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Iron(III)-Promoted Hydrolysis of 4-Nitrophenyl Phosphate

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The roles of metal ions (metal centers) in promoting the hydrolysis of phosphate esters and polyphosphates have been the subject of considerable study, and a great deal is now known about the ways in which metal ions can function in these processes.[2−9] Much of the interest in this area relates to the requirement of metal ions for biological phosphoryl transfer.

In model (nonenzymatic) systems, the most effective metal centers in promoting phosphate ester/polyphosphate hydrolysis have been found to be Co^{III} (in such complexes as $[Co(trpn)(OH)(H_2O)]^{2+}$, $[Co(tamen)(OH)(H_2O)]^{2+}$, $[Co(tn)_2(OH)(H_2O)]^{2+}$, etc.),[2−7] the trivalent lanthanides Ln^{III},[4a,8,9] and, for divalent metals, Cu^{II} [4,8] and Pb^{II}.[8]

In biological systems, iron is an essential component of the purple acid phosphatases (PAPs); these are dinuclear iron enzymes that catalyze the *in vitro* hydrolysis of phosphate esters under weakly acidic conditions.[10−12] While the *in vivo* function has not been established,[12a] a great deal of information is now available on these enzymes, as well as on a large number of model systems which have been constructed, at least in part, in attempts to reproduce key elements of the enzymes.[13] For the enzymes the $Fe^{III}-Fe^{II}$ form is active, while the $Fe^{III}-Fe^{III}$ form is inactive or at least is of low activity. Active $Fe^{III}-M^{II}$ forms, where M = Zn, Co, or Cd, have also been prepared.[11] Studies on the PAPs and on model systems have produced a wealth of structural, spectroscopic, and other information, but we are not aware of any model systems that have been shown to promote phosphate ester hydrolysis.

The present study was undertaken to see if we could promote the hydrolysis of 4-nitrophenyl phosphate, in the weakly acidic to neutral pH range, using a simple complex of iron(III). Obviously one could not simply bring the aquairon(III) ion into this pH region, because of its ready hydrolysis with the formation of insoluble hydrated oxides.[14] In the experiments reported here, the 1:1 complex between Fe^{3+} and $EDDA^{2-}$ (H_2EDDA = $HOOCCH_2NHCH_2CH_2NHCH_2COOH$), which we abbreviate as $(EDDA)Fe^{III}$, was prepared in solution and then used to react with NPP (4-nitrophenyl phosphate).

Experimental Section

The complex $(EDDA)Fe^{III}$ was generated in solution by mixing equimolar quantities of iron(III) nitrate (aqueous solution prepared from $Fe(NO_3)_3{\cdot}9H_2O$, ACS reagent grade) and H_2EDDA (ethylenediamine-*N,N'*-diacetic acid, Sigma Chemical Co. reagent), with subsequent adjustment of the pH to the middle range (pH 5−7) in reaction solutions. Stock Fe^{3+}/EDDA solutions[15] (5.0×10^{-2} M in both iron(III) nitrate and ethylenediamine-*N,N'*-diacetic acid) were prepared as needed. Solutions of 4-nitrophenyl phosphate (NPP) were freshly prepared from the disodium salt hexahydrate (Johnson Matthey Co.). The pH of solutions was controlled with Bis-tris/HNO_3 buffer, and in rate measurements the ionic strength was maintained at 0.10 M with KNO_3.

