Oxovanadium(IV) Complexation by Adenosine 5'-Di- and -Tri-phosphate and Nucleotide Building Blocks

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Complex formation between oxovanadium(IV) and the bioligands adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), adenine, adenosine and 2'-deoxyadenosine 5'-diphosphate (dADP) was investigated in aqueous solution by EPR, electronic absorption spectroscopy, and pH-potentiometry. The reaction scheme implies that, in acidic media, nucleotides chelate the metal ion through unshared oxygen atoms of the polyphosphate chain, whereas vicinal *cis*-oriented hydroxyls of the ribose moiety are the binding set in alkaline solution. Species with mixed co-ordination were also detected. Dihydroxo-bridged dimers can be formed by ATP and ADP molecules co-ordinating through both the phosphate and ribose residues; however, formation of dihydroxo-bridged dimers with mixed chelation is the most favoured. Owing to the poor affinity of VO^{2+} towards *N*-donors, adenine is not able to bind VO^{2+} within the whole measurable pH range. In adenosine the ribose unit provides the ligand with pairs of vicinal *cis*-oriented hydroxyls, the right arrangement to form stable five-membered chelated rings. Lack of a C(2')OH group means that dADP does not possess a pair of *cis* hydroxyls and only diphosphate chelation is observed with this ligand.

Owing to its resemblance to phosphate, vanadate(V), which exists predominantly as $H_2VO_4^-$ at physiological pH, is able to pass through cellular membranes by adopting phosphate carrier systems.[1] For the same reason it is mainly responsible for vanadium poisoning inside the cell cytoplasm where it can inhibit or stimulate enzymes that participate in phosphate metabolism.[2] One long and well known example is the inhibition of Na,K ATPase (sodium–potassium pump).[3] The free energy required to transport Na^+ ions out of the cell against the concentration gradient is provided by hydrolysis of ATP catalysed by Na,K ATPase linked to the cellular membrane. Vanadium can interfere with this process because, as vanadate, it can link to the functional group aspartate of ATPase which, in the normal catalytic cycle, is phosphorylated by γ-phosphate of ATP.[4] The five-co-ordinated structure thus formed by vanadate closely resembles the metastable intermediate which occurs during hydrolysis of phosphoric esters, but, being more stable than the latter, it can inactivate the enzyme even at low concentration of vanadium(V). The action of vanadium as a phosphate antagonist is opposed by the presence of low-molecular-weight biomolecules such as the tripeptide glutathione [*N*-(*N*-L-glutamyl-L-cysteinyl)glycine], the principal reducing agent inside the cell, which can reduce vanadate to oxovanadium(IV) and/or form very stable vanadyl complexes. For example, the major part of VO_4^{3-} absorbed by erythrocytes is reduced and then linked by haemoglobin as VO^{2+} which is EPR-active.[5] Noteworthy, vanadyl interacts little with haemoglobin *in vitro* when it is introduced as $VOSO_4$ thus demonstrating that VO^{2+} is hardly able to pass through cellular membranes and is preferably formed inside the cell as a product of vanadate(V) reduction. The oxidation state V^{IV} can be stabilized by complexation. It has been observed that the trapping of VO^{2+} by ATP, presumably through the triphosphate moiety, slows the rate of oxidation of the vanadyl ion to vanadate which can also catalyse the non-enzymatic hydrolysis of the nucleotide.[6]

The complexing behaviour of oxovanadium(IV) towards ATP and ADP has been the subject of several studies. According to Sakurai *et al.*[7] electron spin resonance and electron spectroscopy allow the detection of two VO^{2+}–ATP complexes: a blue complex in acidic and neutral solutions and a green one in alkaline solution above pH 11. The first one has a 1:1 stoichiometry with ATP acting as a chelating tridentate ligand through the oxygens of the α- and β-phosphates and the C(3') hydroxyl group of the ribose moiety. In the second one the vanadyl ion binds two molecules of ATP. For both, the chelating set is a couple of adjacent hydroxyl groups of ribose, one of which is dissociated while the other is undissociated. On the basis of NMR results, the same authors updated their previous paper and suggested the following complexing scheme:[8] (*i*) at acidic pH the prevailing species is a blue complex in which one nucleotide binds VO^{2+} through the oxygens of the β- and γ-phosphates, the C(3') hydroxyl group of the ribose moiety and N(3) of the base; (*ii*) in neutral solution a blue 1:1 complex is also detected but the binding set has changed; the oxygens of the β- and γ-phosphates are still involved together with the undissociated C(2') and C(3') OH groups of the ribose moiety; (*iii*) the green complex observed at higher pH values is the same as above.

According to Baran and co-workers[9,10] who investigated the range pH 4–5, electron, ^{31}P NMR and IR spectroscopy all stress the key role played by the phosphate moiety in the co-ordination of VO^{2+} and exclude any base involvement. In their recent papers,[11,12] however, based on differential UV-spectral measurements, they assumed binding of VO^{2+} to a ring position, likely the N(7) donor, of ADP and ATP (but not AMP) too.

