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Activation of Phosphates to Substitution. 1. Production and Hydrolyses of $[(VO^{3+})_n(P_3O_{10}{}^{5-})]$ and $[(VO^{3+})_n(ATP^{4-})]$

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Hydrolysis rates for ATP (adenosine triphosphate) range over a factor of about 10^{10}, half-times being about 3 months for the uncatalyzed reaction and less than milliseconds for the enzymatic process.[1] We have begun a search for simple activators for phosphate substitution reactions. Results described previously include discovery of a complex of Mo(V), $Mo(O)(P_2O_7)_2{}^{5-}$, in which the 5d electron of Mo(V) interacts with the ^{31}P nucleus,[2] complexes of Mn^{2+}, VO^{2+}, and Fe^{2+} which accelerate triphosphate hydrolysis up to tenfold,[3] and a VO^{2+}-catalyzed decomposition of H_2O_2 coupled to selective hydrolysis of P–O–P bonds[4] at rates up to 10^4 times the uncatalyzed rate. This communication describes the creation of active complexes of VO^{3+} and/or $VO_2{}^+$ with polyphosphates by oxidation of VO^{2+} polyphosphate complexes and subsequent hydrolyses of polyphosphates with up to 10^6-fold acceleration.

Experimental Section

Reagent grade chemicals were used without further purification. $PO_4{}^{3-}$ was measured as phosphomolybdenum blue by use of a modified procedure of Baginski et al.[5,6] Various phosphates were separated and detected by means of TLC techniques developed for this project.[7] Acidity was monitored with a Sargent-Welch LSX pH meter.

Oxidation of VO^{2+} species was accomplished by adding $MnO_4{}^-$ rapidly to give a mole ratio $VO^{2+}/MnO_4{}^- = 5.0$. No competitive oxidation of adenosine occurred.

Absorption spectra were obtained by use of a Cary (14RI) spectrophotometer having thermostated cells.

Stoichiometry of Reactions Observed. A. Oxidation of V(IV).

$$MnO_4{}^- + 5VO^{2+} + 8H^+ \rightarrow Mn^{2+} + 5VO^{3+} + 4H_2O \tag{1}$$

Oxidations of vanadyl polyphosphates are fast enough for easy titration. If stoichiometric mixtures of $MnO_4{}^- + 5VO^{2+}$ are made, reactions are complete on mixing and the products are colorless. Oxovanadium(IV), VO^{2+}, and oxovanadium(V), VO^{3+}, are both coordinated to the phosphates present.[4] The VO^{3+} may hydrolyze to form $VO_2{}^+$ and/or $V_{10}O_{28}{}^{6-}$.

B. Hydrolysis of V(V). As soon as $VOP_3O_{10}{}^{3-}$ at pH 4 is mixed with $MnO_4{}^-$ at 5 °C an absorption band appears at 255 nm, similar to a band observed for $VO_4{}^{3-}$, and slowly decays over a period of about 1 h. Absorbance by adenosine prevents observations of this band in systems involving ATP. For either ATP or PPP_i, the pH rises directly on mixing, from about 4 to 8, as H^+ is consumed by reaction 1 at 5 °C. No yellow color or $PO_4{}^{3-}$ formation is observed for over 50 min. However, the pH decreases slowly from 8 to 4 during that time (see Table I).

In contrast, at room temperature (25 °C) the initial pH is reduced to 2.4 upon mixing; i.e., there is net production of acid. The colorless solution immediately begins to turn yellow as products hydrolyze to form $H_2V_{10}O_{28}{}^{4-}$ and $H_2PO_4{}^-$. With PPP_i, the band at 250 nm is not seen at 25 °C.

A reasonable interpretation of the rapid loss of absorbance at 250 nm accompanied by increasing acidity at 5 °C is that VO^{3+} is hydrolyzed to $VO_2{}^+$ and that both remain complexed to polyphosphate.

$$H_2O + VO^{3+} \rightarrow VO_2{}^+ + 2H^+ \tag{2}$$

This hydrolysis produces more H^+ than is consumed by the permanganate oxidation.

