Mechanistic Studies on the Reactions of Molybdenum(VI), Tungsten(VI), Vanadium(V), and Arsenic(V) Tetraoxo Anions with the $Fe^{II}Fe^{III}$ Form of Purple Acid Phosphatase from Porcine Uteri (Uteroferrin)

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The $Fe^{II}Fe^{III}$ form of purple acid phosphatase (PAP_r) from porcine uteri (uteroferrin) catalyses the hydrolysis of phosphate esters. In a previous paper kinetic studies on the reactions of PAP_r with five different phosphate moieties, including $[H_2PO_4]^-$ as a prototype, were reported. Here these studies have been extended to include the complexing of tetraoxo XO_4 anions of molybdate(VI), tungstate(VI), vanadate(V), and arsenate(V) with PAP_r. UV−vis absorbance changes are small and the range of concentrations is restricted by the need to maximise monomer XO_4 forms. Rate constants k_{obs}(25 °C) were determined by stopped-flow monitoring of the reactions at ∼520 nm. Absorbance changes with arsenate(V) were too small for rate constants to be evaluated. At pH 5.6 the k_{obs} values obtained are independent of $[XO_4]$ (X = Mo, W, V), in the range $(0.4-10) \times 10^{-4}$ M. At concentrations of $VO_4 > 2.0 \times 10^{-4}$ M formation of $[V_3O_9]^{3-}$ occurs, and a decrease in experimentally determined rate constants is observed. In the pH range 3.5−6.3, as in the case of the PO_4 reagents, rate constants decrease with increasing pH due to the slower reaction of Fe(III)−OH as compared to Fe(III)−OH_2. A mechanism involving rapid XO_4 binding at the more labile Fe(II) is proposed. Slower $[XO_4]$-independent bridging to the Fe(III) then occurs with displacement of H_2O at the lower pH's. Nucleophilic attack of the conjugate-base Fe(III)−OH on the phosphate-ester results in hydrolysis which peaks at pH 4.9. At this pH rate constants/s^{-1} are for $[MoO_4]^{2-}$ (1.33), $[WO_4]^{2-}$ (0.77) and $[H_2VO_4]^-$ (1.44), as compared to values of ∼0.5s^{-1} for the five different phosphates. There is therefore little dependence on the identity of XO_4. With O_2 the $PAP_r \cdot MoO_4$ adduct is less reactive than PAP_r, while $PAP_r \cdot AsO_4$ is more reactive. These results are explained by the effect of coordinated XO_4 on the PAP_o/PAP_r reduction potential.

Introduction

To date mammalian purple acid phosphatases (PAP) from porcine uteri and from beef spleen (M_r∼35 kDa) are the most extensively studied and well characterised proteins of this kind. The enzyme has a binuclear Fe site and a single polypeptide chain (318 amino acids).[1] The $Fe^{II}Fe^{III}$ form, PAP_r, catalyses the hydrolysis of phosphate esters (eq 1).[2−6] The intense

$$O_3P(OR)^{2-} + H_2O \rightarrow HPO_4^{2-} + ROH \qquad (1)$$

absorbance of the active pink $Fe^{II}Fe^{III}$ form, peak at 515nm (ϵ = 4000 $M^{-1}cm^{-1}$), and the inactive purple $Fe^{III}Fe^{III}$ form, PAP_o, peak at 550nm (ϵ = 4000 $M^{-1}cm^{-1}$),[7,8] are due to tyrosine (phenolate) to Fe(III) ligand to metal charge-transfer (LMCT).[5,9] Small but perceptible changes in visible range spectra are observed on coordination of PO_4 or other XO_4 anions to PAP_r. Physical techniques including EXAFS, NMR, and EPR have been applied to better understand the active site of the mammalian enzyme.[10−23] It has been suggested that there is one histidine to each Fe and carboxylate coordination of Asp or Glu residues.[10−16] In addition an H_2O is coordinated to each Fe,[17,18] and from EXAFS studies on the $PAP_o \cdot PO_4$ adduct evidence has been obtained for a bridging phosphate.[19] How-