Early experiments demonstrated that $(EDDA)Fe^{III}$ *does* promote the hydrolysis of 4-nitrophenyl phosphate (NPP) to 4-nitrophenol(ate) (NP) and orthophosphate (P_i). The following method was used to establish this promotion, as well as to measure the rates of the process. Reaction solutions were prepared by adding aliquots of Fe^{3+}/EDDA stock solution to freshly prepared solutions containing NPP, Bis-tris buffer, and, in kinetic runs, KNO_3. At appropriate time intervals samples of the reaction solution were added to excess EDTA solution at pH 9.4, and the resulting quenched solution was diluted to a known volume. In the early experiments, absorbance measurements on quenched solutions through the range 360−500 nm established the production of 4-nitrophenolate. For rate measurements, reaction solutions were held at 25 °C. At appropriate time intervals 0.10 mL aliquots were withdrawn; each aliquot was added to 1.0 mL of 0.25 M EDTA solution at pH 9.4, the volume was made up to 5.0 mL, and the absorbance was measured at 400 nm. The hydrolysis studies covered a range of pH (5.0−7.0) and a range of Fe^{3+}/EDDA and NPP concentrations (1.25×10^{-3} to 15.0×10^{-3} M). First-order rate constants were obtained by linear regression from plots of $\ln(A_\infty - A_t)$ *versus* time. Values of A_∞ were calculated using the absorbancy coefficient of 4-nitrophenolate at 400 nm for pH 9.4 (ϵ = 18 700 $M^{-1}\,cm^{-1}$); at this wavelength absorbance contributions from the other components of the quenched solutions could be neglected. This procedure was considered to be more reliable than using experimental values of A_∞, due to possible interference from secondary reactions at longer times. For the faster reactions, which were followed to >80% reaction, the $\ln(A_\infty - A_t)$ *versus* time plots showed good linearity to about 50% reaction; correlation coefficients were routinely >0.99. At longer times there was some fall-off in rates from first-order behavior. The unpromoted and slowest reactions were followed to only 1−2% reaction; in spite of the small degree of reaction, the rate constants are quite reliable due to the high value of the absorbancy coefficient for 4-nitrophenolate.

For comparison purposes, in the discussion which follows, we report values for the *initial rates* of NP production, v_0, in mol $L^{-1}\,s^{-1}$. These values are obtained from the observed first-order rate constants using the relation $v_0 = k_{obs}[NPP]_0$, where the zero subscripts refer to zero time.

Results and Discussion

All experiments were carried out with Fe^{3+} and ethylenediamine-*N,N'*-diacetic acid (EDDA) in a 1:1 molar ratio. A

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(15) EDDA is used here and elsewhere to represent any of the species H_2EDDA, $HEDDA^-$, and $EDDA^{2-}$. The degree of protonation and charges are often omitted for better readability.

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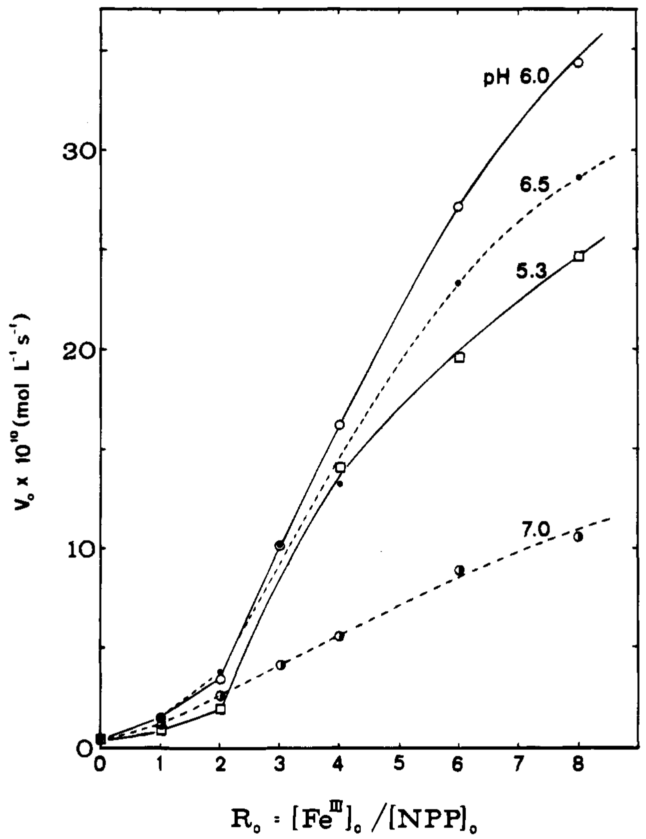


Figure 1. Initial rate of NPP hydrolysis, v_o, as a function of $[Fe^{III}]_0/[NPP]_0$. For all solutions, $[NPP]_0 = 1.25 \times 10^{-3}$ M, $I = 0.10$ M, and $T = 25.0$ °C.

principal purpose for the EDDA was to keep the iron in solution.[16-18] Also, we could expect the EDDA would tie up four coordination sites, leaving two sites per iron, for water and/or hydroxide. On this basis, a simple working hypothesis was that one of the latter two sites would be available for monodentate NPP coordination and the other for hydroxide (or water), which could attack the NPP, leading to hydrolysis. Hydrolysis is indeed observed (see below). Analysis of the results, however, points to a more complex mode of reaction than that suggested above.