Available potentiometric data[13] indicate that, within the interval pH 2.5–5.2, the main species formed in the VO^{2+}–ATP system are $[VO(ATP)]^{2-}$ and $[VO(ATP)_2]^{6-}$. In both complexes ATP chelates the metal ion through the polyphosphate moiety. Around pH 4 the data can be fitted by considering a further species $[(VO)_2(ATP)]$ in which one metal ion is bound to β- and γ-phosphates and the other to one hydroxyl group of ribose.

In the present study we have reinvestigated the co-ordination

mode of VO^{2+} to ATP and ADP within the entire measurable pH range by means of electron spin resonance, electron spectroscopy and potentiometric titrations.

Experimental

Chemicals.—The nucleotides used were Aldrich products of puriss. quality. Their purity was checked and the exact concentration of solutions determined by the Gran method.[14] A stock solution of VO^{2+}, prepared as described in ref. 15, was standardised for metal concentration by permanganate titration and for hydrogen-ion concentration by potentiometry using the appropriate Gran function. The ionic strength was adjusted to 0.2 mol dm^{-3} KCl. In all cases the temperature was 25.0 ± 0.1 °C.

Potentiometric Measurements.—The stability constants of the proton and the oxovanadium(IV) complexes were determined by pH-metric titration of 25.0 cm^3 samples. The nucleotide concentration was 0.004 and 0.002 mol dm^{-3} and the nucleotide to metal ion molar ratio 8:1, 6:1, 4:1, 2:1 and 1:1.

The pH was measured with a Radiometer pHM 84 instrument, with a GK 2322C combined electrode, calibrated for hydrogen-ion concentration according to Irving *et al.*[16] The difference betweeen pH-meter readings and $-\log[H^+]$ was constant in the ranges pH 2–4 and 10–11.6. Thus the junction potential proved to be constant, although not negligible, as discussed in detail previously.[16] The concentration stability constants $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[17] The equilibria corresponding to the formation of the hydroxo complexes of $V^{IV}O$ were taken into account in the calculation of the stability constants of the complexes. The following species were assumed: $[VO(OH)]^+$ ($\log \beta_{1-1} = -5.94$), $[\{VO(OH)\}_2]^{2+}$ ($\log \beta_{2-2} = -6.95$), with stability data calculated from the data published by Henry *et al.*[18] and corrected for the different ionic strengths by use of the Davis equation, and $[VO(OH)_3]^-$ ($\log \beta_{1-3} = -18.0$), taken from ref. 19.

Spectroscopic Measurements.—Isotropic and anisotropic X-band EPR spectra (9.15 GHz) were recorded at 298 or 120–140 K, respectively, on aqueous solutions using a Varian E-9 spectrometer. As usual, the samples for low-temperature measurements were added with a few drops of dimethyl sulfoxide to ensure good glass formation in frozen solutions. Absorption spectra were obtained on a Beckman Acta MIV spectrophotometer.

Results and Discussion

ATP and ADP Complexes.—The proton-dissociation (pK_a) constants measured for adenosine 5′-triphosphate (ATP), 3.89 and 6.32, see Table 1, are in good agreement with the ones reported in previous papers.[13,20] The first pK_a refers to the dissociation of protonated N(1) of the base moiety and the second one to the release of one proton from the polyphosphate chain. This means that three out of the four protons of the triphosphate group dissociate in very acidic media (pH < 1). The least acidic proton probably belongs to γ-phosphate. A similar interpretation applies to the pK_a values, 3.83 and 6.19, of ADP, so, even in that case, a potentially chelating diphosphate moiety is already available at pH < 1.

From the stability constant data listed in Table 1 it can be seen that both oxovanadium(IV)–ADP and –ATP systems could be described by the same speciation model involving mononuclear 1:1 and 1:2 protonated and deprotonated as well as dinuclear deprotonated species. It should be mentioned that dinuclear species $(VO)_2A_2H_{-2}$, $(VO)_2A_2H_{-4}$ and $(VO)_2A_2H_{-6}$ could be substituted by the corresponding monomeric ones, $VOAH_{-1}$, $VOAH_{-2}$ and $VOAH_{-3}$, fairly well; the fitting parameters increased only about 20%. However, EPR measurements indicated unambiguously the almost exclusive formation of EPR-silent species in this formation range. Hence, the model containing dimeric species was adopted. Other deprotonated complexes, such as VOA_2H_{-1}, VOA_2H_{-3}, $(VO)_2A_2H_{-3}$ and $(VO)_2A_2H_{-5}$, were rejected by the computer program. The concentration distribution curves calculated with the data listed in Table 1 at high ATP excess (see Fig. 1) reveal the formation of two mononuclear 1:1 complexes VOAH and VOA below pH 4. Deprotonation of the ligand in the species VOAH probably involves the $N(1)H^+$ group on the nucleic base (*cf.* pK_{base} and pK_{VOAH} values in Table 1), far away from the triphosphate moiety which chelates the metal ion in very acidic media, as inferred from the anisotropic EPR spectra (see Fig. 2). Even at pH 2, the EPR parameters are different from those which identify the aqua-ion: VOAH and VOA (EPR cannot distinguish between the two) have $g_{\|} = 1.930$ and $A_{\|} = 181 \times 10^{-4}$ cm^{-1} (see Table 2), while $[VO(H_2O)_5]^{2+}$ has $g_{\|} = 1.933$ and $A_{\|} = 180 \times 10^{-4}$ cm^{-1}. A decrease in $g_{\|}$ and an increase in $A_{\|}$ in comparison to the aqua-ion values have previously been observed for the diphosphate- and triphosphate–VO^{2+} systems[21] and assumed as indicating the

