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11PPOTENTIALLY PREBIOTIC SYNTHESSES OF CONDENSED
PHOSPHATES

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Abstract. In view of the importance of a prebiotic source of high energy phosphates, we have investigated a number of potentially prebiotic processes to produce condensed phosphates from orthophosphate and cyclic trimetaphosphate from tripolyphosphate. The reagents investigated include polymerizing nitriles, acid anhydrides, lactones, hexamethylene tetramine and carbon suboxide. A number of these processes give substantial yields of pyrophosphate from orthophosphate and trimetaphosphate from tripolyphosphate. Although these reactions may have been applicable in local areas, they are not sufficiently robust to have been of importance in the prebiotic open ocean.

1. Introduction

Adenosine triphosphate is used to drive most biochemical reactions, and it is widely held that there was a prebiotic phosphate compound capable of similar function (Ferris and Usher, 1983; Baltscheffsky, 1993). However, the attempts to demonstrate prebiotic syntheses of condensed phosphates have not given robust syntheses with few exceptions (for reviews see Miller and Orgel, 1974; Lohrmann and Orgel, 1973; Hulshof and Ponnampuruma, 1976; Keefe and Miller, 1995). In view of the importance of a prebiotic free energy source, and because there have been few recent experiments on prebiotic phosphorylations, we have examined a number of new processes. Divalent ions are ubiquitous in natural waters, calcium ions precipitate orthophosphate and calcium and magnesium ions are involved in most enzymatic and non-enzymatic phosphorylation systems, and so the effect of the addition of these ions was also investigated. We have found that some of these are potential prebiotic sources of condensed phosphates. Although these reactions may have been applicable in local areas, they are not sufficiently robust to have been of importance in the prebiotic open ocean.

2. Experimental

Reagents were purchased from Aldrich and used without further purification. Indicated compounds were mixed with an equimolar amount of 0.25 M solution of sodium orthophosphate and the pH was adjusted to 8.0 except where stated otherwise. The reactions were studied in the presence and absence of calcium ions.

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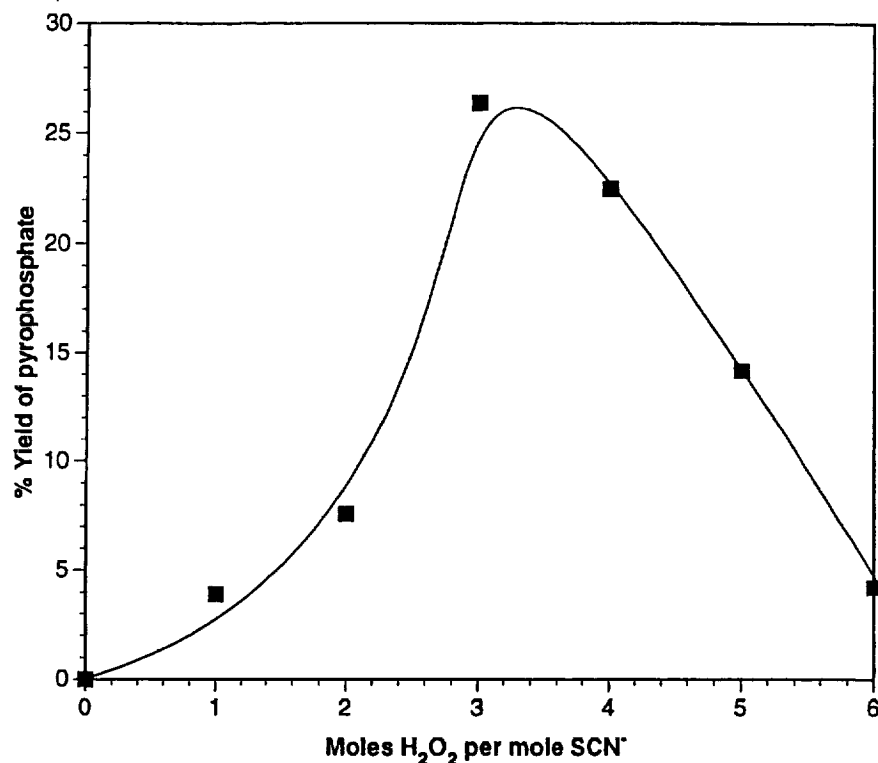


