

in this unsymmetrical azoalkane.

The dimethylallyl radical appears first, as one would predict on the basis that the weaker C-N bond to the dimethylallyl group (27 kcal/mol) should rupture before the stronger C-N bond to methyl (52 kcal/mol).¹ The resulting methyldiazenyl radical persists for 10 to 14 ns under the collisional conditions used here before fragmenting into N₂ plus CH₃[•]. This lifetime is remarkably consistent with the 11-ns value deduced for methyldiazenyl in hydrocarbon solution by Engel and Gerth³ on the basis of thermal activation parameters. Quantum chemical calculations predict that dissociation of methyldiazenyl is an exothermic but activated process with estimated barrier heights of 6.3-17.4 kcal/mol.^{5a,19} Because of this barrier, we expect that the persistence of the methyldiazenyl radical will depend strongly on its internal energy content. In the gas phase, where collisional relaxation of vibrational energy is far slower than in solution, the methyldiazenyl at early times retains the nascent excitation level with which it is formed as the first C-N bond breaks. For MAMB, the large dimethylallyl fragment has 36 vibrational modes and is able to carry away a significant fraction of the excess energy available from the 80.6-kcal/mol excitation photon. This would leave the methyldiazenyl fragment with less vibrational energy, and thus a longer lifetime, than from a similar stepwise photodissociation of azomethane. We suspect that our failure to resolve a stepwise mechanism in previous azomethane vapor studies⁸ may therefore reflect a shorter lifetime for methyldiazenyl rather than a qualitatively different dissociation mechanism. Finally, we note that since photodissociation in azoalkanes is believed to proceed from high levels of the electronic ground state populated by in-

ternal conversion,^{14,15} the stepwise process observed in MAMB should also occur in thermolysis of this compound.

Conclusions

By using time-resolved detection of primary photoproducts, we have kinetically resolved the stepwise breakage of C-N bonds in the ultraviolet-induced dissociation of an unsymmetrical azoalkane. Although the intermediate methyldiazenyl radical was not directly observed, its lifetime was inferred to be 12 ± 2 ns under our gas-phase experimental conditions. We believe that the sequential mechanism applies to thermal as well as photoinduced dissociation of this compound, since both are thought to proceed on the S₀ electronic surface. In addition, it seems quite likely that the studied compound qualitatively represents the entire class of unsymmetrical azoalkanes and that these may now confidently be viewed as dissociating sequentially rather than simultaneously.

These results do not bear directly on the homolysis mechanism of symmetric azoalkanes, such as azomethane. However, if we are able to characterize the nascent rotational and vibrational distributions of the N₂ and CH₃[•] fragments formed from MAMB through dissociation of the methyldiazenyl radical, then these distributions may be compared to those from azomethane and the outcome used to establish or exclude a common intermediate for the two cases. These and related studies are planned for the future.

Acknowledgment. We are grateful to the National Science Foundation and the Robert A. Welch Foundation for research support. Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work. R.B.W. thanks the Alfred P. Sloan Foundation for a Research Fellowship; K.A.B. thanks the A.R.C.S. Foundation for a graduate scholarship.

Registry No. MAMB, 105018-56-2; AMB, 71647-31-9.

(19) (a) Holmes, T. A.; Hutchinson, J. S., Rice University, personal communication. (b) Yamashita, K.; Kaminoyama, M.; Yamabe, T.; Fukui, K. *Chem. Phys. Lett.* **1981**, 83, 78.

Pyrophosphate Formation from Acetyl Phosphate and Orthophosphate Anions in Concentrated Aqueous Salt Solutions Does Not Provide Evidence for a Metaphosphate Intermediate¹

Daniel Herschlag and William P. Jencks*

Contribution No. 1603 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254. Received June 3, 1986

Abstract: The formation of pyrophosphate (PPi) from the anions of acetyl phosphate (AcP) and orthophosphate (Pi) in concentrated aqueous sodium perchlorate is approximately first order in each reactant. The pH dependence of the reaction in the presence of 0.25 M Pi monoanion and 6.4 M sodium perchlorate at 54 °C shows different yields of PPi from AcP monoanion and dianion. This result is inconsistent with trapping of a free metaphosphate intermediate by Pi monoanion and water. The results are consistent with a concerted mechanism for PPi formation and yields of PPi from the different ionic species of 11.7% for AcP²⁻ and Pi²⁻, 7.8% for AcP²⁻ and Pi⁻, and 4.2% for AcP⁻ and Pi⁻. Measurement of rate constants and partitioning with AcP²⁻, 0.25 M Pi²⁻, and 6.4 M sodium perchlorate, in the presence and absence of pyridine, show that the phosphorylated pyridine monoanion yields 40% PPi, whereas AcP²⁻ yields 10% PPi under the same conditions. The formation of PPi from AcP and Pi dianions is at least fourth order in sodium ion in concentrated sodium perchlorate solutions. The corresponding reaction of the monoanions is second order in sodium ion. The order of effectiveness of salts for facilitation of the formation of PPi from the dianions of AcP and Pi is NaClO₄ > NaCl, KCl, CsCl, and NaCl > CsCl. However, increasing the concentration of sodium perchlorate from 0.1 to 6.6 M changes the rate constant for hydrolysis of AcP mono- and dianion by <30% at 39 and 54 °C. Values of $\Delta H^\ddagger = 29$ kcal mol⁻¹ and $\Delta S^\ddagger = +12$ eu for PPi formation from the dianions of AcP and Pi and $\Delta H^\ddagger = 19$ kcal mol⁻¹ and $\Delta S^\ddagger = -20$ eu for PPi formation from the monoanions were obtained from the yields of PPi and the rates of hydrolysis at 39 and 54 °C. The high concentrations of sodium ion decrease electrostatic repulsion between the anions of AcP and Pi and may catalyze the formation of PPi by bridging the anions of AcP and Pi. These results provide no evidence for the existence of a metaphosphate intermediate in reactions in aqueous solution.

The reactions of phosphate monoesters and related compounds with solvent and other nucleophilic reagents involve nearly com-

plete bond breaking with the leaving group and little bond formation to the nucleophile. For example, the rapid hydrolysis of

phosphate monoester monoanions and some dianions proceeds with entropies of activation near zero and no significant solvent isotope effect, as well as a large negative value of β_{lg} for the dianions and a large bridge ^{18}O isotope effect for 2,4-dinitrophenyl phosphate dianion.^{2,3} In addition, phosphate monoesters and phosphorylated guanidine exhibit low selectivity: the partitioning between components of mixed solvents (ROH/HOH) is often equal on a molar basis,⁴ and β_{nuc} for the reaction of pyridines with dinitrophenyl phosphate dianion is zero.^{5,6} The hydrolysis of the mono- and dianions of AcP⁸ proceeds with entropies and volumes of activation close to zero and no significant solvent isotope effect; the hydrolysis of substituted benzoyl phosphate dianions follows a Hammett correlation with $\rho = 1.2$; and the hydrolysis of AcP is accompanied by appreciable formation of PPI in concentrated aqueous sodium perchlorate solutions.^{9,10} With the exception of PPI formation from AcP, all of these results serve to characterize the transition state; they show that the transition state is open and metaphosphate-like.

These observations led workers to support the hypothesis that metaphosphate is an intermediate in the hydrolysis of phosphate monoesters and related compounds.^{2,11,12} For example, DiSabato and Jencks, in 1961, concluded for the hydrolysis of AcP that: "While no single one of these considerations should be taken alone as conclusive proof of the monomolecular or bimolecular nature of a reaction, taken together they constitute strong evidence that the neutral hydrolyses of acyl phosphates occur [via a metaphosphate intermediate]."⁹ Such conclusions are not justified by the data; transition states are not intermediates and the characteristics of a transition state do not usually demonstrate the existence of an intermediate. Reactions that exhibit "borderline" behavior proceed through transition states in which the bond to the leaving group is nearly broken, whether or not an intermediate is formed.¹³

Efforts to prove that metaphosphate exists as an intermediate have not been successful for reactions in aqueous solution. Phosphocreatine, the monoanion of phenyl phosphate, and the dianion of 2,4-dinitrophenyl phosphate form methyl phosphate in 50% aqueous methanol with inversion of configuration, and there is no positional isotope exchange of the α - β bridge oxygen atom of $[\beta\text{-}^{18}\text{O}_4]\text{ADP}$ with nonbridge α -oxygen atoms concurrent with hydrolysis. Thus, these reactions are concerted and involve no intermediate, or are preassociation stepwise and involve an intermediate with a lifetime that is insufficient to allow rotation or diffusion in solution.^{14,15} Skoog and Jencks¹⁶ and Bourne and

Williams¹⁷ have provided evidence that the reactions of pyridines with the monoanions of phosphorylated pyridines and isoquinoline do not involve metaphosphate intermediates, although the transition states are open and metaphosphate-like.¹⁸

However, in the gas phase, metaphosphate monoanion has been identified by mass spectroscopy¹⁹ and metaphosphate derivatives have been formed in the gas phase by pyrolysis.²⁰ A metaphosphate intermediate with a significant lifetime could presumably be formed in a solvent with sufficiently low nucleophilicity. The observed low selectivity and absence of nucleophilic assistance in the Conant-Swan fragmentation^{21,22} would be expected if the reaction proceeded through a metaphosphate intermediate or if the reaction were concerted with a metaphosphate-like transition state and no intermediate. However, inversion of configuration for reaction with an alcohol in chloroform shows that there is no long-lived intermediate in this reaction.²³ Reactions in acetonitrile and dioxane, such as the transfer of phosphoryl groups to nucleophilic acceptors in the "three-phase test", the cleavage of phenyl phosphate and ADP with racemization, and the positional isotope exchange of $[\beta\text{-}^{18}\text{O}_4]\text{ADP}$, could represent either a metaphosphate intermediate or reaction with the solvent to form an adduct,^{15,24} as has been suggested for the Conant-Swan fragmentation;²¹ the adduct of SO_3 with dioxane is known.²⁵

Phosphorylation of *tert*-butyl alcohol by a number of mono-substituted phosphoryl compounds^{26,27} could proceed through a metaphosphate intermediate, but could also proceed through an open transition state and no intermediate. In fact, trimethylamine participates in a second-order reaction with *p*-nitrophenyl phosphate dianion,²⁸ and inspection of CPK molecular models suggests that the steric hindrance for trimethylamine and *tert*-butyl alcohol is not grossly different. The fast reactions of 2,4-dinitrophenyl phosphate dianion and *p*-nitrophenyl phosphate mono- and dianion²⁶ reflect the stabilities of the transition states, but are not

(15) Lowe, G.; Tuck, S. P. *J. Am. Chem. Soc.* **1986**, *108*, 1300-1301.