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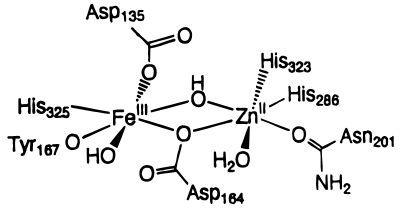

Figure 1. $Zn^{II}Fe^{III}$ active site structure of kidney bean purple acid phosphatase,[25] as a model for the $Fe^{II}Fe^{III}$ active site of the mammalian enzyme.

ever magnetic susceptibility measurements,[5,20−22] do not provide unequivocal evidence for either Fe−O−Fe (in PAP_o) or Fe−OH−Fe (in PAP_r). Also relevant are recent studies on the reaction of labeled adenosine 5′-triphosphate (ATP) with PAP_r, which have demonstrated that transfer of phosphate to an H_2O position on PAP_r occurs with inversion at the phosphorus.[24]

There is as yet no X-ray crystal structure of the mammalian enzyme. However the structure of kidney bean phosphatase ($M_r \sim 55$ kDa per homodimer), which has a $Zn^{II}Fe^{III}$ active site, has recently been determined at a resolution of 2.9Å.[25] The Zn(II) and Fe(III) separation is 3.1 Å, and the metals are bridged by a single O-atom of the carboxylate of Asp-164. A terminal hydroxo ligand (to Fe), terminal aqua (to Zn), as well as a μ-hydroxo bridge have been modeled into the active site structure, Figure 1. When Zn is exchanged for Fe the active site shows spectroscopic behavior nearly identical to the mammalian enzyme,[21,23b] and similarities of the active sites are indicated. However there are also some differences,[25] and the functional relationship between the kidney bean and mammalian enzymes remains to be established.

An unexpected finding from kinetic studies on the reactions of PAP_r (uteroferrin) with five different phosphates $[H_2PO_4]^-$, phenyl phosphate (and the *p*-nitro derivative), pyrophosphate, tripolyphosphate, and adenosine 5′-triphosphate (ATP), is the observation that first-order rate constants for the uniphasic processes are independent of $[PO_4]$.[26] Particularly relevant in the present case is the study with $[H_2PO_4]^-$, which can be regarded as a prototype reaction with no associated hydrolysis step. At pH's in the range 2.6−6.5 the reaction can be summarized by eq 2, where K_o is believed to involve substitution

$$Fe^{II}\bullet\bullet\bullet Fe^{III}(H_2O)(H_2O) + H_2PO_4^- \overset{K_o}{\rightleftharpoons} Fe^{II}\bullet\bullet\bullet Fe^{III}(HPO_4)(H_2O) \overset{k_f}{\underset{k_b}{\rightleftharpoons}} Fe^{II}\bullet\bullet\bullet Fe^{III}(HPO_4)_2 \quad (2)$$

at the more labile Fe(II), and forward and back rate constants k_f and k_b for bridge closure are as defined. At pH < 3.5 Fe(III)−H_2O is present at the active site and PO_4 bridging (k_f) with displacement of H_2O is observed. At higher pH's as the build-up of Fe(III)−OH becomes more extensive (eq 3), and

