Modeling the Transition State of Biological Phosphoester Cleavage: The Complexation of Vanadate(V) by Nucleosides

Jens Richter and Dieter Rehder*

Institut für Anorganische und Angewandte Chemie der Universität, Martin-Luther-King-Platz 6, D-W-2000 Hamburg 13


Vanadate Nucleosides, $^{51}$V NMR Spectra, Transition State Analogue

The predominant complex species formed between inosine and vanadate in aqueous media is bimetallic and biligate ($\delta^{51}(V) = -523$ ppm; the formation constant at pH 7.9 and an ionic strength $I = 1 \text{ M is } K_v = 2.2(1) \times 10^{-5}$), with essentially a pentagonal-bipyramidal geometry around vanadium, coordination through two adjacent ($2'$ and $3'$) ribose oxygens, and dimerization via alkoxo bridges. The complexation depends upon concentration, pH, temperature, and solvent. The pH range of 7.1–8.8 has been studied. In dry dmsO, the geometry of the vanadate-inosine complex is probably octahedral ($\delta^{51}(V) = -494$ ppm). Adenosine (Ad) shows a behaviour very much comparable to inosine in aqueous media ($K_v = 2.5(1) \times 10^{-3}$), but higher equilibrium concentrations of a 1:2 complex (VAd$_3$) have been detected in this case. There is evidence (by $^1$H NMR) for participation of the NH$_2$ group of the base in coordination.

1. Introduction

Vanadium is an essential trace element probably for all organisms. Its biological role has become increasingly evident during the last few years (reviewed in [1]). Under normal physiological conditions, vanadium is existent in the form of H$_2$VO$_4^-$, which inhibits and stimulates a large variety of phosphorylation enzymes. Among these, K,Na-ATPase [2], ribonuclease-A [3] and ribonuclease-T$_1$ (RNase-T$_1$) [4–6] have been studied comprehensively. The inhibitory function towards RNases is connected with the stability of the transition state for the cleavage/formation of an ester bond in RNA. The transition state (cf. Fig. 1), under usual physiological conditions, is a labile ternary complex between phosphate, substrate and enzyme, with phosphorus in a trigonal-bipyramidal environment. Such an intermediate should be stabilized considerably when vanadate is built in as a competitor to phosphate. The relative stability of the ternary vanadate complex, which results in inactivation of the enzyme, has been documented [3, 5].

RNase-T$_1$ specifically cleaves the phosphoester bond in the 3' position of guanosine [4, 7]. Guanosine itself is only sparingly soluble in water, and we have therefore used inosine (which lacks the NH$_2$ group of guanosine) as a model nucleoside for systematic studies. We have shown, by $^{51}$V NMR, that the ternary complex formed between vanadate, inosine and RNase-T$_1$ has a formation constant of 1.5·10$^5$ M$^{-2}$ [5], with spectroscopic characteristics very similar to those of the binary vanadate-inosine complex, indicating that this binary system remains intact as the enzyme is taken up as a third component. The binary compound therefore describes, in a good approximation, the ternary compound and the inhibitory action as a whole.
The nature of the binary complexes formed between vanadate(V) and nucleosides has been under debate. There is general agreement that vanadate forms (i) weak tetrahedral monoesters of composition HVO$_2$OR$^-$ with the ribose moiety and (ii) more stable cyclic esters with the 2'- and 3'-hydroxyls of ribose, and vanadium in a trigonal-bipyramidal [8] or octahedral environment [9]. This species resonates at $\delta$(V$^{51}$V) = -523 ppm (relative to VOCl$_3$). The metal:ligand stoichiometry is discussed controversially, however. Complexes of composition 1:1, 1:2, 2:1 and 2:2 have been proposed [5, 8–11]. The reasons for these conflicting reports will be addressed, based on a $^{51}$V NMR investigation of the complexation of vanadate by inosine and guanosine. Adenosine is included in this study because of the role of vanadate in ATPase inhibition.