At this stage we assume that polyphosphate complexes of $VO_2{}^+$ are present in solution.

C. Hydrolysis of Polyphosphates Bound to V(V). We observed hydrolysis of products of both the $ATP–VO^{2+}–KMnO_4$ and $PPP_i–VO^{2+}–KMnO_4$ reactions by monitoring the $PO_4{}^{3-}$ concentration vs. time. ATP and PPP_i in aqueous solution at pH ~2 do not significantly hydrolyze over a period of several days. When VO^{2+} is added to either solution the hydrolysis rate is somewhat enhanced ($t_{1/2} \approx$ 2–3 weeks); however, if the VO^{2+} is oxidized by either $KMnO_4$ or H_2O_2[4] the hydrolysis rate increases dramatically. Table II shows that when an equimolar ATP-and-VO^{2+} solution (0.01 M) is oxidized by $KMnO_4$, $t_{1/2}$ for hydrolysis of ATP is shortened to approximately 2 h.

Table I. Changes of pH with Time (0.006 M VO^{2+}, 0.006 M ATP, 0.0012 M $KMnO_4$)

	pH	
t, min	25 °C[a]	5 °C[b]
0.16	2.6[a]	8.0
0.50		7.2
1.0	2.5	6.8
2.0		6.5
4.0		6.0
6.0		5.6
10.0	2.48	5.1
30.0	2.42	4.2
60.0	2.42	4.2[b]

[a] Yellow color appears immediately. [b] Solution colorless for 50 min.

Table II. Hydrolysis of Triphosphates in the Presence of Equimolar V(V)

$[VO^{2+}]_0$	9.7×10^{-3} M	1×10^{-2} M
$[ATP]_0$	9.9×10^{-3} M	
$[PPP_i]_0$		1×10^{-2} M
$[MnO_4{}^-]_0$	1.94×10^{-3} M	2×10^{-3} M

T = 25 °C

Δt, min	$10^3[PO_4{}^{3-}]$, M	$10^3[PO_4{}^{3-}]$, M
5	0.67	0.90
15	2.05	1.85
30	4.00	2.51
50	5.70	3.50
60	6.30	
250		6.61
1440	11.00	8.75[a]

[a] Unreacted PPP_i observed.

Furthermore, the fact that the amount of $PO_4{}^{3-}$ produced is larger than the original ATP concentration indicates that more than one $PO_4{}^{3-}$ is hydrolyzed off some of the ATP. TLC separations as described by Scott[6] also show that both AMP and ADP are present after rapid hydrolysis is complete (24 h). A small $P_2O_7{}^{4-}$ impurity in the ATP disappears during ATP hydrolysis. These results differ from those obtained with the $H_2O_2–VO^{2+}–ATP$ system, in which only ADP and P_i were produced at room temperature. Moreover, $KMnO_4$, unlike H_2O_2,[4] immediately oxidizes VO^{2+} with polyphosphate hydrolysis proceeding after oxidation. Similarly, PPP_i hydrolysis is enhanced by oxidation of VO^{2+} with $KMnO_4$. However, when VO^{2+} and PPP_i are present in equimolar amounts (0.01 M), the PPP_i is incompletely hydrolyzed to $PP_i + P_i$. We infer that an equilibrium

$$PPP_i + VO^{2+} \rightleftarrows (VO^{2+} \cdot PPP_i) \tag{3}$$

is established such that when oxidation occurs, the PPP_i is not all coordinated and hence is not all subsequently hydrolyzed. We have, in fact, detected unreacted PPP_i by TLC, after 24 h. If the VO^{2+} is present in excess the equilibrium is forced to the right and all the PPP_i is hydrolyzed. In this case the ratio of product $[P_i]$ to $[PPP_i]_0$ is greater than unity as is observed with ATP.

