14−19,24] although a 1:1 stoichiometry has also been proposed.[32,33] However, a −2 charged 2:2 species alone does not fit the data satisfactorily. Since deviations were prevalent for data acquired at neutral to alkaline pH, a deprotonated complex was added to the speciation scheme. This lowered the U value considerably, and most of the deviations disappeared. A $V_2Ur_2^{4-}$ complex does not fit data as well as $V_2Ur_2^{3-}$, although one may have expected the pK_a value for the coordinated uridine to be similar on both uridine residues. At alkaline pH small but systematic deviations remained. As the −507 ppm resonance should arise from a more alkaline species than the $V_2Ur_2^{3-}$ complex (charge −1.5 per vanadium), a (−1, 1, 1, 0) complex (charge −2 per vanadium) was included in the model. This complex was able to explain the −507 ppm resonance. The high 3σ reflects the low complex fraction in the solutions. As the −470 ppm resonance was too small to be accurately evaluated, it was not included into the calculations. Thus, experimental data on the V−UrH_2 system are completely explained by two 2:2 vanadate-to-ligand complexes, $V_2Ur_2^{2-}$ and $V_2Ur_2^{3-}$ (−523 ppm resonance), and a 1:1 complex VUr^{2-} (−507 ppm resonance). Formation constants are summarized in Table 1 and give a pK_a value of 8.75 for $V_2Ur_2^{2-}$. Deprotonation takes place without any noticeable change in the ^{51}V NMR chemical shift indicating a protonation site far away from the vanadium atom, presumably the amine proton.

Resolution enhancement of the −523 ppm resonance reveals three peaks at −521.7, −523.3, and −526.9 ppm. Since the evaluation of combined potentiometric and NMR data unambiguously gave complexes of 2:2 composition for this resonance, the three signals represent isomers. The relative amounts of vanadium in the three subpeaks are difficult to evaluate accurately, but an approximate ratio of 6:1 for the sum of the −521.7 and −523.3 ppm peaks versus the −526.9 peak was found. In earlier publications, evidence for very weak 1:1 complexes (in addition to a 2:2 complex) at −523 ppm has been reported.[16,17] Therefore, the presence of a possible pentacoordinated monomeric ester superimposed on the 2:2 resonance was tested. However, such a (0, 1, 1, 0) species was rejected in the LAKE calculations. The formation of a weak tetrahedral monoester with its ^{51}V NMR resonance close to, or superimposed on, the monomeric vanadate resonance has also been inferred from ^{51}V NMR studies.[16,17] Therefore, the monomeric vanadate integral was carefully evaluated in the whole pH range studied, but no additional species contributing to this resonance was necessary to explain the data. However, we were able to observe a very small signal or shoulder close to the monomeric resonance (upfield) in solutions with pH between 8 and 10. As low vanadium concentrations and very high excess of ligand have been employed in the past for studies presenting tetrahedral monomeric species, spectra from solutions with $[V]_{tot}$ = 0.5 mM and $[Ur]_{tot}$ = 320 mM at pH 5.2, 7.3, and 8.5 were recorded. At pH 5.2 the minor signal is absent, but for the two more alkaline solutions a very small (<1%) but rather distinct resonance is seen. However, when the same experiments were repeated using recrystallized uridine, this resonance was absent. Thus, the formation of a tetrahedral vanadate−uridine ester could not be confirmed in the present study, even under conditions which should favor such a species. This observation is likely to have important consequences with respect to the potency of a 1:1 V−Ur complex as an inhibitor.

To illustrate equilibrium conditions, distribution diagrams for $[V]_{tot}$ = 10 mM were calculated and plotted using the formation constants given in Table 1 and the vanadate formation constants reported in ref 30. In Figure 2a, the distribution for an 8-fold

(33) Geraldes, C. F. G. C.; Castro, M. M. C. A. *J. Inorg. Biochem.* **1989**, *35*, 79.