Experimental results from this study are summarized in Figures 1 and 2. Figure 1 provides a plot of initial rates for production of 4-nitrophenol(ate) (NP), v_0, versus the *ratio* of initial concentrations, $R_0 = [Fe^{III}]_0/[NPP]_0$, for constant $[NPP]_0$. The rates observed in the absence of Fe^{III} (i.e., for $R_0 = 0$) are in reasonable agreement with values extrapolated from the literature.[19] Adding (EDDA)Fe^{III} obviously promotes the hydrolysis (Figure 1), although in a manner that is highly dependent on R_0 as well as on pH. In Figure 2 we show the dependence of v_0 on pH for two values of R_0.

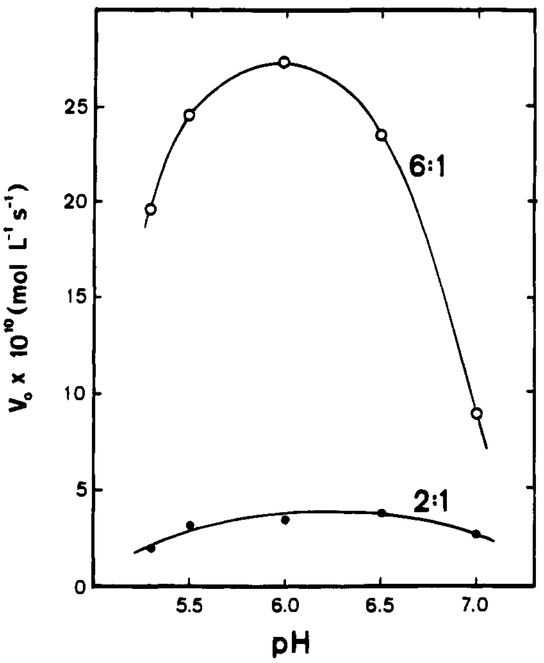
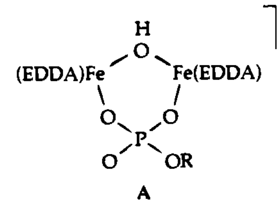


Figure 2. Initial rate of NPP hydrolysis, v_0, as a function of pH. For all solutions, $[NPP]_0 = 1.25 \times 10^{-3}$ M, $I = 0.10$ M, and $T = 25.0$ °C.

Figure 1 displays a marked change in slope at $R_0 = 2$; below this ratio the values of v_0 are notably small. The observations do not allow an interpretation in which the most reactive species, for $R_0 > 2$, is a simple mononuclear complex involving *one* iron (and EDDA) and *one* NPP. The sharp change in slope at $R_0 = 2$, seen for pH 5.3, 6.0, and 6.5, in fact supports the view that a 2:1 iron to NPP complex is formed, fairly quantitatively at $R_0 = 2$, that this complex has a low reactivity toward hydrolysis, and that a considerably more reactive species, which contains three irons per NPP, is developed by reaction of a third iron with the 2:1 complex at R_0 values of >2.[20]

Our results are consistent with the suggestion that the 2:1 complex has a structure of type **A**.



A

Structure **A** relates to the long known $[Fe_2(OH)_2]^{4+}$ aqua ion,[21] and, more particularly, to several very recently established hydroxo-bridged diiron(III) complexes[13] including the hydroxo/

