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Condensation Reactions of Aqueous Vanadate with the Common Nucleosides

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^{51}V and ^{1}H NMR studies of the interactions of adenosine, inosine, cytidine, guanosine, and uridine with vanadate have been undertaken. In disagreement with statements in the recent literature, the major products formed are isomeric binuclear bis(ligand) products that give rise to ^{51}V NMR signals near −523 ppm. These products are not formed with either 2′-deoxyuridine or 2′-deoxythymidine. At pH 7.0 the formation of the −523 ppm products is very favorable, with the overall formation constants varying between 3×10^7 M^{-3} and 7×10^7 M^{-3}, dependent on the nucleoside. Some evidence for a cyclic mononuclear tetrahedral ester with an NMR signal at −560 ppm was obtained. A dimerization constant of 3×10^6 M^{-1} to afford the −523 ppm product from the above monomer was estimated. Proton magnetic resonance indicated the presence of three predominant products, the major one being symmetrical and consisting of about 70% of the total product. In addition to the products mentioned above, other materials corresponding to vanadate esters at the various hydroxyl groups of the ribose ring are formed and include for instance a vanadate analogue of adenosine monophosphate.

Introduction

The interactions that vanadate undergoes when in the presence of metabolic products have been a topic of increased interest as a result of the fact that vanadate oxoanions give rise to a number of biological responses, both in vivo and in vitro.[1-4] In addition to this, vanadium occurs in the prosthetic group of various bromo- and iodoperoxidases from a variety of marine brown and red algae[5] and in certain nitrogen-fixing enzymes of the bacteria genus *Azotobacter*.[6,7]

It seems highly likely that many of the biological effects of vanadate derive from participation of vanadate in enzymic reactions as a phosphate analogue. In this role, vanadate apparently can activate the function of some enzymes by spontaneously forming vanadate esters of hydroxyl-bearing compounds, which are then accepted by the enzyme in lieu of the normal biological phosphate derivatives.[8,9] Inhibition of other types of enzymes, notably a number of ribonucleases,[10,11] may be a result of the function of vanadate as a transition-state analogue. This possibility was suggested from the results of kinetics studies utilizing ribonuclease A and both V(IV) and V(V) complexes of uridine.[10] X-ray and neutron diffraction studies of a crystalline uridine/vanadate/ribonuclease A complex provided a detailed picture of the previously proposed inhibitor.[12] From this study a distorted trigonal-bipyramidal coordination about the vanadium nucleus was observed. Such a geometrical arrangement has also recently been observed in the crystalline triester of orthovanadate, $(VO(OCH_2CH_2Cl)_3)_2$.[13]

The formation of pentacoordinate vanadium products occurs readily in aqueous solution when vanadate is in the presence of 1,2-diol functionalities such as glycols,[14] cyclohexanediols,[15] pyranose glycosides[15] and various derivatives of ribose including uridine,[16,17] adenosine,[17] adenosine monophosphate,[16] inosine,[18] and others.[19]

Of considerable interest concerning the formation of products in these systems are those materials giving rise to ^{51}V nuclear magnetic resonance (NMR) signals at −523 and −510 ppm, since it has been proposed that these signals derive from pentacoordinate, possibly trigonal-bipyramidal, products. These products form from condensation of vanadate with the 2′- and 3′-hydroxyls of the ribose ring. It is imperative that the products giving rise to the −510 and −523 ppm signals be properly identified, since this information is critical for the determination of the formation constants of the products and is also necessary for the analysis of enzyme inhibition studies.

Unfortunately, there is considerable ambiguity in the literature concerning the identification of the products. The signal arising at −510 ppm has been recently assigned to a monomeric 1:1 complex between vanadate and ligand.[18] However, an earlier report indicated that a product giving rise to this chemical shift derives from a quaternary complex of tris(hydroxymethyl)-aminomethane buffer, ligand, and two vanadate ions.[20] Similarly, the −523 ppm signal has been identified as deriving from a mixture of $V_1\ell_1$ and $V_1\ell_2$ complexes,[17-19] where ℓ refers to the diol ligand. In this case intensive earlier work had shown that the −523 ppm product signals derive from $V_2\ell_2$ complexes with little or none of the $V_1\ell_1$ products being present, this for a variety of ligands, including ethylene glycol,[14] the *cis*- and *trans*-1,2-cyclohexanediols,[15] adenosine monophosphate,[16] and uridine.[16] However, it should be noted that the situation changes if the ligand is an α-hydroxy carboxylate instead of a *cis*-diol. Such carboxylate ligands form mononuclear vanadium complexes with chemical shifts near −517 ppm.[21]

Since there is disagreement between the various reports and because these are important systems for biochemical studies, we have undertaken a study of vanadate interactions with a variety of the common nucleosides including inosine, cytidine, adenosine, and 2′-deoxythymidine and as well have repeated our previous investigation of uridine.

Experimental Section

Materials. Inosine, adenosine, guanosine, cytidine, uridine, 2′-deoxythymidine, 2′-deoxyuridine, and tris(hydroxymethyl)aminomethane (Tris) buffer were purchased from Sigma Chemical Corp. Microanalysis of the nucleosides showed these materials contained no water. All ma-

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terials were used as provided. Vanadium(V) oxide (99.99%) from Aldrich Chemical Co. and *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) buffer (from Boehringer Mannheim GmbH) also were used as provided.

Stock solutions of materials were prepared according to established procedures.[16] Stock solutions of ligands were used for the low ligand concentration studies, while the ligands were directly weighed out for the high concentration studies. Adenosine stock solutions and samples required warming (≈40 °C) for dissolution. Crystallization from solution (30 mM adenosine) occurred after 6–8 h. Separation of adenosine did not occur when in the presence of 5 mM vanadate.