Fig. 1. Yields of pyrophosphate from the oxidation of thiocyanate with hydrogen peroxide. The orthophosphate and thiocyanate were 5 mM, and the hydrogen peroxide was 5 to 30 mM

Reaction mixtures were heated in thermostatically controlled heating blocks or were studied at room temperature. Sealed glass vials were used for solution state studies and open glass tubes were used for solid state studies. Solutions were dried by evaporation under reduced pressure, and the dry mixtures were shaken with D₂O and EDTA and the yield of pyrophosphate or other new phosphorus-containing compounds was determined by ³¹P NMR. The identities of new peaks in the NMR were confirmed by spiking solutions with small amounts of pyrophosphate and higher condensed phosphates. Yields are expressed as % conversion of orthophosphate or tripolyphosphate to the specified product, and yields as small as 0.1% can be detected using the described procedure. A number of reagents were examined, these will be considered individually.

2.1. CONDENSATION REACTIONS OF ORTHOPHOSPHATE

2.1.1. Ammonium Formate

Ammonium formate would have been abundant on the prebiotic Earth as it is formed by the hydrolysis of cyanide which is the central prebiotic reagent. It is

extremely soluble (24.8 molal at 25 °C), (Linke and Seidell, 1958) and might give formyl phosphate under sufficiently concentrated conditions. Ammonium formate was mixed with aqueous trisodium orthophosphate and the pH brought to 8.0 with 1 M HCl. The sample was heated to 100 °C for 3 days, having dried out in one day. The yield of pyrophosphate was 53%. Repeating the experiment in the presence of one equivalent of magnesium chloride reduced the yield to 10%, while the addition of one equivalent of calcium chloride gave no pyrophosphate, this difference may be due to the fact that calcium orthophosphate is less soluble than magnesium orthophosphate. The same experiments at 80 °C gave no pyrophosphate in 3 days. The strong temperature dependence of this reaction is interesting and may be due to the onset of melting of the ammonium formate near 100 °C.

2.1.2. *Oxidation of Thiocyanate*

Thiocyanate has the potential of being an important prebiotic compound since it forms rapidly and quantitatively from cyanide and elemental sulfur (Bartlett and Davis, 1958). It is relatively stable to hydrolysis because its pK_a is approximately -1 (Morgan *et al.*, 1969; Crowell and Hankins, 1968). One of the sinks for thiocyanate on the prebiotic Earth would have been oxidation with hydrogen peroxide. Hydrogen peroxide would have been produced in small amounts in the atmosphere by the photolysis of water vapour and by ionizing radiation in liquid water, e.g. from ^{40}K . The chemistry of this oxidation is complex and poorly understood (Wilson and Harris, 1960) and (Wilson and Harris, 1961). Aqueous solutions of 5 mM phosphate, 5–30 mM hydrogen peroxide and 5 mM thiocyanate gave yields of pyrophosphate of up to 26% at room temperature as shown in Figure 1. The ratio of hydrogen peroxide to thiocyanate is crucial to this experiment, the yield has a maximum at three equivalents and falls off rapidly in each direction. Neither thiocyanate or hydrogen peroxide cause the condensation of phosphate alone. This would appear to suggest that the condensing agent is an intermediate in the oxidation of the thiocyanate. Possibilities such as thiocyanogen (NCSSCN) or sulfenic acids such as cyanosulfenic acid (HOSCN) are attractive, but we have no direct evidence in this regard.

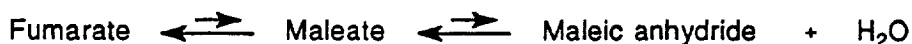
2.1.3. *Carbon Suboxide*

Carbon suboxide (C_3O_2) is produced by passing an electric discharge through carbon monoxide (Ott, 1928) and therefore is likely to be present in atmospheres containing substantial amounts of CO (Abelson, 1966; Rubey, 1955). Carbon suboxide reacts with two molecules of water to give malonic acid (Wilson, 1935) and has been suggested as a constituent of comet Halley (Huntress *et al.*, 1991). We therefore synthesized carbon suboxide by the dehydration of malonic acid by phosphorus pentoxide at 160 °C (Diels and Meyerheim, 1907). The carbon suboxide was distilled directly onto the surface of a solution of orthophosphate frozen in liquid nitrogen and the mixture was allowed to thaw. No new phosphorus contain-

ing products were observed after reaction with orthophosphate in the presence and absence of magnesium and calcium ions.

