Peptide and RNA contributions to iron–sulphur chemical gardens as life’s first inorganic compartments, catalysts, capacitors and condensers

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Hydrothermal chimneys and compartments comprising transition metal sulphides and associated minerals have been proposed as likely locations for the beginnings of life. In laboratory simulations of off-axis alkaline springs, it is shown that the interaction of a simulated alkaline sulphide-bearing submarine vent solution with a primeval anoxic iron-bearing ocean leads to the formation of chimney structures reminiscent of chemical gardens. These chimneys display periodicity in their deposition and exhibit diverse morphologies and mineralogies, affording the possibilities of catalysis and molecular sequestration. The addition of peptides and RNA to the alkaline solution modifies the elemental stoichiometry of the chimneys—perhaps indicating the very initial stage of the organic takeover on the way to living cells by charged organic polymers potentially synthesized in this same environment.

Keywords: chemical gardens; geo-mimicry; origin of life; periodicity; transition metal catalysis; hydrothermal vents

1. Introduction

Life is a processor of energy and materials made available through convective forces on our planet, augmented by the sunshine at the surface. It maintains itself through homeostasis (i.e. through chemiosmosis and the proton motive force), by ‘drinking orderliness from a suitable environment’, by continual reference to genetic information and by exploratory channelling [1–5]. Organisms alive today can be thought of as decorating unbroken mutable threads of the genetic code stretching back, and tied, to the last universal common ancestor. But

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the contiguous, although evolving membrane plumbs even greater depths of the evolutionary tree to its base at the emergence of life, with iron sulphide clusters and phosphates constituting common motifs throughout [2].

In the study of life, we are best served not only by studying life ‘in the act’, but also the collective processes underlying its existence. It is through the effort to explore and understand the means by which matter and energy were first channelled to beget life, i.e. ‘to arrive where we started’, that we may grow to understand its underlying properties and raison d'être. We begin with questions broadly relating to matter, energy, enthalpy and entropy. How do the physical underpinnings of material existence order themselves to form the propagative, dissipative structures that we are and that live around us? Where best to realize the first such far-from-equilibrium systems with the propensity to focus matter and energy so that they could become evolutionarily sustainable, i.e. what type of chemical system on the early Earth would afford the entrainment and concentration of material flux, as well as a facility for waste disposal that life first adopted and recapitulates today? We will argue that the process was accomplished through a focused, yet probable set of material and energetic interactions that occurred on geochemical and geological scales, so giving ‘birth’ to biochemistry—a process that supervenes upon the laws of physics and chemistry. Yet, biochemistry is not simply better geochemistry. There is a discontinuity between the two. Biochemical reactions make products beyond the scope of geochemistry for they are not restricted to ‘one pot’. Two pots—one tiny, inside another large—is life’s trick [6–8]. A possible interface between these is a semipermeable, semiconducting membrane, one that allows the use of pumps and turbines to maintain the interior far-from-equilibrium, yet in a stable and low-entropy state while exporting entropy to the exterior [9]. A chemical garden behaves like that—for a while. Replacing Glauber’s seed crystal (Glauber 1649, quoted in [10]) with a continuous stream of sodium sulphide and silicate venting into a large reservoir of acidulous ferrous-iron-bearing solution will generate a plethora of tubular mineral precipitates and associated morphologies. Scaling this up to our, and even other globes in time and space, submarine chemical gardens constitute the kind of microfluidic flow reactors and affinity columns best able to explain life’s onset.

2. Initial conditions

Throughout its history, the Earth has existed in states of disequilibrium. Thermal disequilibrium results in convection, and convection eventuates and focuses chemical disequilibrium. In turn, a chemical disequilibrium is dissipated by metabolism in an aqueous environment. After all, the Earth is a giant (photo) electrochemical cell or battery, whereas the solid surface acts as the anode and the oxidized entities in the volatisphere act as the dispersed cathode [11]. The convective process switches on the current by offering electrons—gained from reduced metals in the ocean crust—in the form of hydrothermal hydrogen and methane generated through the reduction of water and carbon dioxide, to potential electron acceptors such as nitrate and ferric iron and, of course, that same carbon dioxide dissolved in the ocean. Autotrophic anaerobic life realizes this potential by mediating electron transfer.
The last universal common ancestor potentially delivered electrons to all three of these acceptors, but because carbon dioxide was overwhelmingly dominant in the atmosphere and ocean, this was (and is) the main recipient. The ultimate reduction or waste product was probably initially acetate \([12–15]\), and the womb or *omphalos* of life a chemical garden-like precipitate over a submarine alkaline spring, especially where it vented into the acidulous, iron-bearing Hadean Ocean \([16–18]\). Much as predicted, a comparable spring—the so-called Lost City—was discovered in the North Atlantic in 1999 \([16,19,20]\).