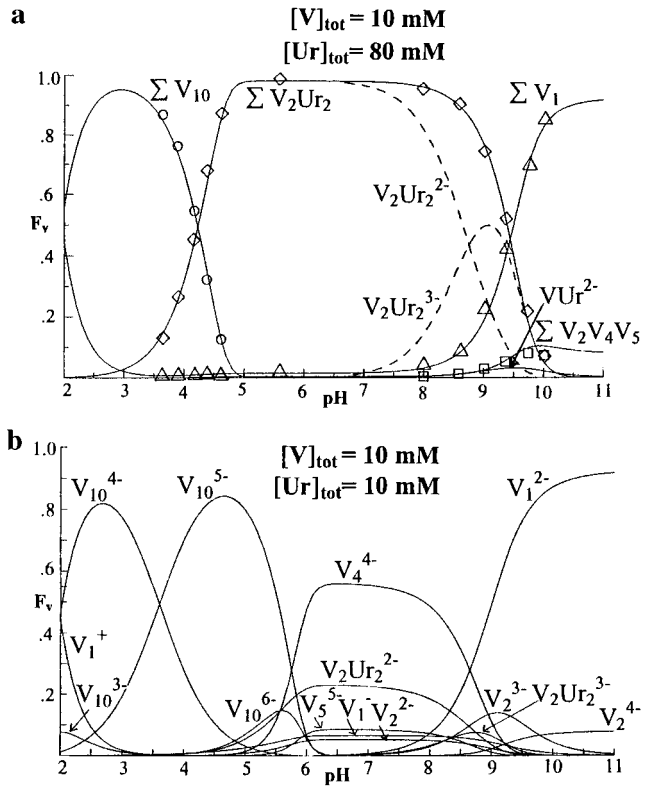

Figure 2. Diagrams showing the distribution of vanadium, F_V, vs pH. F_V is defined as the ratio between [V] in a given species and $[V]_{tot}$ in the solution. (a) $[Ur]_{tot}/[V]_{tot} = 8$. The sums for the decavanadate, oligovanadate, monovanadate, and $V_2Ur_2^{n-}$ species are shown. The symbols represent experimental NMR data points. The $V_2Ur_2^{2-}$ and the $V_2Ur_2^{3-}$ complexes are shown by dashed curves. (b) $[Ur]_{tot}/[V]_{tot} = 1$. All vanadium-containing species are shown except those containing <5% of $[V]_{tot}$.

excess of uridine is shown (the same conditions as in Figure 1). At neutral pH as much as 98% of $[V]_{tot}$ is complexed to uridine. The experimental ^{51}V NMR data points are indicated by symbols, and as seen the fit is excellent. Figure 2b illustrates the distribution prevailing at a ratio $[V]_{tot}/[Ur]_{tot} = 1:1$ (the same as in the vanadate−uridine species formed). At this ratio the amount of vanadium complexed to uridine is, as expected, dramatically lower.

Speciation in the Vanadate−Uridine−Imidazole System. Mixed ligand complexes are expected to form between vanadate, uridine and imidazole, as those formed in the vanadate−adenosine−imidazole system.[14] Therefore, imidazole was added as a fourth component. ^{51}V NMR spectra show the presence of a new broad resonance at −483 ppm, from pH ∼ 5.5 to ∼10.5 with maximum area at pH ∼ 8.5. To obtain the same amount of vanadium bound in these V−UrH_2−ImH complexes as in the V−UrH_2 complexes, a large excess of ImH is needed. The ^{51}V NMR spectra for different imidazole concentrations in a solution containing 10 mM vanadate and 40 mM uridine are shown in Figure 3. The coordination of imidazole to vanadium is strongly favored when uridine is present. Variations in pH, concentrations, or concentration ratios do not change the chemical shift, the line width, or the shape of the resonance. The signal is clearly asymmetric and resolution enhancement shows two peaks indicating the presence of two isomers, one at −483.2 (major) and one at −480.1 ppm (minor).

From LAKE calculations (Table 2b) the −483 ppm resonance was characterized as a −1 charged, mononuclear, mixed ligand species, $VUrIm^-$, with the same stoichiometry as in the corresponding adenosine system.[14] However, because uridine has a deprotonation site, it is possible for an additional −2 charged V−UrH_2−ImH complex to form. This species was tested and resulted in a lower *U* value. Thus, the combination of two monomeric species, $VUrIm^-$ and $VUrIm^{2-}$, represents the model that best explains experimental data.