2. Results and Discussion

The aqueous vanadate-inosine system

For the quantitative evaluation of the degree of complexation between inosine and vanadate, the equilibrium concentrations of the various species present in solution are determined via the integral intensities of the resonance signals in the $^{51}$V NMR spectra. The following factors influencing complex formation have to be considered: Total concentration of vanadate, c(V); total concentration of inosine, c(In); the ratio c(V)/c(In); pH; ionic strength I; temperature T. In order to determine the complex stoichiometry, the latter three parameters were kept constant (pH 7.9, I = 1 M, T = 25 °C for the concentration range c(V) = 1–10 mM; pH 7.4, I = 0.2 M, T = 25 °C for c(V) = 0.05–0.9 mM).

Examples for spectra are contained in Fig. 2, 4 and 5. Apart from the sharp signals for free, tetrahedral vanadates at -588 (V$_6$O$_{18}$$^{6-}$; V$_6$), -584 (V$_2$O$_{15}$$^{5-}$; V$_2$), -576 (V$_4$O$_{12}$$^{4-}$; V$_4$), and around -572 (H$_2$V$_2$O$_7$$^{2-}$ \( \rightleftharpoons \) HV$_2$O$_5$$^{3-}$ + H$^+$; V$_2$) and -556 ppm (H$_2$VO$_4$$^{3-}$ \( \rightleftharpoons \) HVO$_2$$^{2-}$ + H$^+$; V$_1$) (cf. ref. [12] for assignments, and ref. [13] for the exchange dynamics), there is a broad signal at -523 ppm, the intensity of which increases as c(In)/c(V) increases. This signal indicates complexation of vanadate by inosine and is attributed in the literature to a cyclic ester. The considerable broadening (line widths at half-height, $W_{1/2}$, are ca. 800 Hz) is mainly a consequence of effective quadrupole relaxation (the nucleus $^{51}$V has a spin of 7/2 and a quadrupole moment of $-0.04 \times 10^{-28}$ m$^2$). The resonance signal at -523 ppm exhibits an asymmetry, sometimes visible as a shoulder, at high magnetic field ($\delta$(V$^{51}$V) = -525 ppm), indicating that there are at least two structurally closely related vanadium species present.

In order to determine the complex stoichiometry, four data sets were evaluated: (a) c(V) = constant = 10 mM, c(In) variable (10–100 mM); (b) c(V) variable (1–10 mM), c(In) = constant = 10 mM; (b') c(V) variable (0.05–0.9 mM), c(In) = constant = 10 mM; (c) c(V)/c(In) = constant = 1/4, c(In) and c(V) variable (c(In) = 4–40 mM; c(V) = 1–10 mM [14]). Since a direct participation of V$_2$, V$_4$ and V$_5$ has not been observed, only the equilibrium concentrations [-523] = [VIn] (the sum of the -523 signal and its asymmetry at -525 ppm), [V$_1$] and [In] were taken into account. At pH 7.4–7.9, V$_1$ mainly corresponds to diprotonated monovanadate, H$_2$VO$_4$$^-$. This signal also envelops the tetrahedral ester HVO$_2$In$^-$. The complex formation constants of simple tetrahedral esters are of the order of magnitude of 0.1–1 M$^{-1}$ [11, 15]. The correction of [V$_1$] for the presence of small amounts of ester is therefore not explicitly considered here. [V$_1$] and [-523] are obtained from the $^{51}$V NMR spectra directly, the [In] values are calculated from c(In) and [VIn]$_0$. To establish the complex formation constants, the following equilibria were considered:

\[ \text{V}_1 + \text{In} \rightleftharpoons \text{VIn}_0; \quad [\text{VIn}_0] = K_1[\text{V}_1][\text{In}] \quad (1a; b) \]
\[ \text{VIn}_0 + \text{In} \rightleftharpoons \text{VIn}_2; \quad [\text{VIn}_2] = K_2[\text{VIn}_0][\text{In}] \quad (2a; b) \]
\[ 2\text{VIn}_0 \rightleftharpoons (\text{VIn}_0)^2; \quad [\text{VIn}_0^2] = K_3[\text{VIn}_0]^2 \quad (3a; b) \]
\[ 2\text{V}_1 + 2\text{In} \rightleftharpoons (\text{VIn}_2)^2; \quad [\text{VIn}_2]^2 = K_4[\text{V}_1]^2[\text{In}]^2 \quad (4a; b) \]