### 3. Hydrothermal vents and the origin of life

The discovery of deep-sea hydrothermal vents and their associated ecosystems forced the recognition that the steep geothermal and chemical gradients at these locations could not only generate sulphide ore deposits, but also sustain a diversity of life \([21–28]\). With these examples of hydrothermal chimneys to go on, smaller, fossil specimens—comprising pyrite \((\text{FeS}_2)\)—were soon discovered, although in mineral sulphide deposits generated at lower temperature \([16,29–33]\). Since these discoveries, active submarine hydrothermal vents have been found to exist with a wide array of attributes and can be divided into the high-temperature acidic ‘Black Smokers’ and the cooler, alkaline ‘off-axis’ systems, the Lost City type \([20]\). Although black smokers are directly driven by magmatic intrusion at oceanic spreading centres or over mantle plumes, off-axis vents result from thermal mining and exothermic rock–water interactions during natural open system convective circulation within mantle rocks exposed at the ocean floor \([34,35]\). Such systems are known to persist for tens of thousands of years \([36]\) and were likely present in much greater number than today on the young Earth. On this anaerobic Hadean Earth, the volcanic black smoker-type vents, also occurring in larger numbers, would have delivered large quantities of iron directly into the acidulous ocean \([37]\).

In contrast, where alkaline hydrothermal fluids at off-axis vents traversed sulphide accumulations, sulphide itself would likely have been mobilized and upon interaction with dissolved metal ions in the ocean, precipitated with them to form inorganic chimneys \([16,38]\). This notion is based on the discovery of fossilized pyrite structures at what were relatively shallow water vent locations and the known reactivity of sulphide with transition metals (in particular, iron) \([29,30,39]\). Thus, one could imagine how chemical gradients could be sustained and how reactions and molecular sequestration in a relatively low-temperature environment insulated from the turbulent conditions of the surface could operate \([2]\). On the early ocean floor, locations would have existed that channelled the exergonic products of serpentinization, locations where reduced minerals (mafic and ultramafic crust) were oxidized, and water and carbon dioxide were concomitantly reduced to hydrogen and methane \([16,40]\). From a chemical perspective, off-axis spring localities, where low-potential (reducing) electronic species are exhaled from the Earth, may have provided a portion of the necessary energy required for early hydrogenation reactions that were crucial in the accumulation of biologically relevant molecules and electronic equivalents at the origin of life \([41,42]\).
Given considerations of the Earth’s early atmosphere, as well as the difference in redox potential of a reducing vent and a carbon dioxide-rich volatilosphere, it has been suggested that the first organisms operated autotrophically, sequentially reducing carbon dioxide to acetate as realized by the acetogenic bacteria today [13]. This concept of a geochemically driven autotrophy is an entirely different notion from that espoused in the so-called ‘soup’ theories. The latter take no account of local chemical and electronic disequilibria as the primary driver of molecular evolution [43]; it was not in the diffuse seascape of the open ocean where life likely began but at the focused regions of energetic frustration and dissipation on the ocean floor, a much more plausible site for its forced emergence [44,45].

Other than the fossilized remnants of pyrite chimneys and an early report of the formation of such chimneys in vitro [2,16,30], little is known as to the properties of chimney formation as it would have occurred on the early Earth. Given the existence of alkaline hydrothermal chimneys as probable localities for a geochemically and electrochemically driven emergence of life, they are urgent targets for research into the processes that govern their formation, as well as for empirical testing of their ability to act as flow-through reactors and culture chambers for chemical interconversions leading at least to the onset of metabolism [16]. Thus, we have begun the systematic investigation of metal sulphide and oxide chimneys in the laboratory, with the intention of probing in detail the determinants of their formation and characteristics [18]. Recently, we have shown that the passage of high-pressure alkaline fluid through iron sulphide and basaltic rock is sufficient for the mobilization of appreciable quantities of both sulphide ions as well as silica and that the resultant fluid is sufficient to support the formation of a chimney upon introduction to a ferrous-iron-bearing solution [38]. Here, the presence of silica in solution was observed to greatly enhance the durability and size of obtained structures. Adding to these experiments, we have investigated the formation of iron sulphide chimneys in vitro in an effort to determine the lower bounds of reagent concentration needed to support growth, as well as the propensity of these to be modified by the addition of charged organic polymers relevant to the evolution of biological complexity.