Table 1 Stability constants (log β) of the proton and oxovanadium(IV) complexes of ADP and ATP at $I = 0.2$ mol dm^{-3} (KCl) and 25 °C[a]

	ADP	ATP
HA	6.19(1)	6.32(1)
H_2A	10.02(1)	10.21(1)
VOAH	10.09(1)	10.00(3)
VOA	6.68(2)	6.49(2)
VOA_2	10.80(6)	10.42(5)
VOA_2H_{-2}	−4.45(9)	−3.76(13)
VOA_2H_{-4}	−22.34(8)	−22.57(10)
$(VO)_2A_2H_{-2}$	4.96(16)	3.82(4)
$(VO)_2A_2H_{-4}$	−10.53(16)	−9.27(4)
$(VO)_2A_2H_{-6}$	−28.03(25)	−28.84(29)
No. of points	310	403
Fitting (cm^3)[b]	1.59×10^{-2}	1.36×10^{-2}
$\log K_{VOA_2}$[c]	4.12	3.93
$\log (K_{VOA}/K_{VOA_2})$	2.56	2.56
$\log K_{VOA} - \log K_{HA}$	0.39	0.17
$\log K_{VOA_2} - \log K_{HA}$	−2.07	−2.39
$\Delta\log \beta_{VOA_2H_2}$[d]	1.02	2.02
$\Delta\log \beta_{(VO)_2A_2H_4}$[e]	0.71	2.94
$VOA_2 \rightleftharpoons VOA_2H_{-2} + 2H^+$	−15.25	−14.18
$VOA_2H_{-2} \rightleftharpoons VOA_2H_{-4} + 2H^+$	−17.89	−18.81
$(VO)_2A_2H_{-2} \rightleftharpoons (VO)_2A_2H_{-4} + 2H^+$	−15.49	−13.09
$(VO)_2A_2H_{-4} \rightleftharpoons (VO)_2A_2H_{-6} + 2H^+$	−17.50	−19.57

[a] The standard deviations are reported in parentheses. [b] The average difference in the calculated and experimental titration curves expressed in cm^3 of the titrant. [c] For triphosphate: 4.07 (ref. 21). [d] $\log \beta_{VOA_2H_2} - \frac{1}{2}(\log \beta_{VOA_2} + \log \beta_{VOA_2H_4} + \log 4)$. [e] $\log \beta_{(VO)_2A_2H_4} - \frac{1}{2}(\log \beta_{(VO)_2A_2H_2} + \log \beta_{(VO)_2A_2H_6} + \log 4)$.

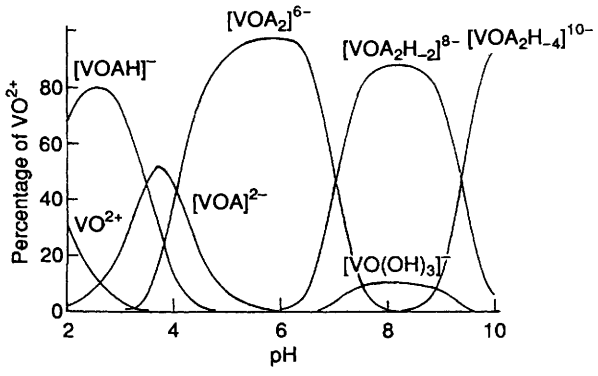

Fig. 1 Speciation curves for complexes formed in the VO^{2+}–ATP system; $c_{VO^{2+}} = 0.004$, $c_{ATP} = 0.04$ mol dm^{-3}

formation of monochelated complexes with a diphosphate-like binding mode. So, a similar chelating set, which is also supported by the bathochromic shift to 800 nm of the band at 760 nm typical for the aqua-ion, is assigned to VOAH and VOA.

