Supplementary Material Available: Tables of complete crystallographic data, all atom coordinates including all hydrogen atoms, anisotropic thermal parameters, bond distances and angles involving both non-hydrogen and hydrogen atoms, intermolecular distances, and selected dihedral angles between least-squares planes and PLUTO steropair and packing diagrams for **5** (35 pages); a listing of observed and calculated structure factors (17 pages). Ordering information is given on any current masthead page. A complete listing of previously reported transition metal B_3 complexes is also available from the author.

An NMR Investigation of the Interactions Occurring between Peroxovanadates and Peptides

Alan S. Tracey* and Jaswinder S. Jaswal

Contribution from the Department of Chemistry, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada. *Received June 24, 1991*

Abstract: ^{51}V nuclear magnetic resonance has been used in the investigation of the reactions which occur between peroxovanadates and simple peptides and other small molecules in aqueous solution. Variation of the concentration of the reactants and changes in the pH of the solutions allowed product stoichiometry, protonation states, and equilibrium constants to be determined. It was found that the diperoxovanadate $VO(OO)_2(H_2O)_2^-$ readily formed monodentate complexes with both the amino and carboxylate functionalities of the peptides. A linear free energy relationship between the formation constants of the products and the pK_a values of the conjugate acids of the reactant groups was observed. This relationship showed that product formation was favored by stronger electron-donating ligands. Inhibition of the vanadate-catalyzed decomposition of hydrogen peroxide by peptides was studied by ^{51}V NMR and UV spectroscopy. The results were consistent with the formation of a monoperoxopeptidovanadate as the inhibiting species. It was found that the terminal amino and carboxylate functionalities and an amide N-H in the peptide bond were all required for the peptide-induced inhibition to occur. The relevance of these results to some aspects of the biochemical function of peroxovanadates is discussed.

Introduction

It has been known for many years that vanadium oxoanions give rise to many biological responses.[1] Many of these responses have their origin in the activation or inhibitions of the function of a number of different enzymes. It is becoming increasingly clear that (oxo)peroxovanadium(V) anions similarly generate a number of biological and biochemical responses. These responses include the following: activation of the insulin receptor[2] and other tyrosine kinases;[3] enhanced binding of insulin-like growth factor II receptor binding to adipocytes;[4] mediation of Na^+ transport by (Na^+, K^+) ATPase;[5] and antitumor activity.[6]

The molecular mechanisms by which these responses are generated are not known, nor is there sufficient evidence available to make a credible hypothesis. Several processes which might lead to the observed biochemical responses come readily to mind. Peroxovanadates might directly activate or inhibit the enzymic system responsible for the observation or may give rise to covalent modification of the proteins by promoting oxidation, thus influencing the function of the system. An additional possibility is that peroxovanadate might provide a "slow-release" mechanism, enhancing the concentration of vanadate in locations not otherwise readily accessible. In this case vanadate would generate the observed response. Alternate possibilities certainly exist.

The identification of the actual processes involved requires an understanding of the basic chemistry of peroxovanadates, in particular their interactions with amino acids, peptides, other metabolites and small molecules. In order to address these problems we have recently initiated a study of the formation of peroxovanadium(V) complexes and the subsequent reactions of these materials.

The chemistry of the vanadate/peroxide system can be quite complex since vanadate in the presence of hydrogen peroxide forms a number of products, their relative concentrations being dependent on vanadate and peroxide concentration and on pH.[7-11] However, at near neutral pH and with concentrations of a few millimolar for both reactants, the predominant products are the mono-, di-, and triperoxovanadates and the tetraperoxodivanadate.[9] All of the above materials are of interest and a study of their reactions with some simple di- and tripeptides and related molecules is reported here.

The study of the chemistry of the peroxovanadates is often complicated by the vanadate-catalyzed disproportionation of hydrogen peroxide to oxygen and water. This topic has been recently reviewed and the mechanism of action discussed.[12] Fortunately, this decomposition does not present a serious problem for this study since the decomposition is slow relative to the time taken to finish the experiments. Furthermore, many of the ligands utilized in this study strongly inhibit the disproportionation.

Experimental Section

Materials. All reagents, unless mentioned otherwise, used in this study were reagent or higher quality materials purchased from major suppliers. Of note in this regard is that vanadium(V) oxide 99.99% was purchased form Aldrich Chemical Co. and hydrogen peroxide from Fischer Scientific Co. The peptides were obtained from Sigma Chemical Co. Proton NMR spectra were obtained for the peptides and other ligands of this study, as deemed necessary. All were used as supplied. Glycylglycyl-