4. Analysis of in vitro iron sulphide chimneys in off-axis submarine hydrothermal vent simulations

On the basis of hydrothermal simulations of serpentinization reactions of ancient oceanic crust hosting sulphide deposits, we infer that certain off-axis hot (approx. 100°C) springs would have supplied not only hydrogen, but also HS\(^-\) ions to the Hadean Ocean in millimolar concentrations [38]. In the experiments referred to herein, we introduced solutions into visijars containing simulacra of the Hadean Ocean. In these reactions, tubular precipitates of iron sulphides were realized comparable to chemical gardens. Analysis of these precipitates was carried out with environmental scanning electron microscopy (ESEM), and relative elemental abundances obtained with energy-dispersive X-ray spectroscopy (EDAX). ESEM was carried out on an FEI XL30 FEG at 4.0T, an accelerating voltage of 20kV and at a working distance of 10.2–10.7mm with a gaseous secondary
Figure 1. ESEM images of a chimney and chimney walls obtained from the introduction of a 5 mM sulphide solution to a 10 mM Fe$^{2+}$ solution. NaCl was 35 g l$^{-1}$ in both solutions (cf. [19]) and anaerobicity was maintained by N$_2$ sparging. Scale bars, (a) 100 μm, (b) 20 μm and (c) 5 μm.

electron detector. The experimental set-up is comparable to that described previously [18,38,46], and the resulting structures may be considered a subset of chemical garden phenomena.

In our experiments, a 5 mM HS$^{-}$-bearing solution was introduced into a 10 mM ferrous iron solution (representing the hydrothermal fluid and the Hadean Ocean, respectively) at a flow rate of 0.12 ml min$^{-1}$, so producing highly porous chimney-like mound structures (figure 1a). These porous, high-surface-area
structures exhibit diverse mineralogical morphologies (figure 1b,c). As judged by EDAX, Fe:S ratios approached unity at some locations (Fe:S = 1.3). Indeed, in materials from similar experiments in which silicate was also added at standard temperature and pressure, mackinawite (FeS) was generated and greigite (Fe₃S₄) was formed at higher temperature (70°C) as analysed by X-ray diffraction and Raman spectroscopy [18,38,47] (and see [39]). A striking observation from these experiments was the general, although not invariable, appearance of crenulated patterns on both the exterior and interior of the chimneys (Fe:S ∼ 4) with ridges and rills aligned normal to the apparent direction of chimney growth (figure 1b). Such a periodic deposition of mineral flanges bears some resemblance to previously characterized tubular inorganic assemblies [10,46,48] (cf. [49]).

To investigate the possible effects of biologically relevant soluble charged polymers on the formation and patterning of FeS chimneys, short peptides or RNA were added to the alkaline spring solution. This had the effect of modifying the chemistry and structure of the small chimneys. For example, in the cases of addition of 100 µM of peptide (GGGGG, ANASPEC) or 100 µg ml⁻¹ RNA (Saccharomyces cerevisiae, Sigma R7125) to the sulphide solution in separate experiments, average ratios were Fe:S = 5.9 (n = 52, peptide) and 5.8 (n = 57, RNA) versus 3.1 (n = 71) in the cases of no added polymer. Upper limits of Fe:S ratios observed were 18 and 37 for peptide and RNA, respectively, whereas in the absence of polymers, the Fe:S ratio never exceeded 8. Other experiments with iron-bearing chimneys in the presence of polymers have shown that the stability of the membranes was enhanced proportionally to the polymer length, a phenomenon attributed to the change of viscoelastic solution properties [50]. Here, we assume that peptides and RNA polymers exert a significant effect on overall chimney structure, durability and architecture, as a result of their ability to chelate metal cations at the chimney–solution interface. This is because both the short peptides and RNA exist as multi-dentate ligands that can bind metal ions, or their sulphides or oxides, directly through the functional groups that comprise the backbones and, except pentaglycine, the side chains of the polymers. These include hydroxide, carboxyl, carbonyl and amine groups and—in the case of RNA—phosphate [51–55]. Indeed, such properties of RNA have led to the demonstration of RNA binding to freshly precipitated mackinawite [56]. Given the increase of Fe:S ratios with the addition of these polymers, they might also direct epitaxial growth of iron rich minerals by specific metal ion chelation and crystallization, whereby sulphide has been displaced by surrogate polymer-derived ligands. These interactions shed new insights as to how peptides may have taken over from minerals to comprise the first organic membranes [54] (cf. [57]).