Eqs (1) and (2) describe the formation of mononuclear, eqs (3) and (4) the formation of binuclear compounds. Assuming that the data sets represent the formation of mononuclear species (we will show later that this assumption is not realistic) allows to estimate apparent constants (indicated by a prime) K'$_1$ and K'$_2$ from eq. (5), a combination of eqs (1b) and (2b):

\[ ([\text{VIn}_0] + [\text{VIn}_2]) = [\text{V}_1][\text{K}'_1][\text{In}] + \text{K}'_2[\text{In}]^2 \quad (5) \]

A plot of [-523]/[In] vs. [V$_1$] (low concentration range, i.e. data set b’; Fig. 2) yields an almost straight line, from which K'$_1$ = 235(14) M$^{-1}$ is obtained. This K'$_1$ agrees with other values from the literature [5, 10], but is two orders of magnitudes
larger than what has been reported for the effective constant $K_1$ in ref. [11] ($= 3 \text{ M}^{-1}$). A fit of all of the data ($a$, $b$ and $b'$) to a 2nd order polynomial provides $K_1'K_2' = 44(1) \text{ M}^{-1}$, from which $K_2' = 0.12(2) \text{ M}^{-1}$ may be calculated, indicating negligible formation of a complex with more than 1 inosine ligand per vanadium.

A closer look at the data shows that the above assumption (formation of a complex which is mononuclear in vanadium) cannot be maintained at least at $c(\text{V}) > 0.7 \text{ mM}$. Evaluation of the data set $c$ (constant $c(\text{V})/c(\text{In})$) clearly demonstrates that a binuclear and biligate complex is the dominating species. The results obtained from an evaluation of the data (Table I) are represented graphically in Fig. 3, showing how the equilibrium concentrations $[-523]$ and $[\text{V}_2]$ vary with $c(\text{V})$ and $c(\text{In})$. If we now evaluate the data sets $a$ and $b$ along the line defined by eqn. (4) (formation of a 2:2 complex), i.e. by plotting $[-523]/[\text{V}_2]^2$ vs. $[\text{In}]^2$, $K_4 = 2.2(1) \times 10^{-7} \text{ M}^{-1}$ is obtained from the slope of a straight line. This complex formation constant, determined at pH 7.9, is smaller than $K_4 = 7.0(5) \times 10^{-7} \text{ M}^{-1}$ reported for pH = 7.0 [11]. Employing the literature value $K_1 = 3 \text{ M}^{-1}$, the dimerization constant $K_3$ is calculated according to $K_3 = K_4/(K_1^2) \approx 7 \times 10^6 \text{ M}^{-1}$.

We have already indicated that the extent of complex formation also is a function of pH. The spectra and the graphical presentations in Fig. 4 convey an optical impression of this pH dependence. Along with the decrease of $[-523]$ with increasing pH, a low-field shift of the signal for $\text{V}_1$ is observed, due to a shift of the equilibrium $\text{H}_2\text{VO}_4^- \rightleftharpoons \text{HVO}_2^{2-} + \text{H}^+$ towards the monopro-

Table I. Equilibrium concentrations of the vanadium species (related to the number of vanadium atoms) for constant $c(\text{V})/c(\text{In}) = 1/4$ but variable $c(\text{V})$ and $c(\text{In})$ ((mM); data set $c$).

<table>
<thead>
<tr>
<th>$c(\text{V})$</th>
<th>$c(\text{In})$</th>
<th>$[-523]_b$</th>
<th>$[\text{V}_2]_b$</th>
<th>$[\text{V}_3]$</th>
<th>$[\text{V}_4]$</th>
<th>$[\text{V}_5]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>11.0</td>
<td>33.9</td>
<td>11.0</td>
<td>37.0</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>26.9</td>
<td>22.4</td>
<td>7.3</td>
<td>38.8</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>46.9</td>
<td>16.3</td>
<td>4.6</td>
<td>28.1</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>57.6</td>
<td>11.7</td>
<td>3.4</td>
<td>24.6</td>
<td>2.6</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>66.7</td>
<td>10.6</td>
<td>2.9</td>
<td>18.3</td>
<td>1.9</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>74.1</td>
<td>8.4</td>
<td>2.1</td>
<td>13.3</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>77.1</td>
<td>7.6</td>
<td>2.2</td>
<td>12.0</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>83.9</td>
<td>5.6</td>
<td>1.2</td>
<td>8.1</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>84.9</td>
<td>5.8</td>
<td>1.2</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>86.2</td>
<td>6.9</td>
<td>1.7</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The data are noted in %, related to the sum over all detectable vanadium species = 100%. Minor amounts of other species than those given in the Table may be present. The effective equilibrium concentrations, $[\text{V}_n]_{\text{eff}}$ (mM), of the oligovanadates $\text{V}_n$ is $[\text{V}_n]_{\text{eff}} = c(\text{V}) \cdot [\text{V}_n]/100n$; \(^b\) represents the sum of all pentavalent vanadate-inosine complexes; \(^c\) includes tetrahedral ester $\text{HVO}_4\text{In}^-$.