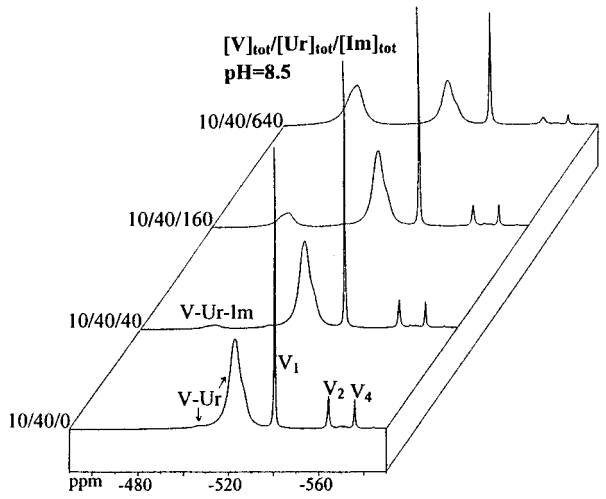

Figure 3. ^{51}V NMR spectra of aqueous solutions containing 10 mM vanadate, 40 mM uridine and varying imidazole concentrations at pH = 8.5. Spectra are plotted in an absolute intensity mode.

The distribution of vanadium-containing species, at the same $[V]_{tot}$ and $[Ur]_{tot}$ as in Figure 2a but with a 2-fold excess of ImH over UrH_2, is shown in Figure 4a and b. As seen, the $V_2Ur_2^{n-}$ species predominate and it is only in the very alkaline pH range (>10) that $VUrIm^{n-}$ is more prevalent than $V_2Ur_2^{n-}$. However, for μM vanadium concentrations and equimillimolar concentrations of uridine and imidazole, the $VUrIm^-$ species is the predominant organic ligand containing species (Figure 5). At alkaline pH (>8.9) the predominant species is the −2 charged monomeric vanadate−uridine complex (VUr^{2-}).

Solution Structure and Lability of the $V_2Ur_2^{n-}$ Complexes. The five earlier proposed structures of 2:2 vanadate−diol complexes (reviewed in ref 24) are shown in a simplified version for vanadate−uridine in Figure 6. Oxygen atoms that can exchange with $H_2{}^{17}O$ are numbered. Only one possible isomer (cis) is shown, but other isomers will have oxygen atoms with very similar structural features. Three complexes (Figure 6a−c) can be described as generated from a μ-O bridged vanadate dimer, with the two uridine ligands coordinating to only one trigonal bipyramidal vanadium atom each. These three structures contain three types of oxygen atoms that would be labeled by the ^{17}O-enriched water (O1, O2, and O3). While O1 and O2 are not identical terminal oxygen atoms, the pairs of atoms O1 and O1′ and O2 and O2′ are also not identical given the chirality in the uridine ligand. However, the latter atoms may or may not be distinguished in the ^{17}O NMR spectrum. The chemical shifts of the O1 and O2 atoms are expected to occur around 1000 ppm. O3 is a bridged oxygen atom with an expected chemical shift value around 700 ppm. Consequently, these types of structures are likely to give spectra containing two or three resonances with a ratio of 4:1 or 2:2:1.

The structure shown in Figure 6d contains octahedral vanadium, with uridine coordinated to both vanadium atoms. This structure contains six exchangeable oxygen atoms and depending on the conformation of the uridine rings, O1 may be very similar to O1′ and O2 similar to O2′. However, given the chirality of uridine, O1 is likely to be different from O2 and O1′ from O2′. All these oxygen atoms should have