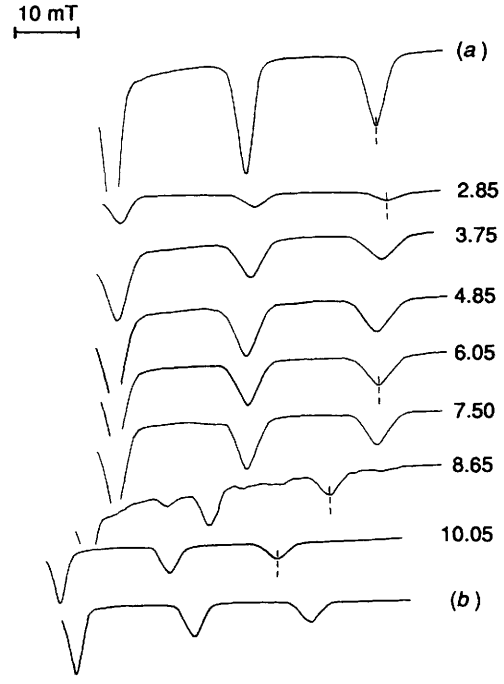

Fig. 2 High-field parallel resonances of the EPR spectra recorded at 140 K on aqueous solutions of VO^{2+} (0.004 mol dm^{-3}) and ATP at the nucleotide-to-metal molar ratio of 10:1 and varying pH. Spectra (*a*) and (*b*) are those of $[VO(H_2O)_5]^{2+}$ and $[VO(OH)_3]^-$, respectively

With increasing pH the trend of spectral parameters is reversed: there is a progressive decrease in $A_{\|}$ while the hyperfine multiplet is shifted to lowfield as expected for 'normal' vanadyl complexation in solution. Moreover, a non-resolved superhyperfine coupling of the unpaired electron with ^{31}P nuclei gives rise to greater band width. Around pH 6, $g_{\|} = 1.934$ and $A_{\|}(^{51}V) = 178 \times 10^{-4}$ cm^{-1} identify VOA_2 which, according to potentiometry, is the only species at this pH.

The EPR spectrum recorded at pH 8.6 shows the presence of two coexisting species. The magnetic parameters $g_{\|} = 1.957$ and $A_{\|}(^{51}V) = 151 \times 10^{-4}$ cm^{-1} characterise the complex VOA_2H_{-4} which becomes the sole species in very alkaline solution (pH $\geqslant$ 10). Information on the structure of this species can be drawn from the comparison with the D-ribose–VO^{2+} system which, within the same pH range, gives rise to a complex with similar EPR parameters.[22] Analysis of the complexing behaviour of D-ribose and other simple sugars towards VO^{2+} allows one to postulate VOA_2H_{-4} as a bis-chelated complex in which each ribose moiety binds the metal ion through its deprotonated hydroxyl groups at C(2′) and C(3′). The latter are *cis* oriented relative to each other, properly arranged for the formation of stable five-membered chelated rings. The high stability of this species is demonstrated by the fact that, over the whole measurable pH range, hydrolysis does not take place. Besides, potentiometric data rule out VOA_2H_{-3}, thus suggesting that both sugar hydroxyls dissociate and bind the metal ion co-operatively, the driving force being the formation of such a stable complex. Even d–d absorptions, as compared to those of the analogous species formed by D-ribose, support the above ligand arrangement: 420, 500 and 675 nm with ε = 22, 10 and 35 dm^3 mol^{-1} cm^{-1}, respectively. The admission of ribose into the metal

Table 2 The EPR parameters and chelating sets for oxovanadium(IV) complexes formed by nucleoside di- and tri-phosphates as well as their building blocks

Species[a]	$g_{\|}$	$10^4 A_{\|}$/cm^{-1}	Chelating set[b]
Diphosphate			
VOA_2[c]	1.937	176	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_3^{2-}})(\mathbf{PO_3^{2-}}–O–\mathbf{PO_3^{2-}})$
Triphosphate			
VOA_2[c]	1.937	176	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_3^{2-})(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_3^{2-})$
ATP			
VOAH, VOA	1.930	181	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_2^{-})$
VOA_2	1.934	178	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_2^{-})(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_2^{-})$
VOA_2H_{-2}	1.947	166	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–O–PO_2^{-})(\mathbf{O^-},\mathbf{O^-})$
VOA_2H_{-4}	1.957	151	$(\mathbf{O^-},\mathbf{O^-})(\mathbf{O^-},\mathbf{O^-})$
ADP			
VOAH, VOA	1.928	182	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)$
VOA_2	1.933	178	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)$
VOA_2H_{-2}	1.947	166	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)(\mathbf{O^-},\mathbf{O^-})$
VOA_2H_{-4}	1.958	150	$(\mathbf{O^-},\mathbf{O^-})(\mathbf{O^-},\mathbf{O^-})$
dADP			
VOAH, VOA	1.928	181	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)$
VOA_2	1.934	179	$(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)(\mathbf{PO_3^{2-}}–O–\mathbf{PO_2^{-}}–)$
Adenine[d]			
Adenosine			
VOA_2H_{-4}	1.958	150	$(\mathbf{O^-},\mathbf{O^-})(\mathbf{O^-},\mathbf{O^-})$
D-Ribose			
VOA_2H_{-4}	1.958	149	$(\mathbf{O^-},\mathbf{O^-})(\mathbf{O^-},\mathbf{O^-})$

[a] A indicates the ligand with fully deprotonated phosphate groups. [b] Groups taking part in chelation are indicated in bold. [c] Ref. 21. [d] No complex formation.

