Potentiometric and Electron Spin Resonance Investigation on the Interactions between Adenosine 5'-Triphosphate (atp) and Triphosphate Ligands with Vanadyl Ion. Synthesis and Characterization of Solid Vanadyl-atp Compounds from Acidic Aqueous Solution

Renzo Cini,* Gianluca Giorgi, and Franco Laschi
Dipartimento di Chimica della Università di Siena, Pian dei Mantellini 44, 53100 Siena, Italy
Michal Sabat, Antonio Sabatini, and Alberto Vacca
Dipartimento di Chimica della Università di Firenze, Via Maragliano 77, 50144 Firenze, and Istituto per lo Studio della Stereochimica ed Energetica dei Composti di Coordinazione, C.N.R., Via J. Nardi 39, 50132 Firenze, Italy

The VO^{2+}–atp [atp = adenosine 5'-triphosphate(4−)] and VO^{2+}–tp (tp = triphosphate) systems were studied by potentiometry and *X*-band e.s.r. spectroscopy in the pH ranges 2.5—5.2 and 2.5—6.5 respectively. The systems were investigated in the presence of 0.1 mol dm^{-3} $NaClO_4$ at 25.0 ± 0.1 °C in the ligand–metal molar ratio range 3—1.5. Analysis of the data reveals that VO^{2+} is able to form stable species with both ligands. The logβ values relevant to the 1 : 1 complexes are 6.67 ± 0.02 and 9.87 ± 0.02 for atp and tp respectively. Species $[(VO)(atp)]^{2-}$, $[(VO)(atp)_2]^{6-}$, and $[(VO)_2(atp)]$ were found in all the mixtures investigated. The complex $[(VO)_2(atp)]$ has a logβ value equal to 10.21 ± 0.02; VO^{2+}–tp species with a 2:1 molar ratio were not found in the mixtures investigated. A structure in which the VO^{2+} ion interacts just with the triphosphate chain is proposed for the complex $[(VO)(atp)]^{2-}$ while metal co-ordination to the phosphate chain and to the ribose hydroxyl group is suggested for $[(VO)_2(atp)]$. Solid $[(VO)_2(atp)]$ was isolated from aqueous solutions containing VO^{2+} and atp in a 2:1 molar ratio at pH 2.5. An oxygen only co-ordination mode for the metal cation was determined from spectroscopic data. A solid compound containing VO^{2+}, atp, and bipyam [bipyam = bis(2-pyridylamine)] in a 1 : 1 : 1 molar ratio was also isolated from acidic aqueous solution.

Vanadium has a significant role in many biological processes. It is a constituent of living plants and animals and it has important effects for normal growth.[1] There is evidence that vanadium is important in the glucose metabolism, muscle contraction, renal and bladder functions as well as in many other physiological processes.[2] Interest in vanadium has greatly increased since the discovery of the inhibition of Na^+, K^+-ATPase.[3]

Vanadium can be involved in accumulation phenomena by living systems. It was found that vanadium is accumulated in the natural product Amavadin which was isolated from *Amanita muscaria* (a poisonous mushroom).[4,5] The molecule responsible for the metal accumulation is probably the anion of *N*-hydroxy-α,α'-iminodipropionic acid $HON[C(CH_3)CO_2H]_2$ which is able to form very stable complexes with the VO^{2+} ion.[6] The role of the –OH group seems to be important in stabilizing the metal–ligand interaction.

Of the four common oxidation states only V^{III}, V^{IV}, and V^{V} are important for the cell. Vanadium(II) is a very strong reducing agent and it cannot exist in any living organism. The similarity between the chemistries of the vanadates (V^V) and phosphates plays an important role in the biological activity of this metal. However the vanadyl cation can compete with other bivalent cations like Mg^{II}, Ca^{II}, Mn^{II}, Cu^{II}, and Zn^{II} for ligand binding sites. It has to be pointed out that from *in vivo* studies the distribution of vanadium among tissues is not dependent on the +3, +4, or +5 oxidation state of the salt supplied. These data suggest 'that all the metabolic vanadium is converted to a common oxidation state in the circulation, probably to the vanadyl ion, before being assimilated by most tissues'.[2] Further information indicates that vanadates may be reduced to vanadyl in serum which then links to transferrin.[2] The equilibrium between vanadates and vanadyl is very important for living processes. The reduction from vanadate to vanadyl produces a change from a negative to a positive ion. Vanadium is a unique element for living systems in this respect. Furthermore the species involved in the V^V–V^{IV} redox couple are labile.[1] On the other hand vanadyl plays an important role in vanadium chemistry as it can be used as a physicochemical marker of the binding sites.

Vanadyl must be involved in complex formation to exclude precipitation as a hydroxo species at physiological pH. However it forms hundreds of stable species with different ligands.[7] Thus vanadyl can link the functional groups of many biological molecules such as nucleotides, amino acids, nucleic acids, or proteins. Previous studies about the non-enzymatic hydrolysis of adenosine 5'-triphosphate(4−) (atp) showed that atp protects vanadyl from oxidation by H_2O_2 owing to complex formation between the metal and the nucleotide.[8]

Because the interaction between metal ions and nucleic acids (or their constituents) is very important in the genetic information transfer mechanism, a series of investigations on metal–nucleotide model compounds have been carried out in this laboratory over the past decade.[9–12] As vanadyl plays an interesting role in the living cell, and to continue studies about the nature of possible linkages between the nucleotide donor centres and suitable metal cations, we carried out the present investigation on the VO^{2+}–atp and VO^{2+}–tp (tp = triphosphate) systems both in aqueous solution and in the solid state.