$$Fe(III)-H_2O \overset{K_a}{\rightleftharpoons} Fe(III)-OH + H^+ \quad (3)$$

there is a slowing down in PO_4 bridge closure due to the greater difficulty in displacing the hydroxide. However from activity measurements the hydrolysis of $O_3P(OR)^{2-}$ increases due to the more extensive participation of Fe(III)−OH, consistent with a mechanism involving nucleophilic attack on the phosphate by the coordinated hydroxide.[26] The latter activity can be studied by monitoring absorbance changes due to the release of ROH = α-naphthol or *p*-nitrophenol from the phosphate esters $O_3P(OR)^{2-}$, and maximizes at pH 4.9 which we believe corresponds to the maximum buildup of Fe(III)−OH. At higher pH values the enzymic activity decreases presumably because of other effects of pH, e.g. on amino acids. In the present work the tetraoxo-anions XO_4, (X = Mo, W, V) and As,[7,12−15] are considered, with the aim of elaborating/establishing further the mechanism.[23,26] Recent kinetic investigations on the oxidation of PAP_r to PAP_o with $[Fe(CN)_6]^{3-}$ have provided evidence for association of anionic complexes within electron-transfer range of the active site.[27] In other studies the effect of replacing the Fe(II) of uteroferrin with Zn(II) has been explored, when the reaction with $[HPO_4]^{2-}$ at pH 4.9 is ∼35-fold faster.[28]

At pH 4.9 both molybdate(VI) and tungstate(VI) act as strong inhibitors for the hydrolysis of *p*-nitrophenyl phosphate by PAP_r uteroferrin (K_i's both 4 μM), whereas arsenate is a weaker inhibitor (K_i = 2−15 mM).[29] In the present work a limiting factor is the tendency of XO_4 anions to oligomerize at the lower pH values, and the need to control conditions so as to maintain monomeric XO_4 forms as the dominant species. Effects which two of the XO_4 reactants have on air oxidation of PAP_r to PAP_o are also explored.

Experimental Section

Protein. Purple acid phosphatase (uteroferrin) was obtained from the allantoic fluids of porcine conceptuses at midpregnancy, and purified according to procedures already described.[7,26]

Tetraoxo Anions. Sodium molybdate, $Na_2[MoO_4]\cdot 2H_2O$ (Sigma), sodium tungstate $Na_2[WO_4]\cdot 2H_2O$ (BDH, Analar), sodium orthovanadate, $Na_3[VO_4]$ (Sigma), and disodium hydrogen arsenate $Na_2H[AsO_4]\cdot 7H_2O$ (Aldrich) were used. At pH < 7 the formation of $[HMoO_4]^-$ and $[HWO_4]^-$ hydroxo forms initiates oligomerization of $[MoO_4]^{2-}$ and $[WO_4]^{2-}$. Relevant acid dissociation constants K_{1a} and K_{2a}/M are as defined in (4) and (5), where pK_a (25 °C) values are respectively 3.65

$$H_2XO_4 \overset{K_{1a}}{\rightleftharpoons} HXO_4^- + H^+ \quad (4)$$

$$HXO_4^- \overset{K_{2a}}{\rightleftharpoons} XO_4^{2-} + H^+ \quad (5)$$

and 3.55 (Mo; I = 1.0 M)[30] and 3.5 and 4.6 (W; I = 0.10 M).[31] In the latter case it is noted that pK_{2a} > pK_{1a}. Whereas O-atom exchange reactions of the d^o ions $[MoO_4]^{2-}$ and $[WO_4]^{2-}$ with H_2O and OH^- are known to be quite fast,[32] those for PO_4 ions are much slower.[33] In the range of pH explored VO_4 oligomerization is observed.[34] The interconversion of V(V) monomer and dimer forms has been studied by NMR.[35,36] An essential difference is that oligomerization is more pronounced at the higher pH's ≥ 5, and significant amounts of $[V_3O_9]^{3-}$ are formed with $[VO_4]$ at levels ≥ 0.20mM. Relevant pK_a's are for H_3VO_4 (3.2) and $H_2VO_4^-$ (3.8) at I = 0.50 M ($NaClO_4$). In all cases the tendency for oligomerization to occur is minimized by working at

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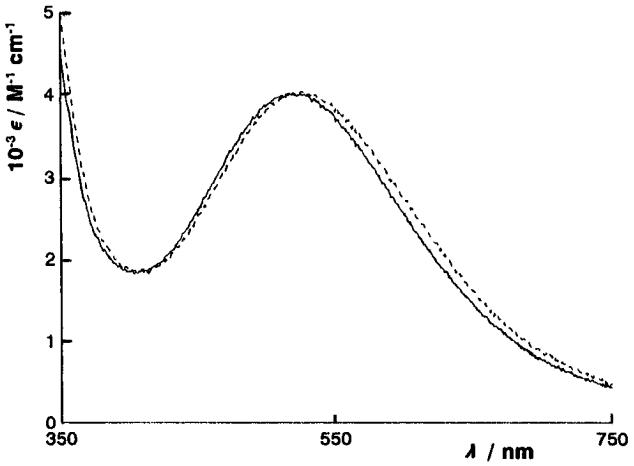