$$(18 + n)H_2O + 10VO_2ATP^{3-} \rightarrow H_2V_{10}O_{28}{}^{4-} + nAMP + (10 - n)ADP + (10 + n)PO_4{}^{3-} + (34 + n)H^+ \tag{4}$$

$$(18 + n)H_2O + 10VO_2P_3O_{10}{}^{4-} \rightarrow H_2V_{10}O_{28}{}^{4-} + (10 + 2n)PO_4{}^{3-} + (10 - n)P_2O_7{}^{4-} \tag{5}$$

That coordination of the ATP or PPP_i, before oxidation occurs, is necessary for enhancement of the hydrolysis rate was verified as follows. Firstly, EPR data[4] clearly indicate that rapid coordination of polyphosphates to the VO^{2+} occurs. Secondly, if a stronger ligand (acetate ion) is added to the VO^{2+} solution before the PPP_i or ATP and then the VO^{2+} is oxidized, no enhancement of hydrolysis occurs. Excess PPP_i inhibits hydrolysis, a fact suggesting that complexes containing more than one V atom are more active. Thirdly, if ATP

Table III. Hydrolysis of $V^V(PPP_i)$ at 20 and 16 °C

Δt, min	0.33	0.67	1	2	10	30	60	(24 h)
$10^3[P_i]$, M								
20 °C	0.56	0.93	1.22	2.09	4.31	5.22	6.0	7.0
16 °C	0.85	1.31	1.44	1.93	3.75	4.76	5.16	8.0

$[VO^{2+}]_0 = 9.7 \times 10^{-2}$ M; $[PPP_i]_0 = 5 \times 10^{-3}$ M; $[MnO_4^-]_0 = 1.94 \times 10^{-2}$ M

Table IV. Half-Times for Hydrolysis of Polyphosphates ($T = 25$ °C)

System	Concn, M	$t_{1/2}$, s	Ref
ATP in H_2O	10^{-2}	$\sim 10^7$	3
(Mn^{2+} ATP)	10^{-2}, 10^{-2}	$\sim 8 \times 10^6$	4
(VO^{2+}, ATP)	10^{-2}, 10^{-2}	$\sim 2 \times 10^5$	6
(VO^{2+}, ATP) + H_2O_2	10^{-2}, 10^{-2}, 10^{-1}	$\sim 1 \times 10^3$	6
(VO^{3+}, ATP)	10^{-2}, 10^{-2}	$\sim 2 \times 10^3$	This work
(VO^{3+}, PPP_i)			
A		2×10^3	This work
B	10^{-1}, 10^{-2}	2×10	This work

or PPP_i is added to an ammonium vanadate solution and pH adjusted to ~2, the hydrolysis rate is significantly slower than in the VO^{2+}–ATP–$KMnO_4$ system ($t_{1/2}$ of several days). Ammonium vanadate does not catalyze hydrolysis of PP_i or ADP at all at room temperature over 24 h. Hydrolyses of both PP_i and ADP are enhanced by VO^{2+} + $KMnO_4$ at room temperature but proceed at very much slower rates than ATP or PPP_i.

When excess VO^{2+} was initially present the pH (2.5) at 25 °C remained nearly constant. With ATP and excess VO^{2+}, a precipitate occurred several minutes after oxidation; analysis showed it to be a mixture of adenosine, AMP, and vanadyl polymer. However, with PPP_i no precipitate occurred; therefore, we chose it for quantitative study. Table III reports typical results of experiments in which phosphate concentration was monitored as a function of time for a 20-fold excess of VO^{2+} over PPP_i. Only enough $KMnO_4$ was used to oxidize all the vanadyl ion in the solution. Variation of $KMnO_4$ concentration showed it to have no effect on the hydrolysis rate as long as enough was added to oxidize all vanadyl ion present. Table III reveals that the PO_4^{3-}/PPP_i ratio became greater than 1 and thus indicates that some PPP_i was completely hydrolyzed to 3 P_i. Furthermore, TLC experiments showed PP_i to be formed only in the first 10 min after oxidation at room temperature.

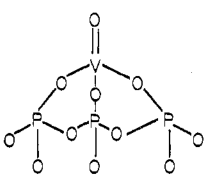
At temperatures above 35 °C the PPP_i was completely hydrolyzed to 3 P_i in 2–3 h.