2.1.4. *Maleic Anhydride*

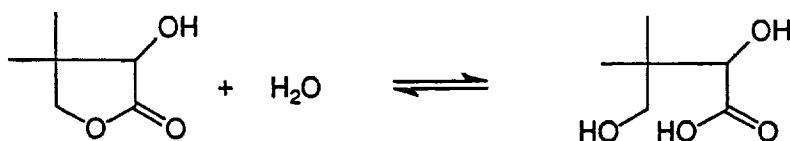
Maleic anhydride has the potential of being a significant high energy prebiotic compound. It is volatile (vapor pressure = 10 mm Hg at 79 °C) and so if it was produced on the early Earth it might have become concentrated in a local area. Its production is plausible, the equilibrium between fumarate and maleate favours fumarate and the equilibrium between maleate and maleic anhydride favours maleate.



However, if the anhydride evaporates then the equilibrium might be drawn over. Thus maleic anhydride, once generated, might act as a condensing agent. The product would be maleic acid, which could be recycled. Our studies show that heating a dry mixture of maleic anhydride and sodium dihydrogen phosphate at 100 °C for 3 days gave 5% pyrophosphate.

2.1.5. *Pantoyl Lactone*

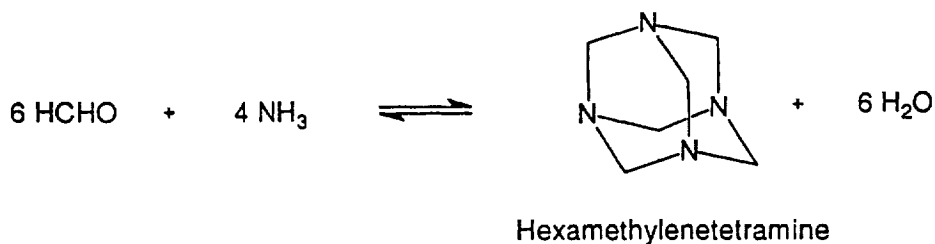
Pantoyl lactone takes up a molecule of water in the ring opening reaction shown below



The equilibrium constant for this reaction is 3.7×10^{-3} at pH <4 but is 37 at pH 8. The dihydroxy acid would have been present in the prebiotic environment as it is readily produced from isobutyraldehyde which is the precursor to valine in spark discharge experiments (Schlesinger and Miller, 1993). Since pantoic acid is a product of the hydrolysis shown above, it may be recycled. Our experiments showed that the dry heating of pantoyl lactone and sodium dihydrogen phosphate gave a yield of pyrophosphate of 5%, repeating the experiment with the addition of one equivalent of magnesium chloride caused a precipitate to form and reduced the yield to 0%.

2.1.6. *Hexamethylenetetramine*

Hexamethylene tetramine is a very stable product of the reaction of formaldehyde and ammonia (Walker, 1964). It can also be made by the action of ionizing radiation on methanol and ammonia (Bernstein *et al.*, 1994) a process that may be important in comets or interplanetary dust particles.



If the reaction is run in the reverse direction by removing volatile formaldehyde and ammonia or by sequestering the ammonia as ammonium ions, then six molecules of water could be removed from phosphate or other prebiotic molecules. Since the starting materials formaldehyde and ammonia are regenerated on hydrolysis of the hexamethylene tetramine, they can be recycled, so the process might be considered catalytic. Our investigations showed that the dry heating of hexamethylenetetramine and monohydrogen or dihydrogen phosphates did not result in the formation of condensed phosphates. Repeating the experiment with the addition of one equivalent of magnesium chloride also gave no condensed phosphates. Some success was obtained with the dehydration of succinic acid. Dry heating of succinic acid and hexamethylene tetramine at 120 °C gave an 8% yield of succinic anhydride and a 16% yield of succinimide. However, heating succinic anhydride with sodium monohydrogen orthophosphate did not give any condensed phosphate, and hexamethylene tetramine was unable to dehydrate maleic acid to give maleic anhydride.