In the case of the addition of RNA, the appearance of a mineral bilayer was observed, with EDAX measurements indicating a lower Fe:S ratio inside than outside (figure 2a,b). Thus, as to be expected, the low ratios are observed proximal to the injected sulphide solution (inside the tube) and high Fe:S ratios are observed distal to the emerging sulphide solution (high iron concentration and low sulphide concentration at the chimney exteriors). In a separate experiment in the presence of RNA, the lighter material of the bilayer had apparently been washed out, indicated by the observation of only a single layer (figure 3). The obtained material was similar in elemental abundance to that observed in the dark layer in figure 2b. These experiments also show that crenulations are not
Figure 2. ESEM images of chimney walls obtained from the introduction of a 5 mM sulphide solution containing 100 μg ml⁻¹ RNA into a 10 mM Fe²⁺ solution. NaCl was 35 g l⁻¹ in both solutions (cf. [19]) and anaerobicity was maintained by N₂ sparging. (a) A half chimney and (b) zoom in where two distinct layers are observed (inside is on the left). The spherical structures are comparable with greigite framboids [29,58]. The light material on the left of (b) has Fe : S = 2.6 and the dark material on the right has Fe : S = 8.6 as judged by EDAX. Scale bars, (a) 100 μm and (b) 5 μm.

limited to the outside chimney structures and that this layer is ribbed on both sides. Observation of these structures along fracture edges revealed crystalline chards traversing the thickness of the wall, with nanoscale spheres sited at their proximal termini (figure 4).

5. Discussion and prospectus

The tubular chemical garden structures fabricated in the laboratory offer themselves as a model for ocean floor mounds growing at alkaline springs on the early Earth. Such a composite mound would have comprised a surplus of compartments with the potential, in the presence of various promoters and electrochemical gradients, of quickening the hydrogenation of carbon dioxide, i.e. the mound as the hatchery or womb of life. The process of serpentinization provided a source of low-potential electrons; FeS minerals and their derivatives comprising the gardens had the potential to catalyse reductions and interconversions; chimney walls would have sequestered some of the products; and high pH, redox and thermal differences between inside and outside the chimney structures could have driven condensations and polymerizations [16,40,59]. In the experiments described herein, sulphide solution was measured at a pH of 4.1, and the iron solution was measured to be 11.6. While the Hadean Ocean may, on giant volcanic exhalations of sulphur dioxide, have occasionally veered to such a low pH, a pH of around 5.5–6 may have been a likely average [17] (cf. [60]). Thus, conservatively we estimate a pH potential operating across the sides of a chimney wall of around 300 mV—comparable to natural gradients obtaining across cells today [61–63]. Added to this would be the volt of redox energy between H⁺/H₂ on the inside and Fe⁴⁺/Fe²⁺ as well as NO₃⁻/NO₂⁻ on the outside of the chimneys [64]. Thus, chemiosmosis—a fundamental and universal trait of biology—is seen to be an aspect of the initial conditions of life's
emergence [2,3]. How the energy flows were organized in these chemical gardens remains unclear. Perhaps the crenulated pattern—a feature of the chimneys with or without the organic additives—is a clue, the rills and ridges defining the original importing and exporting sites across the inorganic membrane [18]. Such periodic depositions are not unique to these experiments and have been observed by other groups [10,46,48] (cf. [49]). In particular, Collins et al. [10] reported

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the analysis of silica chemical gardens and found four distinct hierarchical levels of material organization, which they suggest to be a property associated with the multi-step assembly of structure in non-equilibrium phases. Remarkably, the tubes and associated rills or columns shown here are very similar in spatial scale (approx. 160 μM and approx. 4.4 μM for the macroscopic ‘whiskers’ and associated columns in Collins et al.).

As is well known, precipitate structures—produced where a solution containing an acidic salt of a metal precipitates upon interaction of a dissolved solute in an alkaline aqueous phase—comprise semipermeable osmotic membranes [6,7,66–68]. Such membranes are known to act as selective diffusion barriers across which an electrical potential occurs with the separation of ionic species, and exhibit ionic diffusion with an applied potential [67]. In the cases of BaSO₄ and calcium oxalate membranes, OH⁻ permeation was found to exceed H⁺ permeation by four- or fivefold [68] and may be thought of as capacitors insofar as they maintain charge separation. A similar situation may be involved in the FeS structures presented here, and demonstration of this is a challenge for future experimentation. An additional route for charge transfer across the membrane may be expedited through the mackinawite comprising the walls of the chemical gardens [18], which may allow for electronic communication (figure 5) [61,72–74]. Electron transfer along the tetrahedral planes as shown in figure 5a could permit electron shuttling and, with the addition of ligand modification at plane termini or at mineral defect sites, catalysis akin to some modern-day enzymes.