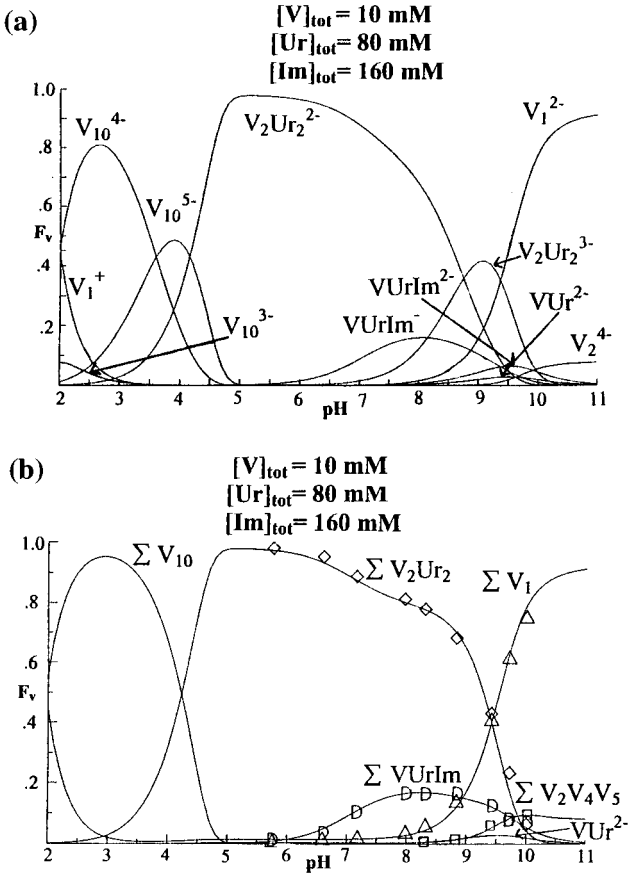

Figure 4. Distribution of vanadium vs pH in a solution containing vanadate, uridine, and imidazole at $[Im]_{tot}/[Ur]_{tot} = 2$. F_V is defined as in Figure 2. (a) Illustrates the complete speciation of the vanadate−uridine−imidazole system. For clarity, vanadium-containing species with <5% of $[V]_{tot}$ are not shown, except the VUr^{2-} complex. (b) Shows the sums for the decavanadate, oligovanadate, monovanadate, $V_2Ur_2^{n-}$ and $VUrIm^{n-}$ species and the fit of experimental data points (symbols).

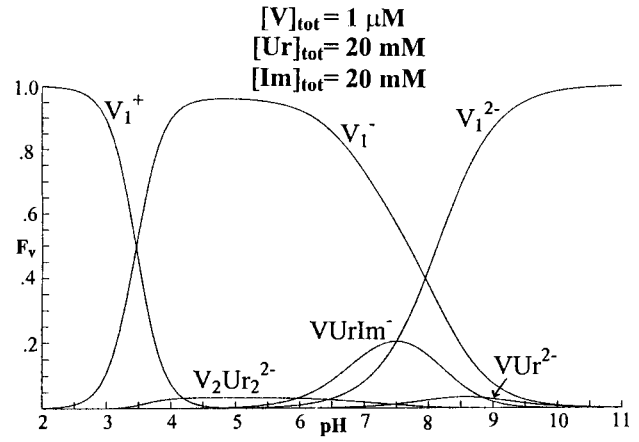

Figure 5. Diagram showing the distribution of vanadium at low vanadate concentration. F_v is defined in Figure 2.

chemical shifts around 1000 ppm. The expected ratio between these resonances would be 2:2:1:1 or 2:2:2 (4:2 would be less likely). The final structure shown in Figure 6e will contain four oxygen atoms with very similar chemical shifts; since O1 is similar to O1′ and O2 is similar to O2′, the observed signal may indeed be a 2:2 doublet around 1000 ppm.

The partial ^{17}O spectrum at 298 K of a solution containing 310 mM vanadate and 620 mM uridine at pH 7.0 is shown in Figure 7a. A broad asymmetric resonance is present at about

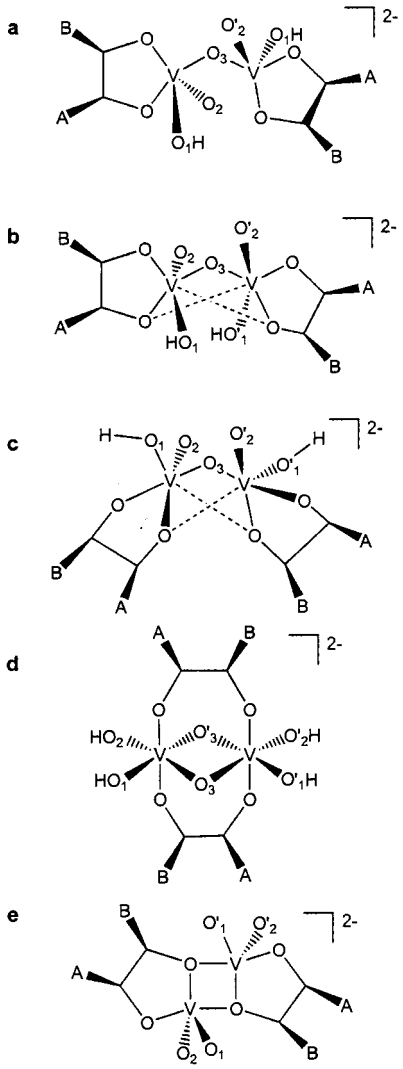

Figure 6. Proposed simplified structures for the 2:2 vanadate−uridine complexes. (a) Initial proposal of Gresser and Tracey;[22] (b) conformationally restricted structure proposed by Tracey and Leon-Lai;[17] (c) conformationally restricted structure proposed by Richter and Rehder;[36] (d) proposal by Crans et al.;[37] and (e) structure based on X-ray crystallographic evidence by model compounds and the vanadate−adenosine complex.[23,24]

1010 ppm. No other resonances, apart from that due to water, were observed. The ^{51}V NMR spectrum of the same solution contains the expected group of 2:2 resonances centered at −523 ppm and no other observable resonances. ^{17}O NMR spectra recorded at lower Ur_{tot}/V_{tot} ratios contained an additional resonance at the chemical shift reported previously for the vanadate tetramer (928 ppm). The addition of excess uridine to low-ratio solutions made this resonance disappear, which is consistent with the assignment of this signal to vanadate tetramer. Thus, the ^{17}O resonance centered at 1010 ppm can umambiguously be assigned to the 2:2 species.

