Alkaline hydrothermal vents and the origins of life

Nature’s electrochemical flow reactors?

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Understanding the evolution and beginnings of biochemistry is a fundamental problem which needs to be addressed in origins of life research. The development of highly complex chemical systems from simple inorganic beginnings is difficult to comprehend and has resulted in much heated scientific debate. The debate is further fuelled by the fact we know very little about conditions present on the early Earth at the time life began. Owing to the highly dynamic nature of the Earth, the geological record for the earliest period of Earth's history when life began is practically non-existent. Without geochemical indicators, we have no idea about the composition of the atmosphere or oceans, when or how much water was present on the Earth's surface or the chemical inventory present before the emergence of life. There has been much speculation and argument around all of these points about what could be acceptably deemed 'prebiotically plausible' environmental conditions. We do know that life started somewhere, but the where, when and how may only be solved by a process of elimination by experimentation.

The Miller–Urey experiment was the birthplace of modern experimental origins of life chemistry. It proved the plausibility for production of biological molecules from simple organic and inorganic precursors. This one experiment inspired many to investigate methods of preparation for every important biomolecule used by life from ATP to DNA and RNA. An issue highlighted by this research method is the wide variety of different conditions required to make each different molecule. There is also the requirement for each building block of each biomolecule to be introduced in the right quantity, at the right time and in the right order for successful completion of each reaction. This method of experimentation is simple and methodical in a laboratory environment, but not really feasible in the natural environment.

The biochemist Christian de Duve hoped to address this problem by looking at the processes of life itself for inspiration. Life can be considered as an assembly of autocatalytic systems reactions and developed to what we know today through the process of evolution.

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by natural selection. While considering metabolism specifically, de Duve postulated that the enzymes responsible for metabolism must have been selected because they optimized the process, but must also have worked within the system that already existed, a proto-metabolism. So, if metabolism was selected over proto-metabolism as it offered greater benefit, then, by the same reasoning, proto-metabolism must also have been selected over purely geochemical processes as it offered the same inherent benefit to the proto-cell. If we then assume that the proto-metabolism was selected above, but was also compatible with, the geochemical process, then most parsimoniously all systems chemistry utilised by life may have developed under similar circumstances.

In attempts to address issues of the huge variety of reaction conditions and be in keeping with de Duve’s theory of congruence, a naturally occurring dynamic flow reactor system may have been utilized in the production of these necessary biomolecules. The discovery of deep-sea hydrothermal vents, known colloquially as black smokers, in 1977 was thought to answer this problem due to the sheer abundance of life which was found in their immediate vicinity. The black smoker systems provided a dynamic far-from-equilibrium system and a wealth of catalytic minerals which could be used by life. The German chemist Günter Wächtershäuser proposed a theory of pyrite-pulling, based around these acidic hydrothermal systems. The theory proposed the use of catalytic iron–nickel sulfide minerals (such as mackinawite) to reduce CO$_2$ to form simple organics, with further chemical complexity introduced by reaction of these organics on the surfaces of these catalytic metal sulphides. Huber and Wächtershäuser were able to show experimentally the production of acetate (CH$_3$COO$^-$) and methyl thioacetate (CH$_3$SCOCH$_3$) under black smoker conditions from CO to CH$_2$SH, but were unsuccessful in proving that the pyrite-pulling theory worked under laboratory conditions from CO$_2$.

Proposed in a series of papers through the late 1980s and 1990s, the geochemist Mike Russell discussed a different type of subsurface system, alkaline vents (see Russell and Hall as an example). Until 2000, when Deborah Kelley discovered the Lost City system, 30 km off-axis from the Mid-Atlantic Ridge (Figure 1), only extant examples of these systems had been found. These vent systems produced cooler fluids than black smokers (40–90°C) and at pH ~9–11, which is far more in keeping with conditions required to undertake organic chemistry. These systems were powered by a process called serpentinization, the reaction of water with the mineral olivine, producing a hydrated mineral (serpentinite) and releasing warm alkaline fluids rich in hydrogen (~15 mM concentration). The seepage of these alkaline fluids into a weakly acidic Hadean ocean (pH 5–6), would precipitate thin inorganic mineral barriers and potentially provide a source of a natural proton gradient within the hydrothermal system. Analysis of the structure showed a labyrinthine collection of micropores within the main chimney structure constructed of calcium carbonate, though ancient ocean systems would have had a different composition because of the acidity of the ocean and are thought to have contained metal sulfide crystals.