All investigations were done at pH 7.00 ± 0.03, and unless indicated otherwise pH was controlled with 30 mM HEPES buffer. pH calibration was done with freshly opened standard buffer solutions. The ionic strength was maintained at 1.0 M with KCl. Solutions for ^{1}H NMR spectroscopy were prepared in D_2O.

Spectroscopy. All NMR spectra were obtained from a Bruker AMX-400 NMR spectrometer operating at ambient temperature. ^{51}V chemical shifts are reported relative to an external $VOCl_3$ reference assigned to 0.0 ppm. Pulse widths of 60°, sweep widths of 40 kHz, and acquisition times of 0.05 s were used for all acquisitions. Typically 5000 transients were averaged to provide the vanadium spectra, although up to 40000 were used for the more dilute solutions. Base-line corrections were applied to all spectra before the integrals were obtained.

Methods. The results were analyzed by using the procedures briefly outlined in the text. Equations were cast into their linear forms, and the least-squares parameters and errors were determined by using a standard least-squares program.

Results

The vanadate ion in aqueous solution undergoes self-condensation to form a variety of oligomeric forms including a dimer and tetramer. The bulk of the work reported here was done at an ionic strength of 1.0 M maintained with KCl and at pH 7.0. Under these conditions vanadate exists predominantly as the $VO_4H_2^-$ ion and has a pK_a of 8.16.[22] At pH 7, vanadate and its oligomers give rise to ^{51}V nuclear magnetic resonance (NMR) signals in the range of about −560 to −585 ppm. Tables giving the concentrations of reactants determined from the various studies are provided in the supplementary material.

Aqueous Inosine/Vanadate System. When tris(hydroxymethyl)aminomethane (Tris) was used as the buffer for pH 7.00 regulation in the presence of inosine, ^{51}V NMR signals at −510, −523, and −542 ppm were observed in addition to those mentioned above. The −542 ppm signal derives from a vanadate/Tris complex,[20] while the −510 and −523 ppm signals are dependent on the presence of inosine. However, successive partial replacements of Tris with the alternative buffer, *N*-(2-hydroxymethyl)-piperazine-*N*-ethanesulfonic acid (HEPES), resulted in the progressive elimination of both the −510 and −542 ppm signals. Within the context of already broad signals, no additional signal broadening accompanying the decrease in intensity of the −510 ppm signal was observed. This indicates that signal loss resulting from coalescence with the much more intense signal at −523 ppm does not occur. The decrease in intensity of the −510 ppm signal was found to be directly proportional to the concentration of the Tris buffer, a behavior identical with that reported for the cyclohexanediol case.[20] Figure 1 shows vanadium NMR spectra obtained from solutions containing Tris or HEPES buffers under similar conditions to those employed in a previous study of this system.[18]

Inosine and vanadate concentration studies were carried out for solutions containing only HEPES buffer and under conditions of 1.0 M ionic strength maintained with KCl and at pH 7.0. If the premise[17,18] that only Vℓ and Vℓ_2 products give rise to the −523 ppm signals is accepted, then the equilibria can be written as eqs 1 and 2, where T_1 refers to mononuclear tetrahedral va-

$$T_1 + \ell \rightleftharpoons V\ell \qquad [T_1][\ell]K_2 = [V\ell] \tag{1}$$

$$V\ell + \ell \rightleftharpoons V\ell_2 \qquad [V\ell][\ell]K_3 = [V\ell_2] \tag{2}$$

nadate, ℓ refers to the ligand, and Vℓ and Vℓ_2 refer to the −523 ppm products. The symbols T and V are used to indicate the difference in coordination between the tetrahedral (T) and pentacoordinate (V) vanadium derivatives.

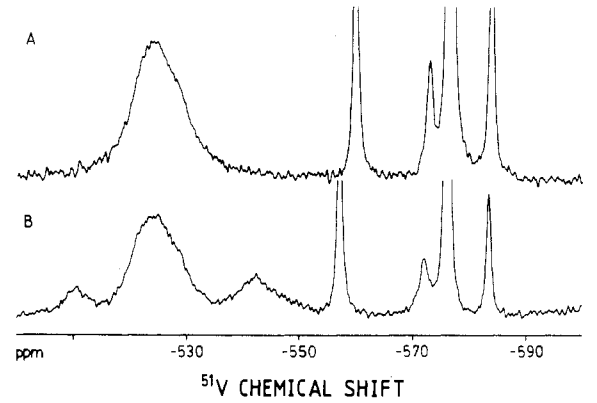

Figure 1. Loss of the ^{51}V NMR signals at −510 and −542 ppm when Tris buffer (spectrum B) is replaced by HEPES buffer (spectrum A). The conditions of the experiment were 1.0 M ionic strength with KCl, pH 7.00, 5 mM total vanadate, and 100 mM HEPES (spectrum A) or Tris (spectrum B).

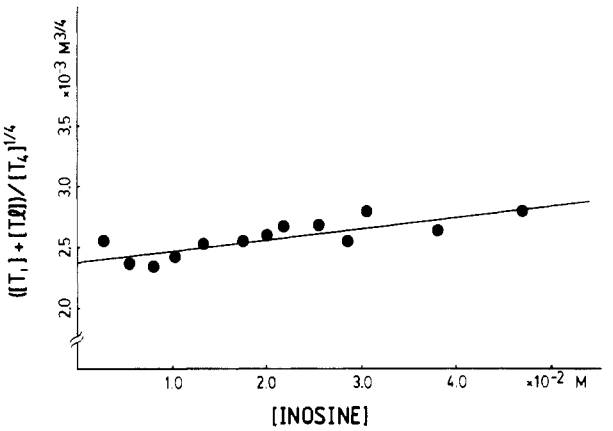

Figure 2. Graph demonstrating the formation of inosine/vanadate products giving rise to signals not separated from the $VO_4H_2^-$ signal. The intercept, $K_0^{-1/4}$, and slope, $K_0^{-1/4}K_1$, provided the formation constants of the vanadate tetramer, K_0, and the products, K_1. The conditions of the experiments are given in the Experimental Section.