(16) Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597-7606.

(17) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591-7596.

(18) It has been suggested that the volume of activation of $-4.8 \text{ cm}^3 \text{ mol}^{-1}$ for the hydrolysis of 2,4-dinitrophenyl phosphate dianion is evidence for nucleophilic participation of water in the transition state: Ramirez, F.; Marecek, J.; Minore, J.; Srivastava, S.; le Noble, W. *J. Am. Chem. Soc.* **1986**, *108*, 348-349. However, small negative volumes of activation do not provide rigorous evidence for a bimolecular reaction mechanism because of uncertainty regarding the effects of solvation and electrostriction of the reactants and anionic leaving groups in this class of reactions, in which there is a large amount of charge development in the leaving group in the transition state. It is unlikely that 2,4-dinitrophenyl phosphate dianion reacts by a mechanism different from that for acetyl phosphate dianion, which has a leaving group of similar basicity, but ΔV^\ddagger for the latter compound is only $-1.0 \text{ cm}^3 \text{ mol}^{-1}$. These volumes of activation provide information about the properties of the transition state relative to the ground state, and are consistent with a metaphosphate-like transition state, but do not establish whether or not there is an intermediate on the reaction path.

(19) Harvan, D. J.; Hass, J. R.; Busch, K. L.; Bursey, M. M.; Ramirez, F.; Meyerson, S. *J. Am. Chem. Soc.* **1979**, *101*, 7409-7410.

(20) Clapp, C. H.; Westheimer, F. H. *J. Am. Chem. Soc.* **1974**, *96*, 6710-6714. Clapp, C. H.; Satterthwait, A.; Westheimer, F. H. *Ibid.* **1975**, *97*, 6873-6874. Sigal, I.; Loew, L. *Ibid.* **1978**, *100*, 6394-6398.

(21) Satterthwait, A.; Westheimer, F. H. In *Phosphorus Chemistry Directed Toward Biology*; Stec, W. J., Ed.; Pergamon Press: New York, 1980; pp 117-124.

(22) Satterthwait, A.; Westheimer, F. H. *J. Am. Chem. Soc.* **1981**, *103*, 1177-1180. Calvo, K. C.; Westheimer, F. H. *Ibid.* **1983**, *105*, 2827-2831. Calvo, K. C.; Westheimer, F. H. *Ibid.* **1984**, *106*, 4205-4210.

(23) Calvo, K. C. *J. Am. Chem. Soc.* **1985**, *107*, 3690-3694.

(24) Rebek, J., Jr.; Gaviña, F.; Navarro, C. J. *J. Am. Chem. Soc.* **1978**, *100*, 8113-8117. Friedman, J. M.; Knowles, J. R. *Ibid.* **1985**, *107*, 6126-6127. Cullis, P. M.; Rous, A. J. *Ibid.* **1986**, *108*, 1298-1300.

(25) Sisler, H. H.; Audrieth, L. F. *Inorg. Synth.* **1946**, *2*, 173-175. Gilbert, E. E. *Chem. Rev.* **1962**, *62*, 549-589.

(26) Ramirez, F.; Marecek, J. F. *Tetrahedron* **1979**, *35*, 1581-1589. Ramirez, F.; Marecek, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 1460-1465. Ramirez, F.; Marecek, J. F. *Pure Appl. Chem.* **1980**, *52*, 1021-1045. Ramirez, F.; Marecek, J. F.; Yemul, S. S. *Tetrahedron Lett.* **1982**, *23*, 1515-1518.

(27) Ramirez, F.; Marecek, J. F. *Tetrahedron* **1980**, *36*, 3151-3160. Ramirez, F.; Marecek, J. F.; Yemul, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1345-1349.

(28) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209-3216.

(1) This research was supported in part by grants from the National Institutes of Health (GM 20888) and the National Science Foundation (PCM 81-17816).

(2) Kirby, A. J.; Warren, S. G. *The Organic Chemistry of Phosphorus*; Elsevier: New York, 1967; pp 284-292.

(3) Gorenstein, D. G.; Lee, Y.-G.; Kar, D. *J. Am. Chem. Soc.* **1977**, *99*, 2264-2267.

(4) Haake, P.; Allen, G. W. *Bioorg. Chem.* **1980**, *9*, 325-341, and references therein.

(5) Kirby, A. J.; Varvoglis, A. G. *J. Chem. Soc. B* **1968**, 135-141.

(6) The β_{nuc} of zero has been considered to represent the borderline between mono- and bimolecular reaction mechanisms (ref 5; Kirby, A. J. In *Phosphorus Chemistry Directed Toward Biology*; Stec, W. J., Ed.; Pergamon Press: New York, 1980; pp 79-83). However, negative values of β_{nuc} have been observed for second-order reactions of quinuclidines with phosphorylated substrates; the negative values have been attributed to a requirement for desolvation of the nucleophile prior to attack and a decrease in the equilibrium constant for desolvation with increasing basicity of the nucleophile.⁷ Furthermore, catalysis requires participation of the nucleophile in the transition state.

(7) Jencks, W. P.; Haber, M. T.; Herschlag, D.; Nazaretian, K. L. *J. Am. Chem. Soc.* **1986**, *108*, 479-483.

(8) The following abbreviations are used: AcP, acetyl phosphate; Pi, orthophosphate; PPI, pyrophosphate.

(9) DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400-4405.

(10) DiSabato, G.; Jencks, W. P.; Whalley, E. *Can. J. Chem.* **1962**, *40*, 1220-1224.

(11) Cox, J. R., Jr.; Ramsey, O. B. *Chem. Rev.* **1964**, *64*, 317-352. Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313-326.

(12) Jencks, W. P. *Brookhaven Symp.* **1962**, *15*, 134-153.

(13) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345-375.

(14) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438-1440. Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *Ibid.* **1984**, *106*, 4911-4916.

evidence for a metaphosphate intermediate. In contrast, the partial racemization of the phosphoryl moiety upon reaction of an asymmetrical PPi derivative with 2-*O*-benzylpropane-1,2-diol in methylene chloride may provide evidence for metaphosphate monoanion in solution.²⁹

While there is no convincing evidence for the existence of a metaphosphate intermediate in dilute aqueous solution, it has been suggested that the formation of PPi from AcP and from an aminophosphonate in concentrated aqueous sodium perchlorate provides evidence for a metaphosphate intermediate.^{9,12,30} We have examined the partitioning of AcP mono- and dianions between reaction with water and with Pi anions and have obtained no evidence that supports the existence of a metaphosphate intermediate. Electrostatic repulsion between the anions of AcP and Pi is decreased in the presence of high concentrations of sodium ion, which allows the formation of PPi by a reaction that is first order with respect to both AcP and Pi. It is suggested that the formation of PPi involves concerted phosphoryl transfer from AcP to Pi in a complex of the reacting anions that is bridged by bound sodium ions.

Experimental Section

Materials. Aqueous solutions of AcP with potassium as the counterion were prepared in 95–100% yield by a modification of the method of Avison.³¹ Acetic anhydride, 1.7 mL, was added slowly to 8.2 mL of 1.95 M Pi (1:1 monopotassium/dipotassium salt) and 0.28 M pyridine at 0 °C with the pH maintained at >6.1 by addition of 4 M KOH; pyridine was removed by extraction with ether.³² A solution of the sodium salt was obtained by addition of sodium perchlorate followed by centrifugation. The lithium, potassium salt of AcP, Sigma Chemical Co., was used for pK_a determinations. Sodium perchlorate (>99%) was from Aldrich, and solutions of sodium perchlorate were passed through Millipore filters. Pyridine was redistilled and acetohydroxamic acid was recrystallized. Water was glass distilled.