Figure 2. UV−vis absorbance spectra for (a) the active $Fe^{II}Fe^{III}$ form of PAP_r peak at 515nm (—), and (b) the molybdate adduct $PAP_r{\cdot}MoO_4$ peak at 525nm (- - -) formed by addition of molybdate (2.8×10^{-4} M) to PAP_r (2.6×10^{-5} M) at pH 5.6.

low total XO_4 concentrations. The dependencies of rate constants on $[XO_4]$ were explored at the higher pH of 5.6 compared to 4.6 for the studies with the phosphates.[26] At pH 5.6 "safe" upper limits for XO_4 were found to be 1.0×10^{-3} M (Mo), 4.0×10^{-4} M (W), and 2.0×10^{-4} M (V). To study the effects of pH concentrations 1.5×10^{-4} M (Mo) 3.0×10^{-5} M (W) and 1.6×10^{-4} M (V) were adopted. Literature pK_a's (25 °C) for H_3AsO_4 are $pK_{1a} = 2.24$, $pK_{2a} = 6.94$, and $pK_{3a} = 11.50$, at an ionic strength I extrapolated to zero.[37] The dominant forms present under the conditions adopted for these studies are accordingly $[MoO_4]^{2-}$, $[WO_4]^{2-}$, $[HVO_4]^-$, and $[H_2AsO_4]^-$, with some tendency for protonation to occur at the lower pH's.

Formation Constants XO_4 with PAP_r. Inhibition constants (K_i) from the effect of XO_4 anions on PAP_r (uteroferrin) phosphate ester hydrolysis, enable formation constants K/M^{-1} as defined in (6) to be

$$PAP_r + XO_4 \overset{K}{\rightleftarrows} PAP_r{\cdot}XO_4 \qquad (6)$$

determined (by taking reciprocals). At pH 4.9 literature K/M^{-1} values are 2.5×10^5 (Mo), 2.5×10^5 (W), and 500 (As) and at pH 5.5 are 2.0×10^5 (Mo), 1.4×10^6 (W), 1562 (As), and 2.5×10^4 (V).[7,29] These are more favorable than the corresponding value for phosphate (83 M^{-1} at pH 4.9), and it was possible therefore to work at lower concentrations. Thus with $[PO_4] < 1$mM there are no detectable absorbance changes, whereas in the present studies with e.g. $[WO_4]^{2-}$ it was possible to use concentrations as low as 3×10^{-5} M.

Kinetic Studies. These were carried out at 25.0 ± 0.1 °C, with ionic strength $I = 0.100 \pm 0.001$ M adjusted using NaCl. Acetate/acetic acid buffer (40mM) was used to vary the pH in the range upto pH 5.8, and Bis/Tris buffer (Sigma) at pH > 5.8. Reactions were monitored at peak positions/nm for the respective products $PAP_r{\cdot}MoO_4$ (525),[18] $PAP_r{\cdot}WO_4$ (520),[38] $PAP_r{\cdot}VO_4$ (520),[39] which compare with PAP_r (515). In the case of $PAP_r{\cdot}AsO_4$ the product peaks at 510 nm.[7] All these peaks have ϵ values close to 4000 $M^{-1}cm^{-1}$. A Dionex D-110 stopped-flow spectrophotometer was used, and rate constants k_{obs}/s^{-1} were evaluated using OLIS programs (Bogart, GA) giving a nonlinear regression fit. In the case of arsenate absorbance changes were not large enough to enable rate constants to be determined. Absorbance changes observed in the case of molybdate are illustrated in Figure 2. In all oxidation studies, $PAP_r \rightarrow PAP_o$, ethylenediaminetetraacetate (0.10 mM) was added to complex any free Fe ions. This is particularly important at the higher oxyanion concentrations when slight denaturation of the enzyme occurs.