In order to determine the concentrations of T_1, which gives rise to a ^{51}V NMR signal at −560 ppm, it is necessary to first examine the possibility that there are other signals under the T_1 signal, by virtue of chemical shift overlap or rapid chemical exchange. The products giving rise to these signals are designated as Tℓ, since they are presumed to retain tetrahedral coordination about the vanadium nucleus. In order to examine the possibility of formation of such products, a suitable reference is required. The tetrameric oligomer of vanadate, T_4, seems suitable for this, since it seems not to react readily with possible ligands and its formation is quite favorable. In this case eqs 3 and 4 give eq 5, which can

$$4T_1 \rightleftharpoons T_4 \qquad [T_1]^4K_0 = [T_4] \tag{3}$$

$$T_1 + \ell \rightleftharpoons T\ell \qquad [T_1][\ell]K_1 = [T\ell] \tag{4}$$

$$\frac{[T_1] + [T\ell]}{[T_4]^{1/4}} = K_0^{-1/4} + K_0^{-1/4}K_1[\ell] \tag{5}$$

be plotted to give $K_0^{-1/4}$ and K_1. The result is displayed in Figure 2. Evidently there are Tℓ products formed, although their formation, $K_1 = 3.8 \pm 0.6$ M^{-1}, is not highly favorable. These products, at least in part, will correspond to vanadate esters formed with the various hydroxyls of the ribose ring. With the above-determined values of $K_0^{-1/4}$ or K_1, the T_1 concentration can be obtained, either from $[T_4]$ or from $[T_1(t)]$ and the ligand concentration, where $[T_1(t)]$ refers to $[T_1]$ plus the concentrations of all T_1 products. Of course, the free ligand concentrations must

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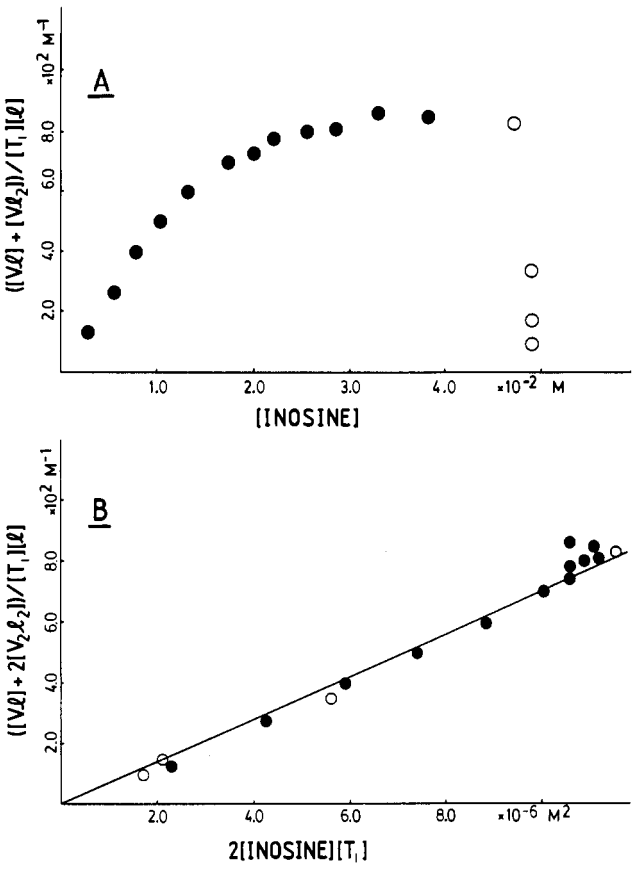

Figure 3. Graphical representations of the formation of the inosine complexes giving rise to the −523 ppm signal. Graph A demonstrates that the product NMR signal does not derive from a mixture of mono- and bis(ligand) mononuclear vanadium products. Graph B supports the hypothesis that the product is a bis(ligand) binuclear vanadium species with no detectable mononuclear product. The conditions for the experiments were pH 7.00, 1.0 M ionic strength maintained with KCl, 30 mM HEPES buffer, and varying concentrations of vanadate (○) or inosine (●).

be determined from the conservation equation, since they do depend on the product concentrations. Fortunately, the perturbation is small enough that establishing reasonably accurate equilibrium concentrations is almost model independent and anyway the concentrations can readily be established from ^{1}H NMR spectroscopy. Free and bound ligand concentrations, calculated on the basis of the ^{51}V NMR spectra, and those determined from proton magnetic resonance were always found to agree within the error of the experiments.