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glycine ethyl ester hydrochloride was prepared according to literature procedures[13] and characterized by ^1H NMR spectroscopy and elemental analysis. The stock solution of hydrogen peroxide was prepared in distilled water and then standardized against potassium permanganate the day of use. Peptide stock solutions were prepared by dissolving the desired peptides in distilled water, warming slightly is required, and adjusting the pH to about 7. Hydrogen peroxide (100 mM), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid) buffer (1 M), vanadate (100 mM),[14] KCl (2 M), and peptide (300 mM to 1 M depending on solubility) stock solutions were initially prepared. From these a H_2O_2 (75 mM)/HEPES (25 mM) intermediate stock solution was prepared and the pH adjusted to pH 7 immediately prior to use. Appropriate amounts of the stock vanadate, buffer, and KCl solutions at pH 7 were combined in proportions appropriate to give the final desired concentrations after the peptide and peroxide stock solutions were added. The conditions for these studies, unless stated otherwise in the text, were the following: 3.0 mM total vanadate; 9.0 mM total hydrogen peroxide; 20 mM HEPES buffer; μ = 1.0 M with KCl; pH 7 or variable for the pH studies. To minimize the H_2O_2 decomposition the required amount of the H_2O_2/HEPES solution was added to the vanadate solution only as the solutions were required. The peptide stock solution also at pH 7 was added last. Mixing in this order allowed equilibrium to be established relatively quickly since it prevents formation of peptide complexes of vanadate which decay slowly, often requiring 24 h or more, dependent on the peptide. For the pH studies the pH of the stock solutions was adjusted as appropriate to give a pH close to the target value after mixing all solutions. No further pH adjustment was done. Freshly opened pH buffers, pH 10.0 and 4.0, were used to calibrate the pH meter. The calibration was confirmed with a pH 7.0 buffer.

Spectroscopy. UV spectra were obtained form a Hewlett-Packard 8932 A diode array spectrometer using cells of 1 cm path length. The UV spectra were obtained under ambient temperature conditions, ~21 °C. Spectra for the kinetic studies were obtained every 5 min for time periods up to 10 h. Each experiment was repeated at least once.

^{51}V NMR spectra were obtained from a Bruker AMX-400 NMR spectrometer operating at 105.2 MHz at ambient temperature. Vanadium chemical shifts are relative to the external reference $VOCl_3$ assigned to 0 ppm. Baseline corrections were applied to all spectra before integrals were obtained. NMR spectral parameters were the following: pulse widths, 60°; spectral widths, 80 KHz; acquisition times, 0.05 s; line broadening, 40 Hz; frequency domain size, 16K data points.

Methods. Equilibrium equations were put into a linear form as outlined in the text and the results analyzed using standard least-squares procedures. The reported errors represent three standard deviations, the 95% confidence level.

Results

If vanadate in its two protonation states, $VO_4H_2^- + VO_4H^{2-}$ (pK_a = 8.16 under conditions of 1 M ionic strength with KCl[12]), is represented as V and hydrogen peroxide as ℓ then the predominant vanadate/peroxide equilibria can be represented as in eqs 1–4.[9] Detailed NMR experiments have previously allowed

$$V + \ell \overset{K_1}{\rightleftharpoons} V\ell \qquad [V][\ell]K_1 = [V\ell] \qquad (1)$$

$$V + 2\ell \overset{K_2}{\rightleftharpoons} V\ell_2 \qquad [V][\ell]^2K_2 = [V\ell_2] \qquad (2)$$

$$V + 3\ell \overset{K_3}{\rightleftharpoons} V\ell_3 \qquad [V][\ell]^3K_3 = [V\ell_3] \qquad (3)$$

$$2V\ell_2 \overset{K_4}{\rightleftharpoons} V_2\ell_4 \qquad [V\ell_2]^2K_4 = [V_2\ell_4] \qquad (4)$$

^{51}V chemical shifts to be assigned to the products observed in this study.[7,9] These assignments have been confirmed by detailed concentration and pH studies and the equilibrium constants have been determined.[9] The formation constants, K_1 through K_4, have the values $(3 \pm 1) \times 10^3$ M^{-1}, $(5 \pm 2) \times 10^8$ M^{-2}, $(5 \pm 2) \times 10^9$ M^{-3}, and (49 ± 5) M^{-1}, respectively for conditions similar to those of this study.[9]

Figure 1 illustrates the effect of addition of the simple small peptide, glycylglycine, to an equilibrium mixture of peroxovanadates. Two major products with ^{51}V chemical shifts near −713 and −747 ppm are formed. Variation, in independent experiments,

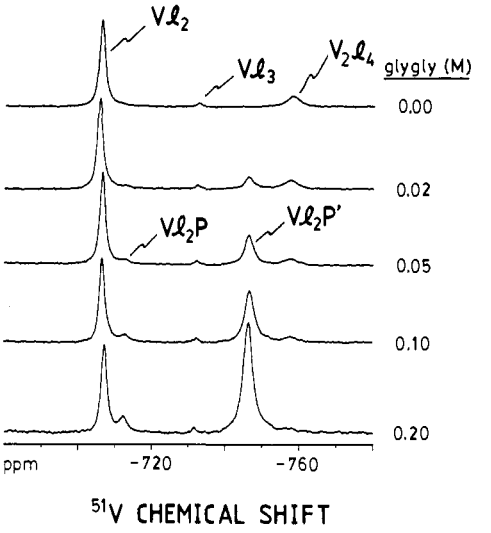

Figure 1. ^{51}V NMR spectra demonstrating the condensation of peroxovanadium(V) with the simple peptide glycylglycine to yield two major products. Conditions of the experiments: 3.0 mM total vanadate; 9.0 mM total H_2O_2; μ = 1.0 M KCl; 20 mM HEPES buffer; pH 6.80; the indicated concentrations of added ligand. The $V\ell_2$ signal is scaled to a constant amplitude for all the spectra.

