Biomimetic phosphoryl transfer catalysed by iron(II)-mineral precipitates

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Abstract—Iron(II) minerals have been found to catalyse the formation of pyrophosphate from activated phosphate compounds and inorganic phosphate at near neutral pH in water. Iron(II) phosphate catalyses the formation of pyrophosphate, from either acetyl phosphate or phosphoenolpyruvate as the activated phosphoryl donor, in yields of up to 25% and 14% respectively. Under similar conditions, these minerals also retard the hydrolysis of pyrophosphate. The catalysis of pyrophosphate formation is tolerant of sulfide ions: pyrophosphate being produced from acetyl phosphate in 12% yield in the presence of equimolar amounts of sulfide. These reactions mimic the extant biosynthesis of ATP from acetyl phosphate or phosphoenolpyruvate; they counter the concern that hydrolysis of polyphosphates would out-compete their accumulation under prebiotic conditions; and hence suggest a possible prebiotic route to polyphosphates under conditions that may have occurred on the early earth. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

Hydrothermal vent systems have been proposed as a possible site for the origin of life on Earth (Corliss et al., 1981; Wächtershäuser, 1988; Martin and Russell, 2003). The iron sulfide minerals released at these sites provide a continuous input of redox energy which is harnessed by contemporary organisms to provide luxuriant oases of life (Kelley et al., 2002). Iron sulfide systems have also been found to catalyse a range of biomimetic transformations (Cody et al., 2000; Cody et al., 2001; Huber and Wächtershäuser, 2003) including carbon fixation (Heinen and Lauwers 1996; Huber and Wächtershäuser 1997) and nitrogen fixation (Dörr et al., 2003) reactions that mimic extant iron-sulfur-dependent processes of anaerobic metabolism (Beinert et al., 1997; Rees and Howard, 2003). The importance of iron sulfide in the origin of life is further suggested by the structure of the metastable iron sulfide mineral greigite, which may be formed under low temperature hydrothermal conditions and resembles that of iron-sulfur clusters found in carbon fixation and other enzymes (Russell et al., 1998). In contrast to the study of iron(II) mineral catalysis of biomimetic processes of carbon and nitrogen metabolism, little is known about possible iron(II) mineral catalysis of biomimetic phosphate chemistry. Indeed, understanding the emergence of the central role of polyphosphates in bioenergetics remains a problem for prebiotic chemistry (Keefe and Miller, 1995) in both hydrothermal and other environments.

Acyl phosphate metabolites, e.g., acetyl phosphate (AcPi), and phosphoenolpyruvate (PEP), are used as phosphoryl donors in central metabolism for the generation of ATP. In addition, polyphosphate generation at membranes occurs via acyl phosphate intermediates. In some cases, pyrophosphate and other inorganic polyphosphates can substitute for ATP, and it has been proposed that inorganic polyphosphates were the evolutionary precursors of ATP (Baltscheffsky et al., 1966; Baltscheffsky et al., 1999; Kornberg, 1999). In biochemical systems, polyphosphate generation from inorganic phosphate and phosphoryl donors often has an absolute requirement for divalent metal ions, typically magnesium(II). The abiological catalysis of phosphoryl transfer reactions of acetyl phosphate by divalent metal ions has been known for some time. For example, calcium(II) and strontium(II) have been found to promote the formation of pyrophosphate from acetyl phosphate and inorganic phosphate (Vieyra et al., 1985). Very high concentrations of monovalent metal ions (>6 mol/L) also promote phosphoryl transfer (Herschlag and Jencks, 1986). There is also a report of metal ion-catalysis of phosphoryl transfer from phosphoenolpyruvate to inorganic phosphate (Hermes-Lima and Vieyra, 1992). There are no reports, to our knowledge, of the catalysis of polyphosphate synthesis by iron(II) minerals.

Most models for prebiotic syntheses of condensed phosphates are characterized by low yields or forced conditions (Yamagata et al., 1991; Keefe and Miller, 1996): involving some combination of high temperatures (>>100°C); abiological dehydrating agents; and/or drying cycles to increase polyphosphate yields, in contradistinction to reactions taking place at moderate temperatures in a fully aqueous environment. In addition, many of these syntheses have no counterpart in extant biochemistry and hence would present unwelcome discontinuities in the emergence of metabolism. The instability of polyphosphates towards hydrolysis poses further challenges to understanding ways in which polyphosphates could have accumulated in the environment to levels that may have been useful for a primordial metabolism. Precipitation of phosphate species at hydrothermal vent sites provides a possible route to concentration of phosphates (Wheat et al., 1996; Arrhenius et al., 1997; Kakegawa et al., 2002) and iron sulfide minerals have been found to contain appreciable amounts of phosphate (Stanton, 1976). It has been speculated that precipitation of phosphate species on the surface of iron sulfide mineral surfaces could have played a role in prebiotic diphosphate chemistry (Russell and Hall, 1997). In light of the possible importance of

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iron(II) for the origin of metabolism, here we report biomimetic studies of phosphoryl-transfer processes and accumulation of pyrophosphate by iron(II) minerals under related chemical conditions.