2.1.7. *Polymerizing Hydrogen Cyanide*

Hydrogen cyanide is the principal organic nitrogen-containing compound formed when a mixture of reducing gases is sparked (Schlesinger and Miller, 1983). It is involved in many prebiotic syntheses and may be concentrated from dilute solution to eutectic mixtures by freezing (Sanchez *et al.*, 1966). Our experiments have shown that aqueous solutions of polymerizing 2 M hydrogen cyanide can cause the condensation of phosphate to pyrophosphate in 4% yield. This reaction does not proceed in the presence of calcium ions. In experiments where ferric and ferrous ions replaced the calcium ions, the reaction does still occur, but the yield was reduced to 1%.

2.1.8. *Polymerizing Hydrogen Cyanide Tetramer*

The structure of the product formed by polymerizing hydrogen cyanide is not well understood, but it is known that some of the intermediates are small oligomers such as the tetramer (Sanchez *et al.*, 1967). The hydrogen cyanide tetramer has also been used in peptide synthesis (Chang *et al.*, 1969). Our experiments showed that no new phosphorus-containing products were formed when a solution of orthophosphate was exposed to polymerizing 2 M hydrogen cyanide tetramer at pH 9.0.

2.1.9. *Polymerizing Glycine Nitrile*

Conditions under which hydrogen cyanide is produced may also lead to the synthesis of smaller amounts of other nitriles. These are also prone to polymerization (Moser and Matthews, 1968) and hydrolysis and are also relatively high energy compounds (the free energy of hydrolysis is approximately 15 Kcal/mol), and in principle could remove water from orthophosphate to produce pyrophosphate. Our experiments showed that no new phosphorus-containing products were formed when a solution of orthophosphate was exposed to polymerizing 2 M glycine nitrile at pH 9.0.

2.1.10. *Polymerizing Lactonitrile*

Lactonitrile was investigated for the same reasons as glycine nitrile. Our experiments showed that no detectable new phosphorus-containing products were formed when a solution of orthophosphate was exposed to polymerizing 0.5 M lactonitrile at pH 9.0.

2.1.11. *Polymerizing Formaldehyde*

Formaldehyde is another organic compound which is formed in large amounts in sparked mixtures of reducing gases (Schlesinger and Miller, 1983). It is invoked in many prebiotic scenarios including the syntheses of amino acids in the Strecker reaction and sugars in the formose reaction. Solutions of orthophosphate were exposed to conditions under which the formose reaction proceeds (0.15 M formaldehyde, pH 12, Ca(OH)_2 and trace amounts of glycoaldehyde at 40 °C). After one week there was no detectable yield of new phosphorus-containing products.

2.1.12. *Imidazole*

Imidazole has been shown to be a prebiotic compound (Ferris and Kuder, 1970; Oró *et al.*, 1984; Ferris, *et al.*, 1968) and is widely used as an activating group for the synthesis of RNA and DNA from nucleotides (Lohrmann and Orgel, 1973). Imidazole was investigated in 0.15 M solution at both 100 °C and 80 °C. In the absence of calcium there was no detectable yield of new phosphorus containing products, while pyrophosphate was observed to form in the presence of calcium which formed a precipitate on addition. After one week at 100 °C the yield of pyrophosphate was 4.3%. Repeating the experiment with magnesium chloride in place of calcium chloride reduced the yield to zero.