of the total vanadate (1–5 mM), total hydrogen peroxide (9–30 mM), and total peptide (P) (20–600 mM) concentrations suggested that the two products both had the stoichiometry $V\ell_2P$. Their formation is then represented as in eq 5 where the ^{51}V

$$V\ell_2 + P \overset{K_5}{\rightleftharpoons} \text{ppm} \qquad [V\ell_2][P]K_5 = [\text{ppm}] \qquad (5)$$

chemical shifts (ppm) are used to identify the individual products. Figure 2 shows the results of plotting the experimental data according to eq 5. Excellent linear correlations are obtained for both products. The constant ratios [ppm]/[$V\ell_2$], shown in Figure 2, A and B, as open circles were obtained when the glycylglycine concentration was maintained constant at 300 mM and the hydrogen peroxide concentration was varied from 9.0 to 30.0 mM. This constancy demonstrates that the −713 and −747 ppm products have the same peroxide stoichiometry as $V\ell_2$. Similarly, vanadium concentration studies showed that these three materials had the same number of vanadium nuclei, which is known to be 1[7–9] for the product identified as $V\ell_2$. These three concentration studies are therefore fully consistent with the proposed $V\ell_2P$ stoichiometry of the two products.

Independent studies were carried out with ethylammonium and acetate ligands in a further attempt to characterize the products being formed. These materials each gave rise to a single product with chemical shifts at −720 (acetate) and −744 ppm (ethylammonium). In order to more fully understand these reactions, the hydrogen ion concentration was varied and this allowed further analysis to proceed as outlined in eq 6 for acetic acid, for which proton release was indicated from preliminary studies of the pH

$$\begin{array}{ccc} V\ell_2H_2^- & + \; RCO_2H \overset{K_5'}{\rightleftharpoons} & \text{ppm} + n\text{H}^+ \\ \Big\downarrow K_{a2} \; H^+ & & \Big\downarrow K_a \; H^+ \\ V\ell_2H^{2-} & + \; RCO_2^- & \end{array} \qquad (6)$$

variation results. In this equation $V\ell_2H_2^-$ refers to $VO(OO)_2(H_2O)_2^-$. In order to carry out the final analysis it was first necessary to determine the pK_a's of $V\ell_2H_2^-$ and acetic acid for the conditions of this study. The pK_a of acetic acid was determined by titration; the value determined was 4.75, in good agreement with the literature value.[15]

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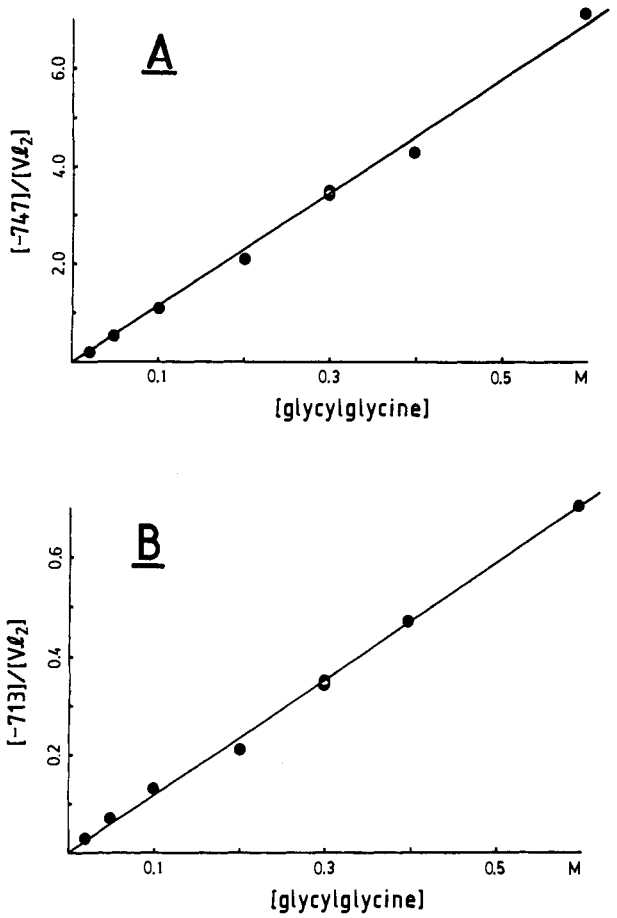


Figure 2. Variable peroxide and peptide concentration studies at constant pH. The linear relationships illustrated in these graphs demonstrate that both the −713 and −749 ppm products have the same Vℓ_2P stoichiometry. Conditions of the experiments: 3.0 mM total vanadate; μ = 1.0 M KCl; 20 mM HEPES buffer; pH 6.80; variable peptide (●) concentration with 9.0 mM total H_2O_2 and variable hydrogen peroxide (O) concentrations with 300 mM total glycylglycine.

Diperoxovanadate has a pH-dependent chemical shift. If the reasonable assumption is made that this dependence derives from protonation–deprotonation reactions,[7,8] then the chemical shift dependence yields the pK_a directly, according to eq 7, where δ_L

$$\mathrm{pH} = \mathrm{p}K_a + \log\left(\frac{\delta_L - \delta_o}{\delta_o - \delta_H}\right) \qquad (7)$$

is the limiting chemical shift at low pH, corresponding to $V\ell_2H_2^-$, and δ_H is the analogous limiting chemical shift at high pH, corresponding to $V\ell_2H^{2-}$. The observed chemical shift, δ_o, will vary between these limits which are δ_L = −693 ppm and δ_H = −765 ppm. The pK_{a2}, determined for $V\ell_2H_2^-$, was 7.42 ± 0.09 under the conditions here of 1.0 M KCl and 0.20 M acetate. This value is similar to that previously determined: 7.2 ± 0.1 in 1.0 M KCl[9] and 6.98 ± 0.25 in 2.0 M $NaClO_4$.[7]