Product Analysis. The initial concentration of AcP in reaction mixtures was determined after conversion to acetohydroxamic acid³³ with acetohydroxamic acid as a standard. Reaction mixtures containing AcP were incubated at 38.5 ± 0.2 , 55.4 ± 0.2 , or 75.0 ± 0.3 °C for $>10t_{1/2}$ and were assayed for PPi by a slight modification of a published procedure.³⁴ Reaction mixtures were titrated to pH ~ 4.8 . Aliquots, usually 0.6 mL, were diluted to 1.5 mL, 0.6 mL of 1 M sodium acetate/acetic acid, pH 4.6, and 0.6 mL of 0.1 M $MnCl_2$ was added; the samples were centrifuged after 30 min. The precipitate was washed with 3 mL of 0.01 M $MnCl_2$, centrifuged, and dissolved in 4.5 mL of 0.5 M H_2SO_4 . Pi was analyzed³⁵ before and after hydrolysis of PPi (100 °C, 30 min) to measure Pi that coprecipitated with PPi and the total Pi from PPi + Pi, respectively. Determinations of PPi were carried out in triplicate; standard deviations were typically <5%. Standards of PPi typically gave $96 \pm 5\%$ recovery for 2.4 μmol of PPi, $92 \pm 8\%$ for 1.2 μmol , and $71 \pm 8\%$ for 0.6 μmol . The yield of PPi is expressed as: $\% \text{ PPi} = ([\text{PPi}]_{\text{final}}/[\text{AcP}]_{\text{initial}}) \times 100$.

Kinetic Studies. Reactions of AcP were carried out at 39.0 ± 0.1 and 54.4 ± 0.1 °C. Pseudo-first-order rate constants for the disappearance of AcP were determined by assay of the remaining AcP as the hydroxamic acid.³³ Endpoints were determined after $>10t_{1/2}$ and the disappearance of AcP was first order for $\geq 3t_{1/2}$.

Measurement of pH and pK_a . Measurements of pH were typically performed at 25 °C using a glass combination electrode containing a saturated solution of lithium trichloroacetate.³⁶ Values of pH and pK_a in solutions containing salts other than sodium perchlorate were found to agree with values determined using an electrode containing a saturated solution of potassium chloride. Deviation from theoretical behavior was observed in determination of the pK_a values of compounds in solutions containing high concentrations of sodium perchlorate; plots of $\log [A^-]/[HA]$ against pH had slopes of ~ 0.8 . Therefore the results of

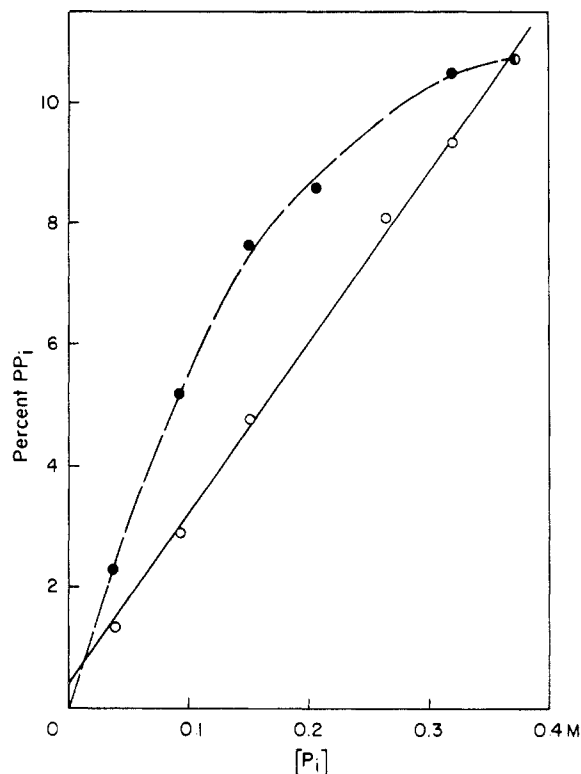


Figure 1. The effect of the concentration of Pi dianion on the yield of PPi from 0.07 M AcP dianion in sodium perchlorate at 54.4 °C. Reactions with varying $[Pi]$ were carried out at a constant concentration of 6.4 M sodium perchlorate (O) or at a constant ionic strength of 7.6 maintained with sodium perchlorate (●). The final pH values were ~ 6.5 . The averages of the initial and final concentrations of Pi are used. The solid line is a linear least-squares fit to the open circles.

Table I. Effect of AcP Concentration on the Yield of PPi from AcP in the Presence of 7.0 M Sodium Ion and 0.25 M Pi Dianion at 54.4 °C^a

[AcP], M	PPi, ^b %
0.023	8.7 (± 0.3)
0.046	8.8 (± 0.01)
0.091	9.3 (± 0.2)
0.160	9.7 (± 0.01)

^a With perchlorate as the counteranion. The final pH values were 6.7 to 7.2. ^b Average and range of values determined from two reaction mixtures.

titrations and pH determinations in these concentrated salt solutions are apparent pK_a and pH values.

Results

Figure 1 shows that the yield of PPi from AcP dianion increases as the concentration of Pi dianion increases at constant sodium perchlorate concentration (solid line) and at constant ionic strength (dashed line); the intercepts are within experimental uncertainty of zero. Table I shows that the percent yield of PPi is almost independent of AcP concentration over the range 0.02 to 0.16 M in the presence of 0.25 M Pi dianion at constant sodium ion concentration. Thus, the formation of PPi is predominantly from a reaction involving AcP and Pi. The small increase in % PPi with increasing AcP concentration (Table I) can be accounted for by the release of Pi from the hydrolysis of AcP. The concave downward curvature in the yield of PPi with increasing concentration of Pi dianion (dashed line, Figure 1) may be attributed to the decrease in the concentration of sodium ion as the concentration of Pi increases at constant ionic strength (see Figure 3).

The dependence on pH of the yield of PPi from AcP and 0.25 M Pi in 6.4 M sodium perchlorate is shown in Figure 2. Apparent pK_2 values for AcP and Pi of 4.1 and 5.7, respectively, were determined by titration in 6.6 M sodium perchlorate at 25 °C.

- (29) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6721–6723.
 (30) Warren, S. G. *J. Chem. Soc. C* **1966**, 1349–1350. Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991–4001.
 (31) Avison, A. W. D. *J. Chem. Soc.* **1955**, 732–738.
 (32) Bodley, A.; Jencks, W. P., unpublished results.
 (33) Lipmann, F.; Tuttle, L. C. *J. Biol. Chem.* **1945**, *159*, 21–28.
 (34) Kornberg, A. *J. Biol. Chem.* **1950**, *182*, 779–793. Jones, L. T. *Ind. Eng. Chem., Anal. Ed.* **1942**, *14*, 536–542.
 (35) Taussky, H. H.; Shorr, E. *J. Biol. Chem.* **1953**, *202*, 675–685.
 (36) Willard, H. H.; Merritt, L. L., Jr.; Dean, J. A. *Instrumental Methods of Analysis*, 5th ed.; Van Nostrand: New York, 1974; p 571.

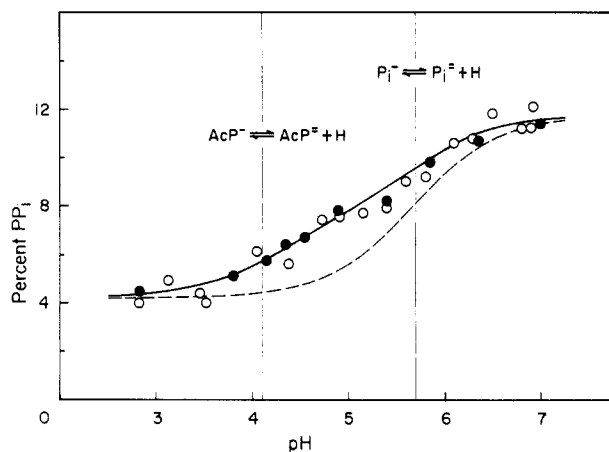


Figure 2. The dependence on pH of the yield of PPI from AcP at 54.4 °C in 6.4 M sodium perchlorate, 0.09 M AcP, and 0.25 M Pi, as the di- and monosodium salts and as the acid. The pH was maintained throughout the reaction by addition of sodium hydroxide. Open and closed symbols are from different experiments. The lines are calculated for pK_2 values of 4.1 and 5.7 for AcP and Pi, respectively, as described in the Discussion.

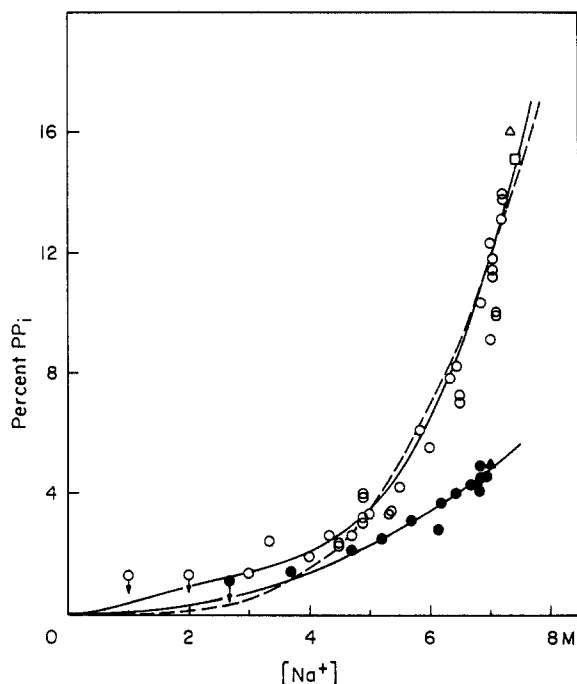


Figure 3. The effect of the concentration of sodium ion on the yield of PPI from the dianions of AcP and Pi (open symbols, $pH_r \sim 6.7$) and from the monoanions of AcP and Pi (closed symbols, $pH_r \sim 3.0$) at 54.4 °C. The reactions were carried out with 0.25 M Na_2HPO_4 or NaH_2PO_4 ; 0.1 M (○, ●), 0.04 M (△, ▲), and 0.02 M (□) Na_2AcP or $NaHAcP$; and varying concentrations of sodium perchlorate. The data are from several different sets of experiments. The lines were calculated from the general equation: $\% PPI/100 = k_p[Pi]/(k_p[Pi] + k_{HOH})$ using values of k_p at various concentrations of sodium ion that were calculated as described in the Results and values of k_{HOH} interpolated from the rate constants in Table III.