2.1.13. *Concentrated Salt Solutions*

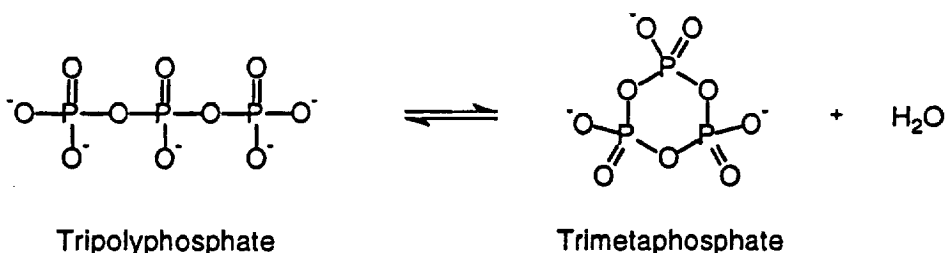
It has been recently reported that concentrated salt solutions cause the formation of glycine peptides (Eder *et al.*, 1993) especially in the presence of copper (II). We therefore investigated whether a similar dehydration could take place with orthophosphate. Heating a solution which was 5 M in NaCl and 1 M in orthophosphate at 100 °C for one week gave no detectable yield of new phosphorus-containing compounds.

TABLE I
Percent yields of condensed phosphates using various reagents as reported in the text

Condensing agent	Percent yield of pyrophosphate from orthophosphate		Percent yield of trimetaphosphate from tripolyphosphate	
	without Ca^{2+}	with Ca^{2+}	without Ca^{2+}	with Ca^{2+}
$\text{NH}_4^+ \text{HCOO}^-$	53	0	—	—
H_2O_2 and SCN^-	26	—	—	—
Carbon Suboxide	0	0	—	—
Maleic Anhydride	5	—	0	38
Pantoyl Lactone	5	—	0	20
Hexamethylenetetramine	0	0	—	—
Polymerizing HCN	4	0	—	—
Polymerizing HCN Tetramer	0	0	—	—
Polymerizing Glycine Nitrile	0	0	—	—
Polymerizing Lactonitrile	0	0	—	—
Polymerizing Formaldehyde	0	0	—	—
Imidazole	0	4	—	—
5 M NaCl	0	0	—	—
Cyanogen	—	—	1	0
Acetic Anhydride	—	—	25	0

2.2. CONDENSATION REACTIONS OF TRIPOLYPHOSPHATE

It is possible that orthophosphate was not the starting point for prebiotic phosphorylations, but rather it was a polyphosphate that could be efficiently recycled. The best example is tripolyphosphate which could be converted to cyclic trimetaphosphate, as shown below.



Cyclic trimetaphosphate is an efficient phosphorylating agent that could in principle be converted back to tripolyphosphate. Under these conditions the tripolyphosphate/trimetaphosphate could be considered a catalyst, and so a small amount of tripolyphosphate synthesized by some other route could be an effective system. If the trimetaphosphate added to compounds such as adenosine to make ATP, then the

trimetaphosphate would be a reagent rather than a catalyst. Beck and Orgel (1965) found that water soluble carbodiimides gave good yields of trimetaphosphate from tripolyphosphate, and cyanate gave 1% yields but only in the presence of calcium phosphate.

We examined a number of routes that might give trimetaphosphate from tripolyphosphate that were not tried by Beck and Orgel. Carbon suboxide, polymerizing formaldehyde, polymerizing lactonitrile, polymerizing hydrogen cyanide, hexamethylene tetramine, succinic anhydride were all investigated using analogous procedures to those described for orthophosphate. None of these systems gave detectable yield of new phosphorus-containing products. Systems which did yield trimetaphosphate are discussed in turn below.

2.2.1. *Maleic Anhydride*

A solution 0.22 M in sodium tripolyphosphate, 0.22 M in calcium chloride and 0.36 M in maleic anhydride was adjusted to pH 7.8 with 1 M HCl left to stand at room temperature for 20 hours. The yield of trimetaphosphate was 38%. Similar experiments with magnesium in place of calcium gave a yield of trimetaphosphate of 40% and with imidazole in place of calcium gave a yield of 10%, omitting the calcium gave no detectable yield of trimetaphosphate. Buffering the solutions with acetate at pH 5 or 8 in the presence of calcium ions also gave no detectable yield of trimetaphosphate.