With the above determined dissociation constants and the known concentrations of diperoxovanadate and acetate the concentrations of $V\ell_2H_2^-$ and RCO_2H are readily calculated for any pH. The formation constant, as defined in eq 6, is then given by eq 8. On

$$\frac{[\mathrm{ppm}][\mathrm{H}^+]^n}{[V\ell_2H_2^-]} = K_5'[RCO_2H] \qquad (8)$$

plotting the data according to eq 8 as depicted in Figure 3, it was found that a linear relationship was obtained only for a value of n equal to 1. Therefore it is evident that the formation of the −720 ppm product occurs from acetic acid with the release of 1 proton. The formation constant, K_5', had the value (9.6 ± 1.0) × 10^{-5}. If product formation is taken to occur from the acetate ion, then

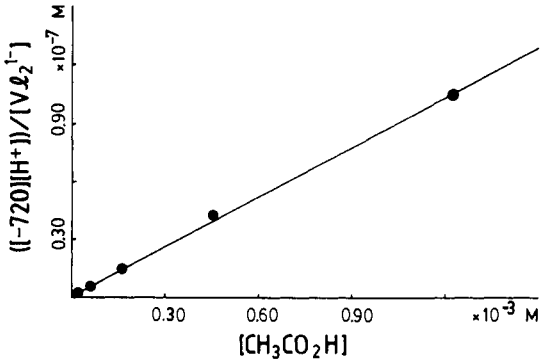


Figure 3. Variable pH study of the peroxovanadate reaction with acetic acid. The linear correlation depicted in this graph illustrates the release of protons during the reaction of diperoxovanadate with acetic acid. Conditions of the experiments: total acetate, 200 mM; total hydrogen peroxide, 9.0 mM; total vanadate, 3.0 mM; KCl, 1.0 M; HEPES buffer, 20 mM; pH, variable.

no proton is released and the formation constant has the value 4.8 ± 0.5 M^{-1}.

A study similar to that outlined above was also carried out for ethylammonium for which $R'NH_3$ replaces RCOOH in eq 6. The pK_a determined for $V\ell_2H_2^-$ under the 1.0 M $CH_3CH_2NH_3Cl$ conditions of this latter study was 7.33 ± 0.10, which is not significantly different from the previously determined value with acetate present. The literature value of 10.75[15] was used for the pK_a of ethylammonium.

The results of the pH study showed that the formation of the ethylammonium product was accompanied by the loss of a single proton with the formation constant k_5'' = (4.4 ± 0.4) × 10^{-8}, where k_5'' is written for ethylammonium which replaces acetic acid in eqs 6 and 8 and n = 1. If the formation is considered to occur from the amine, n = 0 and the value of k_5'' = (2.5 ± 0.3) × 10^3 M^{-1} is obtained.

In order to confirm that the terminal ammonium and terminal carboxylate groups of peptides underwent reactions with peroxovanadate similar to those of ethylammonium and acetate, pH variation studies were carried out with glycylglycine, triglycine, and prolylglycine. For these studies the total ligand concentration was maintained constant at a suitable level predetermined from ligand concentration studies. The pK_a values of the di- and triglycine ammonium groups were determined by titration for utilization in eq 6. The values were found to vary between 8.1 and 8.2 with an estimated error in the individual determinations of about 0.2 pK_a units. A value of 8.15 was assumed for the pK_a's of the two simple peptides of this study. A pK_a value of 9.2 ± 0.2 was determined for prolylglycine. The pK_{a2} value for $V\ell_2H_2^-$ was also determined for the various peptide–peroxovanadate solutions. This value was found to be insensitive to the particular solution and averaged to 7.36 ± 0.08, and this was used in eq 6 for all the ligands studied.

The experimental results were put into the form required for eq 8 using the pK_a values discussed above. The formation of the two glycylglycine products is demonstrated graphically in Figure 4. The result of this pH study showed that the proton stoichiometry of the individual products is the same as for those formed separately with acetate and ethylammonium. Reaction with the carboxylate group of the peptide proceeded with a formation constant similar to that for acetate while product formation with the ammonium group of the peptide was about 100 times larger than that for ethylammonium. The correlations obtained gave little indication that the products themselves had a pK_a within the range of pH values of this study, pH 6.2–9.5.

The results of the pH studies are summarized in Table I. This table provides the formation constants from the ammonium form of the ligand because this is the predominant form of the peptide at pH 7. Similarly, the acid functionality is normally deprotonated. The constants can as easily be written for the amino (or acid) functionality since the formation constants are simply related by the K_a of the ammonium (acid) group as $K = K_5''/K_a$. These

Table I. Constants for Formation (M^{-1}) of Products between Diperoxovanadate and Selected Ligands from pH Variation Studies[a,b]

ligand	$V\ell_2H_2^- + RCO_2^{n-} \rightleftharpoons V\ell_2P^{(n+1)-}$	ppm	$V\ell_2H_2^- + R'NH_3^{n+} \rightleftharpoons V\ell_2P^{(2-n)-} + H^+$	$V\ell_2H_2^- + R'NH_2^{n-} \rightleftharpoons V\ell_2P^{(n+1)-}$	ppm
AcO	4.8 ± 0.5	−720			
$EtNH_3$			$(4.4 \pm 0.4) \times 10^{-8}$	$(2.5 \pm 0.3) \times 10^{3}$	−744
glygly	1.2 ± 0.5	−713	$(1.9 \pm 0.3) \times 10^{-6}$	$(2.7 \pm 0.4) \times 10^{2}$	−747
glyglygly	1.4 ± 0.4	−712	$(2.7 \pm 0.3) \times 10^{-6}$	$(3.8 \pm 0.4) \times 10^{2}$	−743
progly[c]	1.4 ± 0.5	−713	$(8.3 \pm 0.4) \times 10^{-7}$	$(1.3 \pm 0.1) \times 10^{3}$	−750
			$(2.4 \pm 0.3) \times 10^{-7}$	$(3.8 \pm 0.4) \times 10^{2}$	−728