Fig. 2. Upper part: $^{51}$V NMR spectra of aqueous (H$_2$O/D$_2$O 2:1) solutions containing varying amounts of vanadate (indicated on the right-hand margin) and a constant inosine concentration of 10 mM. The pH/D is 7.4, ionic strength 0.2 M (NaCl), the buffer concentration 30 mM (Hepes). The broad signal at $-523$ ppm, indicated $\text{VIn}$, corresponds to the inosine-vanadate complex. The $\text{V}_n$ are free (tetrahedral) oligovanadates, with $\text{V}_1$ probably also covering small amounts of tetrahedral monoester $\text{HVO}_4\text{In}^-$. Lower part: Graphical evaluation of the data obtained from the spectra. $[\text{VIn}], [\text{In}]$ and $[\text{V}_1]$ are the equilibrium concentrations of complex, inosine and monovanadate, respectively. The data were fitted according to a linear relationship ($r^2 = 0.993$); from the slope, the complex formation constant $K'_1$ (apparent) = $235(14) \text{ M}^{-1}$ is obtained.
Fig. 3. Variations of the relative equilibrium concentrations of complex, [−523], and monovanadate, [V1]. The ratio c(V)/c(In) has been kept constant; pH/D = 7.9, I = 1 M (NaCl), H2O/D2O 2/1, 0.3 M Hepes buffer. The scale for [V1] is expanded with respect to that of [−523] by a factor of 2.3. This graphical presentation demonstrates that the dominant complex represented by the −523 ppm signal is dinuclear in vanadium.

The aqueous systems vanadate-guanosine and vanadate-adenosine, and studies in non-aqueous media

The guanosine-(Gu)-vanadate complexes are only sparingly soluble in water. In contrast to In (and adenosine, Ad; see below), a second resonance at −530 ppm, clearly distinct from the −523 ppm signal, arises in a saturated solution (c(Gu) = 10.3 mM, c(V) = 1 mM; pH 7.6, I = 0.2). Under these conditions, [V1] is 0.44 mM and the ratio [Σ(VGu)]/[V1] amounts to 0.25, from which a
Fig. 4. pH/D dependence of complex (V\text{In}, \delta^{51}\text{V} = -523 \text{ ppm}) formation at c(V) = 5 mM, c(In) = 20 mM and I = 1 M (NaCl); solvent H\text{2}O/D\text{2}O 2/1, 0.3 M Hepes buffer. Left: The decrease of [V\text{In}] and the low-field shifts of the signals for V\text{1} and V\text{2} with increasing pH are clearly discernible. At pH/D = 8.8, a signal at -571.1 ppm (*) is uncovered, possibly representing a tetrahedral ester [15]. Right: Block diagramme, showing the variations with pH/D of [-523] and [V\text{1}]. The [V\text{1}] scale is expanded relative to that of [-523] by a factor of 2.

Fig. 5. Temperature dependence of complex (V\text{In}) formation. c(V) = 5 mM, c(In) = 20 mM, pH/D = 7.9, I = 1 M (NaCl), 0.3 M Hepes, H\text{2}O/D\text{2}O 2/1. The signals for free vanadates broaden as a consequence of increased chemical exchange between the V\text{n}. The signals for the V\text{In} sharpen, owing to quadrupole relaxation as the dominant relaxation mechanism for the complexes, cf. Table II for the raw data.

