Complexation of histidine and alanyl-histidine by vanadate in aqueous medium

Martina Fritzsche, Vassilios Vergopoulos and Dieter Rehder*
Department of Chemistry, University of Hamburg, D-20746 Hamburg 13 (Germany)

(Received December 7, 1992)

Abstract

At physiological pH and ionic strength, histidine (H-His-OH) and vanadate form at least two weak complexes with the following characteristics: $\delta(^{51}V)$ (ppm) (K (M^{-1})): **1** −546 (0.7), **2** −561 (1.6). K is the formation constant, and a 1:1 stoichiometry with the ligand coordinating through the amino group is assumed. Type **1** complexes are also formed with H-His-OR (R = Me, Bz), but not with BOC-His-OH. An additional, very weak complex **3** (δ = −591 ppm at pH 7.2, −573 ppm at pH 8.8) is present at $c(V) > 5$ mM. α-Alanyl-histidine gives rise to a relatively strong, possibly dinuclear monoligate complex **4** ($\delta(^{51}V) = -519$ ppm, $K = 5 \times 10^5$ M^{-2} at pH 7.2). Crucial for the formation of **4** are the deprotonated amide of the peptide linkage, the NH_2 group (as part of a chelate-5 ring; β-alanyl-histidine does not provide a type **4** complex), and the carboxylate unit. The complex stoichiometries and modes of coordination have been evaluated on the basis of ^{13}C, ^{14}N and quantitative ^{51}V NMR spectroscopy.

Introduction

Vanadium is now a well recognized biometal [1–4]. Nonetheless, little is known of the structure and function of vanadium in living organisms. A possibly general physiological importance as a trace metal has been inferred from the fact that vanadate can act as a phosphate antagonist. Many phosphate metabolizing enzymes are indeed inhibited or stimulated by vanadates [5], and this effect may well be traced back to vanadate binding to the (active site of the) protein matrix, thus blocking off enzymatic action. Binding of histidine residues to vanadium, either directly or via a hydrogen bond network, has been shown, or made plausible, to exist for, *inter alia*, transferrin [6], xylose-isomerase [7], ribonucleases [8], vanadate-dependent haloperoxidase [9] and carboxypeptidase [10]. The only structurally characterized histidine vanadium complex known to this date contains the histidine derivative *o*-naphthalidene-His, which coordinates V^V through one of the carboxylate oxygens [11].

The most common forms of 'free vanadium' under physiological conditions are vanadate ($H_2VO_4^-$) and vanadyl (VO^{2+}). Vanadate forms relatively weak associates with biogenic ligands. Only a few studies on the interaction between vanadate and amino acids or small peptides have so far been undertaken [12–15], and no systematic investigation with peptides containing histidine as a constituent has yet appeared (for a preliminary report see ref. 16). We have therefore carried out a detailed multinuclear NMR investigation of the aqueous system containing vanadate and alanyl-histidine as a model peptide, supplemented by a study of the (weak) interaction between vanadate and histidine itself.

Experimental

Materials were obtained from commercial sources (sodium orthovanadate: Janssen; L-histidine, L-α-alanyl-L-histidine, β-alanyl-L-histidine = carnosine: Serva) or prepared according to published procedures (N- and O-protected histidines [17]).

Samples for ^{51}V measurements were prepared by adding to 3 ml of H_2O/D_2O 2/1 weighed amounts of the ligand and, via a micro-pipette, appropriate amounts from a stock solution of 0.2 M sodium orthovanadate. The ionic strength was 0.2 M (NaCl). The pH/D was adjusted with NaOH and HCl, using an Ingold micro glass electrode. The final pH values were calculated [15, 18] from the position of the signal for monovanadate. Since complex formation between vanadate and peptides can be a slow process [19], all samples were prepared 4 days prior to measurement to allow for complete equilibration, kept at room temperature and (to prevent

*Author to whom correspondence should be addressed.