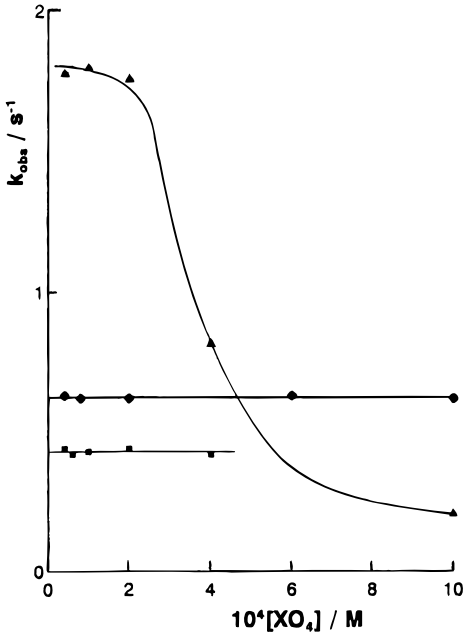

Figure 3. First-order rate constants k_{obs}(25 °C) for the reaction of the $Fe^{II}Fe^{III}$ form of PAP_r (uteroferrin) with a given concentration of molybdate $[MoO_4]^{2-}$ (●), tungstate(VI) $[WO_4]^{2-}$ (■), and vanadate-(V) $[HVO_4]^{2-}$ (▲) at pH 5.6, $I = 0.100$ M (NaCl).

Table 1. Variation of First-Order Rate Constants (25 °C) for the Reactions of Tetraoxo Anions XO_4 with Active $Fe^{II}Fe^{III}$ PAP_r (uteroferrin) (3.0−8.0) × 10^{-6} M, at Different pH's, $I = 0.100$ M (NaCl)

Reaction with Molybdate(VI) (1.5×10^{-4} M)										
pH	3.88	4.20	4.30	4.45	4.63	4.78	5.10	5.25	5.38	5.60
k_{obs}/s^{-1}	7.7	5.7	3.9	2.5	1.90	1.44	1.09	0.96	0.75	0.62
pH	5.92									
k_{obs}/s^{-1}	0.53									

Reaction with Tungstate(VI) (3.0×10^{-5} M)									
pH	3.54	3.74	4.10	4.20	4.40	4.62	4.80	5.10	5.38
k_{obs}/s^{-1}	10.7	9.5	4.0	2.50	1.30	0.99	0.80	0.70	0.51
pH	5.72	5.79	6.30						
k_{obs}/s^{-1}	0.38	0.37	0.26						

Reaction with Vanadate(V) (1.6×10^{-4} M)								
pH	3.56	3.90	4.20	4.40	4.76	5.20	5.62	6.01
k_{obs}/s^{-1}	8.4	7.0	4.2	2.33	1.58	1.22	0.79	0.70

Results

Kinetics of PAP_r with XO_4. First-order rate constants k_{obs} were determined at pH 5.6 with concentrations of XO_4 as illustrated in Figure 3. No variations in k_{obs} are observed in the case of MoO_4, $k_{obs} = 0.62 \pm 0.01$ s^{-1}, or WO_4, $k_{obs} = 0.43 \pm 0.02$ s^{-1}. With vanadate(V) as H_2VO_4-, $k_{obs} = 1.78 \pm 0.02$ s^{-1}, but at concentrations > 2.0×10^{-4} M there is an apparent decrease in k_{obs} due to formation of $[V_3O_9]^{3-}$,[34] Figure 3.

A listing of rate constants k_{obs} with pH in the range 3.5−6.3 is given in Table 1. The variations in rate constants with pH are shown in Figure 4. A single uniphasic decrease is observed in each case with increasing pH, and no additional steps are apparent as in the case of some PO_4 reactants, which exhibit other protonation equilibria in the pH range studied.[26] The lines drawn in Figures 3 and 4 are to indicate trends and are not computer fits since no quantitative treatment is given.