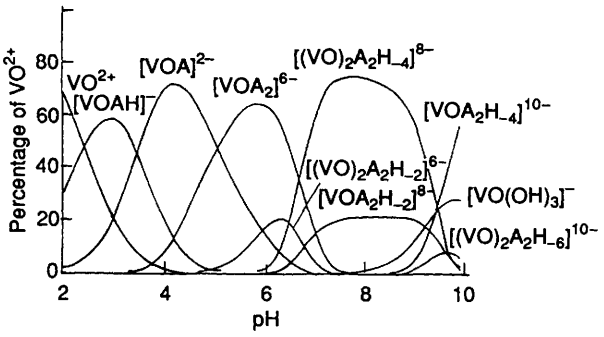

Fig. 3 Speciation curves for complexes formed in the VO^{2+}–ATP system; $c_{VO^{2+}} = 0.004$, $c_{ATP} = 0.008$ mol dm^{-3}

co-ordination sphere drastically changes the appearance of the visible spectrum when compared to that which typifies the sole diphosphate-like binding mode.

By applying the additivity relationship of Holyk[23] to the other set of magnetic parameters measured at pH 8.6, it is possible to get an insight into the structure of the corresponding species. Their values, $g_{\|} = 1.947$ and $A_{\|}(^{51}V) = 166 \times 10^{-4}$ cm^{-1}, are intermediate between those of VOA_2 and those of VOA_2H_{-4} and, according to potentiometry too, can be assigned to VOA_2H_{-2}, a 'mixed' complex with one ATP co-ordinated through the polyphosphate chain and the other through the ribose moiety. The speciation diagram in Fig. 1 and EPR spectra indicate that this species is a dominant one in solution at pH 8.0–8.5 and an absorption spectrum with maxima at 410, 550 and 695 nm (ε = 20, 10 and 30 dm^3 mol^{-1} cm^{-1}) can be assigned to the complex.

The EPR spectra recorded on a frozen solution having $[VO^{2+}] = 0.004$ mol dm^{-3} and a nucleotide-to-metal ratio = 2:1 allow the detection of further species. In alkaline solution there is a gradual decrease in signal intensity and loss of hyperfine resolution. Between pH 8.0 and 9.5 only a broad and weak resonance is observable, whereas the solution is EPR silent at room temperature. These spectral features characterise hydrolytic oligonuclear species in which OH bridges connect paramagnetic oxovanadium(IV) ions. The lack of precipitation, however, indicates that the nucleotide is still participating in metal co-ordination. Investigation of room-temperature spectra confirms the EPR-inactive nature of the above species. Visible spectra and comparison with the D-ribose–VO^{2+} system are essential in order to understand the ligand arrangement about the metal ion. The absorption maxima for these hydrolytic species at 420, 555 and 755 nm (ε = 40, 19 and 19 dm^3 mol^{-1} cm^{-1}) are rather similar to those of the dihydroxo-bridged dimeric species of D-ribose, 410, 535 and 665 nm (ε = 26, 18 and 17 dm^3 mol^{-1} cm^{-1}). In this complex monomeric 1:1 units with chelated deprotonated alcoholate groups are linked through two OH^- bridges forming a species with stoichiometry $(VO)_2A_2H_{-6}$. However, potentiometry reveals that hydrolysis sets in at lower pH values ($\approx$6, see Fig. 3) and three different dimeric species $(VO)_2A_2H_{-2}$, $(VO)_2A_2H_{-4}$ and $(VO)_2A_2H_{-6}$ are formed in the range pH 6–10, $(VO)_2A_2H_{-4}$ being the most significant and dominant at pH 8–9 where EPR indicates the almost exclusive formation of a magnetically coupled species. Thus, it can be assumed that dihydroxo-bridged dimers can be formed by ATP molecules co-ordinating through both the phosphate and ribose residues, resulting in complexes $(VO)_2A_2H_{-2}$ and $(VO)_2A_2H_{-6}$, respectively. However, formation of $(VO)_2A_2H_{-4}$ with mixed chelation is the most favoured process. This was found for the monomeric mixed chelated complex VOA_2H_{-2} too, and the favourable arrangement in both species is clearly demonstrated by the positive values of $\Delta\log\beta_{VOA_2H_{-2}}$ and $\Delta\log\beta_{(VO)_2A_2H_{-4}}$ stabilization constants listed in Table 1. The enhanced formation of these 'mixed' complexes may be due to the favourable combination of diphosphate six-membered and ribose five-membered chelate rings.[24]