Table II. Temperature dependence of the chemical shifts, line widths and equilibrium concentrations for the vanadate-inosine complex at -523 ppm.a

<table>
<thead>
<tr>
<th>T (K)</th>
<th>[-523] (%)b</th>
<th>\delta (ppm)</th>
<th>W_{1/2} (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>66.9</td>
<td>-523.7</td>
<td>1180</td>
</tr>
<tr>
<td>300</td>
<td>52.1</td>
<td>-522.1</td>
<td>1020</td>
</tr>
<tr>
<td>310</td>
<td>35.7</td>
<td>-522.1</td>
<td>910</td>
</tr>
<tr>
<td>320</td>
<td>20.9</td>
<td>-521.1</td>
<td>830</td>
</tr>
<tr>
<td>330</td>
<td>12.1</td>
<td>-519.4</td>
<td>470</td>
</tr>
<tr>
<td>340</td>
<td>8.7</td>
<td>-518.2</td>
<td>470</td>
</tr>
<tr>
<td>350</td>
<td>8.1</td>
<td>-518.9</td>
<td>280</td>
</tr>
<tr>
<td>360</td>
<td>2.7</td>
<td>-515.4</td>
<td>270</td>
</tr>
</tbody>
</table>

a c(V) = 5 mM, c(In) = 20 mM, pH = 7.9, I = 1 M; b percentage of all species detectable in the 51V NMR spectrum.

rough estimate for K' \equiv K_1 = 24 M^{-1} is obtained.

In contrast to the Ad and In systems, there is an additional, high field signal in the vanadate-Gu system at -625 ppm ([-625] = 0.03 mM), which we tentatively assign to a tri-ester “VO(OR)₃”, for which \delta^{51}\text{V} values of this magnitude have been reported for bulkier substituents R such as iPr [16].
The maximum concentration that could be reached with adenosine was $c(\text{Ad}) = 25$ mM. Complexation of vanadate with adenosine is very much the same as with inosine. Again, the predominant species is a dimer of 2:2 stoichiometry. The following complex formation constants have been obtained: $K'_1$ (low concentration range) = 225(40) M$^{-1}$, $K'_2 = 3.4$ M$^{-1}$, $K_4 = 2.5(1) \cdot 10^7$ M$^{-3}$. The main difference to the vanadate-inosine system is the more pronounced role of a complex with a (1:2)$_n$ metal:ligand stoichiometry: The apparent constant $K'_2$ for the formation of a complex of composition $\text{VAd}_2$ is 30 times as large as $K'_2$ for $\text{VIn}_2$. It is very suggestive to trace back this fact to the $\text{NH}_2$ of adenosine. As has been demonstrated in the case of ethanolamine derivatives [19] and dipeptides [20], amino groups do coordinate to vanadate in aqueous media, if they can be involved in chelate ring formation. $\text{NH}_2$ coordination is supported by $^1\text{H}$ NMR evidence: While, in the vanadate-inosine complexes, a coordination shift $\Delta \delta$ of the $^1\text{H}$ resonances is observed for the ribose hydrogens only, there is a distinct $\Delta \delta$ also for the hydrogens at C2 and C8 of the base in the case of vanadate-adenosine complexes (Table III).

An additional argument comes from the $\delta(^{51}\text{V})$ resonances of the complexes in dry dimethyl sulfoxide (dmso). Owing to the insolubility of sodium vanadate in dmso, we have employed tetrabutylammonium vanadate in this case. With the exclusion of water, oligonucleation does not occur as a reaction path competitive to coordination. Consequently, complexation between vanadate and nucleoside is almost complete already at a molar ratio of 1:1 of the two reactants. The $^{51}\text{V}$ chemical shift for $\text{VIn}$ in dmso is $-494$ ppm, hence a low-field shift of 29 ppm with respect to $\text{VIn}$ in water. Signals in this region have been reported for octahedral complexes [21]. The $-494$ signal also arises in the systems vanadate/adenosine/dmso and vanadate/guanosine/dmso, where an additional resonance at $-465$ is observed (Fig. 6), corresponding to a complex of coordination number 4–7 with mixed O,N coordination [19, 21].

| Inosine | $\delta$ | $\Delta \delta$ | Adenosine | $\delta$ | $\Delta \delta$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>8.44</td>
<td>a</td>
<td>8.51</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>H8</td>
<td>8.32</td>
<td>a</td>
<td>8.38</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>H1'</td>
<td>6.50</td>
<td>0.32</td>
<td>6.47</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>H2'</td>
<td>4.16</td>
<td>0.14</td>
<td>4.16</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>H3'</td>
<td>4.12</td>
<td>0.19</td>
<td>3.93</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>H5''</td>
<td>5.35</td>
<td>b</td>
<td>5.33</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

Table III. $\delta(^1\text{H})$ and $\Delta \delta$ values for the ligand hydrogen atoms of the vanadate-inosine and vanadate-adenosine complexes (see Scheme for numbering).