Discussion

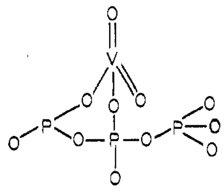
Oxidation of vanadyl triphosphates to V(V) is slow with H_2O_2 but rapid with MnO_4^-.[10]

The band at 255 nm (at 5 °C for [V] = [PPP_i]), characteristic of the colorless V(V) product, suggests a tetrahedral V(V) species (I),[11] since VO_4^{3-} is colorless and exhibits a similar band. Loss of the 255-nm band accompanied by H^+ production suggests addition of oxygen from H_2O to the V(V) center to give II.[11]

Hydrolysis patterns of II with excess V(V), discussed below, suggest that two complexes are present. The fact that VO_2^+ aquo species are yellow perhaps argues against dioxo V(V) species for II. The yellowing of solutions is not visible until triphosphate hydrolysis occurs; it probably involves polyvanadate formation ($V_{10}O_{28}^{6-}$).



I



II

The hydrolysis of these complexes is very complicated, involving at least three steps producing P_i at temperatures between 16 and 26 °C. A fourth step involving $P_2O_7^{4-}$ hydrolysis becomes important at temperatures above 35 °C. Even though further study is required to sort out these steps and evaluate their rate parameters, some discussion of species in which P(V) is activated to substitution is in order.

Two complexes of VO_2^+ and PPP_i appear to hydrolyze to give P_i. One gives simply P_i and PP_i; the other gives 3 P_i, probably in two steps:

$$\underset{a}{VO_2PPP_i} \xrightarrow{H_2O} P_i + PP_i + {}^1/_{10}V_{10}O_{28}^{6-} \tag{A}$$

$$\underset{b}{VO_2PPP_i} \xrightarrow{H_2O} VO_2PP_i + P_i \tag{B}$$

$$VO_2PP_i \xrightarrow{H_2O} 2P_i + {}^1/_{10}V_{10}O_{28}^{6-} \tag{C}$$

Steps B and C lead to $P_i/PPP_i > 1$. This ratio increases at lower temperature as does the initial rate of production of P_i (Table III). This observation indicates increased formation of complex b vs. a at lower temperatures. Reaction B appears to be 10–20 times as fast as reaction A. Reaction C is another order of magnitude slower but still faster than reactions of PP_i initially present.

The design of this preliminary study of $P_3O_{10}^{5-}$ hydrolysis was predicated on the vain hope that excess VO^{2+} would yield one complex ($VOP_3O_{10}^{3-}$) that would on oxidation hydrolyze simply to $P_2O_7^{4-}$ and PO_4^{3-}. In fact, at least five hydrolysis steps appear to take place at room temperature and a sixth at >35 °C. Sorting out these steps would require extensive study.

At present we can report that both ATP and PPP_i bound to freshly oxidized V(V) hydrolyze with separation of a terminal PO_4^{3-} and can partially hydrolyze by cleavage of a second P–O–P linkage. This second step is faster than the hydrolysis of either PP_i or ADP initially present. Present indications are that, with each tripolyphosphate, two complexes are formed with V(V), one giving rapid hydrolysis (B) of the terminal PO_4^{3-}, followed by slower hydrolysis (C) of the remaining P–O–P linkage. The second complex hydrolyzes only the terminal phosphate (A). Approximate half-times at room temperature are given in Table IV.

Other Oxidants. As previously reported,[4] H_2O_2 oxidizes VO^{2+} complexes of triphosphates, diphosphates, and phosphate diesters to form hydrolyzable V(V) complexes. Orthophosphate monoesters are *not* hydrolyzed. Preliminary studies show that Br_2, Cl_2, and BrO_3^- all oxidize V(IV) polyphosphates to V(V) polyphosphates with dramatically enhanced hydrolysis rates. Hydrolysis patterns differ from those resulting from MnO_4^- oxidations and will require extensive, separate study.