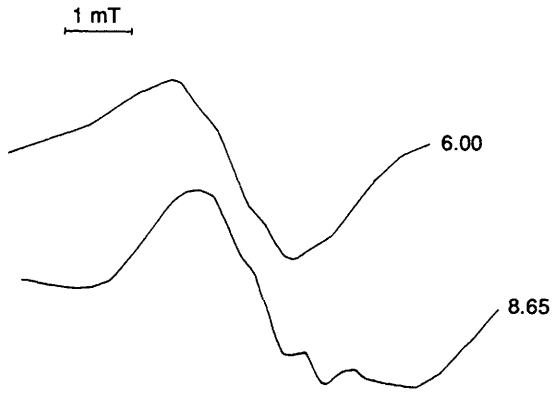

Fig. 4 Hyperfine perpendicular resonances $M_I = -\frac{3}{2}$ in the EPR spectra recorded at 120 K on solutions of VO^{2+} (0.004 mol dm^{-3}) and ADP in D_2O at the nucleotide-to-metal molar ratio of 10:1 and varying pD

Noteworthy is that there is no evidence for the species $(VO)_2A$ suggested by Cini *et al.*[13] Fitting of potentiometric data shows that $(VO)_2A$ can be replaced by VOAH, as was found for weak metal ion–ligand interactions in other systems.[25] When both species are taken into account in the same complexing scheme, $(VO)_2A$ is invariably rejected by the computer program. If the two species are considered separately, the best fit of the data is obtained with VOAH.

The complexing scheme so outlined is the same for both nucleotides, the only differences being due to the higher negative charge of free ATP which makes its complexes less stable than those formed by ADP (see Table 1).

Analysis of potentiometric data shows that the stability constant of VOA_2 (see Table 1), which compares well with those for the analogous species formed by diphosphate and triphosphate,[21] is rather small for a bis-chelated complex. Accordingly, the ratio of the stepwise stability constants K_1 and K_2, which refer to co-ordination of the first and second ligands, respectively, is greater than expected. This might lead to the conclusion that the second ligand is just acting in a monodentate manner, as was assumed for the corresponding aluminium systems,[20] on the basis of the rather similar log K_{AlA_2} stepwise stability constants obtained for the complexes of AMP (only being monodentate), ADP and ATP. In the case of the VO^{2+}–AMP system reliable stability constants for the bis complex could not be obtained due to early precipitation.

Further details as to the arrangement of the polyphosphate moiety around the metal ion have been drawn from a closer EPR investigation of the ADP–VO^{2+} system. Spectra were also recorded on a D_2O–CD_3OD (1:1 v/v) cosolvent mixture with $[VO^{2+}] = 0.004$ mol dm^{-3} and a nucleotide-to-metal molar ratio of 10:1. This solution was titrated with D_2SO_4 and NaOD. In the anisotropic spectrum of the species VOA_2 at pD 6 (see Fig. 4) the $M_I(^{51}V) = -\frac{3}{2}$ perpendicular absorption feature shows an underlying superhyperfine structure with a quintet pattern due to coupling of the unpaired electron with four equivalent ^{31}P nuclei. The experimental coupling constant is 0.69 mT and the intensity ratio for the five components is close to the theoretical one of 1:4:6:4:1. The magnetic and so the geometric equivalence of the four phosphorus atoms with respect to the metal centre can only be achieved if each diphosphate moiety chelates the metal ion. Since the stability constant for the complex VOA_2 formed by ADP is of the same order of magnitude as that of ATP, a chelating binding mode can be assumed for the latter ligand too. The stability of the ATP bis complex, however, is reduced due to repulsive electrostatic effects which hinder co-ordination of a tetraanion to a negatively charged complex (see also Table 1).

On raising the pD to 8.6 to yield the 'mixed' complex

VOA_2H_{-2}, the quintet pattern of the $M_I(^{51}V) = -\frac{3}{2}$ perpendicular absorption feature is gradually replaced by a triplet with $A(^{31}P) = 0.76$ mT and an intensity ratio 1:2:1. This indicates that two equivalent ^{31}P nuclei are now interacting with the metal ion, in accord with the previous hypothesis that in VOA_2H_{-2} only one nucleotide binds through the chelating polyphosphate moiety. In the spectrum of VOA_2H_{-4} there is no evidence of hyperfine coupling due to ^{31}P nuclei suggesting that, in very alkaline solution, diphosphate units are no longer present in the metal co-ordination sphere. Superhyperfine coupling for the species VOA_2 had been observed previously by Makinen and co-workers.[26] Also in the same paper is a reference to a three-line pattern with an approximately 0.67 mT splitting and an intensity ratio of 1:2:1. This was detected for a solution with an ADP to VO^{2+} molar ratio of 1:1, presumably at pH 6.5: even though no indication is given as to the possible structure of this species, the experimental conditions under which it was observed suggest a three-line pattern for VOA.