With $[T_l]$ having been determined, eqs 1 and 2 can be summed to give eq 6, and a plot of the ratio on the left versus the free ligand

$$\frac{[V\ell] + [V\ell_2]}{[T_l][\ell]} = K_2 + K_2K_3[\ell] \qquad (6)$$

concentration should give a straight line if the hypothesis leading to eq 6 is correct. Figure 3A shows the result obtained. In this figure the closed circles represent the points obtained for a constant total vanadate concentration of 5.0 mM and varying proportions of inosine. The open circles represent points from essentially a constant total free ligand concentration ~50 mM, with varying amounts of total vanadate, 0.1, 0.6, 1.0, and 5.0 mM for the four points. Given that the experimental procedures are correct, it is evident that eqs 1 and 2 do not correctly describe the equilibria that are established in this system. Furthermore, at 50 mM ligand and varying total vanadate concentration the ratio of eq 6, indicated by the open circles at Figure 3A, is not constant. This ratio must be nearly constant if the product contains only one vanadium nucleus. It is not exactly a constant, since the free ligand concentration is dependent on the formation of the product complex. This latter effect is small for the vanadate concentrations employed. Clearly the principle component giving rise to the −523 ppm signal is an oligonuclear product.

Formation of a binuclear bis(ligand) product can be represented by eq 7, which, on combination with eq 1 to take into account the possibility of formation of some mononuclear product, gives eq 8. In this equation, $K_2^2K_4$, which can be represented as K_5,

$$2V\ell \overset{K_4}{\rightleftharpoons} V_2\ell_2 \qquad [V\ell]^2K_4 = [V_2\ell_2] \qquad (7)$$

$$\frac{[V\ell] + 2[V_2\ell_2]}{[T_l][\ell]} = K_2 + 2K_2^2K_4[T_l][\ell] \qquad (8)$$

is the overall formation constant of $V_2\ell_2$ from 2 T_l and 2 ℓ. Figure 3B shows a plot of the relevant parameters of eq 8. The plot is linear with an intercept near zero and a slope of $K_2^2K_4 = (7.0 \pm 0.5) \times 10^7$ M^{-3}. Unlike for the previous graph (Figure 3A) in this plot all experimental points are close to a straight line. This result indicates that the −523 ppm signal derives almost completely from binuclear products. Under the conditions of this study no monomer was observed.

Because of the possibility that the 1.0 M ionic strength of the medium might have an unexpected effect on the equilibria established in the system, this inosine/vanadate system was also investigated at pH 7.0 but with no added KCl. The ambient ionic strength was then about 0.035 M.

The analysis of the results proceeded similarly to the procedure outlined above for the 1 M ionic strength solutions. There was a small change in the equilibrium constants, but there was no indication that the equilibrium situation had changed. The values obtained for the various constants were $K_0 = (4.7 \pm 0.3) \times 10^8$ M^{-3}, $K_1 = 3.9 \pm 1.5$ M^{-1}, and $K_2^2K_4 = (6.5 \pm 0.5) \times 10^7$ M^{-3}. Quite clearly, although the ionic strength had a large effect on the oligomerization of vanadate, it did not affect the inosine complexation constants significantly.

Aqueous Uridine/Vanadate System. As for inosine, it has been reported that both $V\ell$ and $V\ell_2$ complexes are formed with uridine and give rise to ^{51}V NMR signals at −521 ppm[17] although in this case an earlier report had identified a $V_2\ell_2$ complex as the source of this signal.[16] Uridine would not be expected to react very differently from inosine, since the ribose rings of the two molecules will have a similar conformation. However, in view of the discrepancy between the two reports we have studied this system at pH 7.0 under conditions of 1.0 M ionic strength with KCl.

When the results of the study were plotted according to eq 5 (Figure 4A), the values obtained were $(2.7 \pm 0.1) \times 10^{-3}$ M$^{3/4}$ for $K_0^{-1/4}$ and 4.5 ± 1.1 M^{-1} for K_1. The value for the formation of $V_2\ell_2$ from two vanadates and two uridines, that is, $K_2^2K_4$ of eq 8, was $(4.1 \pm 0.3) \times 10^7$ M^{-3} from this study, as depicted in Figure 4B. The result obtained here does not differ significantly from that previously reported[16] ($K_2^2K_4 = (2.8 \pm 0.3) \times 10^7$ M^{-3}) at pH 7.5 and are fully consistant with a $V_2\ell_2$ product.

Aqueous Adenosine/Vanadate System. A detailed study of adenosine monophosphate has previously been reported,[16] while brief reports of the interactions of vanadate with adenosine and the adenosine di- and triphosphates have been made elsewhere.[17,19] Not unexpectedly, adenosine behaves similarly to inosine and uridine when in the presence of vanadate. The products, $T\ell$, are formed with an overall formation constant of 5.8 ± 1.4 M^{-1} when determined from a graph plotted according to eq 5, as depicted in Figure 5A. From the value determined for K_0 or K_1, from the above graph, $[T_l]$ can be obtained and eq 8 can then readily be applied, as for inosine. The results are shown in Figure 5B. A good linear correlation was obtained, and here as for the other nucleosides, an intercept with a zero value within the experimental error was observed. The value determined for the formation of $V_2\ell_2$ ($K_2^2K_4$) was $(4.1 \pm 0.2) \times 10^7$ M^{-3}.

Aqueous Cytidine/Vanadate System. Although the interactions of vanadate with cytidine have been briefly described,[17] the details of this system have not previously been investigated. The graphs that were obtained for this study are presented in Figure 6A,B. Good linear relationships have been obtained. The results give the value $K_1 = 4.2 \pm 1.0$ for the formation of the $T\ell$ products and the value $K_2^2K_4 = (2.5 \pm 0.3) \times 10^7$ M^{-3} for the formation of the $V_2\ell_2$ products from 2 $VO_4H_2^-$ and 2 cytidines.