Experimental

Materials.—Vanadyl sulphate trihydrate or pentahydrate was obtained from Aldrich or Fluka respectively. Vanadyl sulphate stock solutions were prepared by dissolving the salt in

0.1 mol dm^{-3} $HClO_4$. The vanadyl concentration was determined by performing volumetric titrations with permanganate.

New samples of adenosine 5′-triphosphate and triphosphate sodium salts were purchased from Sigma. The purity of atp was checked by performing alkalimetric titrations and determination of total and inorganic phosphate.[13] The amount of free phosphate was less than 0.5%.

The pentasodium salt of tp was absorbed on a well washed Dowex 1X8 (H^+ form) 30-cm column and eluted with water. The content of triphosphoric acid in the solution collected was determined by alkalimetric titration. The amount of orthophosphate was also checked,[13] which was less than 0.5%. The solution of triphosphoric acid was used immediately after preparation.

Solutions of $HClO_4$ and NaOH were prepared by diluting more concentrated solutions contained in Merck ampoules. The concentrations of the diluted solutions were measured with cross titrations and the calibration of the potentiometric cell in terms of hydrogen ion concentrations was obtained following the Gran procedure.[14,15] Twice distilled water was used for preparing the solutions. The ionic strength of the titrant and that of the sample solution was adjusted to 0.1 mol dm^{-3} by adding $NaClO_4$. The ionic product of water was determined as $K_w = 1.65 \times 10^{-14}$ mol^2 dm^{-6} (pK_w = 13.78) in 0.1 mol dm^{-3} $NaClO_4$ solution at 25 °C.

Potentiometric Titrations.—The potentiometric titrations were automatically carried out at 25.0 ± 0.1 °C by using a Metrohm 665 Dosimat 10-cm^3 burette and a Metrohm 654 digital potentiometer equipped with a Metrohm glass electrode and a Metrohm calomel reference electrode. The apparatus was connected to an Olivetti M24 PC and the titration experiments were performed under the control of a purposely written Basic program. All the solutions were maintained under an atmosphere of ultrapure nitrogen during the titrations.

The acidity constants of the ligands were determined by titrating freshly prepared and carefully decarbonated solutions containing known amounts of $Na_2(H_2atp)$ or H_5tp and by employing 0.02 mol dm^{-3} NaOH in 0.1 mol dm^{-3} $NaClO_4$ as titrant. Aqueous solutions of atp and tp at pH 3 and 5 (and 7 for tp) were tested for the amount of free phosphate after at least 45 min from the preparation of the mixtures. The analysis revealed that the orthophosphate content was less than 1%. As the calculated junction potential values[14] were less than 0.2 mV in the pH range investigated, no correction on the measured e.m.f. values was applied.

The VO^{2+}–atp and VO^{2+}–tp binary systems were studied by acid–base titrations performed with an experimental procedure similar to that used for the above cited acidity constants. The solutions contained known amounts of atp or tp (*ca.* 0.002 mol dm^{-3}, 25 cm^3) and $VO(SO_4)$ (sometimes $HClO_4$ was added to the system containing atp). The ligand–vanadyl molar ratio ranged between 3 and 1.5. Many trials to study the hydrolysis of the VO^{2+} cation, by titrating solutions containing known amounts of the metal and $HClO_4$ with NaOH, did not produce results of good accuracy. For this reason the stability constants for the $[VO(OH)]^+$ and $[\{VO(OH)\}_2]^{2+}$ species included in our equilibria models as fixed parameters were those previously reported (logβ = −5.36 and −6.82 respectively).[16,17] However, as the atp and tp ligands are able to form stable species with vanadyl, the formation of $[VO(OH)]^+$ and $[\{VO(OH)\}_2]^{2+}$ complexes was negligible for ligand–vanadyl molar ratios greater than 1.5.

During the titrations, the e.m.f. values were stable within a few seconds (*ca.* 10) from the titrant addition. No backward shift was revealed for a period of at least 30 min up to e.m.f. values corresponding to pH 5.5 and 6.5 for the VO^{2+}-atp and VO^{2+}–tp systems respectively. Measurements to test the hydrolysis of the triphosphate chain were performed at pH 3 and 5 (and 7 for tp) in order to determine the effect of the VO^{2+} cation. The ligand–vanadyl molar ratio was 1.0 in all the experiments. At pH 3 the phosphate–total triphosphate molar ratio was below 1% after 45 min from mixing for both systems. On the other hand, at pH 5 the phosphate–total triphosphate molar ratio was *ca.* 3% after 45 min from the vanadyl addition. As the titrations were carried out with an overall time of *ca.* 45 min we did not take into account the presence of phosphate during the computation of the stability constants.

The calculation of the acidity constants as well as that of the formation constants for the metal complexes were performed by means of the least-squares computer program SUPERQUAD,[18] which minimizes the sum of the weighted squared residuals between observed and calculated e.m.f. values. The program was implemented on a IBM 4361 computer. The weighting of the experimental observations takes into account the estimated errors of both e.m.f. and titrant volume. We estimated these errors to be ±0.2 mV and ±0.002 cm^3 respectively in our system. The most probable equilibria model was selected by following a strategy based on statistical inferences applied to the variance of the weighted residuals, σ^2;[19] values of σ^2 lower than 9 were obtained for all the equilibria models.