The lines in Figure 2 were calculated as described in the Discussion.

Figure 3 shows the effect of sodium ion concentration on the yield of PPI from the reaction of the monoanions of AcP and Pi and from the reaction of the dianions of AcP and Pi. The increase in % PPI from reaction of the monoanions is consistent with an observed rate constant for PPI formation, k_p , that is second order in sodium ion concentration; the lower solid line in Figure 3 was calculated from

$$k_p = k_p' [Na^+]^2 \quad (1)$$

Table II. Effect of Concentrated Salt Solutions on the Yield of PPI from the Dianions of AcP and Pi at 54.4 °C

	conditions ^a	PPI, %
A	4.1 M NaCl ^b	1.7
	4.1 M NaClO ₄ ^b	3.2
B	5.9 M CsCl ^c	<1.9
	5.7 M NaClO ₄ ^b	8.2
C	3.6 M KCl ^c	<1.5
	3.5 M NaClO ₄ ^b	2.6
D	3.7 M NaClO ₄ ^b	2.4
	3.7 M NaClO ₄ + 1.7 M NaCl ^b	4.9
	5.5 M NaClO ₄ ^b	7.8
E	4.7 M NaClO ₄ ^b	4.2
	4.7 M NaClO ₄ + 1.0 M NaCl ^b	7.2
	5.7 M NaClO ₄ ^b	8.2
F	4.1 M NaCl ^b	1.7
	4.1 M CsCl ^c	<1.8
	5.9 M CsCl ^c	<1.9
G	3.7 M NaClO ₄ ^b	2.4
	3.7 M NaClO ₄ + 1.7 M NaCl ^b	4.9

^a All reaction mixtures contained 0.25 M Na_2HPO_4 . ^b Na_2AcP , 0.1 M. ^c K_2AcP , 0.1 M.

with $k_p' = 3.5 \times 10^{-6} M^{-3} s^{-1}$. The increase in % PPI from reaction of the dianions at very high concentrations of sodium ion is consistent with a rate constant for PPI formation that is fourth order in sodium ion concentration; the dashed line in Figure 3 was

$$k_p = k_p' [Na^+]^4 \quad (2)$$

calculated from eq 2 with $k_p' = 1.3 \times 10^{-7} M^{-5} s^{-1}$. A better fit to the data for formation of PPI from the dianions over the entire range of sodium ion concentration is obtained using a rate constant that depends on two sodium ions that bind with an association constant K_a and five additional sodium ions in the transition state. The upper solid line in Figure 3 was calculated for this stoichiometry from eq 3, with $k_p' = 3.9 \times 10^{-5} M^{-1} s^{-1}$, $k_p'' = 1.7$

$$k_p = \frac{k_p' K_a [Na^+]^2}{1 + K_a [Na^+]^2} + k_p'' [Na^+]^5 \left(\frac{K_a [Na^+]^2}{1 + K_a [Na^+]^2} \right) \quad (3)$$

$$K_a = [Pi \cdot Na_2] / [Pi^{2-}] [Na^+]^2$$

$\times 10^{-8} M^{-6} s^{-1}$, and $K_a = 0.25 M^{-2.37}$

Table II shows the effects of sodium perchlorate, sodium chloride, potassium chloride, and cesium chloride on the yield of PPI from the dianions of AcP and Pi. The comparisons of Table II, A–C, show that sodium perchlorate is more effective at facilitating the formation of PPI than the other salts. In the presence of 3.7 or 4.7 M sodium perchlorate the yields of PPI are increased by the addition of sodium chloride, but the increase is less than that obtained with additional sodium perchlorate (Table II, D and E).

Comparison of the yields of PPI in 4.1 and 5.9 M cesium chloride and 4.1 M sodium chloride in Table II, F, shows that sodium ion is of greater or about equal effectiveness compared with cesium ion for promoting the formation of PPI. The solubility limit prevents the examination of 5.9 M sodium chloride. However, the addition of 1.7 M sodium chloride to 3.7 M sodium perchlorate doubles the yield of PPI (Table II, G), so that comparison of the limit of PPI formation for 5.9 M cesium chloride and the observed value for 4.1 M sodium chloride (Table II, F) shows that sodium ion is more effective than cesium ion at promoting the formation of PPI.

The effects of lithium perchlorate and sodium fluoride on PPI formation could not be investigated because of the limited solubility of lithium phosphate and sodium fluoride.

(37) The data could also be accounted for by the kinetically equivalent association of two sodium ions with AcP dianion or one sodium ion with AcP dianion and one sodium ion with Pi dianion, with similar association constants.

Table III. Observed Rate Constants for the Hydrolysis of 0.03 M AcP Monoanion and Dianion in Concentrated Sodium Perchlorate Solutions at 39.0 and 54.4 °C

conditions		$10^3 k, \text{s}^{-1}$	
	[NaClO ₄], M	AcP ⁻	AcP ²⁻ ^a
39.0 °C	0.1	18.9 ^b	7.2 ^c
	5.0	16.7 ^d	7.2 ^d
	6.6	16.4 ^b	7.1 ^c
	6.6		6.8 ^e
	0.1 + 0.54 M pyr		15.0 ^c
	6.6 + 0.54 M pyr		12.4 ^c
conditions		$10^4 k, \text{s}^{-1}$	
	[NaClO ₄], M	AcP ⁻	AcP ²⁻ ^a
54.4 °C	0.1	11.8 ^b	4.9 ^c
	4.9		5.3 ^c
	6.6	8.7 ^b	5.7 ^c
	6.6		5.9 ^f

^a Buffer catalysis was estimated to be <10% in all cases, from the small effects of varying pH values and buffer concentration. ^b 0.1 M NaH₂PO₄, 0.03 M sodium acetate, pH ~3.3. ^c 0.1 M Tris, pH 8.5. ^d From ref 9. ^e 0.1 M Tris, pH 7.9. ^f 0.15 M Tris, pH 8.5.

Table IV. Effect of Temperature on the Yield of PPI from the Monoanions and from the Dianions of 0.07 M AcP and 0.25 M Pi in 5.7 M Sodium Perchlorate

	temp, °C	PPI, %	$10^4 k_{\text{Pi}}, \text{M}^{-1} \text{s}^{-1}$
AcP ²⁻ /Pi ²⁻ ^b	38.5	6.1	0.15
	54.4	7.1	1.5
	75.0	8.1 (8.4) ^c	
AcP ⁻ /Pi ⁻ ^d	38.5	3.4	0.19
	54.4	2.8	0.86
	75.0	1.8 (2.2) ^c	

^a The second-order rate constant for PPI formation, k_{Pi} , was calculated from % PPI of this table, $k_{\text{H}_2\text{O}}$ of Table III with 6.6 M NaClO₄ at 54.4 °C and at 38.5 °C, extrapolated from 39.0 °C, and the equation: % PPI = $k_{\text{Pi}}[\text{Pi}]/(k_{\text{Pi}}[\text{Pi}] + k_{\text{H}_2\text{O}})$; the average concentration of Pi of 0.29 M was used. ^b pH_{final} ~6.7. ^c Calculated from values of E_a listed in Table V and rate constants for phosphorolysis and hydrolysis in this table and in Table III. ^d pH_{final} ~3.2.

The data of Table III show that increasing the concentration of sodium perchlorate from 0.1 to 6.6 M changes the rate constants for hydrolysis of the mono- and dianions of AcP by <30% at 39.0 and 54.4 °C. The rate constants obtained at low ionic strength and 39 °C agree with those obtained previously under similar conditions.⁹ The observed rate constant for the reaction in the presence of 0.54 M pyridine is 32% lower in 6.6 M sodium perchlorate than in 0.1 M sodium perchlorate (Table III); it is possible that this decrease results from an increase in the self-association of pyridine in the presence of concentrated salt.²⁸

The effect of temperature on the yield of PPI from the monoanions and from the dianions of AcP and Pi is shown in Table IV. The yields of PPI at 39 and 54 °C and the rate constants for hydrolysis under similar conditions (Table III) were used to calculate the rate constants for the reactions with Pi listed in Table IV. The small effect of the salt concentration on the rate constants for hydrolysis (Table III) allows calculation of the rate constants for phosphorolysis using rate constants for hydrolysis obtained under similar, but not identical conditions. The observed yields of PPI at 75 °C show fair agreement with the yields calculated from the rate constants and energies of activation for hydrolysis and phosphorolysis obtained at 39 and 54 °C (Table IV).