The broad signal centered at 1010 ppm gives rise to several signals when analyzed using signal enhancement. Variable temperature ^{17}O NMR studies revealed that as the temperature increased, the broad asymmetric resonance sharpened and at 321 K two distinct resonances emerged (Figure 7b). This spectrum was sufficiently resolved to be simulated (Figure 7c), giving each signal a Lorentzian fraction ranging from 0.5 to 1.0. The chemical shifts of the resonances were 1007 and 1032 ppm, respectively. Integrating the simulated spectrum showed that although the ratio of the peaks varied ±10% for different

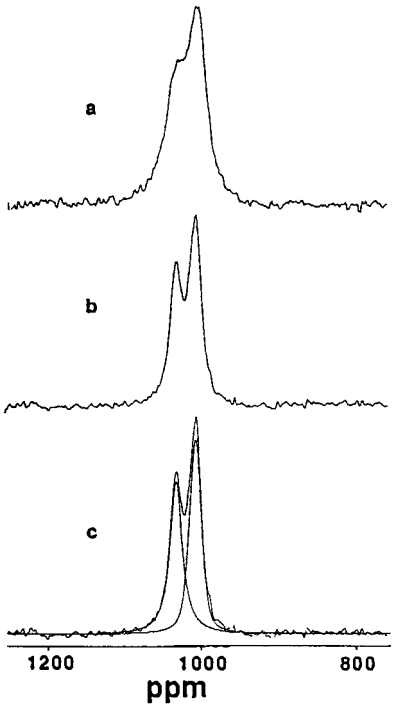

Figure 7. Partial ^{17}O spectra at $[Ur]_{tot}/[V]_{tot} = 2$. (a) 298 K and (b) 321 K. Relevant parameters: spectral width 125 kHz; 8 ms acquisition time; (a) 147 000 and (b) 146 000 transients. (c) Simulation of the 321 K ^{17}O spectrum using the program WinNuts 2D Version 5.093 for Acorn NMR 1993, 1994, 1995. The spectrum was fitted employing Lorentzian line shape fits.

simulations and different spectra, an average of 1:1 was obtained. Recording the spectrum at different spectral widths changed the integrated ratio slightly. Given the nature of ^{17}O NMR spectra and the error in such simulations, the observed signal ratio is within the range expected for a compound having two almost identical oxygen atoms. In addition, the variable temperature study shows that at ambient temperature these complexes are sufficiently stable for the four oxygen atoms to exhibit different chemical shifts. The ^{17}O NMR studies suggest that the solution structure for the vanadate−uridine 2:2 complexes is closely related to the vanadate−adenosine 2:2 complexes found in the solid state (Figure 6e).[23] This conclusion is consistent with the observations reported recently for related systems in aqueous solution, including the vanadate-ethylene glycol system.[32] Furthermore, the chemical shift difference between the two signals (25 ppm) is similar to the difference between the two oxygen atoms (one equatorial and one axial) in the complex formed between vanadate and triethanolamine which also contains a five-coordinate vanadium in a distorted trigonal bipyramidal arrangement.[34]

The series of ^{13}C NMR spectra recorded with V/Ur ratios from 1:1 to 1:2 in the pH region from 7 to 10 show that free ligand and several complexes are present in solution. Since the ^{51}V NMR spectrum shows only the broad asymmetric −523 ppm signal, we conclude that the resonances from several species in the ^{13}C NMR spectra are due to different isomers. Given the broadness of the isomer signals, it furthermore appears that the isomers exchange on the ^{13}C NMR time scale. The isomer exchange increases as the temperature increases and at 323 K several resonances have coalesced and broadened into the baseline. The isomer lability demonstrated by ^{13}C NMR spectroscopy is thus consistent with signal coalescence observed in the ^{17}O NMR spectra.