Spectroscopic Measurements.—Infrared spectra for the complexes in the solid state, as Nujol mulls between CsI plates or as KBr pellets were measured on a Perkin-Elmer model 597 spectrometer.

E.s.r. spectra were recorded with a ER 200-SRC Bruker spectrometer operating at *X*-band frequency (9.78 GHz). The external magnetic field H_0 was calibrated with a microwave bridge ER041 MR Bruker wavemeter and the temperature was controlled with a ER 4111 VT Bruker apparatus (the accuracy was ±1 K). In order to estimate accurate *g* values diphenylpicrylhydrazyl (dpph) was used as a suitable field marker [g_{iso}(dpph) = 2.0036, ω_0 = 9.78 GHz].

Titrations for the VO^{2+}–atp and VO^{2+}-tp systems were carried out using the following procedure. A solution (20 cm^3) of $VO(SO_4)$ (1×10^{-3} mol dm^{-3}) and atp (or tp) (1×10^{-3} mol dm^{-3}) (initial pH 2.80, adjusted with $HClO_4$) was titrated with NaOH under nitrogen in a cell fitted with a Metrohm glass electrode. Samples were taken periodically and transferred to a nitrogen-flushed quartz cell. Each titration was performed twice to test the reproducibility. The e.s.r. spectra were recorded up to pH 5.5 and 7.5 for the VO^{2+}–atp and VO^{2+}–tp systems respectively. For both systems eight-line spectra were obtained for all the measurements. No additional lines appeared in the pH ranges investigated.

The vanadyl–ligand interactions in the solid state were investigated by isolating the Mg^{II} or Zn^{II} compounds containing the vanadyl complexes as doping reagents.

Simulations of the e.s.r. spectra were carried out by using the SIM14A program[20] implemented on a VAX-750 computer and the computed lineshape was dimensioned to fit the experimental spectrum. Spin–spin interactions for the solution phase at both room and liquid-nitrogen temperatures were ruled out by using VO^{2+} concentrations not greater than 1×10^{-3} mol dm^{-3}.

Ultraviolet and visible spectra were measured with a Perkin-Elmer model 200 spectrophotometer equipped with a Hitachi recorder; 1-cm path length quartz cells were used for all the determinations in solution. Reflectance spectra for the solid compounds were obtained from a thin layer of powder smeared on a S&S filter paper strip.

Synthesis of the Solid Compounds.—$[(VO)_2(atp)]\cdot6H_2O$ (**1**). $Na_2(H_2atp)$ (605 mg, 1×10^{-3} mol) was dissolved in water (8 cm^3) and the pH was adjusted to 5.0. $VO(SO_4)\cdot5H_2O$ (500 mg, 2×10^{-3} mol) was dissolved in water (2 cm^3). The vanadyl solution was added quickly to the atp solution with stirring at room temperature. A fine sky-blue solid immediately formed. The pH was 2.5. The mixture was stirred for 10 min. The resulting solid was filtered off, washed three times with a small amount of water (1 cm^3), and twice with ethanol then collected and stored over silica gel at room temperature. Yield 40% (Found: C, 17.00; H, 3.00; N, 9.70; P, 11.90; V, 12.95. Calc. for $C_{10}H_{24}N_5O_{21}P_3V_2$: C, 16.10; H, 3.25; N, 9.40; P, 12.45; V, 13.65%). Attempts to obtain single crystals suitable for *X*-ray analysis were unsuccessful.

$[(VO)H_2(atp)(bipyam)]\cdot 7H_2O$ (**2**). Bis(2-pyridyl)amine (bipyam) (171 mg, 1 × 10^{-3} mol) was dissolved in 95% ethanol (5 cm^3). The solution was mixed with an aqueous solution (5 cm^3) of $Na_2(H_2atp)$ (605 mg, 1 × 10^{-3} mol). The resulting mixture was added to a solution (5 cm^3) of $VO(SO_4)\cdot 5H_2O$ (250 mg, 1 × 10^{-3} mol). A sky-blue powder precipitated. The pH of the mother-solution was 3.5. The mixture was stirred for 10 min. The resulting solid was collected, washed three times with water and twice with ethanol, then stored on silica gel. Yield 50% (Found: C, 26.70; H, 3.75; N, 12.25; P, 10.80; V, 5.70. Calc. for $C_{20}H_{37}N_8O_{21}P_3V$: C, 27.60; H, 4.30; N, 12.90; P, 10.70; V, 5.85%). Several attempts to prepare single crystals for *X*-ray analysis failed.

Results and Discussion

Solution Studies.—The stepwise protonation constants of H_2atp^{2-} and H_3tp^{2-} are reported in Table 1. The pK_a value for H_2atp^{2-} was in good agreement with previous data.[21,22] As far as the pK_a values for H_5tp are concerned, the values for H_2tp^{3-} and Htp^{4-} are in good agreement with the values of 5.6 and 7.9 in 0.1 mol dm^{-3} $NaNO_3$ at 25 °C reported in ref. 23. However, the first acidity constant of H_3tp^{3-} was higher than that previously reported for 0.1 mol dm^{-3} $NaNO_3$ (pK_a = 2.6).[23]

$$VO + L \rightleftharpoons [(VO)L];\ \beta^{VO}_{[(VO)L]} = [(VO)L]/[VO][L] \quad (1)$$

$$VO + L + H \rightleftharpoons [(VO)HL];\ \beta^{VO}_{[(VO)HL]} = [(VO)HL]/[VO][H][L] \quad (2)$$

$$VO + 2L \rightleftharpoons [(VO)L_2];\ \beta^{VO}_{[(VO)L_2]} = [(VO)L_2]/[VO][L]^2 \quad (3)$$

$$2VO + L \rightleftharpoons [(VO)_2L];\ \beta^{VO}_{[(VO)_2L]} = [(VO)_2L]/[VO]^2[L] \quad (4)$$