Table V lists activation parameters for hydrolysis and phosphorolysis of the monoanion and the dianion of AcP. The entropies of activation for hydrolysis are calculated from first-order rate constants and also from second-order rate constants, in order to allow comparison with second-order rate constants for phosphorolysis. The activation parameters for hydrolysis at low salt concentrations agree with those found by DiSabato and Jencks under slightly different conditions and over a larger temperature range.⁹

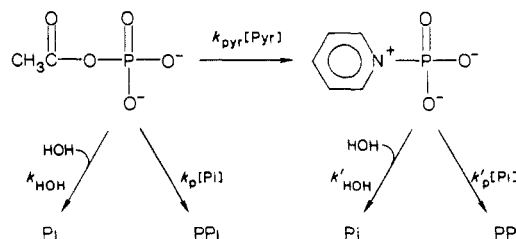
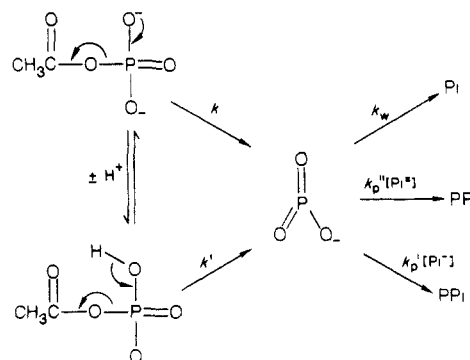
Scheme I**Scheme II**

Table VI shows that pyridine catalyzes the formation of PPI from the dianions of AcP and Pi, as well as the hydrolysis of AcP in aqueous sodium perchlorate. Catalysis of AcP hydrolysis by pyridine involves a phosphorylated pyridine intermediate,^{38,39} and the data of Table VI suggest that the formation of PPI from AcP is also catalyzed via a phosphorylated pyridine intermediate, as shown in Scheme I. The yield of PPI from the monoanion of phosphorylated pyridine and 0.25 M Pi dianion was calculated to be 40 and 41% at 0.14 and 0.21 M pyridine, respectively, from the rate and product data of Table VI and from Scheme I; the yield of PPI from the dianion of AcP is 10% under the same conditions. The ratio of the rate constant for PPI formation to that for Pi formation from the phosphorylated pyridine monoanion of $k_{\text{p}}'/k_{\text{HOH}} = 2.7 \text{ M}^{-1}$ is six times larger than the corresponding ratio for PPI and Pi formation from AcP dianion of $k_{\text{p}}/k_{\text{HOH}} = 0.44 \text{ M}^{-1}$.

Discussion

Mechanism of Pyrophosphate Formation. The previously proposed mechanism for hydrolysis and phosphorolysis of the anions of AcP, shown in Scheme II,^{9,12} predicts that the partitioning of a metaphosphate intermediate between reaction with Pi monoanion and with water will be the same for metaphosphate generated from the monoanion and from the dianion of AcP in concentrated sodium perchlorate solutions. The dashed line of Figure 2 was calculated according to Scheme II, from eq 4 and

$$\frac{\% \text{ PPI}}{100} = \frac{k_{\text{p}}'[\text{Pi}^-] + k_{\text{p}}''[\text{Pi}^{2-}]}{k_{\text{w}} + k_{\text{p}}'[\text{Pi}^-] + k_{\text{p}}''[\text{Pi}^{2-}]} \quad (4)$$

the apparent $\text{p}K_2$ for Pi in 6.6 M sodium perchlorate of 5.7. Values of $k_{\text{p}}'/k_{\text{w}} = 0.18 \text{ M}^{-1}$ and $k_{\text{p}}''/k_{\text{w}} = 0.53 \text{ M}^{-1}$ for eq 4 were determined from the formation of 4.2 and 11.7% PPI at low and high pH, respectively (Figure 2). The calculated yield of PPI for the mechanism of Scheme II, which invokes a free metaphosphate intermediate, is significantly less than the observed yield in the central pH region of Figure 2. Therefore, a common free metaphosphate intermediate is not responsible for the formation of PPI.

A concerted mechanism for PPI formation, shown in Scheme III, can account for the pH dependence of PPI formation. The solid line in Figure 2 was calculated for the concerted mechanism of Scheme III according to eq 5, with $k_1/k_2 = 1.5$ (Table III),

(38) Park, J. H.; Koshland, D. E., Jr. *J. Biol. Chem.* **1958**, *233*, 986–990.

(39) DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393–4400.

Table V. Activation Parameters for Hydrolysis and Phosphorolysis of the Monoanion and of the Dianion of AcP^a

reaction	conditions	E_a , kcal mol ⁻¹		ΔH^\ddagger (39 °C), kcal mol ⁻¹		ΔS^\ddagger (39 °C), eu	
		AcP ⁻	AcP ²⁻	AcP ⁻	AcP ²⁻	AcP ⁻	AcP ²⁻
hydrolysis ^b	0.1 M NaClO ₄ ^c	24.1	25.3	23.5	24.7	-0.5	+1.6
	$\mu = 0.6$ (KCl) ^d	22.9	26.7	22.3	25.9	-3.6	+3.7
	6.6 M NaClO ₄ ^c	22.1	27.5	21.4	26.8	-7.3	+8.4
phosphorolysis	5.8 M NaClO ₄ ^f	20	30	19	29	-15 ^e	+1.1 ^e
						-20 ± 6	12 ± 3

^a Calculated from the relationships $\Delta H^\ddagger = E_a + RT$, $\Delta G^\ddagger = -RT \ln (kh/k_B T)$, and $S^\ddagger = (\Delta H^\ddagger - \Delta G^\ddagger)/T$. ^b Values were calculated from the first-order rate constants unless otherwise noted. ^c From the data of Table III. ^d From ref 9. ^e Calculated from the second-order rate constant for hydrolysis, using $[H_2O] = 38$ M in 6.6 M NaClO₄. ^f From the data of Table IV at 39 and 54 °C.

Table VI. Effect of Pyridine on the Yield of PPi from the Dianions of AcP and Pi and on the Observed Rate Constant for Hydrolysis of AcP²⁻ in Aqueous Sodium Perchlorate at 54.4 °C

pyridine, M	$10^4 k_1$, s ⁻¹	PPi, % ^a
0	5.7	9.9 (±0.1)
0.14	6.6	13.6 (±0.2)
0.21		15.0 (±0.4)
0.28	7.2	
0.54	8.1	
0.13 M dioxane	5.9	10.3
0.20 M dioxane		10.3

^a Varying amounts of pyridine or dioxane were added to solutions with 0.1 M Tris HCl, pH 8.5, 6.6 M NaClO₄, and 0.03 M Na₂AcP.

^b Varying amounts of pyridine or dioxane were added to solutions with 6.4 M NaClO₄, 0.25 M Na₂Pi, and 0.09 M Na₂AcP. ^c The parentheses indicate the range from two reaction mixtures.

$k_2''/k_2 = 0.53$ M⁻¹, $k_2'/k_2 = 0.34$ M⁻¹, $k_1'/k_1 = 0.18$ M⁻¹, and $k_1'' = 0$. The concentrations of the mono- and dianions of AcP

$$\frac{\% \text{ PPi}}{100} = \frac{\{k_2''(\text{Pi}^{2-})(\text{AcP}^{2-}) + k_2'(\text{Pi}^-)(\text{AcP}^{2-}) + k_1'(\text{Pi}^-) \times (\text{AcP}^-) + k_1''(\text{Pi}^{2-})(\text{AcP}^-)\}}{\{k_2''(\text{Pi}^{2-})(\text{AcP}^{2-}) + k_2'(\text{Pi}^-)(\text{AcP}^{2-}) + k_1'(\text{Pi}^-)(\text{AcP}^-) + k_1''(\text{Pi}^{2-})(\text{AcP}^-) + k_1(\text{AcP}^-) + k_2(\text{AcP}^{2-})\}} \quad (5)$$

and Pi were calculated from their apparent pK_2 values of 4.1 and 5.7, respectively. The yields of PPi from AcP and Pi dianions, AcP dianion and Pi monoanion, and AcP and Pi monoanions calculated from these rate constants are 11.7, 7.8, and 4.2%, respectively, in the presence of 0.25 M Pi and 6.4 M sodium perchlorate.⁴⁰ The calculated line gives a satisfactory fit to the data.

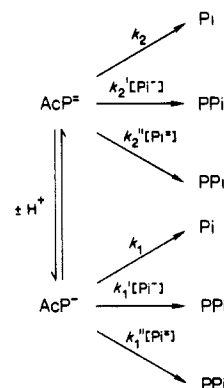
Several kinds of evidence are consistent with, although they do not prove, a concerted nucleophilic reaction of phosphate with AcP to form PPi. There is evidence for a concerted displacement mechanism for the reaction of phosphorylated pyridines and isoquinoline with pyridine,^{16,17} which is a weaker base than Pi dianion, and for catalysis of the hydrolysis of dinitrophenyl phosphate by 3-nitropyridine,⁵ which is a weaker base than Pi monoanion. Thus, it is reasonable that anions of Pi should act as nucleophiles toward anions of AcP, if the sodium ion concentration is high enough to overcome electrostatic repulsion. The

(40) The reaction of AcP²⁻ with Pi⁻ is kinetically indistinguishable from the reaction of AcP⁻ with Pi²⁻:

$$k_2'(\text{AcP}^{2-})(\text{Pi}^-) = k_1''(\text{AcP}^-)(\text{Pi}^{2-}) = k_1'(K_P/K_{\text{AcP}})(\text{AcP}^{2-})(\text{Pi}^-)$$

$$k_2' = 0.025k_1''$$

For example, if no reaction between AcP²⁻ and Pi⁻ occurred ($k_2 = 0$; Scheme III), the above equation and the rate constants used to calculate the solid line in Figure 2 would give $k_1''/k_1 = 0.1$ M⁻¹. The reaction of AcP⁻ and Pi²⁻ would then yield 69% of the AcP⁻ as PPi, in the presence of 0.25 M Pi²⁻ and 6.4 M sodium perchlorate, a sixfold larger yield of PPi than that from the dianions of AcP and Pi, and the value of the rate constant for reaction of Pi²⁻ with AcP⁻ (k_1'') would be 26-fold larger than the rate constant for the reaction of the dianions of AcP and Pi (k_2''). The rate constant for the reaction of AcP⁻ and Pi²⁻ (k_1'') could be larger than the rate constant for the kinetically indistinguishable reaction of AcP²⁻ and Pi⁻ (k_2') because Pi²⁻ is a stronger nucleophile than Pi⁻ and acetic acid is a better leaving group than acetate ion.