0020-1693/93/$6.00

light-induced redox reactions) in the dark. ^{13}C and ^{14}N NMR measurements of the system vanadate/H-His-OH were carried out in equimolar 50 mM solutions in H_2O/D_2O at pH 7.2.

^{51}V NMR spectra were obtained on a Bruker AM 360 spectrometer at 94.73 MHz in 10 mm diameter rotating vials at ambient temperatures. Typical measuring parameters: sweep width 125 kHz, time domain 8 K, pulse angle 60°, no relaxation delay, line broadening factor 20 Hz. $\delta(^{51}V)$ values are quoted relative to external $VOCl_3$. ^{13}C NMR spectra (90.6 MHz) and ^{14}N NMR spectra (28.9 MHz) were scanned on the same instrument, and are referenced against TMS (^{13}C) and liquid NH_3 (^{14}N).

Results and discussion

Histidine, as other amino acids [15], forms rather weak complexes with vanadate in aqueous solution, where complex formation competes with the condensation of monovanadate ($H_2VO_4^- \rightleftharpoons HVO_4^{2-} + H^+$; V_1) to divanadate ($H_2V_2O_7^{2-} \rightleftharpoons HV_2O_7^{3-} + H^+$; V_2), cyclic tetravanadate ($V_4O_{12}^{4-}$; V_4, the predominant species at $c(V) > 1$ mM), and cyclic pentavanadate ($V_5O_{15}^{5-}$; V_5). The ^{51}V NMR signal pattern in solutions containing histidine and vanadate is dominated by the uncomplexed, tetrahedral vanadates V_1, V_2, V_4 and V_5 (see Figs. 1 and 2). In order to detect vanadate–histidine complexes by ^{51}V NMR [20], a large molar excess of the ligand is necessary. At molar ratios vanadate/His = 1/40 (absolute concentrations: c(vanadate) = 5 mM, c(His) = 200 mM) and pH = 7.2, three weak signals at $\delta(^{51}V) = -546$ (**1a**), -561 (broad; **2**) and -591 ppm (**3**) are observed in addition to the vanadate resonances (Fig. 1). Signals at -546 and -560 have formerly also been observed in the vanadate–glycine system and allocated to 1:1 complexes [15]. It has been suggested that the type **2** (-561 ppm) complexes are tetrahedral, arising from a condensation reaction between vanadate and the carboxylic acid group, hence a mixed anhydride. Since these complexes are not observed with histidine esters, this assignment appears to be a reasonable one. For the type **1** (-546 ppm) complexes, a trigonal-bipyramidal geometry has been proposed, achieved with the amino acid acting as a bifunctional ligand, i.e. condensation (of the carboxylic group) plus coordination (of the amino group). H-Ala-His-OH and BOC-His-OH, where the NH_2 of histidine is no longer available, do not give rise to a complex of type **1**, the histidine esters H-His-OR, however, do (R = Me, **1b**; Bz, **1c**). Hence, histidine and its esters very probably coordinate in a monofunctional fashion through the NH_2 group.

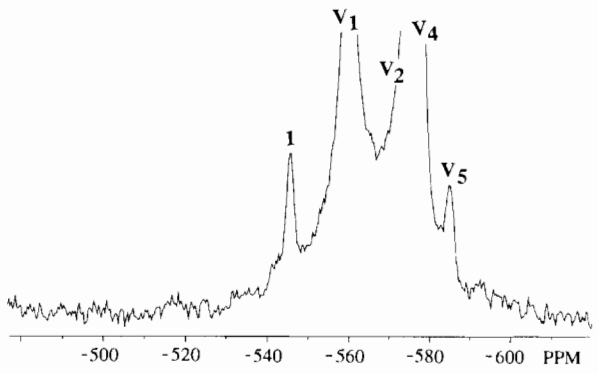
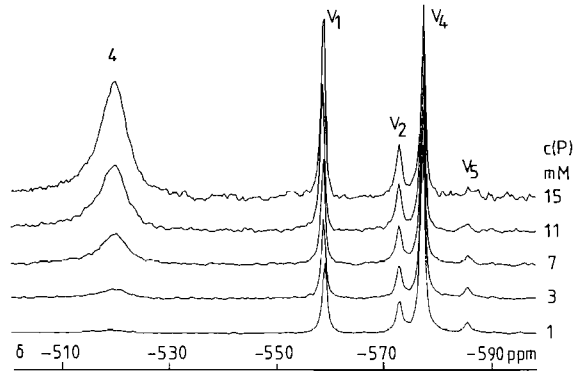