Equations (1)—(4) define the overall stability constants for the vanadyl complexes found in our binary systems (L = atp or tp; overall charges omitted for clarity). The logβ values are listed in Table 1. The acidity constants of the complex species were calculated on the basis of the stability constants. The $\log\beta^{VO}_{[(VO)L]}$ values for atp and tp (6.67 ± 0.01 and 9.87 ± 0.02 respectively) show that vanadyl forms stable complexes with both ligands. The interaction between vanadyl and phosphate oxygen atoms was previously found from solution studies in acidic 1.0 mol dm^{-3} $NaClO_4$ media for VO^{2+}–phosphate and VO^{2+}–pyrophosphate systems.[24,25] The studies were carried out by performing spectrophotometric measurements. In the pH range investigated (1.89—3.40) the principal species were $[(VO)(H_2PO_4)]^+$ (logβ = 3.2), $[(VO)(H_2PO_4)_2]$ (logβ = 5.15), and $[(VO)(H_2P_2O_7)]$ (logβ = 4.4).[25] The authors suggested a bidentate co-ordination for the $H_2PO_4^-$ ligand. The ligand $H_2P_2O_7^{2-}$ is also considered to act in a bidentate fashion but bridging rather than chelating. It was also found, however, that at pH 5 the vanadyl cation is able to give a polynuclear species with pyrophosphate.[26] The species was formulated as $[(VO)_3(P_2O_7)_3]^{6-}$ and a cyclic structure was hypothesized with each ligand molecule bidentate. From our data it is evident that the triphosphate chain forms more stable complexes than orthophosphate and pyrophosphate ligands. The stability constant for the complex $[(VO)(tp)]^{3-}$ is higher than that for the $[(VO)(atp)]^{2-}$ species. This can be due to a higher net negative charge on tp than that on the triphosphate chain of atp. The pK_a value for $[(VO)(Htp)]^{2-}$ complex is 4.19 ± 0.01 indicating a large acidification effect of the vanadyl cation.

Table 1. Stability constants of the binary VO^{2+}–atp and VO^{2+}–tp systems as determined by potentiometric titrations (25.0 ± 0.1 °C; *I* = 0.1 mol dm^{-3} $NaClO_4$). The stepwise acidity constants (pK_a) for the protonated ligands as well as for the protonated complex species are also reported. The values in parentheses are the standard deviations on the least significant figure

	logβ		pK_a	
Species *	atp	tp	atp	tp
[(VO)L]	6.67(1)	9.87(2)		
[(VO)HL]		14.06(2)		4.19(1)
$[(VO)L_2]$	10.32(1)			
$[(VO)_2L]$	10.21(2)			
H_3L				1.87(1)
H_2L			4.08(1)	5.46(1)
HL			6.52(1)	7.81(1)

* L = atp or tp.

From Table 1 and Figure 1 it is evident that different species are found in the VO^{2+}–atp system compared to the VO_2^+–tp system. The analyses of the titration curves obtained for the VO^{2+}–atp system revealed the presence of $[(VO)_2(atp)]$. Moreover this species is one of the major compounds in all the mixtures investigated (and also for VO^{2+}–atp molar ratios lower than 1:3). The species $[(VO)_2(atp)]\cdot 6H_2O$ (**1**) was therefore isolated by mixing the components in a 2:1 molar ratio (see Experimental section). Complexes of the type $[M_2(atp)]$ have already been found in M^{2+}–atp systems containing transition-metal ions.[27–29] The $\log\beta^{[(VO)(atp)]}_{[(VO)_2(atp)]}$ value is 3.54 ± 0.02. For $[Ni_2(atp)]$, $[Zn_2(atp)]$, and $[Cu_2(atp)]$ the $\log\beta^{[M(atp)]}_{[M_2(atp)]}$ values are 2.4, *ca.* 3.0, and *ca.* 4.2 respectively. It has to be pointed out that the complex containing two metal ions per nucleotide molecule is the most reactive species in the diphosphorylation reaction.[27]

Many attempts to include the species $[(VO)_2(tp)]^-$ in the equilibrium model for the VO^{2+}–tp systems did not produce satisfactory results. This indicates that the nucleoside moiety plays an important role for the ligand–vanadyl interaction in the complex $[(VO)_2(atp)]$. Preliminary potentiometric investigations on the VO^{2+}–ctp system (ctp = cytidine 5′-triphosphate) show a pattern of species similar to that found for the VO^{2+}–atp system.