[a] Conditions of the experiments: 3.0 mM vanadate; 9.0 mM hydrogen peroxide; μ = 1.0 M KCl; 20 mM HEPES buffer; variable pH. The appropriate ligand concentration for each pH study was determined from ligand concentration studies. [b] Errors are quoted to the 3σ level. [c] Progly gives three products. Two products derive from the amino functionality possibly because the ring prevents internal rotation or slows isomerization.

Table II. Formation Constants (M^{-1}) for the Products of the Equilibration of Diperoxovanadate with Selected Ligands[a-c]

ligand	$V\ell_2H_2^- + RCO_2^{n-} \rightleftharpoons V\ell_2P^{(n+1)-}$	ppm	$V\ell_2H_2^- + R'NH_3^{n+} \rightleftharpoons V\ell_2P^{(2-n)} + H^+$	ppm
	From Ligand and H_2O_2 Concentration Studies			
glyglyOEt			$(2.8 \pm 0.4) \times 10^{-6}$	−743
glyglyglyOEt			$(3.3 \pm 0.5) \times 10^{-6}$	−743
glyval	2.0 ± 0.2	−718	$(1.8 \pm 0.2) \times 10^{-6}$	−745
valgly	1.2 ± 0.3	−714	$(4.6 \pm 0.6) \times 10^{-7}$	−747
	From Ligand Concentration Studies for Which Only a Few Spectra Were Obtained			
glyglyglygly	1.6 ± 0.6	−713	$(2.1 \pm 0.8) \times 10^{-6}$	−743
CBzglyglygly[d]	2.3 ± 0.8	−715		
glyser	1.3 ± 0.9	−716	$(2.1 \pm 0.7) \times 10^{-6}$	−748
glyasp[e]	3.0 ± 0.7	−717	$(1.3 \pm 0.4) \times 10^{-6}$	−748
valasp[e]	4.0 ± 0.8	−714, −719	$(2.9 \pm 0.7) \times 10^{-7}$	−757
glypro	1.2 ± 0.8	−714	$(2.1 \pm 0.8) \times 10^{-6}$	−750
glysar	1.3 ± 0.5	−712	$(2.3 \pm 0.7) \times 10^{-6}$	−750
NH_4^+			$(1.6 \pm 0.3) \times 10^{-7}$	−750
	Ligands Other than Peptides (Determined at pH 7)			
$(CH_3)_3N$	weak reaction: product −739 ppm			
$H_2NCH_2CH_2NH_2$	strong reaction: product −736 ppm (K_5 = 26 M^{-1} at pH 7.1)			
$HOCH_2CH_2NH_2$	like $CH_3CH_2NH_2$: product −743 ppm (K_5 = 1.4 M^{-1} at pH 7.0)			
$HOCH_2CH_2OH$	no product observed up to 2.5 M			
$HOCH_2CH_3$	no product observed up to 2.5 M			
uridine	minor product observed at 1 M, −740 ppm			
CH_3CONH_2	no product observed up to 1.0 M			

[a] Conditions of the experiments: 3.0 mM vanadate; μ = 1.0 M KCl; 20 mM HEPES buffer; pH 7; variable H_2O_2 and ligand concentrations as indicated. [b] Formation constants were determined at fixed pH then put into the pH-independent form using the following dissociation constants: $pK_{a2}(VO(OO)_2(H_2O)_2^-)$, 7.36; $pK_a(R'NH_3)$, 8.15 for all peptides. [c] Errors are quoted to the 3σ level. [d] CBz = carbobenzoxy ($PhCH_2O(O{=})C-$). [e] Both glycylaspartate and valylaspartate give rise to products deriving from condensation with the individual carboxylate groups. The two signals were not well-resolved. [f] See also ref 24.

values of K are included in Table I, but only for the amino group.

A number of other peptides were studied only at constant pH. On the assumption that the pK_a values of the amino group of these peptides were similar to those measured for the above studies ($pK_a \sim 8.15$ except for the prolylglycine derivatives, $pK_a = 9.2 \pm 0.2$), the formation constants were measured and then calculated according to eq 6. The values are reported in this form in Table II since these values allow calculation of the pH-dependent equilibrium constants for any pH using eq 9 where K_{a2} is the

$$K_5 = \frac{K_5^0}{[H^+](1 + K_{a2}/[H^+])(1 + K_a/[H^+])} \qquad (9)$$

acidity constant for $V\ell_2H_2^-$ ($pK_{a2} = 7.36 \pm 0.08$) and K_a is the acidity constant for the ammonium ion ($K_5^0 = K_5''$) or carboxylic acid ($K_5^0 = K_5'$) group, dependent on the equilibrium of interest. K_5 is the equilibrium constant defined in eq 5 where the charge states of all species are ignored and it represents the observed value. For the sake of comparison, formation constants that would be observed at pH 7.0 and 7.5 have been calculated and put into Table III. As can be seen these values change substantially with even an increase of only 0.5 of a pH unit.