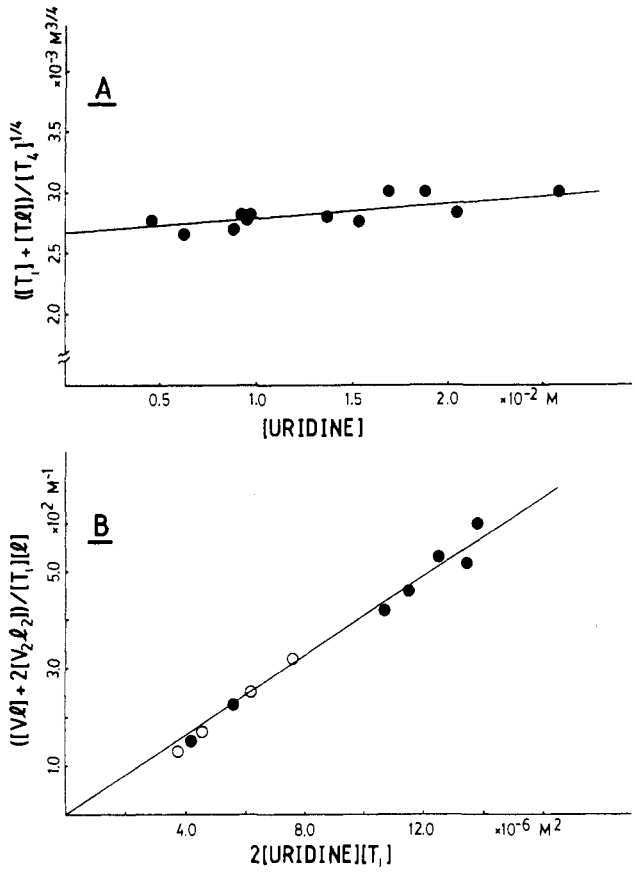

Figure 4. Graphical representations of the formation of the vanadate/uridine complexes giving rise to NMR signals at −560 ppm (graph A) and at −523 ppm (graph B). The conditions for the experiments were pH 7.00, 1.0 M ionic strength with KCl, 30 mM HEPES buffer, and varying proportions of vanadate (○) or uridine (●).

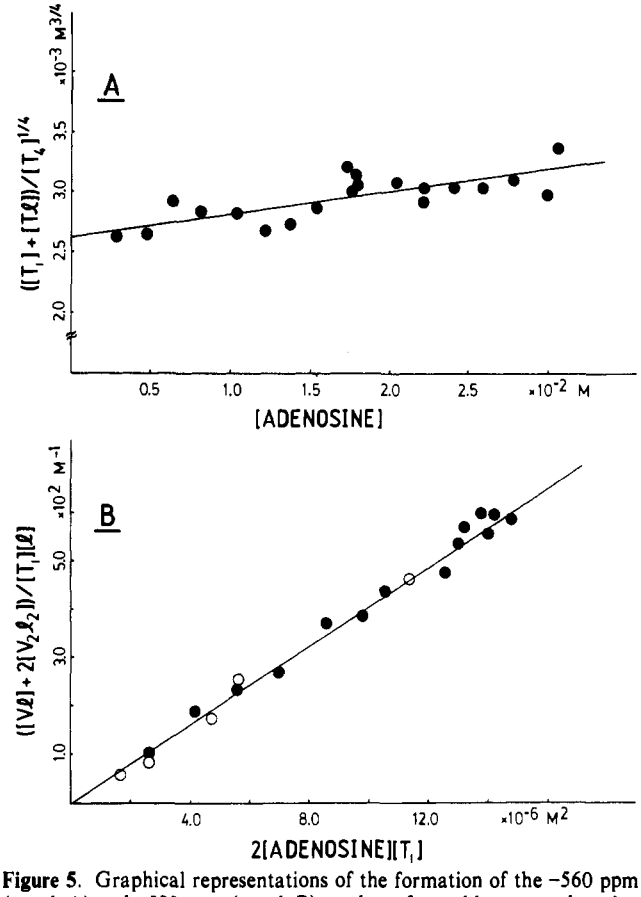

Figure 5. Graphical representations of the formation of the −560 ppm (graph A) and −523 ppm (graph B) products formed between adenosine and vanadate. The experimental conditions were pH 7.00, 1.0 M ionic strength with KCl, 30 mM HEPES buffer, and varying proportions of vanadate (○) or adenosine (●).

Aqueous Guanosine, 2′-Deoxythymidine, and 2′-Deoxyuridine Systems with Vanadate. Guanosine is quite water insoluble, so a detailed study of this nucleoside was not made. As previously noted,[18] a product giving rise to a ^{51}V NMR signal at −523 ppm is observed. From a few spectra obtained at low guanosine concentrations and from the assumption that only $V_2\ell_2$ is formed, a value for $K_2^2K_4 = (3.6 \pm 0.5) \times 10^7$ M^{-3} was estimated. It was not possible to obtain a value for K_1, the overall formation constant for the Tℓ products, but it should be similar to those for the previously discussed nucleosides, ~4 M^{-1}.

2′-Deoxythymidine has no *cis*-1,2-dihydroxyl groups to form $V_2\ell_2$ products. Neither this material nor 2′-deoxyuridine[16] gives rise to a −523 ppm signal in the NMR spectrum. The overall formation constant for the Tℓ products was determined to be 0.0 ± 0.8 M^{-1} and 1.6 ± 1.5 M^{-1}, respectively, for the deoxythymidine and deoxyuridine ligands.

Proton Magnetic Resonance. The −523 ppm vanadium signals of all products are asymmetrical, indicating the presence of stereoisomers.[16] Three major signals in the proportion 1:1.4:4.9 were observed in the portion of the proton NMR spectrum corresponding to the anomeric proton of the inosine nucleoside and its complexes. These signals maintained a constant proportionality with each other as either the vanadate or nucleoside concentration was changed. Some signals were significantly broadened as can be seen in the spectrum of inosine shown in Figure 7. The spectra offer some evidence that the resonance at 6.31 ppm may be a composite signal. Spectra similar to that of Figure 7 were obtained for all but the deoxynucleosides of this study.