^{13}C N.m.r. data previously obtained for the VO^{2+}–atp system at pH 3 show that C(3′), C(4′), and C(8) undergo the largest effects on complexation suggesting that the ribose -OH groups and/or the purine nitrogen atoms [N(7) and N(3)] can act as donors.[30] On the other hand our e.s.r. data (see below) relevant to the VO^{2+}–atp system at pH 3—5 agree with a vanadyl ion experiencing a '4 O' co-ordination mode in the basal plane.[31] Moreover the addition of VO^{2+} to an aqueous solution of atp does not cause any change in the ultraviolet spectra recorded at pH 2.5—5.5, suggesting that the adenine base does not interact with the metal ion. It has to be emphasized that the VO^{2+} cation prefers oxygen over nitrogen donors; it does not bind ammonia in aqueous solution and forms weak complexes with ethylenediamine but very stable complexes with oxalate.[6] These

Table 2. *X*-Band e.s.r. parameters (the values in parentheses are the standard deviations on the least significant figure). Values of a_0 for [(VO)(porphyrinate)] and $[(VO)(H_2O)_5]^{2+}$ are reported for comparison

System	$g_{\parallel}$	$a_{\parallel}{}^{a}$	$g_{\perp}$	$a_{\perp}{}^{a}$	g_0	$a_0{}^{a}$
VO^{2+}-atp[b]	1.933(1)	180.1(8)	1.979(2)	64.9(9)	1.964(2)	103.3(9)
VO^{2+}-tp[b]						103.3(9)
[(VO)(porphyrinate)][c]						88.2
$[(VO)(H_2O)_5]^{2+}$[c]						106.4
$[(VO)H_2(atp)(bipyam)]$[d]	1.930(1)	179.8(8)	1.977(2)	64.7(9)	1.961(2)	103.1(9)

[a] $\times 10^{-4}$ cm^{-1}. [b] Frozen aqueous solutions at 100 K (pH = 5.0 at room temperature). [c] Data from refs. 33 and 34. [d] Measurements for the ternary complex were performed using a solid powder containing VO^{2+} doped in a [Zn(Hatp)(Hbipyam)] matrix.

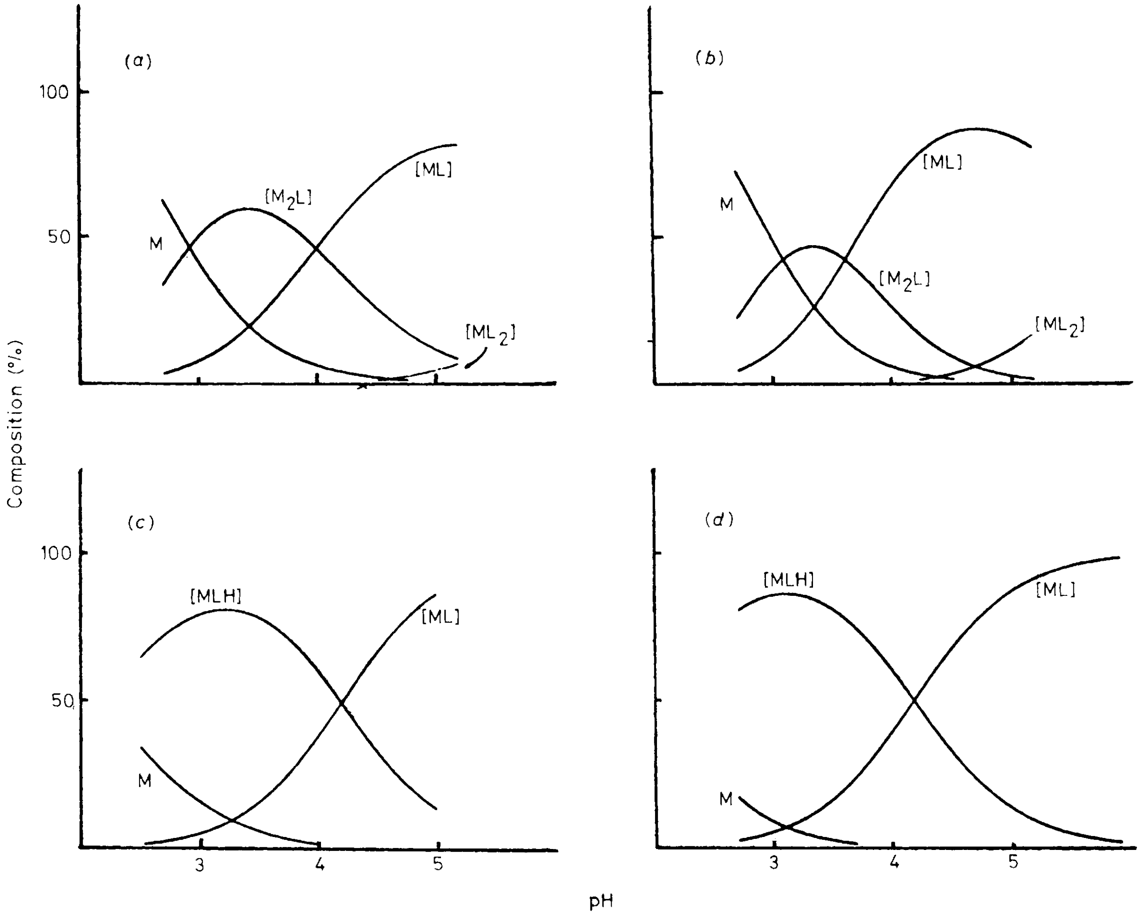

Figure 1. Distribution diagrams for the VO^{2+}–atp [(*a*), (*b*)] and VO^{2+}–tp [(*c*), (*d*)] systems. The percentages are calculated for titration curves where the initial concentrations (mmol dm^{-3}) of ligand, vanadyl, and acid are as follows: (*a*) 2.32, 1.71, 6.79; (*b*) 2.34, 0.76, 6.70; (*c*) 2.32, 1.71, 6.79; (*d*) 2.34, 0.76, 6.70. The initial volume was 25.0 cm^3 and titrant (NaOH) concentration 0.0199 mol dm^{-3}

observations, together with the strong stabilizing effect of the hydroxo group of the *N*-hydroxyiminodiacetate ligand in the interaction with vanadyl,[6] suggest that the adenosine moiety acts as a ligand through the ribose -OH groups in the $[(VO)_2(atp)]$ complex. It is also interesting to note that previous studies on the uridine–vanadyl system in aqueous solution indicate that the *cis*-glycol function of the nucleoside is linked to the vanadium atom.[32]