These thin inorganic barriers could theoretically act like a cell membrane, allowing for a natural proton gradient to be established and potentially harnessed; driving chemistry in an equivalent way, to chemiosmosis driving biochemistry. It was proposed by Lane and Martin that...
(and Lane) that this natural proton gradient could be harnessed across these thin catalytic inorganic barriers, allowing for the reduction of CO$_2$ to formate (CHOO$^-$) then on to formaldehyde (CH$_2$O), methanol (CH$_3$OH) and finally methane (CH$_4$). These products could then undergo further reaction within the vent system to form a suite of potential biological molecules and analogues such as methyl thioacetate (analogue of acetylCo A) and acetyl phosphate (potential energy precursor of ATP).

It is when we consider the basic requirements of cells in the context of these hydrothermal systems that we see congruence of life with the systems chemistry available within vents. There are six essential and interlinked requirements for cells to exist and survive. They need energy to power essential biochemistry, they must fix carbon to create cell biomass and excrete waste to maintain disequilibrium. They require a compartment to maintain structure and allow concentration of the essential organics, catalysts (ultimately enzymes) to minimize energy requirements and direct selectivity of biochemical reactions and finally require the ability to reproduce to allow natural selection. Current life exists in permanent disequilibrium, powered by a process called chemiosmosis which was first proposed by Peter Mitchell and describes a process in which proton gradients are harnessed across a thin membrane to power reactions, specifically the formation of ATP, an energy-rich biomolecule used to power further biochemical processes and also the direct reduction of CO$_2$ via proteins such as Ech.

To investigate fully the properties and potential of alkaline vent systems in the origin of life on Earth, we built a simple hydrothermal vent simulation reactor to laboratory-test these theories (Figure 2). The bioreactor construction is a very simple benchtop design, allowing for a warm alkaline fluid to be diffused into a cool acidic bulk ocean. At the point of interface between the two fluids, an organic precipitate forms containing iron–nickel sulfide minerals, generating a pH gradient and therefore a proton gradient across the thin catalytic inorganic barrier. The contents, pH, temperature and flow rates of the fluids can be altered independently to investigate different natural conditions and environments.

The conditions we have tried to simulate in the bioreactor are the currently accepted geological conditions present on the early Earth. The oceans were understood to be slightly acidic due to a much higher atmospheric CO$_2$ content which dissolved into the prebiotic oceans (typically pH 6.4 for carbonic acid at standard temperature and pressure, although it could be lower, around pH 5, if atmospheric pressure were higher). The interaction of alkaline vent fluids with an acidic ferruginous ocean results in precipitation of thin inorganic mineral barriers, some of which are catalytic iron–nickel–sulfur minerals such as mackinawite, and separate a pH gradient across them. This pH gradient could be considered to be akin to the proton gradient identified by Peter Mitchell in his paper on chemiosmotic coupling which is the universal power source for all living cells. Using Lost City as an example, there is a natural proton gradient, a temperature gradient (between the cold deep ocean and warm alkaline fluids) and high water pressure (7500–10 000 kPa of pressure, although the current reactor only operates at 100 kPa) available within these systems, all of which can provide energy to drive chemical reactions. In considering this, it is plausible to consider alkaline hydrothermal vents as a natural occurring electrochemical flow reactor.