Rate Constants for Reaction with O_2. Whereas an equilibrium mix containing $PAP_r{\cdot}PO_4$ is oxidized more quickly by air than PAP_r,[26] in the case of $PAP_r{\cdot}MoO_4$ at pH 5.0 no reaction is observed over 24 h. Absorbance changes were monitored at ~620 nm. The conditions explored were with PAP_r at 2.65×10^{-5} M and MoO_4 at 0.31 and 0.63 mM. With arsenate on the

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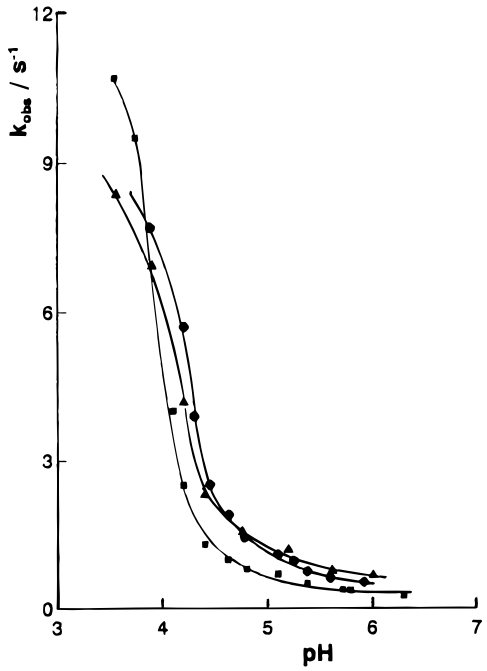


Figure 4. Variation of first-order rate constants k_{obs}(25 °C) with pH for reactions of the active $Fe^{II}Fe^{III}$ form of PAP_r (uteroferrin) with dominant forms of molybdate(VI), $[MoO_4]^{2-}$ (●), tungstate(VI), $[WO_4]^{2-}$ (■), and vanadate(V), $[H_2VO_4]^-$ (▲), I = 0.100 M (NaCl).

other hand it was found that PAP_r, AsO_4 is more rapidly air oxidized than PAP_r. Rate constants $10^5k_o/s^{-1}$ obtained at different arsenate $[AsO_4]$/mM levels, $PAP_r = 3.03 \times 10^{-5}$ M and $[O_2] = 2.64 \times 10^{-4}$ M (air saturated solutions), are as follows: 6.4 (10.1), 6.4 (36.3), and 6.2 (58.2), indicating no dependence on $[AsO_4]$.

Discussion

Within the limits imposed by the need to retain monomeric tetraoxo XO_4 ions it has been possible to determine first-order rate constants for the reaction of XO_4 (in excess) with PAP_r (X = Mo, W, V) and demonstrate that as in the case of PO_4 reactants[26] rate constants are independent of $[XO_4]$, Figure 3. Thus at pH 5.6 it was possible to use a range of molybdate(VI) concentrations up to 1.0×10^{-3} M, and tungstate(VI) to 4×10^{-4} M, but with vanadate(V) (2.0×10^{-4} M) more restrictive conditions were necessary to avoid formation of $[V_3O_9]^{3-}$. Absorbance changes in the case of X = As were too small for rate constants to be determined. It was also possible to vary the pH within the range 3.5−6.3, while retaining mononuclear species, Figure 4. The mononuclear ions which are relevant are $[MoO_4]^{2-}$, $[WO_4]^{2-}$, and $[H_2VO_4]^-$, respectively. The pH at the lower limit was restricted to 3.5 for Mo(VI) and >4.0 for W(VI).