- $^a$ No coordination shift observed; $^b$ not determined due to partial overlap with other signals.

Fig. 6. 94.7 MHz $^1\text{H}$ $^{51}\text{V}$ NMR spectrum of a 1:1 mixture of adenosine and tetrabutylammonium vanadate ($c(\text{V}) = c(\text{Ad}) = 1$ mM) in dry dmso. The half-widths $W_{1/2}$ of the broad signals are 1.8 kHz ($\delta = -456$ ppm) and 2.1 kHz ($\delta = -494$ ppm). The relatively sharp resonances at $-527$ and $-569$ ppm probably belong to species also observed in the aqueous system, indicating that $\text{H}_2\text{O}$ is formed in the course of coordination as described by eq. (6).

**Conclusions**

There are several reports in the literature, dealing with the investigation of complex formation between vanadate and nucleosides such as inosine [5, 8–11]. In most cases, it has been assumed that mononuclear 1:1 complexes are formed, in analo-
gy to the vanadate complexes obtained with diols [22] or sugars [23]. If the data collected in the usual concentration range (c(V) = 0.1–10 mM) are treated with this underlying assumption, apparent formation constants K′ of several hundred M⁻¹ are calculated. Evaluation of the data sets for constant c(V)/variable c(ligand), and variable c(V)/constant c(ligand) excludes the presence of significant amounts of complexes of the stoichiometry (1:2)ₐ or (2:1)ₐ, but does not allow for an unambiguous discrimination between the stoichiometries 1:1 and 2:2. This is possible, however, if the ratio c(V)/c(ligand) is kept constant over a wide range of absolute concentrations of the two components.

In the present case (ligand = inosine, adenosine) we have demonstrated, that the signal at −523 characteristic of complexed vanadate mainly represents a 2:2 species. The dinuclear complex is mainly formed from the pentavalent precursor in the manner described by eq. (6). Guanosine has a significantly less pronounced tendency to coordinate to vanadate than inosine and adenosine, and it is likely that the main components in this case are mononuclear. The differing behaviour between guanosine on the one hand, and inosine and adenosine on the other hand, while not easily explained, points towards the limitations of using inosine as a model nucleoside for, e.g., guanosine.

We point out that, in the case of In and Ad, there is a high field asymmetry in the −523 ppm signal, sometimes visible as a shoulder at −525 ppm, over the whole concentration, pH and temperature ranges under investigation. For Gu, these two signals lie at −523 and −530 ppm. The high-field signal is indicative of a second, minor species, possibly a complex containing two ligands per vanadium, a plausible assumption based on a slight upward curvature of the graphs.

Both the monomeric and the dimeric complexes can be of physiological significance: The average vanadium concentration in human tissue is 0.1–1 µM [24], where presumably only monomeric complexes can form and exert an inhibitory action. Higher concentrations are possible, however, under toxic conditions or by accumulation of vanadate in special cell compartments. It has in fact been shown that oligovanadates do inhibit phosphate metabolizing enzymes [25]. Hence dimeric complexes may be involved. Association through alkoxo bridges [16, 17] favours the formation of dimeric complexes: K₃, the constant for the formation of (VL)₂ from 2 VL, amounts to 10⁶ to 10⁸ [26] and hence is several orders of magnitude larger than the dimerization constant (= 10³) for the formation of divanadate from monovanadate, where dimerization occurs through an oxo bridge.