2. MATERIAL AND METHODS

Unless otherwise stated, all chemicals were purchased from commercially available sources, were of the highest grade, and used without further purification. Acetyl phosphate was synthesized by the method of Avison (1955), and determined to be free of inorganic phosphate by ^{31}P NMR spectrometry.

2.1. General Method for Reactions Involving Acetyl Phosphate

Buffered solutions (0.5M buffer: MES at pH 5.5–6.5, PIPES at pH 7.0, and HEPES at pH 7.5) containing 50mM inorganic phosphate, 50mM acetyl phosphate and 100mM sodium sulfide (where required) were added to iron(II) sulfate at 0°C under argon. The resulting heterogeneous reaction mixture was incubated at 37°C in a water bath. Aliquots were withdrawn by syringe at various times, treated with potassium cyanide (ca. 12 mol equivalent) and the supernatant analyzed by ^{31}P NMR spectrometry.

2.2. General Method for Reactions Involving Phosphoenolpyruvate

Buffered solutions (0.5M buffer: MES at pH 5.5–6.5, PIPES at pH 7.0, and HEPES at pH 7.5) containing inorganic phosphate and 50mM phosphoenolpyruvate were added to iron(II) sulfate at 0°C under argon, and then incubated at 45°C in a water bath. Aliquots were withdrawn by syringe at various times, treated with potassium cyanide (ca. 12 mol equivalent) and the supernatant analyzed by ^{31}P NMR spectrometry.

2.3. Hydrolysis of Pyrophosphate

Buffered solutions (MES, 0.5M, pH 6.5) containing 25mM pyrophosphate and 100mM sodium sulfide (if required) were added to iron(II) sulfate at 0°C under argon, and then incubated at 95°C in a water bath. Aliquots were withdrawn by syringe at various times, treated with potassium cyanide (ca. 12 mol equivalent) and the supernatant analyzed by ^{31}P NMR spectrometry.

3. RESULTS

Phosphate and activated phosphoryl donors were efficiently precipitated from aqueous solution by the addition of iron(II) salts. To study the nature of chemistry occurring within these heterogeneous mixtures, a protocol was devised whereby all the phosphate species present were solubilized by treatment of the reaction mixtures with excess cyanide ions. The composition of the phosphate species was determined directly by ^{31}P NMR spectrometry of the resulting supernatant.

Pyrophosphate formation using acetyl phosphate. Significant amounts of pyrophosphate were formed within one hour (Fig. 1) when aqueous mixtures of acetyl phosphate and phosphate, co-precipitated in the presence of iron(II), were incubated at 37°C. We further analyzed this chemistry by studying the effect of pH, iron(II) levels and temperature.

The reaction was investigated over the pH range 5.5–7.5, with the yield of pyrophosphate showing a pH maximum of 6.5 (Fig. 2). At lower pH the yield dropped; at pH 5.5 the yield of pyrophosphate was reduced four-fold compared to pH 6.5. Increasing the pH above 6.5 also reduced the yield of pyrophosphate; however the reaction could not be studied above pH 7.5 using our methodology, presumably due to the formation of

iron hydroxides and the subsequent difficulty in solubilising the precipitate.

The yield of pyrophosphate was also dependent on the amount of iron(II) present (Table 1). At pH 6.5, no pyrophosphate was produced if the molar ratio of iron(II) to phosphate species was less than 0.25. The yield of pyrophosphate from acetyl phosphate increased to 25% when the molar ratio of iron(II) to phosphate species approached 1, suggesting that pyrophosphate formation is dependent on the specific nature of the phosphate precipitate.

The temperature dependence of pyrophosphate production was measured between 22°C and 67°C. The highest yields of pyrophosphate occurred at 38°C and 51°C. At 22°C, pyrophosphate formation was slower and yields were reduced by ~50%. At 67°C the rate of pyrophosphate formation was similar to the rate observed at 38°C and 51°C but the yields were reduced to ~25% of the maximum yield observed.