Within the hydrothermal system simulator, using a precipitated thin, inorganic barrier with, on one side, an acidic ocean containing CO$_2$ and, on the other, an alkaline fluid rich in H$_2$, we have tried to reduce CO$_2$ to organic carbon. The theory suggests that H$_2$ will give up electrons, based on reduction potential (Figure 3), passing them through the iron–nickel–sulfur catalyst to...
CO₂ causing a reduction reaction, to formate, formaldehyde, methanol or methane. Methanogens and acetogens, thought to be among the oldest organisms on Earth, have a metabolic process very similar to this for fixation of CO₂ while making biomass and either methane or acetate respectively⁵. The conditions inside the alkaline hydrothermal vent are favourable to this reaction when considering the calculated reduction potentials for each half-reaction and have also been calculated to be favourable in the generation of cell biomass and for the production of many organic biomolecules including amines, saccharides, fatty acids (lipids) and amino acids⁶. So far, experimentally, we have seen indications for the production of formate and formaldehyde, but with reliability issues surrounding repeatable sampling, analytical methods and maintaining strict anaerobic conditions, the results have been inconsistent. There is, however, indication that there is an overall increase in organic carbon concentration within the reaction system and work around resolving these issues is ongoing.

Having made simple organic molecules within the reactor, we next looked at increasing the chemical complexity within the system and make biomolecules or potential prebiotic analogues. With this in mind we have conducted experiments on two fronts. The first looked to form activated biomolecules such as thioacetate (CH₃COS⁻) and acetyl phosphate (CH₃COPO₃⁻), whereas the second looked at the formation of sugars within the simulated vent environment. In the first instance, starting with formate, we attempted to form amounts of thioacetate and methyl thioacetate (CH₃COSCH₃) within the reactor. Huber and Wächtershäuser⁷ have previously shown it possible to make methyl thioacetate and acetate under black smoker type conditions, but would this be possible within an alkaline vent environment? Again we have identified small amounts of thioacetate within the system, but the results are inconsistent. We have also, by including phosphate in the reaction system, observed the formation of acetyl phosphate which was identified by 31P-NMR analysis.

The detection of acetyl phosphate is important as it shows the ability of the system to produce a highly activated and energetic organophosphate molecule which can be used to phosphorylate other organics, but also to provide a source of chemical energy, much like ATP does in modern biochemistry.

Secondly, to make sugars, we attempted to undertake the formose reaction within the reactor under simulated vent conditions. Taking formaldehyde in solution at pH 10 and 60°C in the presence of Ca²⁺ ions, it was possible to undertake the formose reaction. Over a period of 5 hours, sugars up to sepioses (seven carbon chain sugars) are generated. During the process of analysis, we were able to identify the production of all enantiomers of ribose and glucose, and also the production of deoxyribose within the reaction system, although only in small quantities (~0.06% of available formaldehyde converted into ribose). Further investigations of conditions to make the formose reaction more selective towards ribose are being conducted using a variety of different metal ions and minerals which are present within alkaline hydrothermal systems.

Although we have shown the ability to make these important molecules in the vent simulator, the issue of losing these vital molecules to the bulk ocean needs to be addressed. The organics produced need to be retained within the vent system, allowing for further reaction and concentration if viable cells are eventually to be produced. The German physicist Dieter Braun has tried to address this issue using a process called thermophoresis⁶. Thermophoresis works by concentrating organics into more stable cooler regions using the processes of thermal

**Figure 3.** Proposed vectorial reduction of CO₂ by H₂ across a thin FeS barrier. The reduction potential (Eₗ) of the H⁺/H₂ couple is ~900 mV at pH 10, whereas the Eₗ of the CO₂/HCOOH couple at pH 6 is ~370 mV, and the Eₗ of the HCOOH/CH₃O couple is ~520 mV. A semiconducting FeS barrier should ‘feel’ the distinct reduction potentials in both compartments and transfer electrons from H₂ to CO₂ to produce simple organics such as formaldehyde (CH₂O). Reproduced with the kind permission of Dr Nick Lane from Cold Spring Harbor Perspectives in Biology 6, 1–18. doi:10.1101/cshperspect.a015982. © 2014 Cold Spring Harbor Laboratory Press.