Scheme III

rapid intramolecular attack of the carboxylate ion of salicyl phenyl phosphate dianion on the phosphoryl monoanion shows that an anion is an effective nucleophile toward the phosphate diester anion if it is held in a reactive position.⁴¹ In fact, the rate acceleration from intramolecularity for the phosphate diester monoanion is greater than that estimated for a triester; the greater rate acceleration may arise from enforced proximity of the carboxylate ion nucleophile and the diester monoanion that overcomes the electrostatic repulsion.

It is reasonable that the selectivity between reaction of metaphosphate ion with Pi and water ($k_{\text{Pi}}/k_{\text{HOH}}$) for metaphosphate generated next to an acetate ion from AcP dianion would not differ greatly from the selectivity for metaphosphate generated next to pyridine, from phosphorylated pyridine monoanion. However, the selectivity for reaction with Pi compared to reaction with water, $k_{\text{Pi}}/k_{\text{HOH}}$, is sixfold larger for phosphorylated pyridine monoanion than for AcP dianion (Table VI; Results). This difference is consistent with nucleophilic involvement in the transition state and no metaphosphate intermediate, based on differences in selectivity for other reactions. The selectivity of $k_{\text{pyr}}/k_{\text{HOH}} = 1.3\text{--}3.0 \times 10^4$ for reactions of phosphorylated pyridine monoanions is greater than $k_{\text{pyr}}/k_{\text{HOH}} = 1.1 \times 10^2$ for reactions of AcP dianion, and values of β_{nuc} for reactions of phosphorylated pyridine monoanions are larger than those for reactions of both *p*-nitrophenyl phosphate dianion and 2,4-dinitrophenyl phosphate dianion, which have oxyanion leaving groups of higher and lower pK_a than AcP dianion.^{5,16,28,39}

In general, if the hydrolysis of phosphate monoester dianions were to occur through a mechanism with a metaphosphate intermediate, then the observed rate constants for hydrolysis should be larger than those expected for a nucleophilic reaction. A comparison of the rate constants for reactions of phosphate monoester dianions with those of phosphate diester monoanions, which are believed to react by nucleophilic substitution,⁴² provides no indication of such behavior. The second-order rate constants for hydrolysis, k_{HOH} , may be compared with the second-order rate constants for reaction with pyridines, k_{pyr} , which presumably represent nucleophilic reactions, by extrapolating observed rate

(41) Bromilow, R. H.; Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1971**, 1091–1097.

(42) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165–1172.

constants for pyridines to $pK_a = -1.74$, the pK_a of H_3O^+ , to give k_{pyr}' . The ratios for the dianions of *p*-nitrophenyl phosphate and 2,4-dinitrophenyl phosphate of $k_{HOH}/k_{pyr} = 1 \times 10^{-2}$ and 6×10^{-3} , respectively, are not significantly larger than the ratios for the monoanions of methyl dinitrophenyl phosphate and bis(dinitrophenyl) phosphate of 8×10^{-3} and 6×10^{-1} , respectively.^{5,28,42,43}

Thus, the available evidence supports, but does not rigorously demonstrate, a concerted mechanism for the phosphorolysis of AcP in aqueous sodium perchlorate solutions. Although we are not aware of any evidence that supports the existence of a metaphosphate intermediate for phosphorolysis or phosphoryl transfer reactions in aqueous solution, the absence of such evidence does not disprove the existence of an intermediate. A preassociation stepwise mechanism, in which metaphosphate is formed as an intermediate but has a lifetime too short to allow diffusion, is not definitely excluded by the data. For example, the pH dependence of the yield of PPI formation from AcP would be consistent with a preassociation mechanism if the association constants for AcP and Pi increase with increasing charge on the anionic reactants because of bridging by sodium ions.

Electrostatic Interactions. The formation of PPI from AcP and Pi can be explained by direct binding of sodium ions to the anionic reactants to overcome electrostatic repulsion. At ordinary salt concentrations PPI is not formed in appreciable amounts from anions of AcP and Pi (Figure 3; see also ref 9). Other reactions of anions of substituted phosphates with anionic nucleophiles are also slow. Comparison of the rates of reaction of anionic and uncharged nucleophiles with the monoanions of phosphate diesters and with an uncharged phosphate triester indicates that anionic nucleophiles react with the monoanions about two orders of magnitude slower than expected in the absence of electrostatic repulsion.⁴² Fluoride ion, a good nucleophile for attack on phosphorus,⁴⁴ reacts with the monoanions of AcP and phosphoramidates, but no reaction with the dianion of AcP was detected.³⁹

The dependence of the yield of PPI on the concentration of sodium ion (Figure 3) is consistent with a reaction of the monoanions of AcP and Pi that involves two sodium ions and a reaction of the dianions of AcP and Pi that involves at least four sodium ions. This high sensitivity of PPI formation to the concentration of sodium ion suggests that sodium ions lower charge repulsion between the anions of AcP and Pi to catalyze the formation of PPI. Lowering of charge repulsion could occur by direct binding of sodium ions to the reactants or by an electrostatic field interaction of sodium ions that shields the repulsion between the reactants.

Although the dependence of the yield of PPI from the dianions of AcP and Pi on the concentration of sodium ion is fit at very high concentrations of sodium ion by a model involving four sodium ions, a better fit over the entire range of sodium ion concentration is obtained by using a model involving two sodium ions with saturation and an additional five sodium ions (Figure 3). Smith and Alberty estimated an association constant for sodium ion and the dianion of Pi of 4 M^{-1} at 25 °C and ionic strength 0.2 from the dependence of the apparent pK_2 of Pi on the concentration of sodium ion, assuming that Pi and *n*-tetrapropylammonium ion do not associate.⁴⁵ This value is in reasonable agreement with an association constant of $7.0 \pm 2.4 \text{ M}^{-1}$ (25 °C, ionic strength 0), which was derived statistically from the effect of sodium chloride on $CaHPO_4$ solubility, with correction for activity coefficient effects by use of the Debye-Hückel equation.⁴⁶ A similar association constant might be expected for the sodium ion and dianion of AcP. Saturation with two sodium ions or the formation of Pi dimers may prevent upward curvature in the yield of PPI with increasing concentration of disodium Pi (Figure 1).

More direct support for specific binding of sodium ions, rather than an electrostatic field effect, comes from the differences in the ability of different cations to promote the formation of PPI and from the entropy of activation for phosphorolysis. Smith and Alberty obtained an order of binding affinities of monovalent cations for Pi dianion of: $Li^+ > Na^+ > K^+ > (CH_3)_4N^+ > (C_2H_5)_4N^+$;⁴⁵ cesium ion is expected to bind less strongly than potassium ion (and sodium ion), as seen in the Eisenman series XI for binding to anionic sites of strong field strength.⁴⁷ The greater effectiveness of sodium than of cesium ions for catalysis of PPI formation from the dianions of AcP and Pi (Table II) is consistent with a requirement for ion pairing.

The fact that the entropy of activation of +12 eu for phosphorolysis of AcP dianions is a large, positive value, which is greater than the value of +1.1 eu for hydrolysis, suggests that ion-pair formation is involved in phosphorolysis (Table V). The change in entropy for ion-pair formation is expected to be positive as a result of the release of water of solvation from the ions,⁴⁸ and the change in entropy upon association of sodium ion and the dianion of Pi (25 °C, ionic strength 0.2) was estimated to be +24 eu by Smith and Alberty from pK_a measurements at 0 and 25 °C.⁴⁵ The observed value of the entropy of activation for formation of PPI should reflect both positive contributions from ion-pair formation and negative contributions from requirements for positioning of AcP and Pi ions in the transition state. The more negative entropy of activation for phosphorolysis of the monoanion of AcP by Pi monoanion than for phosphorolysis of the dianion of AcP by Pi dianion is consistent with involvement of fewer sodium ions in the reaction of the monoanions.

The enhanced ability of sodium perchlorate compared with sodium chloride to stimulate the formation of PPI (Table II) may reflect a greater availability of sodium ion for binding to the anionic reactants in the presence of perchlorate ion. The association constant of 0.37 M^{-1} at ionic strength 4.0 for ion-pair formation between sodium and perchlorate ions, determined by Raman spectroscopy,⁴⁹ is about fourfold smaller than that for sodium and nitrate ions.⁵⁰ Raman spectra of nitrate ion in the presence of silver ion show no evidence for binding of perchlorate ion that competes with the association of nitrate ion with silver ion.⁵¹ In contrast, Raman spectra of nitrate ion in the presence of sodium ion suggest that the apparent association constant for contact ion-pair formation of sodium and nitrate ions decreases in the presence of chloride ion at constant ionic strength.⁵⁰ This may result from competition between chloride and nitrate ions for association with sodium ion.