Pyrophosphate formation using phosphoenolpyruvate. The ability of phosphoenolpyruvate to act as the phosphoryl donor for the formation of pyrophosphate was also investigated. Significant amounts of pyrophosphate were formed in one week at 45°C under the same conditions as described for the experiments with acetyl phosphate, although yields were consistently lower. The maximum yield of pyrophosphate observed when phosphoenolpyruvate was the phosphoryl donor was 14%. The pH dependence for the production of pyrophosphate using phosphoenolpyruvate was similar to that using acetyl phosphate as the donor, with a maximum at pH 6.5 (Fig. 2). Similarly, the yield of pyrophosphate was also dependent on the amount of iron(II) present, although in this case a higher molar ratio of iron(II) to phosphates was required before significant production of pyrophosphate was observed (Table 1).

Pyrophosphate formation in the presence of other metal ions. The specificity of metal ion catalysis was evaluated by running analogous reactions in the presence of nickel(II) and zinc(II) salts. Catalysis of pyrophosphate formation was found to be much more efficient for iron(II) than for either nickel(II) or zinc(II) ions. Nickel(II) and zinc(II) ions catalysed the decomposition of acetyl phosphate at a rate comparable to iron(II), however the amount of pyrophosphate produced was significantly diminished. Under optimized conditions for pyrophosphate formation from iron(II), the yield of pyrophosphate was 25%, while for both nickel(II) and zinc(II) the yield of pyrophosphate was ~4% (Fig. 3).

Pyrophosphate formation in the presence of iron(II) sulfide. To further test the relationship between the above results and the chemistry of hydrothermal vent-type environments, the iron(II)-catalyzed reaction between acetyl phosphate and inorganic phosphate was repeated in the presence of sulfide ions. When the phosphoryl-transfer reaction was carried out in the presence of freshly precipitated iron(II) sulfide (1 equivalent of sulfide/iron(II)), pyrophosphate was obtained in a yield of 12%, approximately ~50% of the yield obtained in the absence of sulfide ions (Fig. 3).

The stability of pyrophosphate in the presence of iron(II) precipitates. To assess whether the conditions used in this work could lead to the accumulation of pyrophosphate, we studied the rate of hydrolysis of pyrophosphate in the presence of iron(II) and iron(II) sulfide, as well as in the absence of any iron(II) at 95°C and pH 6.5. In the absence of iron(II), the
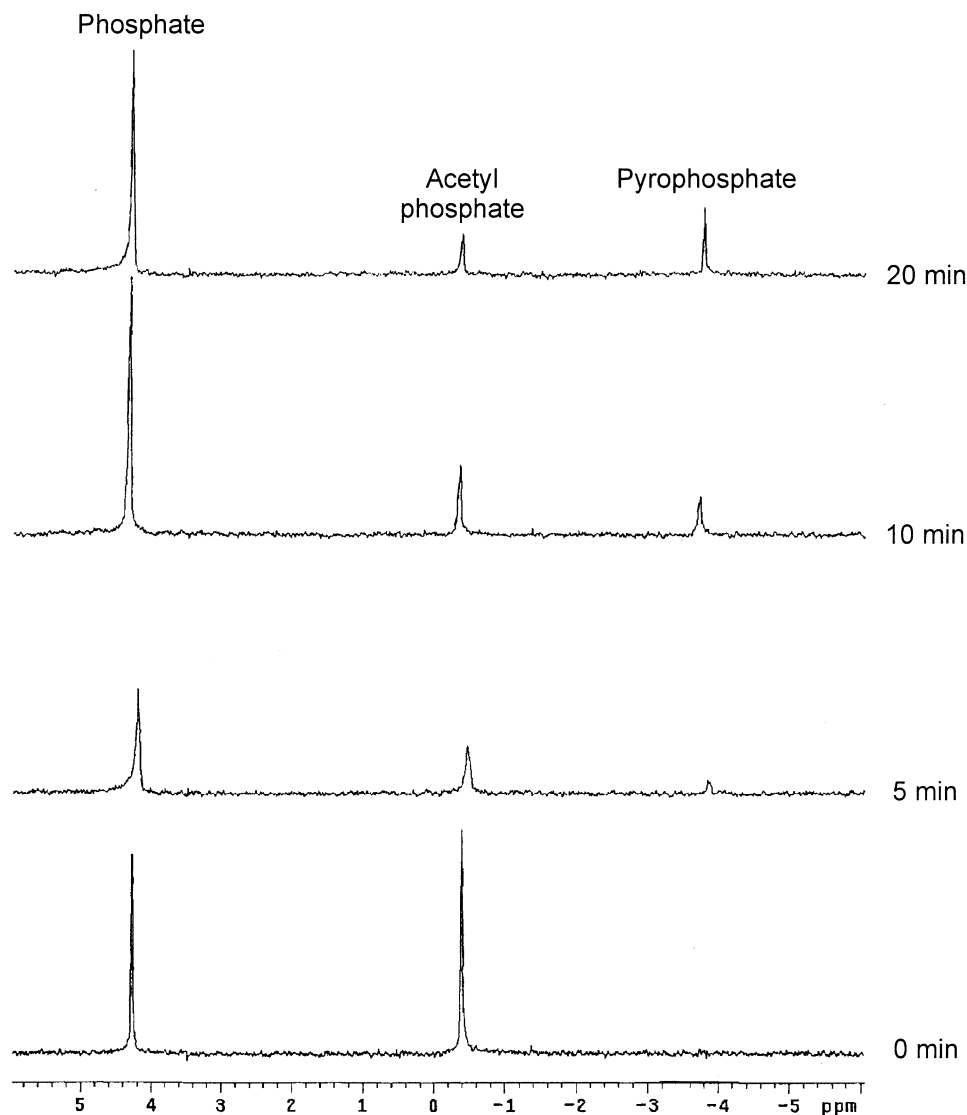
Fig. 1. ^{31}P NMR spectra showing the time course of pyrophosphate formation from acetyl phosphate for a typical reaction. 0.5mmol K_2HPO_4, 0.5mmol AcP_i and 0.75mmol $FeSO_4 0.7H_2O$ in a MES buffer at pH 6.5 was incubated under argon at 37°C. Aliquots were treated with KCN and centrifuged before analysis of the supernatant by ^{31}P NMR.