**Experimental**

⁵¹V NMR spectra were scanned on a Bruker AM 360 spectrometer at 94.73 MHz in 10 mm diameter vials. The solvents were H₂O/D₂O 2/1 or dmso/dmso-d₆ 2/1. Typical measuring parameters: sweep width 125 kHz, time domain 8.2 K, pulse angle 60°, relaxation delay 0 s, line broadening factor 30 Hz, scan number 5,000 to 40,000. ¹H NMR spectra were obtained on a Bruker MSL 300 spectrometer in H₂O/D₂O 2/1 under the usual conditions and with partial suppression of the water signal.

Hepes ((N-2-hydroxyethyl)piperazine-N'-(2-sulfonic acid), hemisodium salt) was used as a buffer. In contrast to Tris buffer, which forms binary complexes with vanadate [5, 27] and a ternary complex with vanadate and inosine [11], Hepes does not show any tendency of complex formation [19, 27].

The following stock solutions were used for the sample preparations for collecting the data sets a, b and c: Hepes 0.3 M in H₂O/D₂O 2/1, pH/D 7.75; an ionic strength of 1 M was achieved by dissolving 2.05 g NaCl in 50 ml of this solution. A vanadate solution of c(V) = 0.5 M was prepared by dissolving 0.92 g Na₃VO₄ (Janssen) in 10 ml of Hepes stock solution. Inosine and adenosine solutions were prepared by dissolving 0.67 g inosine and 0.17 g adenosine, respectively, in 25 ml of Hepes stock solution. For the data collection b' (c(V) = 0.05–0.9 mM, c(In/Ad) = 10 mM), the ionic strengths and buffer concentrations were adjusted to 0.2 M (NaCl) and 0.03 M (Hepes), respectively, in order to minimize matrix effects at these low vanadium concentrations.

The inosine-vanadate and adenosine-vanadate samples were prepared by mixing appropriate amounts of the stock solutions with the use of high precision Eppendorf pipettes. This was done several days before measurement, in order to ensure complete equilibration, and with protection against direct light (to avoid reduction of V⁵⁺ to V⁴⁺). The final pH/D values were 7.9(1) for a, b, and c, and 7.4(1) for b'. For adjustment of other pH values, 1 M HCl or NaOH was added. pH values were adjusted with a single-rod micro glass
electrode (Ingold); the exact determination of the pH was carried out with the use of the 3/V resonance $\delta_0$ of the signal for $V_1$ according to eq. (7):

$$\text{pH} = \text{pK}_a + \log[(\delta_1 - \delta_0)/\delta_0]$$  \hspace{1cm} (7)

$pK_a = 8.16$, $\delta_1$ and $\delta_0$ are the limiting values for $H_2VO_4^-$ ($\sim 561.7$ ppm) and $HVO_3^{2-}$ ($\sim 537.3$ ppm) at an ionic strength (NaCl) of 1 M.

The guanosine-vanadate system was prepared by treating a suspension of guanosine with $0.2 M$ vanadate and $4 ml$ of $0.03 M$ Hepes, $I = 0.05 M$, and successive filtration. The final concentrations for the oligovanadates are $[V_1]/n$, $[V_2]/n$, $[V_3]/n$, and $[V_4]/n$ (the effective equilibrium concentrations for the oligovanadates are $[V_n]/n$).

$^1H$ NMR measurements were carried out in unbuffered solutions. The pH was adjusted to 6.4 with 1 M HCl in these cases in order to provide an optimum (with respect to free nucleoside) complex concentration. In Table III, $\delta(1^H)$ values and coordination shifts $\Delta\delta$ are summarized.

Dimethylosulfoxide, dried by refluxing over CaH$_2$ for 8 h, was distilled in a N$_2$ stream and stored under N$_2$ over 4 Å molecular sieve. Tetra­butylammonium vanadate (tbav) was prepared from $V_2O_5$ and an aqueous solution of [Bu$_4$N]OH, as described in ref. [20]. $\delta(1^V)$ for tbav in dmos is $\sim 499$ ppm, indicating that dmos becomes coordinated to vanadate.

This project was supported by the Deutsche Forschungsgemeinschaft (Re 431/9-1) and the European Community (SC1-CT90-0458).

[14] Only the data for the set $c$ are reported here explicitly (Table I). Tables containing data for the other sets may be obtained from the authors on request.

[26] Tracey and co-workers report a formation constant $K_1 (pH 7.5) = 6.2 \times 10^6 M^{-1}$ (personal communication).