We have previously noted that the vanadate-catalyzed disproportionation of H_2O_2 to water and oxygen is greatly slowed or even stopped in the presence of peptides.[9] Under conditions of 0.4 mM vanadate, 0.4 mM H_2O_2, 1.0 M KCl, and pH 7, UV and NMR spectroscopy studies showed that the peroxide was completely decomposed in about 200 min. This decomposition was not significantly affected by the presence of either 40 mM acetate or 40 mM ethylammonium. However, the decomposition

Table III. Formation Constants (M^{-1}) for Selected Complexes of Peroxovanadate Calculated for pH 7.0 and 7.5

ligand	$V\ell_2 + P \rightleftharpoons V\ell_2P^a$ pH 7.0	pH 7.5	$V\ell_2 + P' \rightleftharpoons V\ell_2P'^b$ pH 7.0	pH 7.5
acetate	3.4	2.1		
ethylammonium			0.3	0.6
glygly	0.9	0.5	13	21
glyval	1.4	0.8	12	19
valgly	0.8	0.5	3.0	5.0
valasp[c]	2.8	1.7	1.9	3.2
glypro	0.8	0.5	14	23
progly[d]	1.0	0.6	5.7, 1.7	10.8, 3.1

[a] Equilibrium is for reaction at the carboxylate group. [b] Equilibrium is for reaction at the amino group. [c] Two products are formed from condensation with the two different carboxylate groups. [d] Progly gives rise to two amino derived products, probably stereoisomers.

essentially was stopped (perhaps 5–10% decomposition in 600 min) with 40 mM glycylglycine. This suggested the presence of a new type of product, not observed in the equilibrium studies described above. A time-course NMR study under conditions similar to those of the UV studies indeed did reveal an additional product signal. The slow formation of this product is demonstrated in Figure 5. The chemical shift of this material, −648 ppm, is well removed from those of the diperoxo derivatives and indicates this is a monoperoxovanadium(V) product. It very clearly is a highly favored product since, as can be judged from the spectra, essentially all of the other peroxoproducts are converted into this material. All other unprotected peptides so far studied also strongly inhibited or prevented peroxide decomposition. On the

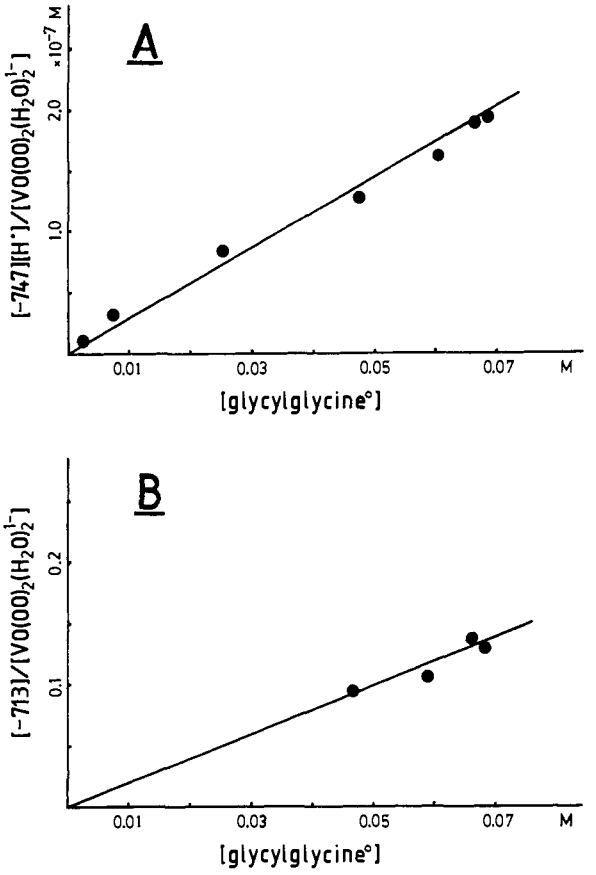


Figure 4. Variable pH study of the peroxovanadate/peptide system at constant total peptide and total peroxide concentrations. These graphs demonstrate that a single hydrogen is released when a vanadodiperoxide product is formed from the ammonium functionality of neutral glycylglycine (plot A) but not released when formed from the carboxylate (plot B). The concentration of glycylglycine^0 is given by glycylglycine^0 $\rightleftharpoons$ glycylglycine$^-$ + H^+, with the pK_a value of 8.1 given in the text. Conditions of the study: vanadate, 3.0 mM; hydrogen peroxide, 9.0 mM; glycylglycine, 70 mM; HEPES buffer, 20 mM; KCl, μ = 1.0 M; pH, variable.