half-life of pyrophosphate was ~100min, compared to half-lives of ~1400min and ~2300min in the presence of iron(II) and iron(II) sulfide respectively (Fig. 4).

4. DISCUSSION

We have shown that iron(II) is an effective catalyst for the production of pyrophosphate from inorganic phosphate and the activated phosphoryl donors acetyl phosphate and phosphoenolpyruvate. The formation of pyrophosphate takes place on, or within, the precipitate that forms and is dependent on both the pH and the amount of iron(II) relative to phosphates present in the reaction. These effects may be explained by chemistry of the precipitate that forms in the reaction.

One would expect the pH to have a significant effect on the nature of the chemistry that occurs between the iron(II) and the phosphate species since this is an ionic interaction between the

oxyanions of the phosphates and the positively charged metal ions. This influences both the precipitation equilibria and dynamics and, potentially, the reactivity of the phosphoryl species. Fully protonated phosphates, phosphoric acids, are soluble in the presence of divalent metal ions. As the pH increases, the degree of ionization of the phosphates increases, leading to an increase in the amount of precipitate formed with iron(II), and thus an increase in the yield of pyrophosphate, since pyrophosphate formation is dependent on the precipitate. However, in alkaline solutions the nature of the precipitated iron species changes since iron(II) forms insoluble polymeric iron(II) hydroxides, resulting in a reduced availability of iron(II) for phosphoryl transfer. The maximal yield of pyrophosphate at pH 6.5 may reflect, in part, a compromise between the beneficial effect of increased precipitation set against the decreased availability of appropriate iron(II) species as the pH is increased; it
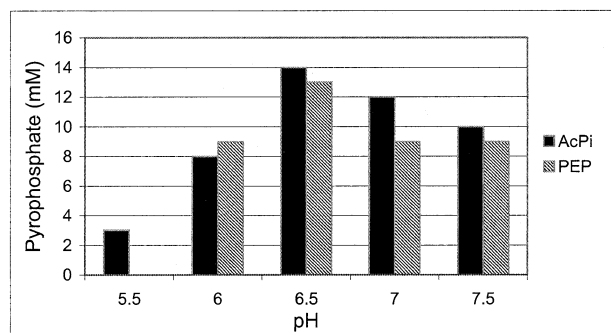
Fig. 2. Effect of pH on the yield of PP_I from AcP_i^a or PEP^b. Yields were determined by integrating the peak signals of the ^{31}P NMR spectrum; $\%PP_I = 100 \times PP_I/\Sigma(P_i + AcP_I/PEP + PP_I)$ and are the average of two experiments. ^{a}Reaction mixtures containing 0.5mmol K_2HPO_4, 0.5mmol AcP_i and 0.5mmol $FeSO_4.0.7H_2O$ in 10mL buffer solution were incubated at 37°C under Ar for 90 min. ^{b} Reaction mixtures containing 0.5mmol K_2HPO_4, 0.5mmol PEP and 0.5mmol $FeSO_40.7H_2O$ in 10mL buffer solution incubated at 45°C under Ar for 10 d.
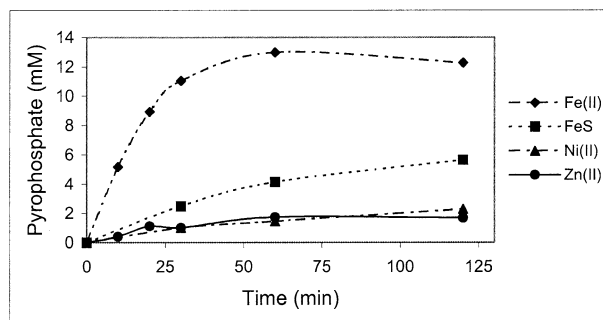