A comparison of the $\Delta\log \beta_{VOA_2H_{-2}}$ and $\Delta\log \beta_{(VO)_2A_2H_{-4}}$ stabilisation constants and the stepwise deprotonation constants listed in Table 1 shows that both in mono- and di-meric complexes the mixed diphosphate–ribose bonding mode is somewhat more favoured with ATP than with ADP. This can be explained by electrostatic reasons. The polyphosphate chain replaced by the dinegative ribose moiety is tetranegative in the case of ATP and trinegative in the case of ADP. Therefore, the rearrangement to the mixed bonding mode decreases the electrostatic repulsion between the co-ordinating sites of the ligands to a greater extent with the ATP nucleotide.

Complexes with Nucleotide Building Blocks.—The nucleotides ATP and ADP are 'good' ligands for the oxovanadium ion. They possess several binding sites which are open to the metal ion at different pH values thus preventing, if a sufficiently high nucleotide-to-metal molar ratio is present, metal hydrolysis and precipitation. In order to verify the binding set for each of the complexes formed by ATP and ADP we have investigated 'simpler' ligands which can be considered as building blocks of ATP and ADP: diphosphate, triphosphate, adenine, adenosine and 2'-deoxyadenosine diphosphate (dADP).

A detailed investigation of the complexing properties of diphosphate and triphosphate towards the vanadyl ion, reported elsewhere,[21] confirms that these moieties are the metal binding sites in acidic media (see the comparison of stability and EPR data in Tables 1 and 2, respectively). However, there is no evidence for the formation of a species analogous to the already reported and well characterised trinuclear complex formed by diphosphate acting as a chelating and bridging ligand through the four unshared oxygens.

On the other hand, adenine is not able to bind VO^{2+} within the whole measurable pH range. This was expected due to the poor affinity of VO^{2+} towards *N*-donors. Adding a ribose unit to give adenosine provides the ligand with pairs of vicinal *cis*-oriented hydroxyls, the right arrangement to form stable five-membered chelated rings. However, this binding set becomes available, *e.g.* hydroxyls are deprotonated, only at very alkaline pH. The lack of other sites which can anchor the metal ion at lower pH allows for hydrolysis and precipitation of $VO(OH)_2$, so a back-titration approach is required for adenosine. Both the metal, as $VOSO_4$, and the ligand are dissolved in very alkaline media (pH > 12–13, meter readings) and then the pH is lowered till precipitation of $VO(OH)_2$ occurs. When the ligand-to-metal molar ratio is 10:1 EPR anisotropic spectra (see Fig. 5) show that only one species is present within the interval pH 13.5–8.3. The magnetic parameters compare well with that of the complex VOA_2H_{-4} formed by ATP and ADP. Below pH 8 the ^{51}V hyperfine structure is gradually replaced by a broad resonance which indicates the formation of oligomeric species (visible spectra confirm that the ligand is still taking part in metal co-ordination) and then precipitation occurs. When the ligand has the same concentration as that of the metal ion a further species

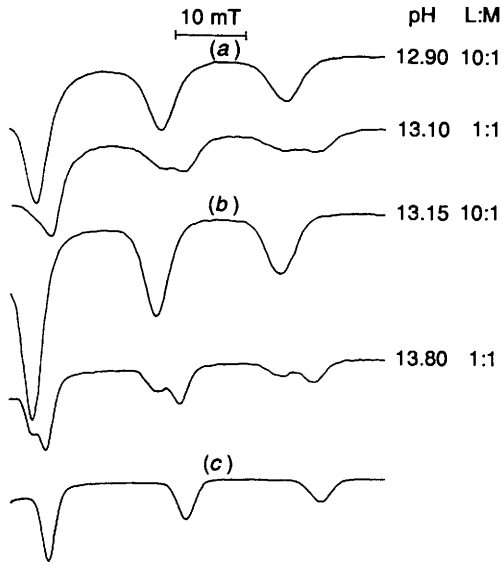

Fig. 5 High-field parallel resonances of the EPR spectra recorded at 140 K on aqueous solutions of VO^{2+} (0.004 mol dm^{-3}) and adenosine (*a*) or D-ribose (*b*) as a function of pH and ligand-to-metal molar ratio (L:M). Spectrum (*c*) is that of $[VO(OH)_3]^-$

is detected at very high pH (> 13, meter reading): $A_{\|}(^{51}V) = 159 \times 10^{-4}$ cm^{-1} and $g_{\|} = 1.954$. Since this complexing scheme reproduces exactly that of D-ribose and other simple sugars possessing pairs of *cis*-oriented vicinal hydroxyls,[20] this complex is assigned the composition $VOAH_{-4}$ or $VOAH_{-2}(OH)_2$ with the metal ion bound to the ribose moiety of one adenosine and two terminal OH^-. Comparison of the systems VO^{2+}–ATP, –ADP and –adenosine, together with the previously reported data on the D-ribose system, further supports the key role played by the sugar moiety at high pH. Moreover, the failure to detect a species such as VOA_2H_{-2} formed by ATP and ADP is in accord with the hypothesis that, in those complexes, the polyphosphate chain is effectively involved in metal complexation.