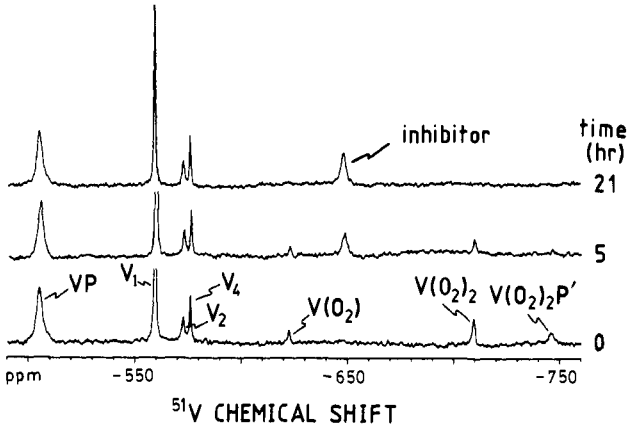


Figure 5. Time-course ^{51}V NMR study showing the slow formation (−649 ppm) of a third type of peroxovanadate/peptide derivative. This product is thought to be the inhibitor responsible for the stabilization of H_2O_2 against vanadate-catalyzed decomposition. In this figure V_1, V_2, and V_4 represent monovandate and its dimer and tetramer, VP a peptidovanadate derivative, and $V(O_2)$, $V(O_2)_2$ and $V(O_2)_2P'$ the peroxovanadates of this study. The minor, carboxylate-derived product $V(O_2)_2P$ is not observed here because of its low concentration. Conditions for the experiment: 0.5 mM total vanadate; 0.2 mM total H_2O_2; 50 mM glycylglycine; pH 7.0, and the variable times after sample preparation indicated.

other hand, if the terminal nitrogen was protected or if the peptide nitrogen was substituted as in glycylproline or glycylsarcosine,

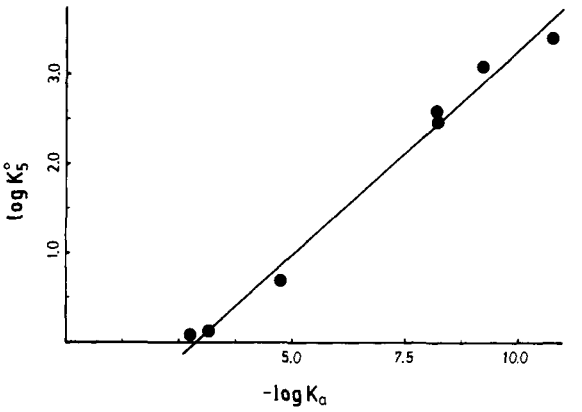


Figure 6. A linear free energy relationship relating the pK_a values of the conjugate acid form of the ligand to the formation constants of the diperoxovanadium(V) products. The formation constants were taken from Table I.

then insignificant changes in the rate of disproportionation of H_2O_2 were observed. The carboxylate-protected derivative glyglyOEt also did not inhibit the decomposition of hydrogen peroxide. Some inhibition was observed, but this was attributed to the result of the partial hydrolysis of glyglyOEt to form glygly. The amino acid glycine was found to slow decomposition to a significant extent but not nearly as effectively as glygly. Under conditions where they were observable, ^{51}V signals occurred within the range of about −650 to −660 ppm whenever peptide inhibitors were in solution. Signals near this region have not yet been observed when the additive does not inhibit decomposition.

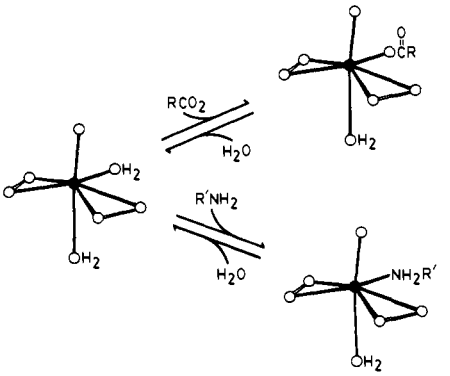
Discussion

Small peptides with non-functionalized side chains, such as glycylglycine or glycylproline, undergo facile condensation reactions with diperoxovanadate ($VO(OO)_2(H_2O)_2^-$) to form two major types of products. Reaction occurs at either the carboxylate or ammonium terminal positions of the peptide. Reaction at the ammonium group is accompanied by release of a proton, indicating that condensation occurs with the amino functionality. Single products, in accord with those mentioned above, were obtained with C-terminal and N-terminal protected peptides while both products were obtained with glycylsarcosine which has a methyl group on the amido nitrogen of the peptide linkage. Both acetate and ethylamine gave products analogous to the above.

It is evident from these results that complexation occurs in a simple monodentate fashion. Since protons are not required to maintain pH, hydroxide ion is not released as condensation occurs. The carboxylate group has no protons so water cannot be formed as a product of the reaction with this ligand although it could be displaced if the diperoxovanadate itself contains water. In fact, if water is not released there must be an adjustment in the coordination shell as it is unlikely that coordination to the two peroxo groups is much affected by incorporation of these ligands. Consequently, if water is not released, expansion of the coordination shell is indicated. This possibility cannot be ruled out. If the diperoxovanadate already has incorporated water then water can be displaced as condensation occurs. Either of these two reaction pathways leads to the same product since the coordination shell would have been expanded when the water was initially taken up.

The acidity constants for the ligands of this study range over about 7 orders of magnitude, the pK_a values varying from about 3.1 (peptide carboxylic acid) to 10.8 (ethylammonium). Over this range of acidities the formation constants vary from about 1.3 to 2.5×10^3 M^{-1}, respectively (Table I). When plotted in energy terms, the pK_a of the ligand versus the formation constant of the product as shown in Figure 6, a reasonably good linear plot was obtained. This linear free energy relationship shows that there is a strong dependency of product formation on the ability of the ligand to inductively donate electrons to the vanadium nucleus of the diperoxovanadate. This contrasts markedly with vanadate