Discussion

Vanadate in aqueous solution reacts readily and spontaneously with a variety of nucleosides to give essentially the same types of products in a similar distribution. The products fall into two general categories. The major products give rise to ^{51}V NMR signals at about −523 ppm. The second type of product gives signals which are not separated from that of $VO_4H_2^-$ and may well be in rapid exchange with this material. Nucleosides that do not have a hydroxyl group at the 2′-position of the ribose ring do not provide −523 ppm products. Table I gives the overall formation constants determined for the two classes of compounds. Wide-scale spectral widths provided no indication that other derivatives were formed. However, if HEPES buffer was replaced by Tris buffer, a mixed-ligand product with an NMR signal at −510 ppm was observed. This product apparently is similar to that formed with cyclohexanediols under similar conditions.[15]

The identities of the products giving rise to signals at the vanadate position (−560 ppm) are of interest. Hydroxyl groups, primary, secondary, or tertiary, react with $VO_4H_2^-$ to provide alkyl vanadate esters with formation constants in the range of about 0.1–0.25 M^{-1}.[23] Simple esters such as this can be expected to form with the hydroxyl groups of the ribose ring, thus giving rise to a vanadate analogue of adenosine monophosphate and other similar products. Unexpectedly, only the 2′-deoxynucleosides show formation constants within the range expected, that is, about 0.4 M^{-1} for the sum of formation constant for the possible isomers (0.6 M^{-1} for the other nucleosides). The values determined for the remaining nucleosides ranged from 3.8 to 5.8 M^{-1}. An explanation for this may be that the 2′-hydroxyl and the base acting together form a kinetically labile cyclic vanadium product. However, the value measured for this formation constant when determined for the β-methyl riboside ligand (Table I) was also larger than expected, 3.3 M^{-1}. An alternative explanation for the large values may lie in the formation of tetrahedral 2′,3′-cyclic vanadate esters with formation constants ranging from 3 to 5 M^{-1}, dependent on the nucleoside. If this is a viable possibility, then it must be questioned as to why such a product was not observed

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Table I. Formation Constants for Condensation Products of Vanadate in the Presence of Common Nucleosides[a]

nucleoside	equil const $4T_i \rightleftharpoons T_4$	$T_i + \ell \rightleftharpoons T\ell$	$2T_i + 2\ell \rightleftharpoons V_2\ell_2$
	Purines		
inosine	$(3.2 \pm 0.3) \times 10^{10}$ M^{-3}	3.8 ± 0.6 M^{-1}	$(7.0 \pm 0.5) \times 10^7$ M^{-3}
inosine[b]	$(4.7 \pm 0.3) \times 10^8$ M^{-3}	3.9 ± 1.5 M^{-1}	$(6.5 \pm 0.5) \times 10^7$ M^{-3}
adenosine	$(2.0 \pm 0.2) \times 10^{10}$ M^{-3}	5.8 ± 1.4 M^{-1}	$(4.1 \pm 0.2) \times 10^7$ M^{-3}
guanosine			$(3.6 \pm 0.5) \times 10^7$ M^{-3}
	Pyrimidines		
uridine	$(2.1 \pm 0.2) \times 10^{10}$ M^{-3}	4.5 ± 1.1 M^{-1}	$(4.1 \pm 0.3) \times 10^7$ M^{-3}
cytidine	$(2.4 \pm 0.3) \times 10^{10}$ M^{-3}	4.2 ± 1.0 M^{-1}	$(2.5 \pm 0.3) \times 10^7$ M^{-3}
2′-deoxyuridine	$(2.6 \pm 0.4) \times 10^{10}$ M^{-3}	1.6 ± 1.5 M^{-1}	
2′-deoxythymidine	$(3.1 \pm 0.2) \times 10^{10}$ M^{-3}	0.0 ± 0.8 M^{-1}	
methyl β-D-ribofuranoside	$(3.1 \pm 0.3) \times 10^{10}$ M^{-3}	3.3 ± 1.3 M^{-1}	$(2.9 \pm 0.3) \times 10^7$ M^{-3}

[a] Unless indicated otherwise, all equilibrium constants were determined from solutions of 1.0 M ionic strength maintained with KCl, at pH 7.00, 30 mM HEPES buffer, and varying concentrations of vanadate and nucleoside. [b] Equilibrium constants were determined under conditions of ambient ionic strength, ~0.035 M, at pH 7.00 and 30 mM Tris buffer.