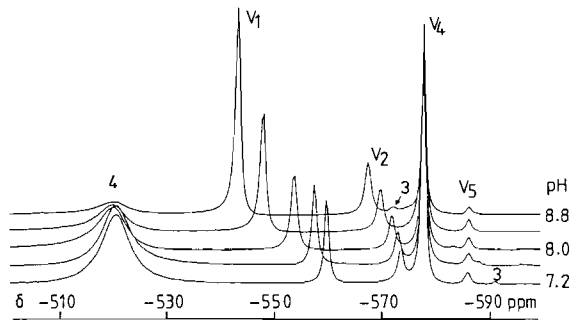
Fig. 1. 94.7 MHz ^{51}V NMR spectrum of a solution containing the histidine ester H-His-OMe (c = 200 mM) and vanadate (c = 5 mM) in H_2O/D_2O 2/1; pH/D 7.2. The region for tetrahedral vanadates has been expanded to visualize the weak components. The V_n are uncomplexed vanadates, **1** is a complex where the ligand coordinates through the NH_2 group.

Based on eqn. (1), complex formation constants K_1 of 0.7 (**1a**), 0.9 (**1c**), 1.1 (**1b**) and 1.6 (**2**) M^{-1} can be estimated. This is in the order of magnitude reported for complexes formed with glycine [15] and ligands coordinating through oxygen functionalities such as alcohols [21], lactate [22], salicylate and nicotinate [23], which usually exhibit $\delta(^{51}V)$ values similar to those of **1** and **2**. The weak complex **3** at $\delta(^{51}V) = -591$ ppm (apparent K c. 0.1 M^{-1}) has not been noted for other amino acids. A $\delta(^{51}V)$ value of -597 ppm has been reported for $VO(pivalate)_3$, where there are, in an overall equilibrium between species differing in the mode of coordination of the carboxylate, also contributions from the carboxylate coordinated in the η^2 mode [24], which may be responsible for a relatively high shielding of the ^{51}V nucleus. The -591 resonance is also present in solutions containing the histidine derivatives, including the esters, and we have also observed this signal in many other systems, provided the overall vanadate concentration exceeds c. 5 mM. It is therefore tempting to assign **3** to a complex formed between an oligonuclear vanadate (V_4 or V_5) and the ligand. The position of the -591 resonance is pH dependent; it goes down to -582 ppm at pH = 8.2 and further to -573 ppm at pH = 8.8.

α-Alanyl-histidine, P, does not exhibit the type **1** signal. Instead, it gives rise to a relatively strong and broad resonance at -519 ppm, indicating formation of a comparatively strong vanadate–peptide complex **4**. This is shown by two series of spectra obtained for constant c(V) and variable c(P) (Fig. 2(a)), and for constant concentrations of the reactants but variable pH (Fig. 2(b)). Apparently, the peptide bond plays a crucial role in complex formation. Since peptides with a tertiary amide function such as sarcosine and glycyl-proline do not coordinate to vanadate [12], a deprotonated secondary amide-N very probably is the coordinating site. The coordination of a deprotonated amide-N has also been suggested for the complex formed



(a)



(b)

Fig. 2. 94.7 MHz ^{51}V NMR spectra for the systems vanadate/Ala-His in $H_2O/D_2O = 2/1$ at an ionic strength of 0.2 M (NaCl). (a) Variable $c(P)$ (P = peptide) constant $c(V) = 3$ mM (V = total vanadate), constant pH/D = 7.2. (b) Constant $c(P) = 20$ mM, constant $c(V) = 5$ mM, variable pH. **4** is the main vanadate–peptide complex ($\delta(^{51}V) = -519$ ppm). A weak complex **3** of unknown nature, the resonance position of which is pH dependent, is also indicated. See footnote [a] of Table 2 for identifying the tetrahedral, ligand-free V_n.