The mammalian $Fe^{II}Fe^{III}$ form of PAP_r has properties in common with the $Zn^{II}Fe^{III}$ enzyme, Figure 1. In particular the H_2O coordinated to each metal is an important feature. A step involving acid dissociation of Fe(III)-H_2O has been suggested,[23,26] in order to explain the decrease in rate constants with increasing pH. Support comes from the observation that, e.g., coordinated and uncoordinated $[H_2PO_4]^-$ do not undergo deprotonation in this range of pH, and therefore the effect of pH must originate from the protein. There are alternative explanations, but if there is an H_2O coordinated to the Fe^{III}, it would be expected to exhibit acid behavior in this range. With vanadate(V) at $\leq 5 \times 10^{-4}$ M, the protonated form $[H_3VO_4]$ is dominant at pH's 3.2−3.8 and $[H_2VO_4]^-$ from 3.8 to 7.8, and this may contribute alongside Fe(III)−OH and Fe(III)-H_2O equilibration to the trends observed for VO_4 in Figure 4. In the previous studies with phenyl phosphate, pyrophosphate, tripolyphosphate and ATP, a second pH effect was clearly apparent suggesting that the degree of protonation of the Fe(II)-coordinated XO_4 may also be relevant.[26] In all other respects the kinetics reported in this paper are similar to those observed for $[H_2PO_4]^-$,[26] and the mechanism can be expressed as in (7). Octahedral high-spin aqua Fe^{II} is 10^2-10^3 times more

$$Fe^{II}(H_2O)\cdots Fe^{III}(H_2O) + XO_4 \rightleftharpoons Fe^{II}(XO_4)\cdots Fe^{III}(H_2O) + H_2O \rightleftharpoons Fe^{II}(XO_4)\cdots Fe^{III}(XO_4) + 2H_2O \quad (7)$$

labile than the corresponding aqua Fe^{III} consistent with these changes.[26] The observation that trimethyl phosphate, with only one oxo group, does not appear to react at the Fe^{III}, but inhibits reaction with $[H_2PO_4]^-$, supports a mechanism in which the initial change is at the Fe^{II}.[26] The second equilibrium is we believe important in determining the kinetic behavior observed. In addition to Fe(III)−OH formation, protonation/deprotonation of XO_4 would be expected to contribute if monodentate XO_4 and $\mu(XO_4)$ forms exhibit different degrees of protonation in the range of pH explored. Since only one process is apparent in the pH profiles in Figure 4 the behavior is like that of $[H_2PO_4]^-$ and simpler than in the case of the other phosphates.[26]

With these additional studies it is clear that all the reactions so far studied are independent of $[XO_4]$ and that this is not a special feature of the PO_4 reagents. The sequence in (7) can explain the independence of $[XO_4]$ if K values for the first stage are large so that $K[XO_4] \gg 1$. The extent of formation of (μXO_4) products in the second stage of (7), gives rise to the different inhibition effects referred to in the experimental section. From redox studies on the oxidation of PAP_r with $[Fe(CN)_6]^{3-}$,[27] saturation kinetics apply and $K = 540$ M^{-1} for association of the oxidant to PAP_r prior to electron transfer. Moreover redox-inactive complexes $[Cr(CN)_6]^{3-}$ ($K = 550$ M^{-1}) and $[Mo(CN)_8]^{4-}$ ($K = 1580$ M^{-1}) inhibit the $[Fe(CN)_6]^{3-}$ reaction and provide further evidence for electrostatically controlled association steps as a part of these studies. There is therefore at least one favorable site for association of anionic reactants within electron-transfer range of the active site of PAP_r. The initial association step can be included in the mechanism to give (8) − (10), where (8) and (9) together give extensive complexation.

$$PAP_r + XO_4 \overset{K_{assoc}}{\rightleftharpoons} PAP_r \cdot XO_4 \quad (8)$$

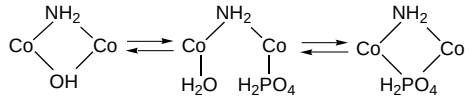
$$PAP_r \cdot XO_4 \overset{K_{Fe2}}{\rightleftharpoons} Fe^{II}(XO_4)\cdots Fe^{III}(H_2O) \quad (9)$$

$$Fe^{II}(XO_4)\cdots Fe^{III}(H_2O) \overset{k_f}{\underset{k_b}{\rightleftharpoons}} Fe^{II}(XO_4)\cdots Fe^{III}(XO_4) \quad (10)$$