Significant e.s.r. parameters for both solution and solid phases are reported in Table 2. The e.s.r. titrations carried out on 1:1 VO^{2+}–atp and VO^{2+}–tp at room and liquid-nitrogen temperature did not show any changes in the a_0 and g_0 parameters on varying the pH in the range 2.8—5.5 (2.8—7.5 for tp). No significant reduction in intensity is shown up to pH 5.2 and 6.5 for VO^{2+}–atp and VO^{2+}–tp respectively (see Figure 2), indicating that e.s.r.-silent species are not formed to any appreciable extent. These species could be multinuclear complexes in which the metal centres are close to each other to allow a fast relaxation, or solid compounds {*e.g.* $[VO(OH)_2]$}.[16] Therefore, the two metal centres in $[(VO)_2(atp)]$ have to be far apart.

The a_0 parameter, which reflects the average environments around the VO^{2+} ion due to the equatorial donors,[31] is equal to 103.3 × 10^{-4} cm^{-1} for both the VO^{2+}–atp and VO^{2+}–tp systems. This value is close to 106.4 × 10^{-4} cm^{-1} found for the $[(VO)(H_2O)_5]^{2+}$ cation[33] which contains four equatorial oxygen donors. On the contrary the complex [(VO)(porphyrinate)] (with four equatorial nitrogen atoms) shows a_0 =

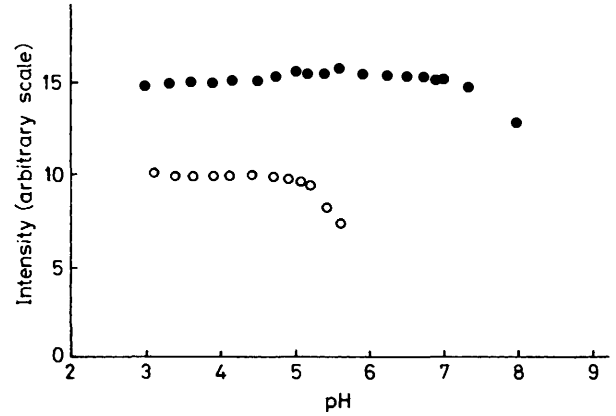

Figure 2. E.s.r. first derivative peak-to-peak intensities (arbitrary scale) of the $M_I = -\frac{7}{2}$ line for the VO^{2+}–atp (○) and VO^{2+}–tp (●) systems at 20 °C as a function of pH. The concentration of the ligand was 2.80×10^{-3} mol dm^{-3}, while that of the VO^{2+} cation was 2.65×10^{-3} mol dm^{-3} for both the systems

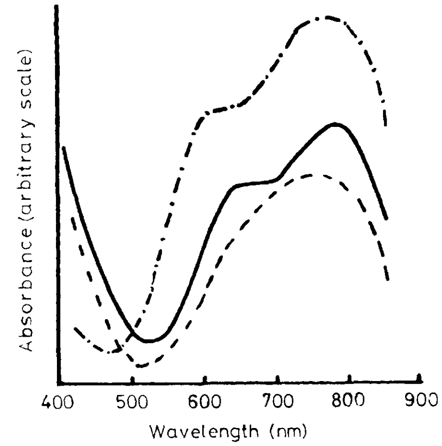

Figure 3. Visible reflectance spectra for: (**1**) (——), (**2**) (— — —), and $VO(SO_4)\cdot5H_2O$ (— · — · —)

88.2×10^{-4} cm^{-1}.[34] Therefore these data are in agreement with an oxygen only co-ordination mode for vanadyl, but the presence of one nitrogen atom in the inner co-ordination sphere cannot be ruled out. Our e.s.r. investigation also excludes the presence of polynuclear compounds like those previously found in the VO^{2+}–pyrophosphate system[26] as no additional lines were shown in the pH range investigated.

Solid-state Studies.—The i.r. absorption maxima for $[(VO)_2(atp)]\cdot6H_2O$ (**1**) and $[(VO)H_2(atp)(bipyam)]\cdot7H_2O$ (**2**) are reported in Table 3. A comparison of the spectra of (**1**) and that of $Na_2(H_2atp)$ shows that the band, which according to previous studies may be attributed to the NH_2 bending vibration,[35] undergoes a shift from 1 705 to 1 695 cm^{-1} on complexation. This can be due to a deprotonation at the N(1) position. The antisymmetric stretching vibration of the α and β $-PO_2-$ groups is shifted from 1 255 cm^{-1} in $Na_2(H_2atp)$[35–37] to 1 220 cm^{-1} in (**1**). The absorption at 980 cm^{-1} in $Na_2(H_2atp)$, attributable to the symmetric stretching vibration of the terminal phosphate group[35–38] shows a shift to 965 cm^{-1}. All these data indicate a metal co-ordination to the triphosphate chain.