A goal of this work is to develop and test postulates for ways in which phosphates may be activated. The results given here are in accord with two postulates of factors which may assist in activation of P(V) centers for phosphorylations and hydrolyses of polyphosphates and phosphate esters.

I. Complexes Which Increase Positive Charge on P(V) Centers Should Enhance Nucleophilic Attack on P. All positive metal ions are mild catalysts for the hydrolysis of ATP.[1] $S_2O_7^{2-}$, in which S(VI) is far more positive than P(V), hydrolyzes very fast.[8] In this work, increasing charge on the coordinated vanadium center clearly labilizes polyphosphates to substitution.

II. Ring Formation Enhances Nucleophilic Attack on P(V). Cyclic triphosphate reacts smoothly with amines in water to give a linear triphosphate bound to nitrogen.[9]

$$P_3O_9^{3-}(\text{ring}) + RNH_2 \rightarrow R\text{-}NH\text{-}P_3O_9H(\text{chain})$$

Vanadium(V) may be linked by O bridging to two P(V) atoms closing a ring in the active complexes. Other postulates have been made which are not tested by the work reported in this communication.

It has been proposed that Mg^{2+} assists hydrolysis of ATP by coordinating to P_1 and P_2, encouraging the terminal PO_3^- to leave. This agrees with the finding that $V^VP_3O_{10}$ at 5 °C, which has a spectrum like VO_4^{3-}, must hydrolyze to give VO_2^+

before $P_3O_{10}^{5-}$ hydrolyzes. Possibly, V(V) bound to all three P's does not enhance hydrolysis while V(V) bound to only two P's frees the third or terminal PO_3^- to depart, dramatically accelerating the hydrolysis.

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Registry No. $(VOATP)^{2-}$, 63956-78-5; $(VOPPP_i)^{3-}$, 63956-79-6; $(VOATP)^-$, 63956-80-9; $(VOPPP_i)^{2-}$, 63956-81-0; MnO_4^-, 14333-13-2.

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(10) Recent observations by Charles Huber suggest that H_2O_2 is catalytically decomposed by VO^{2+} which is stabilized with respect to VO_2^+ by polyphosphate complexes.
(11) Postulated monovanadium complexes are tentative. Complexes of more than one V(V) per polyphosphate are readily conceivable.

Correspondence

Equilibrium Constants for the Metalation of Zinc Porphyrins

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Sir:

The equilibrium between divalent metal ions and free base (PH_2) porphyrins to form metalloporphyrins (MP) is usually represented by the equation

$$M^{2+} + PH_2 = MP + 2H^+ \qquad K_e \tag{1}$$

It is thus surprising to note that few mass law studies on this process have been reported. Dempsey, Lowe, and Phillips[1] investigated eq 1 with zinc sulfate and mesoporphyrin IX dimethyl ester solubilized in the detergent cetyltrimethylammonium bromide (CTAB) and found apparent equilibrium constants K_e^* of 6×10^{-3} M (80 °C), 2.5×10^{-3} M (60 °C), and 1×10^{-3} M (20 °C). No experimental details were published. The K_e value in water differs from K_e^* in detergents by the factor $(1 + K_S^{MP})/(1 + K_S^{PH_2})$ where it is assumed that there is an equilibrium between porphyrin species in the water and detergent phases, characterized by an unknown K_S value.

Table I. Equilibrium Data for the Zinc(II) Tetra(2-*N*-methylpyridyl)porphine Reaction 25.5 °C

$(ZnP)/(PH_2)$	(H^+),[a] M	$10^3(Zn^{2+})$, M	K_e,[b] M
0.195	0.99	1.0	191
0.410	0.99	2.0	191
0.746	0.99	4.0	183
1.39	0.99	8.0	172
1.97	0.99	10.0	193
1.56	0.89	6.4	193
1.82	0.79	6.4	177
2.40	0.70	6.4	183
3.29	0.60	6.4	185
4.41	0.50	6.4	172
8.24	0.40	6.4	206
35.5	0.19	6.4	200
			Av 188 ± 11

[a] $\mu = 1.0\ (LiNO_3/HNO_3)$. [b] $K_e = (ZnP)(H^+)^2/(PH_2)(Zn^{2+})$.