Fig. 3. Time course for the formation of pyrophosphate from acetyl phosphate and inorganic phosphate catalyzed by iron(II), iron(II) sulfide, nickel(II) or zinc(II). Reactions were carried out at pH 6.5 and contained 0.5mmol AcP_i, 0.5mmol P_i and 1mmol metal sulfate in 10mL. Iron(II) sulfide was formed in situ by the addition of 1mmol sodium sulfide.

may also reflect the ionization state of the phosphate species undergoing reaction (Hershlag and Jencks, 1986; Westheimer, 1987). Although in solution the rate of hydrolysis of acetyl phosphate is independent of pH over the range pH 4–9 (Koshland, 1952), the rate of pyrophosphate formation from acetyl phosphate and phosphate is dependent on the ionization state of the phosphate species (Hershlag and Jencks, 1986).

The effect of increasing the amount of iron(II) appears to be two-fold: it increases the rates of both hydrolysis and phosphorolysis of acetyl phosphate and phosphoenolpyruvate; it also increases the relative rate of phosphorolysis compared to hydrolysis. Over and above the issues of pH, considered above, these effects can be rationalized as due to the selective precipitation of different phosphate species since pyrophosphate formation necessarily requires the co-precipitation of both inorganic phosphate and the phosphoryl donor. At low levels of iron(II), the precipitate will consist predominately of iron(II) phosphate, with limited opportunity for pyrophosphate formation since inorganic phosphate is expected to bind more tightly to iron(II) than either acetyl phosphate or phosphoenolpyruvate. As the amount of iron(II) is increased, the phosphoryl donor will also be incorporated into the precipitate, facilitating

the formation of pyrophosphate. Once all of the phosphoryl donor is precipitated, any further increase in the amount of iron(II) is unlikely to affect significantly the yield of pyrophosphate.

The formation of pyrophosphate in the presence of iron(II) sulfide is interesting in that the presence of sulfide ions, which compete with the phosphates for the iron(II), does not preclude the formation of pyrophosphate. This is notable since both cyanide and EDTA, which also bind iron(II), prevent the formation of pyrophosphate. This could be due to the ability of iron(II) sulfide to present sites capable of catalyzing pyrophosphate formation, for example the positive iron(II) centers on the surface of iron(II) sulfide. Alternatively, the phosphate species may be able to displace the sulfide, forming co-precipitates rich in iron(II)-phosphates, and these may be the site of pyrophosphate formation. At present it is difficult to discern between the two alternatives. In addition, the observation that neither the nickel(II) or zinc(II) precipitates are as capable of catalyzing pyrophosphate suggests that this is not simply a feature of any precipitate of divalent metal ions with these phosphate species. In a previous study of the ability of calcium(II) to catalyze

Table 1. Effect of iron(II) on the yield of PP_I from AcP_i or PEP

Fe(II) (mmol)	% PP_i from AcP_i^a	% PP_i from PEP^b
0	0	0
0.25	0	0
0.5	14	0
0.625	20	-
0.75	25	4
1.0	24	14

^{a} Reactions mixtures containing 0.5mmol K_2HPO_4, 0.5mmol AcP_i and $FeSO_4.7H_2O$ in MES buffer at pH 6.5 were incubated under Ar for 90 min at 37°C.

^{b} Reactions mixtures containing 0.5mmol K_2HPO_4, 0.5mmol PEP and $FeSO_4.7H_2O$ in MES buffer at pH 6.5 were incubated under Ar for 10 days at 45°C.
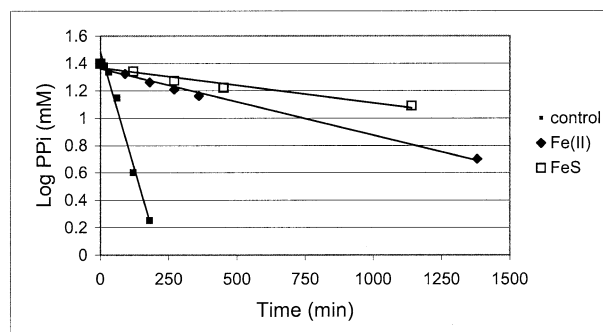

Fig. 4. A semilog plot showing the hydrolysis of pyrophosphate in the presence of Fe(II) and FeS. Pyrophosphate (0.25mmol) was incubated at 95°C and pH 6.5 in the presence of 1mmol Fe(II) or FeS. Aliquots were removed and analysed by ^{31}P NMR after treatment with cyanide solution. Least squares regression was used to estimate the respective half-lives, using the formula $\log [PP_i] = -kt/2.3 + \log [initial\ PP_i]$.