Figure 4. Side views of chimneys obtained from RNA-bearing experiments along fractures. (b) and (d) are zoom in images of (a) and (c), respectively (cf. [65]). Scale bars, (a) 5 μm, (b) 2 μm, (c) 20 μm and (d) 5 μm.
such as hydrogenase (figure 5b) \[11,75\]. Thus, one possibility for harnessing the electronic potential of the abundant hydrogen in these locations would be in H₂ oxidation. The oxidation of hydrogen in stages seems to be one of the basic principles of biological oxidation according to Szent-Györgyi \[76\] (and see \[77\]). Here, the possibility of ligand-modified iron atoms in FeS could allow subsequent bifurcated delivery of the two electrons from hydrogen; the first to Fe³⁺, NO or NO₃⁻ and the remaining, more energetic electron, to carbon dioxide \[78,79\]. More complex enzyme-like structures based on nanometric greigite (Fe₃S₄) may also have played roles in the electron transfer, carbon dioxide reduction and the assembly of activated acetate. Such clusters are known to self-assemble in water in the presence of thiols \[80\]. Our observation here that chemical garden morphologies and FeS stoichiometries are modified by the addition of peptides and RNA suggests that these molecules may be coordinating Fe–S clusters in the manner first assumed by Eck & Dayhoff \[81\] and Bonomi et al. \[80\] (and see \[54\]). However, in contemporary biology, the iron atoms of Fe–S clusters are ligated primarily by the sulphur of cysteine. Carbon, oxygen or nitrogen coordination is comparatively rare. The particular nature of bonding between the organic and inorganic molecules comprising the chemical garden chimneys is a focus for further research. A further point of interest in this regard is the demonstration by Deck et al. \[82\] of the polymerization of nucleotides immobilized on magnetic beads. Their experiments showed that the polymerization of RNA is inhibited by inactivated monomers and that the inhibiting molecules could be removed while the polymers were retained, thus promoting the reaction. We expect the iron sulphides or hydroxides comprising the chimney membranes growing at an alkaline seepage to be capable of performing a similar role as the magnetic beads thus offering a plausible site at the emergence of life for polymerization of RNA through the delivery and removal of nucleotide monomers by convective flow.

Overall, we imagine reactions in these natural catalytic flow reactors falling into step as the first pathways begin to be beaten towards life \[13,83\]. Eventually, some of these cells may have invaded the ocean floor below the hydrothermal mound where they eked out a living in similar, although diminished geochemical and electrochemical circumstances, so inaugurating the deep biosphere \[84\].

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Figure 5. (a) Schematic of possible electron transfer in mackinawite (FeS) highlighting electron transfer and catalysis over a distance. The structure of mackinawite was drawn from Lennie et al. \[69\]. (b) 2Fe subunit of the [FeFe]-hydrogenase from Clostridium pasteurianum (PDB code 3C8Y \[70\]). (Iron is rust, sulphur yellow, nitrogen blue, oxygen red, carbon white and unknown green.) Structures were drawn with PYMOL \[71\]. (Online version in colour.)
Data presented here and elsewhere [18,38] suggest that sulphidic chemical garden structures had the potential to operate as hydrothermal flow-through reactors imagined as the hatcheries of life. A mound of such gardens—ubiquitous on the early Earth—growing on the floor of the acidulous ocean above an alkaline submarine hydrothermal vent provides a plausible setting for the origin of biological complexity [20,44,85,86]. These gardens could have acted as chemical foci and condensers of geochemically derived reducing power and as electrochemical cells. For example, the consequence of thermal and electrochemical gradients on the spontaneous precipitation of the inorganic membranes could have driven (i) phosphate polymerization; (ii) electron transfer; (iii) chemical reductions; (iv) the convective polymerase chain reaction; and (v) polymer concentrations through thermal diffusion [87–90]. Finally, the fine-grained and dendritic morphologies characterizing chimney growth could offer potential catalytic sites (figure 1c). What is true of the early Earth may well hold also for the emergence of life on Mars and the icy wet rocky worlds such as Europa, a moon of Jupiter, and Enceladus, a moon of Saturn [91–93].

Such chimneys would have hosted ‘readymade’ iron–sulphur clusters with affinities to those comprising the active sites of contemporary enzymes [61,62,75]. Biology may have coopted such a pre-existing functionality through the process of geo-mimicry [54,55,75]. Furthermore, these entities could accommodate chemical evolution from the quantum-scale upwards [45]. Indeed, organisms today carry out a myriad of reactions involving protein-bound Fe–S clusters, strongly suggesting that these chemical agents were involved in biology from their very beginning [2,75,81,94–96]. They were there as components of the natural chemical gardens that nurtured the first steps of life and they are still here today as components of mitochondria in our own skins.

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