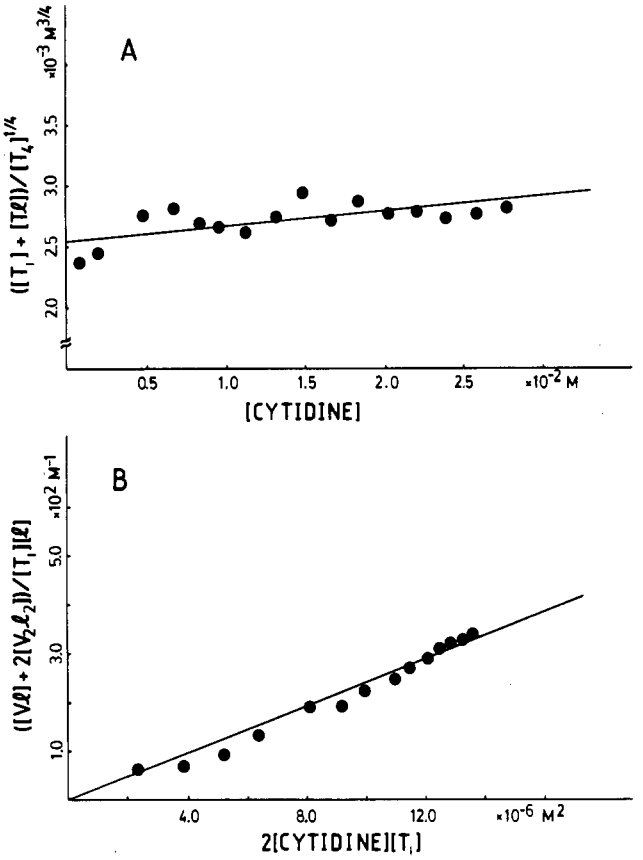

Figure 6. Graphical representations of the formation of the −560 ppm (graph A) and −523 ppm (graph B) products formed between cytidine and vanadate. The experimental conditions were pH 7.00, 1.0 M ionic strength with KCl, 30 mM HEPES buffer, and varying proportions of cytidine.

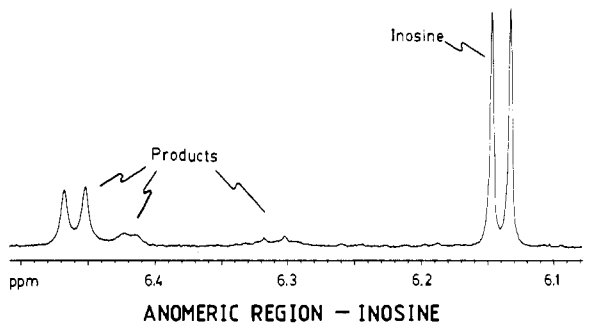

Figure 7. Partial proton NMR spectrum, showing the anomeric proton signals of inosine and its complexes with vanadate. The conditions of the experiment were pH 7.00, 1.0 M KCl, 30 mM HEPES buffer, 30 mM total inosine, and 40 mM total vanadate in D_2O solvent.

with ethylene glycol[14] or the cyclohexanediols,[15] which have been intensively studied. The nature of the product giving rise to the −523 ppm signal may provide an answer. This latter product is binuclear, and there is no evidence for a mononuclear product with a ^{51}V chemical shift at this frequency. Previous evidence does show that the −523 ppm product is pentacoordinate and indicates that it has a trigonal-bipyramidal coordination about each vanadium.[12-14] If the hypothesized cyclic vanadate is the precursor to the binuclear product, then dimerization might be expected to occur essentially independent of the type of ligand, since condensation involves only vanadate (note however, that the previous work[14] did not eliminate the possibility of two simultaneously bridging ligands). In this event, the equilibrium constant for formation of $T\ell$ from T_i plus ℓ, after a small correction for the formation of the acyclic tetrahedral esters, is the equilibrium constant for formation of the cyclic 2′,3′-vanadate diester. It has a value of about 4 M^{-1}, from Table I. The equilibrium constant for dimerization of this cyclic ester to form the −523 ppm product, $V_2\ell_2$, can then be obtained by dividing the value for formation of $V_2\ell_2$ from 2 T_i and 2 ℓ (about 5×10^7 M^{-3}, from Table I) by the square of the value for formation of the cyclic $T\ell$ from T_i and ℓ (about 4 M^{-1}, from Table I) to yield a value of 3×10^6 M^{-1} for the formation of $V_2\ell_2$ from two cyclic esters (see eqs 5,7,8). If this dimerization constant, in fact, is essentially ligand independent, then the formation constant for the cyclic ester K_2 is in the order of 0.1 M^{-1} for *cis*-1,2-cyclohexanediol[15] and is much smaller for the *trans*-cyclohexanediol[15] and for ethylene glycol.[14] On this basis the tetrahedrally coordinated cyclic vanadate ester will not easily be observable in such systems. This of course does not mean that a monomeric pentacoordinate compound does not form. The work here with the nucleosides rules out any significant amount compared to the total corresponding to the −523 ppm signal. However, detailed investigations with dilute vanadate solutions have indicated that, in the case of β-methyl riboside, a pentacoordinate monomeric product having a formation constant of about 1.8 M^{-1} may occur.[24] The present work is not sufficiently precise to measure a value this small, in part because the binuclear products dominate the spectra at −523 ppm. In addition, nucleosides have colligative properties and undergo the so-called base stacking interactions, which undoubtedly affects the precision of determining equilibrium constants, particularly when higher ligand concentrations are utilized. The value of 1.8 M^{-1} (K_2) for formation of the pentacoordinate monomer in conjunction with the overall formation constant of the dimer, 2.9×10^7 M^{-3} ($K_2^2K_4$), gives a value of 9×10^6 M^{-1} (K_4) for this dimerization constant. This value is similar to that estimated by other workers for the dimerization constant in the riboside system.[25]

If the 2′,3′-cyclic vanadate esters in fact are formed as postulated in this work, then these materials might well represent the precursors that are responsible for the potent inhibition of ribo-

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nucleases.[10,11] The nucleophilic attack of a water oxygen on the vanadium nucleus of a lightly bound enzyme/nucleoside/vanadate complex accompanied by rearrangement in the ribonuclease active site to allow efficient hydrogen bonding of the histidine and serine nitrogens would lead directly to a pentacoordinate transition-state analogue of the normal phosphate intermediate. X-ray and neutron diffraction studies have revealed this structure in the uridine/vanadate/ribonuclease A complex.[12]