2.2.2. *Acetic Anhydride*

A solution 0.27 M in sodium tripolyphosphate and 0.39 M in acetic anhydride was adjusted to pH 7.0 with 1 M HCl left to stand at room temperature for 20 hours. The yield of trimetaphosphate was 25%. The addition of one equivalent of either calcium chloride, magnesium chloride or imidazole resulted in no detectable yield of trimetaphosphate.

2.2.3. *Pantoyl Lactone*

A solution 0.11 M in sodium tripolyphosphate, 0.18 M in pantoyl lactone, 0.11 M in calcium chloride and 2.27 M in sodium acetate was adjusted to pH 7.5 with 1 M HCl left to stand at room temperature for 66 hours. The yield of trimetaphosphate was 20%. Omitting either the acetate buffer or the calcium chloride gave no detectable yield of trimetaphosphate.

2.2.4. *Cyanogen*

A solution 0.27 M in sodium tripolyphosphate was adjusted to pH 7.0 with 1 M HCl left to stand at room temperature for 40 hours in a sealed flask with 1.43 mmol of gaseous cyanogen. The yield of trimetaphosphate was 1%. The addition of one equivalent of either calcium chloride, magnesium chloride or imidazole resulted in

no detectable yield of trimetaphosphate. The addition of the calcium or magnesium ions caused a precipitate to form.

3. Discussion

The results reported here are in agreement with previous prebiotic polymerization experiments (Miller and Orgel, 1974; Lohrmann and Orgel, 1973; Hulshof and Ponnamperna, 1976). The yields are generally low and the concentrations employed are too high to be plausible for the early Earth. No formation of polyphosphates (condensed phosphates larger than pyrophosphate) were observed in these experiments. Pyrophosphate is the only condensed phosphate that we were able to generate from orthophosphate. This is not a particularly high energy compound at pH 7 (-3 to -5 Kcal/mol), (Flodgaard and Fleron, 1974; Wood *et al.*, 1966) and consequently is not a very likely precursor to adenosine triphosphate under prebiotic conditions, although it should be stated that the free energy of hydrolysis of condensed phosphate bonds does depend on the solution conditions. Some bacteria grow more rapidly in the presence of pyrophosphate (Wood, 1985), but it has not been demonstrated that they use it as an energy source.

The effects of calcium and magnesium ions are variable. Precipitated calcium orthophosphate is needed for pyrophosphate synthesis from cyanate (Miller and Parris, 1964; Beck and Orgel, 1965; Vieyra *et al.*, 1995). In most of our experiments calcium (and magnesium) ions inhibit the formation of condensed phosphate bonds. Exceptions were the imidazole reaction and trimetaphosphate formation from tripolyphosphate and maleic anhydride or pantoyl lactone. This unpredictability suggests that the prebiotic polyphosphate reactions need to be tested in the presence and absence of calcium and magnesium ions.

We are of two minds over these results. On the one hand the yields and efficiencies of some of these reactions are as good as most that appear in the literature. On the other hand they do not seem sufficiently robust to have been important in the primitive ocean as a whole. However, local environments such as lakes etc. where condensed phosphates might have been concentrated may have been the sites of reactions relevant to the origin of life. This would apply particularly to the volcanic production of phosphorus pentoxide in volcanoes and the appearance of condensed phosphates up to the tetramer in volcanic fumaroles (Yamagata *et al.*, 1991). The same considerations would apply to the high yields of ammonium metaphosphates formed from ammonium dihydrogen orthophosphate in the presence of urea, (Lohrmann and Orgel, 1971; Osterberg and Orgel, 1972; Osterberg *et al.*, 1973). This synthesis is superior to any of our results but it is not clear whether it could have been widespread on the early Earth because ammonium dihydrogen phosphate is not a likely prebiotic compound except in relatively acidic environments. Submarine hydrothermal vents would have provided high temperature acidic environments on the early Earth. However, the rate of hydroly-

sis of condensed phosphate bonds is greatly accelerated at high temperatures and hydrothermal vent fluid contains very low concentrations of orthophosphate.

We have reported these results because they constitute several new approaches to prebiotic polyphosphate synthesis, and it is possible that these positive, but not robust, syntheses could be improved upon.

Acknowledgements

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