Detergents also modify the behavior of the porphyrins that they solubilize. For example, acid–base titrations in CTAB show only the PH_2 and diacid PH_4^{2+} forms, while PH_2, PH_4^{2+}, and the monocation PH_3^+ are stabilized in cationic detergents. The cationic pK behavior approximates that found in water for similar prophyrins.

The only other K_e value that we can find was reported by Brisbin and Balahura[2] using zinc acetate and hematoporphyrin IX in 0.1 M $NaClO_4$ at 25 °C. At pH 7.0 and by assuming eq 1, the ratio $K_e/(H^+)^2$ was experimentally determined to be 1.3×10^7, giving $K_e = 1.3 \times 10^{-7}$ M. The equilibrium was studied at only this one pH. In general, the elucidation of eq 1 is somewhat difficult; the porphyrin should be monomeric and soluble in the acid range, the equilibrium constants between PH_2, PH_3^+, and PH_4^{2+} should be known, and the molar extinction coefficients of the various porphyrin forms are required.

We have simplified this problem by working with tetra(2-*N*-methylpyridyl)porphine,[3,4] which exists as only the PH_2 form from below pH 0 to above pH 7. At 25.5 °C, $\mu = 1.0$ $(LiNO_3/HNO_3)$, mixtures of PH_2, ZnP, HNO_3, and $Zn(NO_3)_2$ were equilibrated in the dark for periods of up to 3 weeks. At constant total porphyrin concentrations, isosbestic points were found in the visible region indicating that PH_2 and ZnP were the major species. The ratio $(ZnP)/(PH_2)$ was determined spectrophotometrically by standard methods. The total porphyrin concentrations were ca. 10^{-5} to 10^{-6} M, while (H^+) and (Zn^{2+}) values were greater than 10^{-3} M, so that $(H^+) = (H^+)_0$ and $(Zn^{2+}) = (Zn)_0$. The typical results shown in Table I indicate that eq 1 is followed with $K_e = 188 \pm 11$ M.

At constant ionic strength in water, the formation rates of zinc porphyrins usually follow the rate law $R_f = k_f(PH_2)\cdot(Zn^{2+})$, while the acid-catalyzed dissociation rates are of the form $R_d = k_d(ZnP)(H^+)^2$. Thus the ratio k_f/k_d should also equal K_e. Such results are shown in Table II. The overall trend is that K_e values decrease with an increase in porphyrin basicity, with the basicity measured by pK_3 (H_3P^+/H_2P). In other words, the less basic the porphyrin ligand, the more "stable" is the corresponding metal–porphyrin complex.

Table II. Equilibrium Constants for Zinc–Porphyrin Metalation Reactions

Reaction	pK_3[g]	k_f, $M^{-1}\ s^{-1}$	k_r, $M^{-1}\ s^{-1}$	K_e, M
Zinc hematoporphyrin IX[a]	6.1			1.3×10^{-7}
Zinc en-protoporphyrin IX[b]	5.0	2.4×10^{-1}	8.2×10^{6}	2.8×10^{-8}
Zinc tetra(4-sulfonatophenyl)porphine[c]	4.95[d]	4.8×10^{-1}	1.1×10^{1}	4.4×10^{-2}
Zinc tetra(4-*N*-methylpyridyl)porphine[e]	2.1	3.7×10^{-2}	8.5×10^{-3}	4.4
Zinc tetra(2-*N*-methylpyridyl)porphine[f]	<0	4.9×10^{-4}	2.4×10^{-6}	1.9×10^{2}

[a] Reference 2. [b] Reference 5. [c] Reference 6. [d] pK_3 and pK_4 (4.86) refer to the trisulfonated tetraphenylporphine. [e] k_f from ref 8, k_r from ref 9. [f] Present work. [g] pK_3 for the H_3P^+/H_2P reaction.