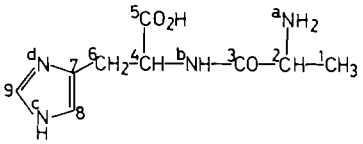
between vanadate and glycyl-tyrosine [20], and is strongly supported by the chemical shift differences $\Delta\delta(^{13}C) = \delta(\mathbf{4}) - \delta(P)$ (see Table 1 and Scheme 1). The carbon atoms adjacent to the nitrogen of the peptide linkage, C(3) and C(4) (Scheme 1), are shifted to low field by 6.7 and 9.9 ppm in the complex with respect to the free ligand. The ^{14}N resonance for the amine-N (N_a) shows a downfield shift of +75 ppm, indicating its coordination (Table 1). Amide coordination has been shown to exist in the V^{IV} complexes formed with pyridine-2-carboxamido ligands [25], but has so far not been documented for V^V.

It is also evident from the ^{13}C NMR results that the NH_2 and CO_2^- groups are involved in coordination, in accord with more indirect results obtained earlier for vanadate complexes of other dipeptides [12, 15]. Since carnosine *does not* give rise to a ^{51}V NMR signal at, or around, −519 ppm typical for a type **4** complex, formation of a chelate-*five* ring seems to be another crucial requirement for a fairly stable complex. The imidazole nitrogens of the histidine moiety do not

TABLE 1. ^{13}C and ^{14}N NMR results

Atom no.[a]	Alanyl-histidine[b]	Complex **4**[c]	$\Delta\delta$[d]
C_1	20.4	21.9	1.5
C_2	52.9	57.6	4.7
C_3	80.1	186.8	6.7
C_4	58.5	68.4	9.9
C_5	174.7	184.7	10.0
C_6	31.7	31.9	0.2
C_7	138.4	137.8	−0.6
C_8	120.8	121.6	0.8
C_9	133.5	135.2	−1.7
N_a	40	115	75
N_b	(130)	[e]	[e]
N_c	188	187	[f]
N_d[g]	209	(220)	[f]

[a]See numbering Scheme 1. c(Ala-His) = c(V) = 50 mM, H_2O/D_2O 2/1, pH/D = 7.2. [b]Uncomplexed ligand in the presence of **4** under the conditions noted in [a]. [c]Cf. eqn. (7). [d]Coordination shift $\Delta\delta = \delta(\mathbf{4}) - \delta$(Ala-His); a positive sign indicates deshielding on coordination. [e]Not observed. [f]$\Delta\delta$ is within the limits of error. [g]Protonated.



Scheme 1.

participate in coordination. At first sight, this is surprising since, in non-aqueous solvents, imidazole coordination to V^V has been established [26a]. Imidazole has also been shown earlier to significantly affect reactions of vanadate with organic molecules [26b], and a variety of N-donating ligands bind to vanadate [27]. On the other hand, the histidine derivative *o*-hydroxy-naphthalidene-His evades coordination through the imidazole nitrogens and rather binds via the carboxylate unit [11]. In this latter case, this is a consequence of protonation of the tertiary N and involvement of the proton in a complex hydrogen bonding network. A similar reason may hold for alanyl-histidine evading complexation through imidazole, a view which is supported by the ^{14}N NMR spectra (Table 1), indicating a low-field shift for the resonance of N_d (Scheme 1; $\delta(^{14}N) = c.$ 220 ppm; the corresponding resonance for the unprotonated tertiary nitrogen in histidine is 139 ppm), typical [28] of a quaternization of this aromatic nitrogen by protonation.