A

$$AcP_i + P_i \xrightarrow{\text{Fe(II)}} \text{Acetate} + PP_i$$

B

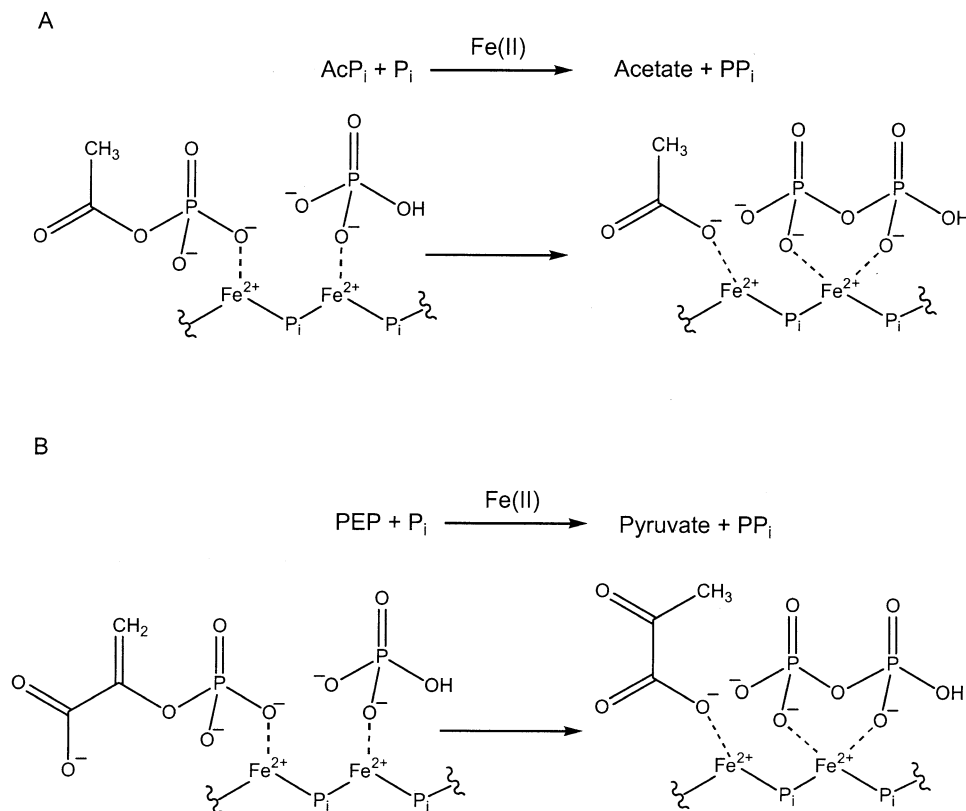$$PEP + P_i \xrightarrow{\text{Fe(II)}} \text{Pyruvate} + PP_i$$

Fig. 5. Possible mechanism for the formation of pyrophosphate on the surface of a iron(II) phosphate precipitate. A, acetyl phosphate as the phosphoryl donor. B, PEP as the phosphoryl donor.

pyrophosphate formation from acetyl phosphate and inorganic phosphate, the appearance of a calcium-phosphate precipitate coincided with a decrease in the yield of pyrophosphate, suggesting that sequestering the calcium and phosphate within the precipitate may reduce pyrophosphate formation (Vieyra 1985). However when large amounts of acetyl phosphate were present in the precipitate, pyrophosphate formation did take place (Meyer-Fernandes and Vieyra 1988).

We can only speculate as to why iron(II) precipitates are better than other, seemingly similar, metal precipitates. Pyrophosphate formation requires that both inorganic phosphate and the phosphoryl donor are brought into close proximity. This could be achieved by binding both species to a single iron(II) center, or perhaps to adjacent iron(II) centers either on the surface of, or within, an iron(II)-phosphate precipitate, leading to pyrophosphate formation as envisaged in Figure 5. The iron(II) can also diminish the electrostatic repulsion between the negatively charged phosphate groups (Westheimer, 1987). The precipitate may also protect acetyl phosphate and phosphoenolpyruvate from hydrolysis by sequestering them within the precipitate where the activity of water would be lowered. However, it is not obvious why other divalent metal ions, such as, calcium(II), nickel(II) or zinc(II) ions, could not carry out the same function equally as well.