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(16) We have not undertaken a detailed study of complex formation between Fe^{3+} and ethylenediamine-*N,N'*-diacetic acid (*sym*-EDDA), and no literature reports on this system are available. However, complex formation between Fe^{3+} and the *isomeric* ethylenediamine-*N,N*-diacetic acid (*unsym*-EDDA) has been the subject of a detailed investigation by Anderegg and Schwarzenbach;[17a] these workers found log $K = 16.92$ at 20 °C and $I = 0.1$ M, where $K = [ML]/[M][L]$ and H_2L represents the free diacetic acid. The stabilities of the 1:1 complexes of Fe^{3+} with *sym*-EDDA and with *unsym*-EDDA should be quite similar, although log K for the former system may be lower by about *one* unit, as indicated by a comparison of stabilities for complexes formed by the two EDDA isomers and various other metal ions.[17b,18] On this basis, we estimate that more than 99.9% of the Fe^{3+} and *sym*-EDDA will be in the form of the 1:1 complex, for 1:1 Fe^{3+}/EDDA mixtures under the concentration and pH conditions used in the present investigation.

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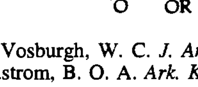
(20) Our results also include the possibility that a 1:1 iron to NPP complex, of even lower reactivity than the 2:1 complex, predominates at $R_o = 1$. Such a 1:1 complex would be likely to have a structure of type



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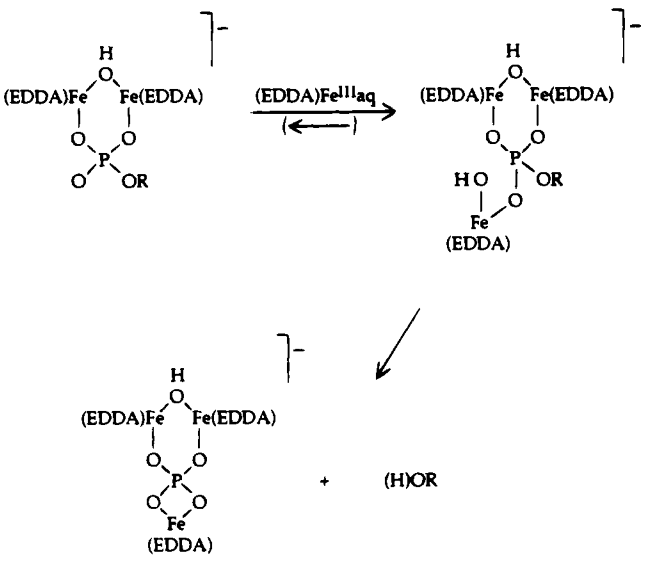
Scheme 1



bis(phosphate ester)-bridged $[Fe_2(OH)\{O_2P(OC_6H_5)_2\}_2(HB(pz)_3)_2]^+$ ion.[13a] Structure **A** would be expected to be unreactive toward hydrolysis of the bridging phosphate ester, whereas it is easy to see how participation of a third (EDDA)Fe^{III} unit could lead to a reactive species. A possible mode of hydrolysis is outlined in Scheme 1. Here the third iron provides a coordinated hydroxide, positioned to attack the phosphorus, hence providing a path for hydrolysis of the ester function.

For R_0 values of >2, the hydrolysis rates rise from low pH to reach a maximum at pH close to 6.0 and then fall off, markedly from pH 6.5 to 7.0. Similar maximization of rates in the middle pH region has been observed for a number of metal ion promoted hydrolysis reactions of phosphate esters and polyphosphates, and the reasons for this behavior have been extensively discussed.[22] In the present case, there are a number of possible reasons for the lowering of rates between pH 6 and 7, including the likelihood that the 2:1 complex loses its integrity by pH 7 (the break at the 2:1 ratio is no longer seen) and the conversion of (EDDA)Fe^{III}(aq) into unreactive dihydroxo and dimeric complexes.

The most significant feature of this study is the demonstration of a set of conditions under which iron(III) promotes the hydrolysis of a simple orthophosphate ester. For the optimal conditions reported here (pH 6.0, $[Fe^{III}]_0/[NPP]_0 = 8$) the rate enhancement is ~160-fold (for pH 6.0, $v_0 = 34 \times 10^{-10}$ M s^{-1} at the 8:1 ratio *versus* 2.1×10^{-11} M s^{-1} for the unpromoted reaction). While the rate enhancements are modest, these results can provide a basis for the further development of models for the purple acid phosphatases (PAPs) that lead to actual phosphate ester hydrolysis.

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[22] See, for example, ref 4b.