Scheme I

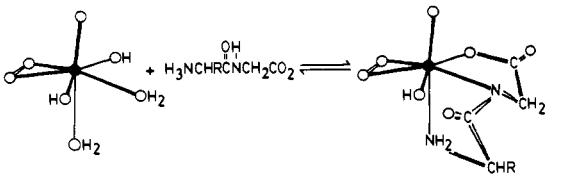


itself, where ester formation is insensitive to the pK_a values of the alcohols.[16,17] The fact that no products from alkyl alcohols were observed follows from the above observations. The protonated alcohols (ROH_2^+) are so strongly acidic that condensation with ROH would occur with a vanishing small formation constant. The pK_a's of the alcohols are large so that condensation with RO^- should be highly favorable. There, however, is insufficient alkoxide present for products to be observed at the pH levels of this study. It seems likely that lower pK_a alcohols such as CF_3CH_2OH would yield observable amounts of products. Phenol, for example, gives a product in quantities predictable from Figure 6. Apparently the vanadium is insensitive to the distinction between nitrogen and oxygen in the ligand other than in the ability to donate electrons. Detailed studies of a homologous series of these compounds should provide considerably more insight concerning complex formation in this system. That the more powerful electron donators promote product formation suggests that the peroxide ligands are effective withdrawers of electron density from the vanadium center, leaving it electron deficient. Electron density at vanadium could be increased by expanding the coordination shell so that electron density can be inductively donated to the coordination center by appropriate ligands. In the absence of better ligands, water would be suitable for this.

Unfortunately, the coordination of $VO(OO)_2(H_2O)^-$ is not known; both 7-[8] and 8-coordination[7] have been proposed. Coordination of this complex could be 6 (no additional water), 7 (one water), or 8 (2 additional waters). Higher coordination than this seems unlikely. The X-ray structures of a number of peroxovanadate complexes with a variety of different ligands indicate that 7-coordination is preferred,[18] including monoperoxo[19,20] and triperoxovanadates.[21] If the peroxide is formally treated as a unidentate ligand then the mono- and triperoxovanadates have octahedral and tetrahedral coordination, respectively, while the diperoxovanadates, in essence, have a pentacoordinate trigonal bipyramidal geometry.[18] This structure is assumed here although it has been argued that the diperoxovanadates have octahedral coordination.[7] Accepting this, formation of the products can be represented as outlined in Scheme I.

X-ray diffraction studies of ammonium oxodiperoxoammine-vanadium(V) have shown that, at least in the crystal, this material has a pentagonal pyramidal structure with the two peroxo groups and the amino group forming the basal plane.[22] The seventh coordination site is not really unoccupied since there are significant long-range interactions between the vanadium and the oxo group of a second molecule. In aqueous solution this site may well be occupied by water. The ^{51}V chemical shift obtained for this product, −750 ppm (Table II), is not significantly different from that of other amino products. This indicates there is a close structural correspondence between the various products in accordance with Scheme I. Interestingly enough, prolylglycine gives rise to three products, one deriving from the carboxylate group and the others from the amino group, as ascertained from the pH dependence of the formation constant. It is difficult to know why two amino products are observed. However, the prolyl ring may provide sufficient steric interactions to slow isomerization enough to allow for the observation of two products.

Scheme II



Glycylglycine was found to form a product in addition to those discussed above. It forms over a period of several hours and apparently derives from the monoperoxovanadate. It has a ^{51}V chemical shift close to that of monoperoxovanadate and is not observed when the peroxide concentration is high enough to convert essentially all of the monoperoxide to diperoxo or higher peroxo derivatives. With certain peptides other than glycylglycine, where product formation is more favorable, products with ^{51}V chemical shifts in the appropriate region (ca. −650 to −660 ppm) were observed as minor products even with high peroxide concentrations. These materials apparently are responsible for the strong peptide-induced inhibition of the vanadium-catalyzed decomposition of hydrogen peroxide. This decomposition is not significantly slowed by acetate, by ethylamine or by carboxylate, terminal nitrogen or peptide nitrogen protected peptides. On the other hand, glycine also inhibits, but much less efficiently than the dipeptide ligand.

Taken together these results suggest that the peptide inhibitor complex is a monoperoxovanadate with one ligand complexed in a tridentate fashion. The formation of this product can then be depicted as shown in Scheme II. The less efficient inhibitor formed with glycine is then assigned to a product with the amino acid complexed in a bidentate manner, similar perhaps to $VO(OO)(picrate)(H_2O)_2$ for which an X-ray structure is known.[19]

Two properties of the inhibitor complex are evident from Figure 5. The first is that its rate of formation is slow, requiring on the order of hours for its half-formation under the condition employed here. Secondly, over time, the product is highly favored which implies that its decomposition rate is exceedingly slow. This property may be very important to the biological function of peroxovanadates. It may for instance allow this product to serve as a complex which slowly releases vanadate as the complex dissociates, leading to vanadate activation or inhibition of enzymic function in biological systems. Of course, over shorter time periods the more rapidly formed products may be equally or, more probably, much more important, perhaps by directly activating or inhibiting enzyme function. It is also possible that the monoperoxo product, if it is structurally similar to the picrate, could lead to covalent modification of proteins since it seems that the picrate, at least, is a good oxidizing agent.[19] This possibility, to an extent, might be tested by studying the effects of the presence of inhibitor ligands on the kinetics of the vanadate-catalyzed hydrogen peroxide oxidation of nicotinamide adenine dinucleotide (NADH).[12,23] The rate of this oxidation is known to follow the concentration of the monoperoxide complex in solution.[12]

Acknowledgment. Thanks are gratefully extended to the Medical Research Council of Canada for its financial support of this work.

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