The ability of iron(II) to catalyze pyrophosphate formation under the conditions described may be of significance to the origin of life. Iron(II) was abundant on the early earth, particularly around hydrothermal vent systems, which have been

implicated as possible sites for the origin of life. The moderate temperatures at which pyrophosphate formation is optimal would easily be found in regions where hot vent fluids mix with the cooler seawater. Similarly the pH and temperature optima are both within a range that may well have been present on the early earth and which have been postulated as possible environments for the origin of life; for instance where alkaline vent fluids (such as found at the Lost City hydrothermal field (Kelley et al., 2001)) met a mildly acidic Hadean ocean water (Russell and Hall, 1997).

These findings are significant to prebiotic theories since acetyl phosphate and phosphoenolpyruvate are plausible prebiotic molecules. Activated carboxylic acid derivatives are implicated as possible intermediates in a range of prebiotic reactions (Liu and Orgel, 1997; Weber, 1981) including the iron-sulfide mediated carbon fixation chemistry that mimics an extant pathway for ATP formation (Huber and Wächtershäuser, 1997). Phosphoenolpyruvate may be formed by the dehydration of 2-phosphoglycerate by analogy with extant glycolysis. This dehydration reaction is thermodynamically favourable in water and biochemical processes of this type are known to be catalyzed by iron-sulfur clusters (Flint and Allen, 1996).

The ability of the iron(II) precipitates formed in this work both to catalyze pyrophosphate formation and stabilize pyrophosphate against hydrolysis is significant as one criticism of hydrothermal-based origin of life hypotheses is that hydrolysis will become overwhelming at the higher temperatures surrounding hydrothermal vents. However, the results are consis-

tent with a moderate temperature for the origin of life, avoiding problems of slow reactions at low temperatures and hydrolytic decomposition at high temperatures.

5. CONCLUSIONS

The work described above demonstrates that the formation of pyrophosphate, by phosphoryl transfer from activated phosphoryl donors to inorganic phosphate, occurs readily in the presence of iron(II). The reaction occurs at an iron(II)-phosphate precipitate, and is influenced by factors which affect the binding of phosphate and acetyl phosphate to the precipitate. The ability of iron(II) to catalyse this reaction is in marked contrast to other metal ions, which typically are poor catalysts for this reaction in an aqueous environment.

The finding that iron(II) precipitates are able to mediate efficiently the accumulation of pyrophosphate in a reaction that is tolerant of sulfide ions may provide a plausible pathway for the entry of polyphosphate chemistry into an emerging heterogeneous metabolism, in hydrothermal vent-type environments. This complements the range of biomimetic processes catalysed by iron-rich metal sulfide systems and suggests an important role for the interactions of phosphorylated compounds and minerals in the early origin of metabolism.

REFERENCES

Arrhenius G., Sales B., Mojzsis S., and Lee T. (1997) Entropy and charge in molecular evolution—the case of phosphate. *J. Theoret. Biol.* **187,** 503–522.

Avison A. W. D. (1955) The synthesis of acyl phosphates in aqueous solution. *J. Chem. Soc.* **158,** 732–738.

Baltscheffsky H., von Stedingk L-V., Heldt H-W., and Klingenberg M. (1966) Inorganic pyrophosphate: formation in bacterial photophosphorylation. *Science* **153,** 1120–1122.

Baltscheffsky M., Schultz A., and Baltscheffsky H. (1999) H$^+$-PPases: a tightly membrane-bound family. *FEBS Lett.* **457,** 527–533.

Beinert H., Holm R. H., and Münck E. (1997) Iron-sulfur clusters: nature's modular, multipurpose structures. *Science* **277,** 653–659.

Cody G. D., Boctor N. Z., Filley T. R., Hazen R. M., Scott J. H., Sharma A., and Yoder H. S.Jr. (2000) Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science* **289,** 1337–1340.

Cody G. D., Boctor N. Z., Hazen R. M., Brandes J. A., Morowitz H. J., and Yoder H. S.Jr.. (2001) Geochemical roots of autotrophic carbon fixation: hydrothermal experiments in the system citric acid, H$_2$O-(±FeS)-(±NiS). *Geochim. Cosmochim. Acta* **65,** 3557–3576.

Corliss J. B., Baross J. A., and Hoffman S. E. (1981) An hypothesis concerning the relationship between submarine hot springs and the origin of life on earth. *Oceanol. Acta* **4,** 59–69.

Dörr M., Kassbohrer J., Grunert R., Kreisel G., Brand W. A., Werner R. A., Geilmann H., Apfel C., Robl C., and Weigand W. (2003) A possible prebiotic formation of ammonia from dinitrogen on iron sulfide surfaces. *Angew. Chem. Int. Edn. Engl.* **42,** 1540–1543.

Flint D. H. and Allen R. M. (1996) Iron-sulfur proteins with nonredox functions. *Chem. Rev.* **96,** 2315–2334.