In order to obtain information on the stoichiometry of complex **4**, the ^{51}V NMR spectra have been evaluated quantitatively. The formalism for such an evaluation in series of the kind shown in Fig. 2 has been reported in detail elsewhere (see, for example refs. 15, 21 and 29). For data see Tables 2–4. Equations (1)–(6) represent the equilibria, which have been employed as the basis

TABLE 2. Concentration of constituents in the system Ala-His/vanadate for variable c(vanadate) and c(peptide)[a]

c(V)	c(P)	[4]	[V_1]	[V_2]	[V_4]	[V_5]
3	1	0.08	0.61	0.38	1.78	0.16
3	3	0.36	0.68	0.43	1.42	0.12
3	5	0.68	0.63	0.35	1.26	0.09
3	7	0.93	0.56	0.33	1.08	0.10
3	9	1.09	0.56	0.26	0.99	0.09
3	11	1.42	0.54	0.28	0.72	0.04
3	13	1.56	0.54	0.25	0.60	0.05
3	15	1.60	0.49	0.27	0.56	0.09
3	17	1.90	0.47	0.21	0.41	0.02
1	9	0.66	0.23	0.06	0.05	0.00
3	9	1.20	0.54	0.27	0.87	0.10
5	9	1.36	0.73	0.53	2.14	0.25
7	9	1.67	0.76	0.65	3.45	0.46
9	9	1.80	0.91	0.74	4.80	0.74
11	9	1.88	0.96	0.90	6.39	0.87
13	9	2.05	0.89	1.10	7.87	1.08
15	9	2.84	1.00	0.93	9.10	1.13
17	9	1.81	1.04	0.99	11.2	1.97

[a]All concentrations in mmol/l. c(i) refers to the starting concentrations, [V_n] to the overall equilibrium concentrations of vanadium within the species V_n. For the concentrations of the individual vanadate species, divide [V_n] by n. $V_1 = H_2VO_4^-/HVO_4^{2-}$, $V_2 = H_2V_2O_7^{2-}/HV_2O_7^{3-}$, $V_4 = V_4O_{12}^{4-}$, $V_5 = V_5O_{15}^{5-}$, **4** is the vanadate–peptide complex at $\delta(^{51}V) = -519$ ppm.

TABLE 3. Concentration of constituents in the system vanadate/Ala-His for $c(V)/c(P) = \text{constant} = 1/4$[a]

c(V)	c(P)	[4]		[V_1]	[V_4]	$\Sigma[V_n]$[b]
		Exp.	Calc.[c]			
1	4	0.40	0.48	0.35	0.09	0.6
2	8	0.94	1.28	0.45	0.40	1.1
3	12	1.45	2.16	0.55	0.69	1.6
5	20	2.9	4.0	0.55	1.04	2.1
7	28	4.4	6.0	0.58	1.40	2.6
9	36	5.9	7.9	0.57	2.00	3.2
11	44	7.2	9.9	0.58	2.46	3.8
13	52	8.5	11.9	0.61	2.91	4.5

[a]See footnote [a] in Table 2. Compare also Fig. 2 for the *percentage* equilibrium concentrations. [b]Sum of all tetrahedral vanadates. [c]Calculated from c(V) and c(P) under the assumption that a mononuclear monoligate complex forms, and using an apparent formation constant $K' = 265\ M^{-1}$.

for the evaluation.