The lack of a C(2')OH group means that 2'-deoxyadenosine diphosphate does not possess a pair of *cis*-oriented vicinal hydroxyls which strongly chelate the vanadyl ion in alkaline solution. Accordingly the complexing behaviour of this ligand is the same as that of ADP and ATP in the acidic pH region wherein the ligand binds the metal ion through the sole polyphosphate moiety. So, below pH 7.5, EPR allows the detection of the species VOAH/VOA and VOA_2 but, when the pH is increased, polyphosphate is not competitive enough with OH^- to prevent hydrolysis. The hyperfine resolution fades away and eventually the EPR signal disappears. At pH > 11 the major species in solution is the totally hydrolysed $[VO(OH)_3]^-$ which is EPR active. Visible spectra, with their absorption maxima at 610 and 810 nm (ε = 12 and 21 dm^3 mol^{-1} cm^{-1}), show that, in the first steps of hydrolysis, the polyphosphate moiety co-ordinates to the metal ion to give, as in the cases of ADP and ATP at lower nucleotide-to-metal ratio, the species $(VO)_2A_2H_{-2}$. The band at 420 nm is not observed, thus confirming that, in the di-μ-hydroxo dinuclear species formed by ATP and ADP between pH 8 and 9.5, the ribose moiety effectively participates in metal co-ordination.

References

1 D. Rehder, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 148.
2 R. Wennig and N. Kirsch, in *Handbook on Toxicity of Inorganic Compounds*, eds. H. G. Seiler, H. Sigel and A. Sigel, Marcel Dekker, New York, 1988, p. 749.
3 L. C. Cantley, jun., L. Josephson, R. Warner, M. Yanagisawa,

C. Lechene and G. Guidotti, *J. Biol. Chem.*, 1977, **252**, 7421.
4 R. L. Post and S. Kume, *J. Biol. Chem.*, 1973, **248**, 6993.
5 L. C. Cantley, jun., and P. Aisen, *J. Biol. Chem.*, 1979, **254**, 1781.
6 G. M. Woltermann, R. A. Scott and G. P. Haight, jun., *J. Am. Chem. Soc.*, 1974, **96**, 7569.
7 H. Sakurai, T. Goda, S. Shimomura and T. Yoshimura, *Biochem. Biophys. Res. Commun.*, 1982, **104**, 1421.
8 H. Sakurai, T. Goda and S. Shimomura, *Biochem. Biophys. Res. Commun.*, 1982, **108**, 474.
9 S. B. Etcheverry, E. G. Ferrer and E. J. Baran, *Z. Naturforsch., Teil B*, 1989, **44**, 1355.
10 G. Urretavizcaya and E. J. Baran, *Z. Naturforsch., Teil B*, 1987, **42**, 1537.
11 P. A. M. Williams and E. J. Baran, *J. Inorg. Biochem.*, 1992, **48**, 15.
12 P. A. M. Williams and E. J. Baran, *J. Inorg. Biochem.*, 1993, **50**, 101.
13 R. Cini, G. Giorgi, F. Laschi, M. Sabat, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1989, 575.
14 G. Gran, *Acta Chem. Scand.*, 1950, **4**, 599.
15 I. Nagypál and I. Fabián, *Inorg. Chim. Acta*, 1982, **61**, 109.
16 H. Irving, M. G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
17 L. Zékány and I. Nagypál, in *Computational Methods for the Determination of Stability Constants*, ed. D. Leggett, Plenum, New York, 1985.
18 R. P. Henry, P. C. H. Mitchell and J. E. Prue, *J. Chem. Soc., Dalton Trans.*, 1973, 1156.
19 A. Komura, M. Hayashi and H. Imanaga, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 2927.
20 T. Kiss, I. Sóvágó and R. B. Martin, *Inorg. Chem.*, 1991, **30**, 2130.
21 P. Buglyó, T. Kiss, E. Alberico, G. Micera and D. Dewaele, unpublished work.
22 M. Branca, G. Micera, A. Dessì and D. Sanna, *J. Inorg. Biochem.*, 1992, **45**, 169.
23 N. D. Chasteen, in *Biological and Magnetic Resonance*, eds. L. J. Berliner and J. Reuben, Plenum, New York, 1987, vol. 3, pp. 53–119.
24 H. Sigel, in *Coordination Chemistry–20*, ed. D. Banerja, Pergamon, New York, 1980.
25 T. Kiss, G. Nagy, M. Pécsi, H. Kozlowski, G. Micera and L. Strinna Erre, *Polyhedron*, 1989, **8**, 52.
26 D. Mustafi, J. Telser and M. Makinen, *J. Am. Chem. Soc.*, 1992, **114**, 6219.

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