Vieyra and co-workers have observed PPI formation from AcP and Pi anions that is sigmoidally dependent on the concentration of Ca^{2+} and Mg^{2+} , suggesting that at least two divalent metal ions bind to the reactants and stimulate PPI formation.⁵² The formation of PPI in the presence of sodium ion may be catalyzed similarly. Ion pairs are also likely to be involved in PPI formation from AcP in 87% acetonitrile and 87% dioxane,⁹ which have lower dielectric constants than water.

It is of interest that these very concentrated salt solutions have little effect on the hydrolysis of acetyl phosphate, in spite of the fact that a large fraction of the water has been replaced by ions at a total concentration of up to ~13 M. The extraordinarily small effect of sodium perchlorate on the rate constants for hydrolysis of AcP monoanion and dianion (Table III) suggests that there is no change in reaction mechanism in these solutions and that the increased rate of PPI formation is not simply the result of electrophilic catalysis by sodium ions; in fact, the reaction with pyridine is slightly decreased in concentrated sodium perchlorate

(47) Eisenman, G. *Biophys. J. (Suppl. 2)* **1962**, 2, 259-323.

(48) Kauzmann, W. *Adv. Protein Chem.* **1959**, 14, 1-63.

(49) Frost, R. L.; James, D. W.; Appleby, R.; Mayes, R. E. *J. Phys. Chem.* **1982**, 86, 3840-3845.

(50) Frost, R. L.; James, D. W. *J. Chem. Soc., Faraday Trans. 1* **1982**, 78, 3249-3261.

(51) Chang, T. G.; Irish, D. E. *J. Soln. Chem.* **1974**, 3, 175-189.

(52) Vieyra, A.; Meyer-Fernandes, J. R.; Gama, O. B. H. *Arch. Biochem. Biophys.* **1985**, 238, 574-583.

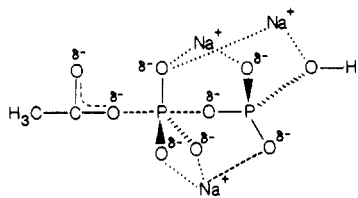
(43) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, 89, 415-423.

(44) Dostrovsky, I.; Halmann, M. *J. Chem. Soc.* **1953**, 508-510. Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B*, **1970**, 1172-1182.

(45) Smith, R. M.; Alberty, R. A. *J. Phys. Chem.* **1956**, 60, 180-184.

(46) Patel, P. R.; Gregory, T. M.; Brown, W. E. *J. Res. Natl. Bur. Std., Sect. A* **1974**, 78, 675-681.

Scheme IV



(Table III). It is unlikely that a decrease in the activity of water accounts for the change in partitioning between reaction with Pi and water with increasing sodium perchlorate concentration because the activity of water in 4 M sodium perchlorate is 0.8.⁵³ Acetonitrile also has very small effects on the rate constants for hydrolysis of AcP anions: 50% acetonitrile at 39 °C gives an 18% decrease and a change of <5% in the rate constants for mono- and dianion hydrolysis, respectively, compared to the rate constants in dilute aqueous solution.⁹

Bridging by Sodium Ions. The reaction of the dianions of AcP and Pi yields more PPi than the reaction of the monoanions of AcP and Pi over the entire range of sodium ion concentration examined, 3.0 to 7.0 M (Figure 3). This difference in the yield of PPi is consistent with facilitation of PPi formation by sodium ions that bridge the negatively charged oxygen atoms of the reactants in ion pairs.

Phosphoryl oxygen atoms from different molecules are bridged by sodium ions in crystals of the salts of phosphoryl compounds such as ATP, β -glycerol phosphate, PPi, and phosphoramidate.⁵⁴ The distances from the sodium ions to the coordinated phosphoryl oxygen atoms are 2.5 ± 0.4 Å, and the geometry about the sodium ions is that of a distorted octahedron. Scheme IV shows some of the possible structures for bridging sodium ions in the transition state for reaction of the dianions of AcP and Pi.

Several observations in the literature render bridging by sodium ions a reasonable mechanism for catalysis of PPi formation from the anions of AcP and Pi. The different effects of different cations on the dismutation of ADP to form ATP and AMP in Me₂SO suggest a role for the cation with specific geometrical constraints, rather than simple electrostatic shielding.⁵⁵ Na⁺, K⁺, Cs⁺, and Rb⁺ ions catalyze the dismutation reaction, whereas Li⁺ and Mg²⁺ ions inhibit the reaction, presumably by competing with other cations for binding to ADP. Furthermore, rate data suggest that the Na⁺ ion binds more strongly than K⁺ ion to ADP, but that two molecules of ADP, each with one K⁺ ion bound to ADP, dismutate faster than those with bound Na⁺ ion. Thus, in addition to charge neutralization through binding, another factor, such as proper geometry for bridging together of reactants, is required for this phosphoryl transfer reaction.

The self-association of Pi anions in solution to form dimers of net charge -2 and -4 is consistent with data from Raman spectroscopy and other techniques⁵⁶ and suggests that even hydrogen bonds are sufficient to overcome the electrostatic repulsion in the complex.⁵⁷ The results of potentiometric titrations of Pi in the presence of divalent metal ions, including Mg²⁺ and Ca²⁺, are consistent with the formation of dimers with net charges of 0 and -1 and bridging of Pi by the divalent metal ion.⁵⁸

Metal ion catalysis of the departure of oxyanion leaving groups provides a precedent for catalysis by sodium ion of the nucleophilic

addition of Pi oxyanions in the reverse direction. The reaction of pyridines with *p*-nitrophenyl phosphate dianion, which has a partial negative charge on the leaving group in the transition state, is catalyzed by Ca²⁺ and Mg²⁺, whereas the reaction with phosphorylated morpholinopyridine monoanion, which has a partial positive charge on the leaving group in the transition state, is inhibited by Ca²⁺ and Mg²⁺. The catalysis of the *p*-nitrophenyl phosphate reaction has been attributed to a favorable electrostatic interaction of the divalent metal ion with the oxygen atom of the leaving group in the transition state, in addition to the interaction with phosphoryl oxygen atoms.⁵⁹ The value of $\beta_{1g} = -0.7$ for the intramolecular reactions of salicylate O-aryl phosphate diesters catalyzed by Zn²⁺ ion, compared with $\beta_{1g} = -1.2$ in the absence of divalent metal ions,⁶⁰ suggests that the charge on the leaving group is partially neutralized in the transition state by an interaction with the divalent metal ion.

The facilitation of nucleophilic attack by Co(III) coordination of *p*-nitrophenyl phosphate dianion and OH⁻ or NH₂⁻ shows that bridging of reactants can increase rates of reaction by inducing intramolecularity.⁶¹ In addition, micelles of dodecylammonium chloride provide rate enhancements of two orders of magnitude for the hydrolysis of AcP, presumably because the phosphoryl anion binds to the positively charged ammonium moieties of the micelle, which induces intramolecularity of the carbonyl moiety of AcP and the amine nucleophile of the micelle.⁶²

The enhanced reactivity of the monocations of primary alkylamines with the anions of monosubstituted phosphoryl compounds appears to be another example of bridging, in this case through hydrogen bonding of the protonated amine to one or more phosphoryl oxygen atoms. These monocations react with *p*-nitrophenyl phosphate dianion and with phosphorylated isoquinoline and substituted pyridine monoanions about twofold faster than predicted from Brønsted-type correlations for a series of primary amines.^{16,17,28} Kirby and Jencks originally suggested that the rate enhancements with primary alkylamine monocations result from an indirect electrostatic attraction, rather than from hydrogen bonding, because the reaction of *p*-nitrophenyl phosphate dianion with the monocation of Dabco, which cannot hydrogen bond to the substrate in the transition state, is faster than predicted from the rate constant for the free base of Dabco and $\beta_{nuc} = 0.1$.²⁸ However, the finding of a negative value of β_{nuc} for attack by quinuclidines on PNPP has removed the basis for the conclusion.⁷

Comparisons of the reactivity of charged and uncharged nucleophilic reagents in several systems indicate that charged groups which are separated by several atoms from the nucleophilic atom have little or no effect on observed rate constants for reaction with phosphoryl groups as the result of electrostatic interaction, at least for reactions in water at moderate ionic strength. This suggests that the rate enhancement observed with flexible diamine monocations is the result of hydrogen bonding to the anionic phosphoryl group.

(1) Dabco monocation shows the same 3.2-fold rate increase as uncharged quinuclidines for reaction with the 2,4-dinitrophenyl phosphate dianion/Ca²⁺ complex compared with free dinitrophenyl phosphate.⁷ (2) Piperazine monocation and three uncharged cyclic secondary amines follow the same Brønsted correlation ($\beta = 0$) for reaction with phosphorylated isoquinoline monoanion.¹⁷ (3) The rate constants for reactions of cyclic diamine monocations with other monosubstituted phosphate compounds are smaller than predicted by Brønsted correlations for structurally related amines.⁶³⁻⁶⁵ (4) Glycine falls on the same Brønsted correlation

(53) Calculated from osmotic coefficients given in: Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*; Academic Press: New York, 1955; p 468.

(54) Kennard, O., et al. *Nature (London)*, **1970**, 225, 333-336. Ul-Haque, M.; Caughlan, C. N. *J. Am. Chem. Soc.* **1966**, 88, 4124-4128. MacArthur, D. M.; Beevers, C. A. *Acta Crystallogr.* **1957**, 10, 428-432. Hobbs, E.; Corbridge, D. E. C.; Raistrick, B. *Ibid.* **1953**, 6, 621-626.