It is proposed that H-bonding as well as overall charge contribute to (8) to account for the favorable nature of the interaction. The overall equilibrium constant for (8) − (10) can be expressed as K_{assoc} K_{Fe2} K_{Fe3}, where $K_{Fe3} = k_f/k_b$. The rate constant k_{obs} has forward k_f K_{assoc} K_{Fe2} and reverse k_b components.

The reaction which models most closely the formation of a μ-phosphato product is that involving the interconversion of $[NH_3]_4Co\mu(NH_2,OH)Co(NH_3)_4]^{4+}$ and $[(NH_3)_4Co\mu(NH_2, H_2PO_4)Co(NH_3)_4]^{4+}$ in the presence of $H^+/[H_2PO_4]^-$, as indicated in (11).[40] Similar reactions of other tetraoxo and related anions

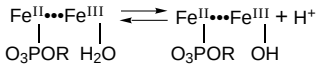
(40) Edwards, J. D.; Foong, S-W.; Sykes, A. G. *J. Chem. Soc., Dalton Trans.* **1973**, 829.



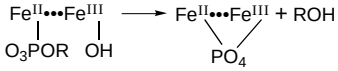
(11)

have been reported.[41] Both equilibria in (11) are rapid for substitution at Co(III), and no buildup of the intermediate was detected. The close proximity of ligands involved in bridge closure accounts for the faster rate processes.

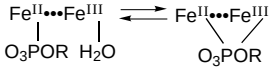
In order to account for phosphatase activity the mechanism (12)−(15) is proposed. In this mechanism conjugate-base formation in (12) leads to hydrolysis of the $O_3P(OR)^{2-}$ ester in (13), but no similar hydrolysis is proposed for (14). Rate



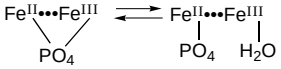
(12)



(13)



(14)



(15)

constants k_{obs} for $[H_2PO_4]^-$, $[MoO_4]^{2-}$, $[WO_4]^{2-}$, and $[H_2VO_4]^-$ show little variation and are in the range $7-12\ s^{-1}$ at pH ∼3.5. This suggests that the rate controlling process for k_f in (10) maybe loss of an H_2O from the Fe(III). At pH 4.9, which corresponds to maximum enzyme activity, (13) will be at its most effective and rate constants k_{obs} for the different XO_4's are also in a narrow range $0.50-1.44\ s^{-1}$. This is surprising in view of the different labilities of XO_4 anions,[32,33] suggesting that some other process may be rate determining and that a further elaboration may be required. One possibility is that equilibration rate constant equal to $k_f + k_b$ may be effective with k_b rather than k_f dominant. Unfortunately there is insufficient information at present to quantify k_f and k_b separately.

Finally the reactivity of $PAP_r \cdot XO_4$ adducts with O_2 at pH 5.0 is accounted for by the different reduction potentials (vs nhe). The latter have been determined for the PAP_o/PAP_r couple (367 mV) and for PAP_r adducts with PO_4 (183 mV), and AsO_4 (262 mV).[18] In the studies with AsO_4 the kinetics are independent of $[AsO_4]$ indicating complexing prior to reaction with O_2. Rate constants for the O_2 reaction with $PAP_r \cdot AsO_4$ ($6.3 \times 10^{-5} s^{-1}$) and $PAP_r \cdot PO_4$ ($6.8 \times 10^{-5}\ s^{-1}$)[26] are very similar. The reduction potential in the presence of MoO_4 is high at 498 mV (pH 6.01) and no reaction of $PAP_r \cdot MoO_4$ with O_2 was observed over ∼24 h. Whereas MoO_4 and WO_4 bind tightly to PAP_r, phosphate and arsenate bind more weakly, which may facilitate O_2 access.[14]

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