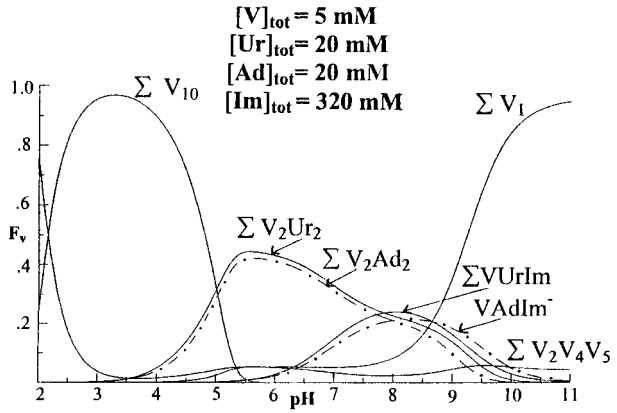

Figure 8. Comparison of the uridine and adenosine systems, illustrated in a diagram showing the distribution of vanadium vs pH. F_V is defined as in Figure 2. The sums for the decavanadate, oligovanadate, monovanadate, $V_2Ur_2^{n-}$,$V_2Ad_2^{n-}$, and $VUrIm^{n-}$ species are shown.

The ^{13}C NMR studies furthermore provide the following mechanistic information. The sharp ligand signals are contrasted to the broad complex signals and suggest that the observed exchange is solely among complex isomers. Interestingly, the lack of exchange between complex and free ligand is different from the patterns observed in most vanadium(V) complexes examined in this manner.[35] These have similar rate constants for complex hydrolysis (i.e. ligand−complex exchange), consistent with the possibility that these complexes are hydrolyzing via a dissociative type of mechanism with a common rate-limiting step. The lack of exchange between free uridine and $V_2Ur_2^{n-}$ complex observed by ^{13}C NMR, suggests that these complexes are formed and hydrolyzed via an alternative mechanism.

Comparison of the Vanadate−Uridine/Adenosine−Imidazole Systems. Figure 8 compares the complexation of vanadate in the AdH and UrH_2 systems when excess of imidazole is present. Since mixed ligand AdH and UrH_2 dimeric vanadate complexes are likely to be formed, it does not reflect the full speciation in the five-component system, but clearly shows the analogy of the two systems and that the base part of the nucleoside is of minor importance for complex formation. In the diagram, only the sum of homonuclear species is shown for clarity. As mentioned above, −2 charged 2:2 complexes are the predominant species. The additional $V_2Ur_2^{3-}$ species is stronger than the $V_2Ad_2^-$ species, binding almost 20% in contrast to at most 5% of $[V]_{tot}$ for the adenosine species (at $[V]_{tot} = 5$ mM, $[Ad/Ur]_{tot} = 20$ mM). The weaker formation constant for $V_2Ad_2^-$ is probably caused by the formation of strong competetive decavanadate complexes in the pH range where $V_2Ad_2^-$ is formed.

Resolution enhancement of the 2:2 (−523 ppm) and 1:1:1 (−483 ppm) ^{51}V NMR resonances reveals three and two peaks, respectively, in both the adenosine and uridine systems. However, the shifts are somewhat closer to each other for uridine than for adenosine (5.2 compared to 6.3 for the 2:2 complexes and 3.1 compared to 4.0 for the 1:1:1 complexes). This is in accordance with the observed trend that a more bulky ligand has more affect on the chemical shifts.[22]

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(36) Richter, J.; Rehder, D. *Z. Naturforsch.* **1991**, *46B*, 1613.
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Intramolecular stacking was one factor suggested to be responsible for the enhanced stability, compared to V−Im complexation, of the ternary 1:1:1 vanadate−adenosine−imidazole complex.[14] Although free adenosine has a greater tendency to stack than uridine, the observed stabilizing interaction in a 1:1:1 vanadium complex will be limited by the stacking partner, imidazole. Optimal stacking effects with imidazole will be obtained when the entire imidazole ring is overlapping with the base part of the nucleoside and the polarity is favorable in such an interaction. Since imidazole is smaller than both the uridine and the adenosine base, both V−AdH−ImH and V−UrH_2−ImH complexes should experience similar stabilizing effects presuming that all other structural factors do not change significantly. Indeed, the stability of the 1:1:1 complexes in the uridine and adenosine systems is found to be remarkably similar in the entire pH range studied, suggesting that this group of complexes is stable. The stability of these ternary complexes are of particular interest, since many enzymes known to interact with vanadate have a histidine residue in the active site.

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