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Figure 5. Heat-driven molecular accumulation in hydrothermal pores. (a) Section through aragonite (CaCO$_3$) from the submarine hydrothermal vent field at Lost City. (b) Simulation of a part of the pore system. If subjected to a horizontal thermal gradient of 30 K, a 1200-fold accumulation of single nucleotides is expected (logarithmic concentration colour scale). A concatenation of three of these pore sections leads to a 109-fold accumulation. (c) The mechanism of accumulation is driven by heat in a twofold way. Thermal convection shuttles the molecules vertically up and down and thermophoresis pushes the molecules horizontally to the right. The result is a strong molecular accumulation from the top to the bottom (linear concentration colour scale). Image used without alteration and with kind permission from Baaske, P., Weinert, F.M., Duhr, S., Lemke, K.H., Russell, M.J. and Braun, D. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 9346–9351. © National Academy of Sciences, USA, 2007.

Convection and thermal diffusion. This process has been shown to concentrate DNA, RNA and lipids up to 1200 times the original concentration, although this has only ever been conducted within sealed systems. Having shown the production of ribose and also the production of acetyl phosphate within the reactor, we looked to use these two molecules to attempt the phosphorylation of ribose under vent conditions. In currently unpublished work, we were able to show $3'$ and $5'$ phosphorylation of ribose using acetyl phosphate at 60°C under simulated vent conditions. Further investigation showed this reaction would also proceed at room temperature, although much more slowly, showing the great phosphorylating power of acetyl phosphate in the vent system.

We have attempted to use the process of thermophoresis to concentrate fluorescein in a simulated open flow vent system with a matrix constructed of alumina foam. The foam matrix constructed for use in the reactor is highly porous (~85% permeable) and chemically inert. Flowing 1 µM fluorescein into the reactor in simulated vent conditions we were able to see by UV–visible light analysis, up to ~5000 times concentration within the open flow system. Although fluorescein is larger than most organic biomolecules, it was chosen for analysis as it is easy to visualize and calculate approximate concentrations visually. Further work is to be conducted with small RNA chains, nucleotides, peptides, amino acids and lipids to confirm the viability of this process in the concentration of organics in the vent environment.

Thus far we have shown the ability of alkaline hydrothermal vents to produce organic molecules which could be used in origin of life reactions. We know that the vents have a natural proton gradient which could have been used to drive essential chemistry in the production of biomolecules and could also then be harnessed by life as it emerged until it was able to develop its own energy sources. We have tentatively shown the reduction of CO$_2$ within the vent environment and the development of more complex organic molecules from simple precursors. We have also shown that vents have the ability to make activated organophosphate molecules for the purposes of phosphorylation and energy storage supply.

With the ability to then concentrate organics in the vent structure, this effect can be used to drive further chemistry including polymerization of amino acids and nucleotides to form DNA and RNA, but also concentrate lipids to promote vesicle formation. In theory, the vent system offers the ability to undertake every process required in the formation of life. There is a strong congruence between the vent systems and the living cell, but there is still a great deal of work to be done to fully investigate and prove the potential role of alkaline hydrothermal vents in the origin of life on Earth. The wide variety of chemistry possible within the vents is due to the variety of conditions and energy sources available within the system and this is why they may be considered to be Nature’s electrochemical flow reactors.

Barry Herschy completed his PhD at the University of Leeds looking at how phosphorus could have been incorporated into biomolecules and the development of vesicles and protometabolism. He is now a Leverhulme-funded postdoctoral researcher, working with Dr Nick Lane at University College London looking at the prebiotic development of biomolecules and investigating the potential of prebiotic chemistry and biochemistry of hydrothermal vent systems. email: b.herschy@ucl.ac.uk.

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