(55) Hopkins, E. A. H.; Wang, J. H. *J. Am. Chem. Soc.* **1965**, 87, 4391-4392.

(56) Preston, C. M.; Adams, W. A. *J. Phys. Chem.* **1979**, 83, 814-821, and references therein.

(57) The association constants for hydrogen-bonded complexes measured by Stahl and Jencks suggest that multiple hydrogen bonds are necessary to explain the observed association constants for Pi dimerization: Stahl, N.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, 108, 4196-4205.

(58) Childs, C. W. *Inorg. Chem.* **1970**, 9, 2465-2469.

(59) Herschlag, D.; Jencks, W. P., manuscript in preparation.

(60) Steffans, J. J.; Siewers, I. J.; Benkovic, S. J. *Biochemistry* **1975**, 14, 2431-2440.

(61) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, 105, 7327-7336. Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *Ibid.* **1984**, 106, 7807-7819. Harrowfield, J. M.; Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *Ibid.* **1980**, 102, 7733-7741.

(62) Melhado, L. L.; Gutsche, C. D. *J. Am. Chem. Soc.* **1978**, 100, 1850-1856. Gutsche, C. D.; Mei, G. C. *Ibid.* **1985**, 107, 7964-7967.

(63) Table I of ref 7 and references therein.

(64) Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53-57, from data for cyclic secondary amines.

line as other primary amines for reaction with *p*-nitrophenyl phosphate dianion and phosphorylated isoquinoline monoanion.^{17,28} (5) Nicotinate anion falls on the same Brønsted correlation line as uncharged pyridines for reaction with phosphorylated pyridine monoanions.¹⁶ An apparent exception is the ≥ 8 -fold decrease in the reactivity of nicotinate anion compared with uncharged pyridines for reaction with 2,4-dinitrophenyl phosphate dianion.^{5,66}

(65) It has been suggested that rate constants for reactions of phosphoryl compounds with monocations of Dabco and piperazine may be greater than expected, as a result of electrostatic attraction: Lloyd, G. J.; Hsu, C.-M.; Cooperman, B. S. *J. Am. Chem. Soc.* **1971**, *93*, 4889-4892; ref 64. However, Brønsted-type correlations that support rate constants that are greater than predicted have not been reported.

The reason for this is not clear, but it may involve an unfavorable interaction between an oxygen atom of the constrained meta carboxylate group of nicotinate with an oxygen atom of the phosphoryl group. Inspection of CPK molecular models suggests that the distance between the phosphoryl oxygen atoms and the carboxylate oxygen atoms of glycine and of nicotinate is nearly the same in the two transition states, but the glycine carboxylate group is free to move to a position in which it is pointed away from the phosphoryl group.

(66) No reaction of nicotinate anion with dinitrophenyl phosphate dianion was observed. A maximum rate constant for the reaction of $7.0 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ at 39 °C was taken from the smallest observed rate constant in Table I of ref 5.

Neutron Profile Refinement of the Structure of FeOCl and FeOCl(TTF)_{1/8.5}

S. M. Kauzlarich,^{†,‡} J. L. Stanton,[‡] J. Faber, Jr.,[‡] and B. A. Averill^{*†}

Contribution from the Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901, Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, and Argonne National Laboratory, Argonne, Illinois 60439. Received April 11, 1986

Abstract: Neutron diffraction studies on powder samples of FeOCl and FeOCl(TTF)_{1/8.5} establish that TTF intercalated into FeOCl contributes to the diffraction pattern and exhibits long-range order. Room-temperature time-of-flight diffraction results are reported. The structure of FeOCl was refined in a space group *Pmmn* with $a = 3.7730$ (1) Å, $b = 7.9096$ (1) Å, and $c = 3.3010$ (1) Å. Least-squares Rietveld refinement (21 parameters, 3296 degrees of freedom) yielded $R = 0.0169$, $R_w = 0.0248$ ($R_{\text{expected}} = 0.0115$). The structure of FeOCl(TTF)_{1/8.5} was refined in space group *Immm* with $a = 3.784$ (2) Å, $b = 3.341$ (2) Å, and $c = 25.97$ (2) Å, using a model in which the TTF molecules lie in the *bc* plane, with equal occupancy of the four possible sites, to give $R = 0.0203$, $R_w = 0.0289$, and $R_{\text{expected}} = 0.0111$ (28 parameters, 1894 degrees of freedom).

Intercalation compounds are of interest not only as catalytic materials¹ but also as low-dimensional conductors.² Neutron diffraction studies on TaS₂- and NbS₂-pyridine intercalates³ showed that the aromatic ring is perpendicular to the host layers and that the C-N axis of the pyridine ring is oriented parallel to the sulfide layers. This result was contrary to initial ideas that the nitrogen lone pair was directed at metal atoms within the host or that the pyridine rings were parallel to the host layers.⁴ A structure similar to that of NbS₂(py)_{0.5} (py = pyridine) has been proposed for FeOCl(py)_{1/3}.⁵ Structural models have been proposed for amine intercalates of FeOCl and other metal oxychlorides based on lattice expansions and Mössbauer,^{5,6} NMR,⁷ and pseudo-single-crystal⁸ studies. The currently accepted model is one in which the threefold axis of the amine is not parallel to the *b* axis of the host, and the amines are "nested" in the chloride layers.^{7a}

A new class of intercalates currently under investigation in our laboratory employs tetrathiafulvalene (TTF) and related compounds as guest species.^{2,9} A layered material such as FeOCl has the potential to enforce a self-stacked structure upon the intercalated electron-donor molecules, a requirement for low-dimensional conductors.¹⁰ In addition, charge transfer occurs between the tetrathiolene guest and the host lattice, resulting in stacks of radical cations between the layers.⁹ Iron K-edge EXAFS (extended X-ray absorption fine structure) spectra show little perturbation of the local Fe environment in the intercalates,¹¹

indicating that the structure of the host layers has not changed significantly. Powder X-ray diffraction data confirm the existence

(1) *Intercalation Chemistry*; Whittingham, M. S.; Jacobson, A. J., Eds.; Academic: New York, 1982.

(2) Averill, B. A.; Kauzlarich, S. M. *Mol. Cryst. Liq. Cryst.* **1984**, *107*, 55-64.

(3) (a) Riekel, C.; Hohlwein, D.; Schöllhorn, R. *J. Chem. Soc., Chem. Commun.* **1976**, 863-864. (b) Riekel, C.; Fischer, C. O. *J. Solid State Chem.* **1979**, *29*, 181-190.

(4) (a) Gamble, F. R.; DiSalvo, F. J.; Klemm, R. A.; Geballe, T. H. *Science* **1970**, *168*, 568-570. (b) Gamble, F. R.; Osieck, J. H.; DiSalvo, F. J. *J. Chem. Phys.* **1971**, *55*, 3525-3530.

(5) Eckert, H.; Herber, R. H. *J. Chem. Phys.* **1984**, *80*, 4526-4540.

(6) (a) Kanamaru, F.; Shimada, M.; Koizumi, M.; Takano, M.; Takada, T. *J. Solid State Chem.* **1973**, *7*, 297-299. (b) Kikkawa, S.; Kanamaru, F.; Koizumi, M. *Physica* **1981**, *105B*, 249-252. (c) Kikkawa, S.; Kanamaru, F.; Koizumi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 963-966. (d) Maeda, Y.; Yamashita, M.; Ohshio, H.; Tsutsumi, N.; Takashima, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3138-3143. (e) Herber, R. H. *Acc. Chem. Res.* **1982**, *15*, 216-224. (f) Herber, R. H.; Maeda, Y. *Inorg. Chem.* **1981**, *20*, 1409-1415.

(g) Fatseas, G. A.; Palvadeau, P.; Venien, J. P. In *Solid State Chemistry, Proceedings of the 2nd European Congress*, Veldhoven, The Netherlands, June 7-9 1982. Metselaar, R., Heijligers, H. J. M., Schoonman, J., Eds. *Studies in Inorganic Chemistry*; Elsevier: Amsterdam, 1983; Vol. 3, pp 627-630.

(7) (a) Rouxel, J.; Palvadeau, P. *Rev. Chim. Min.* **1982**, *19*, 317-332. (b) Clough, S.; Palvadeau, P.; Venien, J. P. *J. Phys. C: Solid State Phys.* **1982**, *15*, 641-655.

(8) Fatseas, G. A.; Palvadeau, P.; Venien, J. P. *J. Solid State Chem.* **1984**, *51*, 17-37.

(9) (a) Antonio, M. R.; Averill, B. A. *J. Chem. Soc., Chem. Commun.* **1981**, 382-383. (b) Averill, B. A.; Kauzlarich, S. M.; Antonio, M. R. *J. de Phys. (Paris)* **1983**, *44* C3-1373-C3-1376. (c) Kauzlarich, S. M.; Averill, B. A.; Teo, B. K. *Mol. Cryst. Liq. Cryst.* **1984**, *107*, 65-74. (d) Averill, B. A.; Kauzlarich, S. M.; Teo, B. K.; Faber, J., Jr. *Mol. Cryst. Liq. Cryst.* **1985**, *120*, 259-262.

* To whom correspondence should be addressed.

[†] Michigan State University.

[‡] University of Virginia.

[‡] Argonne National Laboratory.