$$V_1 + P \rightleftharpoons VP;\ K_1 = [VP]/[V_1][P] \quad (1)$$

$$V_1 + 2P \rightleftharpoons VP_2;\ K_2 = [VP_2]/[V_1][P]^2 \quad (2)$$

$$2V_1 + P \rightleftharpoons V_2P;\ K_3 = [V_2P]/[V_1]^2[P] \quad (3)$$

$$VP + V_1 \rightleftharpoons V_2P;\ K_4 = [V_2P]/K_1[V_1]^2[P] \quad (4)$$

$$2V_1 + 2P \rightleftharpoons V_2P_2;\ K_5 = [V_2P]/[V_1]^2[P]^2 \quad (5)$$

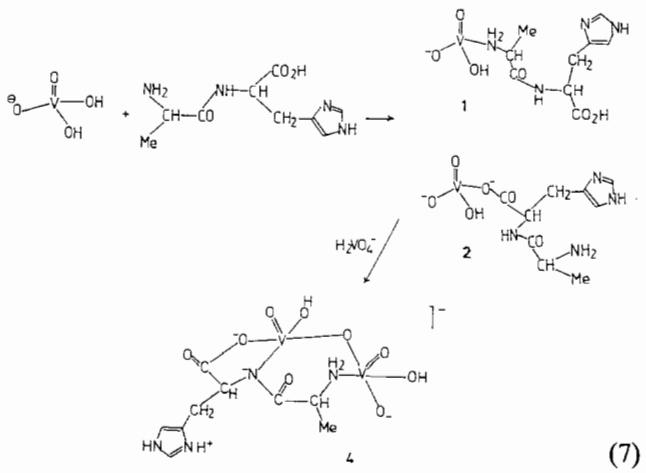
$$2VP \rightleftharpoons V_2P_2;\ K_6 = [V_2P_2]/K_1[V_1]^2[P]^2 \quad (6)$$

TABLE 4. Concentration of constituents in the system vanadate/Ala-His at constant c(V) = 5 mM, c(P) = 20 mM, and variable pH[a].

pH	[4]	[V_1]	[V_2]	[V_4]	[V_5]
7.22	2.71	0.58	0.32	1.31	0.08
7.32	2.34	0.63	0.36	1.47	0.20
7.48	2.59	0.62	0.32	1.37	0.10
7.79	2.30	0.70	0.35	1.49	0.15
7.89	1.86	0.79	0.40	1.73	0.22
8.11	1.70	0.98	0.41	1.76	0.15
8.31	1.30	1.27	0.47	1.79	0.17
8.49	0.82	1.68	0.58	1.74	0.17
8.70	0.65	2.24	0.61	1.30	0.10

[a]See footnote [a] in Table 2 and Fig. 2(b) for a graphical representation.

We note that there is no hidden tetrahedral species present under the resonance for V_1, as has been shown to exist for the complexes formed of vanadate and Gly-Gly [16] or nucleosides [29]. Assuming a 1:1 stoichiometry for complex **4** (eqn. (1)), we arrive at an apparent (indicated by a prime) formation constant $K'_1 = 265\ M^{-1}$. This is substantially more than for any other vanadate–dipeptide complex. In order to check for the formation of dinuclear complexes, we have also evaluated a data set (Table 3) obtained for a constant molar ratio $c(V)/c(P) = 1/4$ in the concentration range $c(V) = 1$–13 mM. With increasing c(V) and c(P), the *percentage* equilibrium concentration of the complex increases at the expense of the percentage equilibrium concentrations of V_1 and ΣV_n (ΣV_n is the sum over all tetrahedral vanadates; see Fig. 3 for a graphical representation), which suggests oligonucleation of the peptide complex. The best fit for the data in Table 3 arises for a dinuclear monoligate complex (eqn. (3)). $K_3 = 5.0(3) \times 10^5\ M^{-2}$ is obtained from the graph of $[(V_2P)]/[V_1]^2$ versus [P] (Fig. 4). No appreciable amounts of a dinuclear, *bi*ligate complex, which might form according to eqns. (5) or (6), are present.



(7)