The reflectance spectra of (**1**), (**2**), and $VO(SO_4)\cdot5H_2O$ in the solid state are reported in Figure 3. The spectrum of (**1**) ($\lambda_{max.}$ = 796 nm) is very similar to that of $VO(SO_4)\cdot5H_2O$ ($\lambda_{max.}$ = 780 nm) which suggests that the co-ordination sphere around the vanadyl cation in (**1**) contains just oxygen donors.

Table 3. Infrared absorption maxima (cm^{-1}) for (**1**) and (**2**). Data relevant to [Mn(Hatp)(Hbipyam)], $Na_2(H_2atp)$, and bipyam are reported for comparison

$[(VO)_2(atp)]\cdot6H_2O$ (**1**)	$[(VO)H_2(atp)(bipyam)]\cdot7H_2O$ (**2**)	[Mn(Hatp)(Hbipyam)]*	$Na_2(H_2atp)$*	bipyam
1 695s	1 690s	1 695 (sh)	1 705s	
	1 600s	1 660s		
	1 605s	1 660s		1 600s
	1 560s	1 560s		1 560s
				1 525s
1 220s	1 240s	1 230s	1 255s	1 310m
1 090s	1 120 (sh)	1 120s	1 110s	1 145m
	1 080s	1 080s	1 065s	
	1 000m	1 000m	1 030m	
965s			980s	985w
920s	890s	895m	910s	
820w	825w	820w	815m	
	780m	770m	725m	765s
720w	725w	710m	705m	730w
			640m	

* From ref. 10.

The i.r. spectrum of (**2**) is very similar to the spectra of the compounds [M(Hatp)(Hbipyam)] (M = Mg^{II}, Ca^{II}, Mn^{II}, Co^{II}, or Zn^{II}).[10] The antisymmetric stretching band of the α and β $-PO_2-$ group is shifted by *ca.* 15 cm^{-1} to longer wavelength on complexation, while the band due to the –P–O–P– stretching vibrations [910 cm^{-1}, $Na_2(H_2atp)$[35–38]] is shifted to 890 cm^{-1}. The intense absorption due to the NH_2- bending vibration shows a shift to longer wavelength (of *ca.* 15 cm^{-1}) and a splitting into two bands.

These data suggest that (**2**) has a structure similar to that of the [M(Hatp)(Hbipyam)] complexes which contain $(M(Hatp)_2$ units, where the metal centre is linked to the two triphosphate chains without any interaction with the nitrogen atoms of the purine and bipyam systems. The similarity between the i.r. spectra of (**2**) and those for the [M(Hatp)(Hbipyam)] compounds prompted us to prepare the vanadyl species doped in a [Zn(Hatp)(Hbipyam)] matrix, to perform e.s.r. studies in the solid state. The *X*-band e.s.r. data are in agreement with an oxygen only co-ordination type (see Table 2). However we must be careful when drawing conclusions about the vanadyl–ligand interactions from these e.s.r. data as the presence of a large amount of the diamagnetic metal can significantly affect the co-ordination sphere of the vanadyl ion.

It has to be pointed out that the analyses of the potentiometric data show the existence of the $[(VO)(atp)_2]$ species in the VO^{2+}–atp binary system (see Table 1). This kind of complex (with a β, γ co-ordination to atp) could co-crystallize with the $[(VO)(H_2O)_5]^{2+}$ ion and bipyam molecules to give lattices similar to those found for the [M(Hatp)(Hbipyam)] compounds. The presence of two different metal centres could not be detected from our e.s.r. measurements for (**2**). On the other hand the metal centres in $[(VO)(H_2O)_5]^{2+}$ and $[(VO)(Hatp)_2]^{4-}$ should have similar e.s.r. parameters if atp links the metal through the oxygen donors. The two acidic protons can reside either on atp {$[(VO)(H_2atp)(bipyam)]$, possibly on γ-phosphate and on N(1)}, or one on atp and the other on bipyam, [(VO)(Hatp)(Hbipyam)], as found for the species [M(Hatp)(Hbipyam)].[10] However it has to be noted that the reflectance spectrum of (**2**) (see Figure 3, $\lambda_{max.}$ = 768 nm) has some differences in respect to those of (**1**) and VO(SO_4). Therefore a structure containing one nitrogen atom linked to the vanadium atom cannot be ruled out for (**2**).

In conclusion the study reveals an interesting behaviour of VO^{2+} in comparison with other first-row transition-metal dipositive ions. It is clear that the vanadyl cation strongly links to the triphosphate chain. Moreover the nucleoside moiety (probably *via* the ribose –OH groups) can interact to some extent with the VO^{2+} cation as: (*i*) the complex $[(VO)_2(atp)]$ is present in the aqueous phase up to an VO^{2+}–atp molar ratio equal to 1:3; (*ii*) (**1**) can be isolated from the solution; (*iii*) the visible reflectance spectrum for (**1**) is very similar to that for vanadyl sulphate; (*iv*) the ultraviolet spectrum of atp is not changed by adding vanadyl in the pH range 2.5—5.5; (*v*) the VO^{2+}–tp system does not show the existence of 2:1 species.

The affinity of vanadyl for the –OH groups could explain the exose transport stimulation phenomenon in the cell and it can rationalize the insulin mimetic effect shown by this cation.