It is clear from this work that the −523 ppm signal is a composite signal corresponding to isomeric binuclear vanadium complexes as originally proposed[14-16] and this original assignment was not incorrect.[17-19] The proton NMR spectra support this conclusion and reveal that there are several types of nucleosides in the product complexes. Evidently from Figure 7, the major product (6.46 ppm) is symmetrical, since only one high-intensity (relative proportion 4.9) signal is observed. The same may be true for the second product (6.42 ppm) of relative intensity 1.4, but line broadening in the spectra indicates chemical exchange occurs in the second or so time scale. ^{1}H NMR spectroscopy may be able to provide further information concerning the equilibria established in this system. A close look at Figures 3-6 suggests there is a small degree of upward curvature in the experimental curves. It is possible that this is a result of the nonideal behavior of the ligands that was alluded to above. However, there is also the possibility that there is additional ligand incorporation into $V_2\ell_2$ to afford $V_2\ell_3$ or $V\ell_2$. If formed, these products should be observable in the proton spectra of high ligand concentration solutions even if the ligands do undergo base-stacking. The presence of significant proportions of $V\ell_2$ (or $V_2\ell_4$) at such high ligand concentrations might account for the discrepancy between the results presented here and the conclusions of other workers. Intensive investigation of these systems by 1- and 2-dimensional NMR techniques should resolve this question and provide detailed evidence concerning the structure of the products observed in this study.

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Registry No. Vanadate, 14333-18-7; inosine, 58-63-9; methyl β-D-ribofuranoside, 7473-45-2; adenosine, 58-61-7; guanosine, 118-00-3; uridine, 58-96-8; cytidine, 65-46-3; 2′-deoxyuridine, 951-78-0; 2′-deoxythymidine, 50-89-5.

Supplementary Material Available: Tables of reactant concentrations determined for the inosine/vanadate, adenosine/vanadate, uridine/vanadate, and cytidine/vanadate systems (7 pages). Ordering information is given on any current masthead page.

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Magnetic Studies of the High-Potential Protein Model $[Fe_4S_4(S\text{-}2,4,6\text{-}(i\text{-}Pr)_3C_6H_2)_4]^-$ in the $[Fe_4S_4]^{3+}$ Oxidized State

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The magnetic susceptibility of the title compound has been examined over the 5-320 K temperature range. The results have been analyzed by use of a spin-system Hamiltonian that includes two Heisenberg AF coupling parameters, $J_{12} = J + \Delta J_{12}$ and $J_{34} = J + \Delta J_{34}$, for the ferric ($\beta$) pair and the mixed-valence (α) pair, respectively, and one resonance delocalization parameter B for the α pair. The $[Fe_4S_4]^{3+}$ core follows the Curie-Weiss law $\chi_{mol} = 0.416/(T + 0.82)$ in the 5-15 K range. The 30-320 K range was well fitted with parameters $J = 571$ cm^{-1}, $B = 598$ cm^{-1}, and $\Delta J_{12} = 144$ cm^{-1} ($\Delta J_{34} = 0$), and $\langle g \rangle = 2.00$. The entire temperature range could be successfully fitted only by allowing the g values for the five lowest energy spin states to vary individually. Best fit parameters settled at $J = 652$ cm^{-1}, $B = 592$ cm^{-1}, and $\Delta J_{12} = 145$ cm^{-1}, and the two lowest lying energy levels $|^9/_2\ ^1/_2\ (4)\rangle$ and $|^7/_2\ ^1/_2\ (3)\rangle$ are nearly degenerate ($\Delta E = 11$ cm^{-1}). The coupling constants $J(Fe^{3+}\text{-}Fe^{3+}) = 797$ cm^{-1} and $J(Fe^{3+}\text{-}Fe^{2+}) = 652$ cm^{-1} are higher than those previously observed for Fe-Fe interactions in related clusters. Possible factors for this increase are the general compression of the $[Fe_4S_4]^{3+}$ core as compared with the $[Fe_4S_4]^{2+}$ core and also the distortion of the core. Further, fits involving both J and B parameters are conceptually distinctive from those using Heisenberg parameters alone.

Introduction

It is now well established that iron-sulfur proteins can exist under the three different redox states $[Fe_4S_4]^{1+}/[Fe_4S_4]^{2+}/[Fe_4S_4]^{3+}$, the first two core oxidation levels being used by ferredoxins, while the latter two are found in the so-called high-potential iron-sulfur proteins (HiPIP). The extensive studies by Holm and co-workers on the synthesis and characterization of the $[Fe_4S_4(SR)_4]^{3-/2-}$ model complexes[2] have given opportunity for detailed analysis of the cluster structural and electronic changes due to the electron transfer. In particular, it has been demonstrated that the reduced clusters[3] $[Fe_4S_4(SR)_4]^{3-}$ possess a more complex structural and ground-state variability than the one-electron-oxidized clusters $[Fe_4S_4(SR)_4]^{2-}$.

Similar studies of the highest core oxidation level have long been precluded by the observed instability[4] of the oxidized (either by chemical or electrochemical means) $[Fe_4S_4(SR)_4]^-$. Only recently, use of sterically encumbered thiolates has allowed for the stabilization[5] and isolation[6] of synthetic analogues with the $[Fe_4S_4]^{3+}$ core. The subsequent Mössbauer studies[7] have been done in the framework of an $S = {}^1/_2$ spin Hamiltonian, and the iron sites have been found to occur in two equivalent pairs. However, an appropriate spin-coupling model for oxidized Fe_4S_4 clusters has so far not been developed, in the sense that electron delocalization gives rise to substantial resonance interactions[8,9]

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