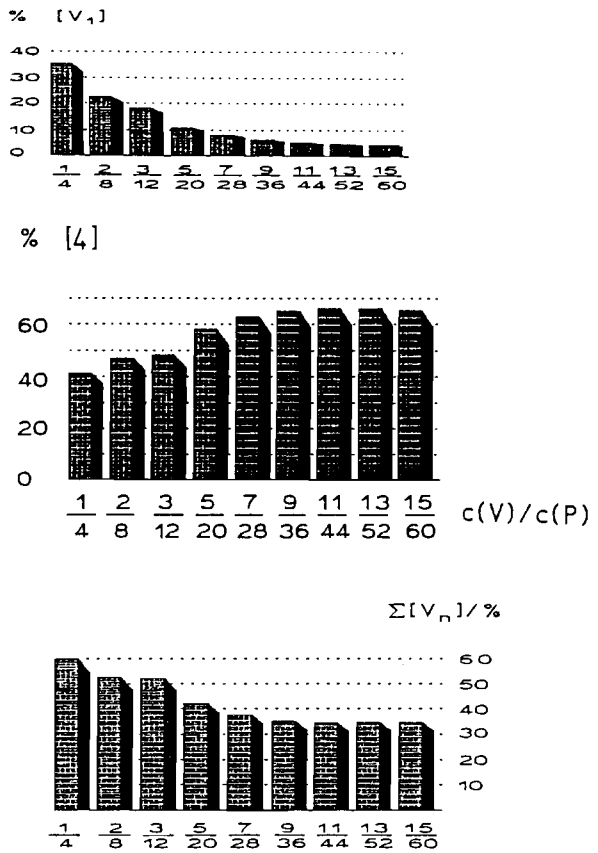


Fig. 3. Graphical presentation of the percentage equilibrium concentrations of V_1, ΣV_n and **4**, in relation to the increasing overall concentrations of vanadate (V) and peptide (P), but constant $c(V)/c(P) = 1/4$. The presentation is based on the data in Table 3.

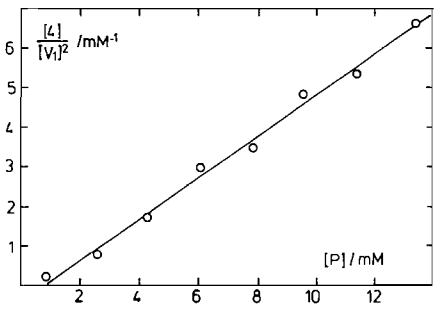


Fig. 4. Plot of $[4]/[V_1]^2$ vs. [P], based on the data in Table 2 (constant $c(V)$, variable $c(P)$). **4** is the complex with $\delta(^{51}V) = -519$ ppm (Fig. 1). The linear regression indicates the formation of a dinuclear, monoligate complex. From the slope, the formation constant $K_3 = 5 \times 10^5$ M^{-2} is obtained.

Dinuclear, biligate complexes have formerly been detected in the system vanadate/inosine and described in terms of oxo- and/or alkoxo-bridged complexes with a trigonal-bipyramidal [29], an octahedral [30] or an intermediate ligand arrangement around vanadium [31]. Equation (7), based on the assumption that **4** is formed via a precursor complex **1** or **2**, includes a tentative formulation for **4** with a 2:1 stoichiometry, an essentially square-pyramidal geometry for the two vanadium centres, and the coordination of the alanyl-histidine ligand as revealed by the ^{13}C, ^{14}N and ^{51}V NMR spectra, discussed in detail above.

The extent of complex formation is also a function of pH (Fig. 2(b) and Table 4) in that increasing pH leads to decreasing amounts of complex, indicative of complex formation necessitating *di*hydrogenvanadate in a condensation reaction as formulated in eqn. (7). The pK_s for $H_2VO_4^- \rightleftharpoons HVO_4^{2-} + H^+$ is 8.1. $H_2VO_4^-$ is therefore increasingly less available as the pH approaches the value of 9.

Conclusions

Complex **4** may be considered to mimic vanadate coordination to histidine sites in proteins. Divanadate (V_2), as other oligovanadates, can be a potent enzyme inhibitor [32]. Since, under normal physiological conditions, V_2 is not readily available, we suggest that inhibition is not directly due to V_2, but rather to a dimer formed by dimerization of a monovanadate–ligand complex as a precursor. The dimerization constant K_4 (eqn. (4)) calculated from K_3 for **4** and K_1 for a potential precursor complex **1** or **2** (*c.* 1 M^{-1}), amounts to *c.* $K_4 = 5 \times 10^5$ M^{-1} and hence is an order of magnitude less than for dimeric vanadate–nucleoside complexes. K_4 is, however, still substantially larger than the dimerization constant for the formation of 'free' (i.e. uncomplexed) divanadate from monovanadate.

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