Heinen W. and Lauwers A. M. (1996) Organic sulfur compounds resulting from the interaction of of iron sulfide, hydrogen sulfide and carbon dioxide in an anaerobic aqueous environment. *Origins Life Evol. Biosphere.* **26,** 131–150.

Hermes-Lima M. and Vieyra A. (1992) Pyrophosphate synthesis from phospho(enol)pyruvate catalyzed by precipitated magnesium phosphate with "enzyme-like" activity. *J. Mol. Evol.* **35,** 277–285.

Herschlag D. and Jencks W. P. (1986) Pyrophosphate formation from acetyl phosphate and orthophosphate anions in concentrated aqueous salt solutions does not provide evidence for a metaphosphate intermediate. *J. Am. Chem. Soc.* **108,** 7938–7946.

Huber C. and Wächtershäuser G. (1997) Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science* **276,** 245–247.

Huber C. and Wächtershäuser G. (2003) Primordial reductive amination revisited. *Tetrahedron Lett.* **44,** 1695–1697.

Kakegawa T., Noda M., and Nannri H. (2002) Geochemical cycles of bio-essential elements on the early earth and their relationships to the origin of life. *Res. Geol.* **52,** 83–89.

Keefe A. D. and Miller S. J. (1995) Are polyphosphates or phosphate esters prebiotic reagents? *J. Mol. Evol.* **41,** 693–702.

Keefe A. D. and Miller S. L. (1996) Potentially prebiotic synthesis of condensed phosphates. *Origins Life Evol. B.* **26,** 15–25.

Kelley D. S., et al. (2001) An off-axis hydrothermal vent system near the Mid-Atlantic Ridge at 30°N. *Nature* **412,** 145–149.

Kelley D. S., Baross J. A., and Delaney J. R. (2002) Volcanoes, fluids and life at mid-ocean ridge Spreading Centers. *Annu. Rev. Earth Pl. Sc.* **30,** 385–491.

Kornberg A., Rao N. N., and Ault-Riché D. (1999) Inorganic polyphosphate: a molecule of many functions. *Ann. Rev. Biochem.* **68,** 89–125.

Koshland D. E. (1952) Effect of catalysts on the hydrolysis of acetyl phosphate. Nucleophilic displacement mechanisms in enzymatic reactions. *J. Am. Chem. Soc.* **74,** 2286–2292.

Liu R. and Orgel L. E. (1997) Oxidative acylation using thioacids. *Nature* **389,** 52–54.

Martin W. and Russell M. J. (2003) On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Phil. Trans. R. Soc. B.* **358,** 59–83.

Meyer-Fernandes J. R., Vieyra A. (1988) Pyrophosphate formation from acetyl phosphate and orthophosphate: evidence for heterogeneous catalysis. *Arch. Biochem. Biophys.* **266,** 132–41.

Rees D. C. and Howard J. B. (2003) The interface between the biological and inorganic worlds: iron-sulfur metalloclusters. *Science* **300,** 929–931.

Russell M. J. and Hall A. J. (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J. Geol. Soc. Lond.* **154,** 377–402.

Russell M. J., Daia D. E., and Hall A. J. (1998) The emergence of life from FeS bubbles at alkaline hot springs in an acid ocean. In *Thermophiles*: *The Keys to Molecular Evolution and the Origin of Life* (eds. J. Weigel and M. W. W. Adams) pp. 77–125. Taylor and Francis, Washington.

Stanton R. L. (1976) Petrochemical studies of the ore environment at Broken Hill, New South Wales: 1-constitution of 'banded iron formation'. *Trans. Inst. Min. Metall. B.* **85,** 33–46.

Vieyra A., Meyer-Fernandes J. R., and Gama O. B. H. (1985) Phosphorolysis of acetyl phosphate with energy conservation in the phosphoanhydride linkage of pyrophosphate. *Arch. Biochem. Biophys.* **238,** 574–583.

Wächtershäuser G. (1988) Before enzymes and templates: theory of surface metabolism. *Microbiol. Rev.* **52,** 452–484.

Weber A. L. (1981) Formation of pyrophosphate, tripolyphosphate, and phosphorylimidazole with the thioester. N, S-diacetyl-cysteamine, as the condensing agent. *J. Mol. Evol.* **18,** 24–29.

Westheimer F. H. (1987) Why nature chose phosphates. *Science* **235,** 1173–1178.

Wheat C. G., Feely R. A., and Mottl M. J. (1996) Phosphate removal by oceanic hydrothermal processes: An update of the phosphorus budget in the oceans. *Geochim. Cosmochim. Acta* **60,** 3593–3608.

Yamagata Y., Watanabe H., Saitoh M., and Namba T. (1991) Volcanic production of polyphosphates and its relevance to prebiotic evolution. *Nature* **352,** 516–519.