References

1 D. W. Boyd and K. Kustin, in 'Advances in Inorganic Biochemistry,' eds. G. L. Eichhorn and L. G. Marzilli, Elsevier, Amsterdam, 1984, vol. 6, p. 311.

2 N. D. Chasteen, *Struct. Bonding* (*Berlin*), 1983, **53**, 105.

3 L. C. Cantley, L. Josephson, R. Warner, M. Yanagisawa, C. Lechene, and G. Guidotti, *J. Biol. Chem.*, 1977, **252**, 7421.

4 E. Bayer and H. Kneifel, *Z. Naturforsch., Teil B*, 1972, **27**, 207.

5 H. Kneifel and E. Bayer, *Angew. Chem.*, 1973, **85**, 542.

6 G. Anderegg, E. Koch, and E. Bayer, *Inorg. Chim. Acta*, 1987, **127**, 183.

7 L. Vilas Boas and J. Costa Pessoa, in 'Comprehensive Coordination Chemistry,' ed. G. Wilkinson, Pergamon Press, Oxford, 1987, vol. 3, p. 453.

8 G. M. Woltermann, R. A. Scott, and G. P. Haight, jun., *J. Am. Chem. Soc.*, 1974, **96**, 7569.

9 P. Orioli, R, Cini, D. Donati, and S. Mangani, *J. Am. Chem. Soc.*, 1981, **103**, 4446.

10 R. Cini, M. C. Burla, A. Nunzi, G. P. Polidori, and P. F. Zanazzi, *J. Chem. Soc., Dalton Trans.*, 1984, 2467.

11 M. Sabat, R. Cini, T. Haromy, and M. Sundaralingam, *Biochemistry*, 1985, **24**, 7827.

12 G. Giorgi and R. Cini, *Inorg. Chim. Acta*, 1988, **151**, 153.

13 E. S. Baginski, P. P. Foa, and B. Zak, *Clin. Chim. Acta*, 1967, **15**, 155.

14 F. J. C. Rossotti and Hazel Rossotti, *J. Chem. Educ.*, 1965, **42**, 375; G. Gran, *Analyst* (*London*), 1952, **77**, 661.

15 M. Micheloni, A. Sabatini, and A. Vacca, *Inorg. Chim. Acta*, 1977, **25**, 41.

16 J. Francavilla and N. D. Chasteen, *Inorg. Chem.*, 1975, **14**, 2860.

17 L. Meites, *J. Am. Chem. Soc.*, 1953, **75**, 6059.

18 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.

19 L. Bologni, A. Sabatini, and A. Vacca, *Inorg. Chim. Acta*, 1983, **69**, 71.

20 J. P. Lozos, B. M. Hoffman, and G. C. Franz, QCPE Program No. 265, Chemistry Department Northwestern University, Evanston, Illinois, 1974.

21 C. F. Naumann, B. Prijs, and H. Sigel, *Eur. J. Biochem.*, 1974, **41**, 209.

22 H. Sigel, K. Becker, and D. B. McCormick, *Biochim. Biophys. Acta*, 1967, **148**, 655.

23 A. Johansson and E. Wänninen, *Talanta*, 1963, **10**, 769.

24 W. C. Copenhafer, M. W. Kendig, T. P. Russel, and P. H. Rieger, *Inorg. Chim. Acta*, 1976, **17**, 167.

25 M. R. Hoffmann, R. A. Stern, P. H. Rieger, and J. O. Edwards, *Inorg. Chim. Acta*, 1976, **19**, 181.

26 C. C. Parker, R. R. Reeder, L. B. Richards, and P. H. Rieger, *J. Am. Chem. Soc.*, 1970, **92**, 5230.

27 H. Sigel, F. Hofstetter, R. B. Martin, R. M. Milburn, V. Scheller-Krattiger, and K. H. Scheller, *J. Am. Chem. Soc.*, 1984, **106**, 7935.

28 H. Sigel and P. E. Amsler, *J. Am. Chem. Soc.*, 1976, **98**, 7390.

29 T. A. Glassman, J. Suchy, and C. Cooper, *Biochemistry*, 1973, **12**, 2430.

30 H. Sakurai, T. Goda, and S. Shimomura, *Biochem. Biophys. Res. Commun.*, 1982, **108**, 474.

31 N. D. Chasteen, R. J. Dekoch, B. L. Rogers, and M. W. Hanna, *J. Am. Chem. Soc.*, 1973, **95**, 1301.

32 R. N. Lindquist, J. L. Lynn, jun., and G. E. Lienhard, *J. Am. Chem. Soc.*, 1973, **95**, 8762.

33 H. A. Kuska and M. T. Rogers, in 'Radical Ions,' eds. K. T. Kaiser and L. Kevan, Wiley, New York, 1968, p. 579.

34 D. Kivelson and S.-K. Lee, *J. Chem. Phys.*, 1966, **41**, 1896.

35 (*a*) A. Epp, T. Ramasarma, and L. R. Wetter, *J. Am. Chem. Soc.*, 1958, **80**, 724; (*b*) M. Tsuboi, Y. Kyogoku, and T. Shimanouchi, *Biochim. Biophys. Acta*, 1962, **55**, 1.

36 H. Britzinger, *Biochim. Biophys. Acta*, 1963, **77**, 343.

37 F. L. Khalil and T. L. Brown, *J. Am. Chem. Soc.*, 1964, **86**, 5113.

38 M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, 1962, **66**, 10.

Received 18*th April* 1988; *Paper* 8/01495G