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Iron and Fire

The Hydrothermal Onset of Life

Microelectrodes

In Situ Environmental Sensors

FROM GEOCHEMISTRY TO BIOCHEMISTRY

Chemiosmotic coupling and transition element clusters in the onset of life and photosynthesis

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Initial Conditions

Convection currents within the Earth dissipate heat, eventually delivering hydrothermal fluids to the cold sink of the ocean. Strong chemical disequilibrium is focused at points of submarine exhalation. This is where metabolism came into its own, dissipating this supply of chemical energy to electron sinks. Metabolism quickens, by many orders of magnitude, oxidation

and reduction reactions on our planet. And just as convection involves juxtaposed molecules behaving in concert as they transfer heat to a lower temperature sink, so too, orderly and commensurate flows through a semi-permeable membrane of electrons, protons and small uncharged molecules but not anions and cations, ensures that metabolic reactions fall into step in the kind of neighbourly co-operation that leads to the rapid dissipation of up to a volt or so of renewable photoelectrochemical energy. Genetically regulated metabolism and convection are coupled on our planet and must have always been so, right back to life's origin (Russell and Hall 1997).

The Emergence of Life

Although the spectacular Black Smokers have been hailed as the likely forge of life, we argue that its hatchery was a humble submarine seepage in the ocean deep (Fig. 1). Here the precipitation of an inorganic semi-permeable membrane

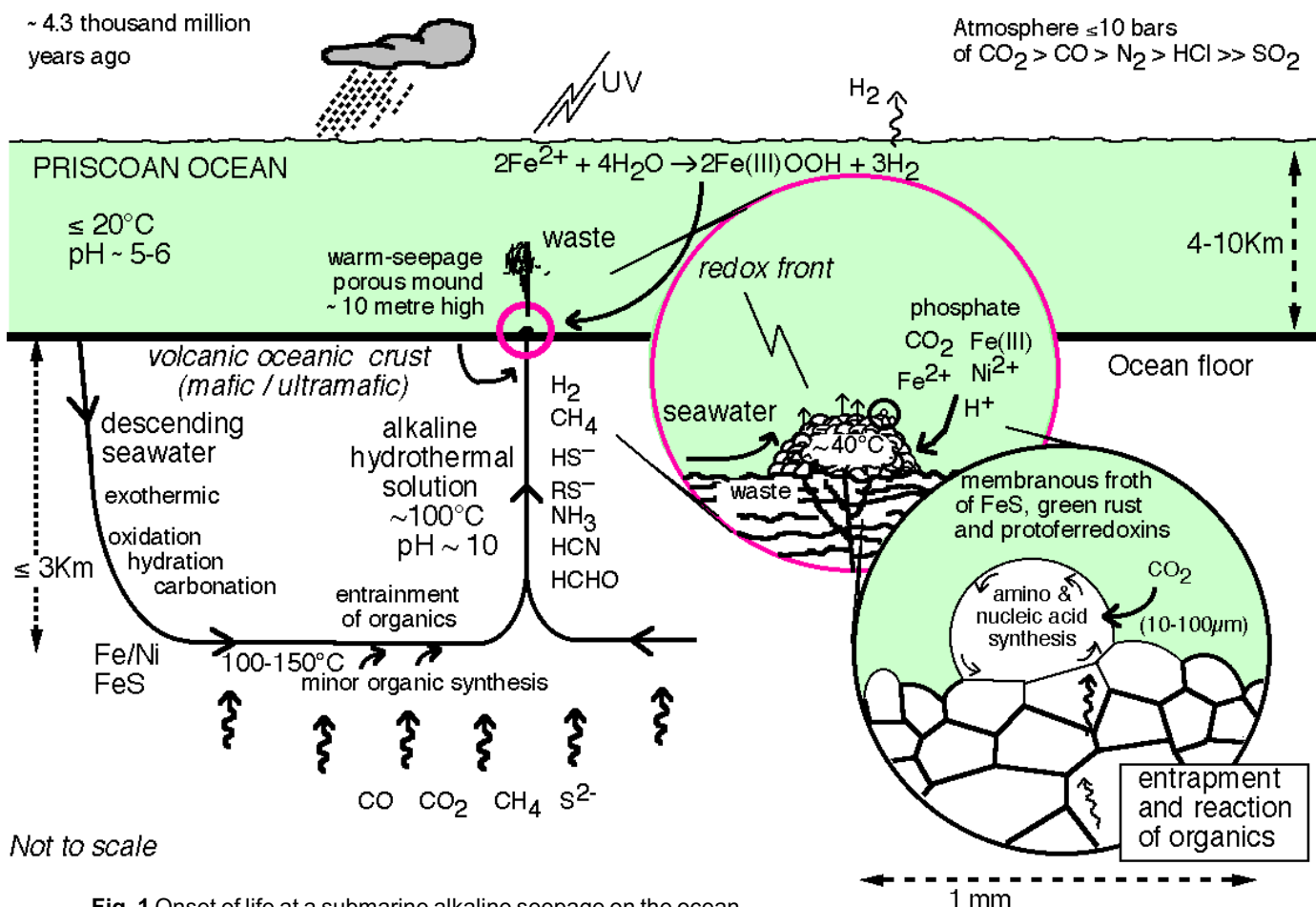


Fig. 1 Onset of life at a submarine alkaline seepage on the ocean floor (Russell et al. 2002).

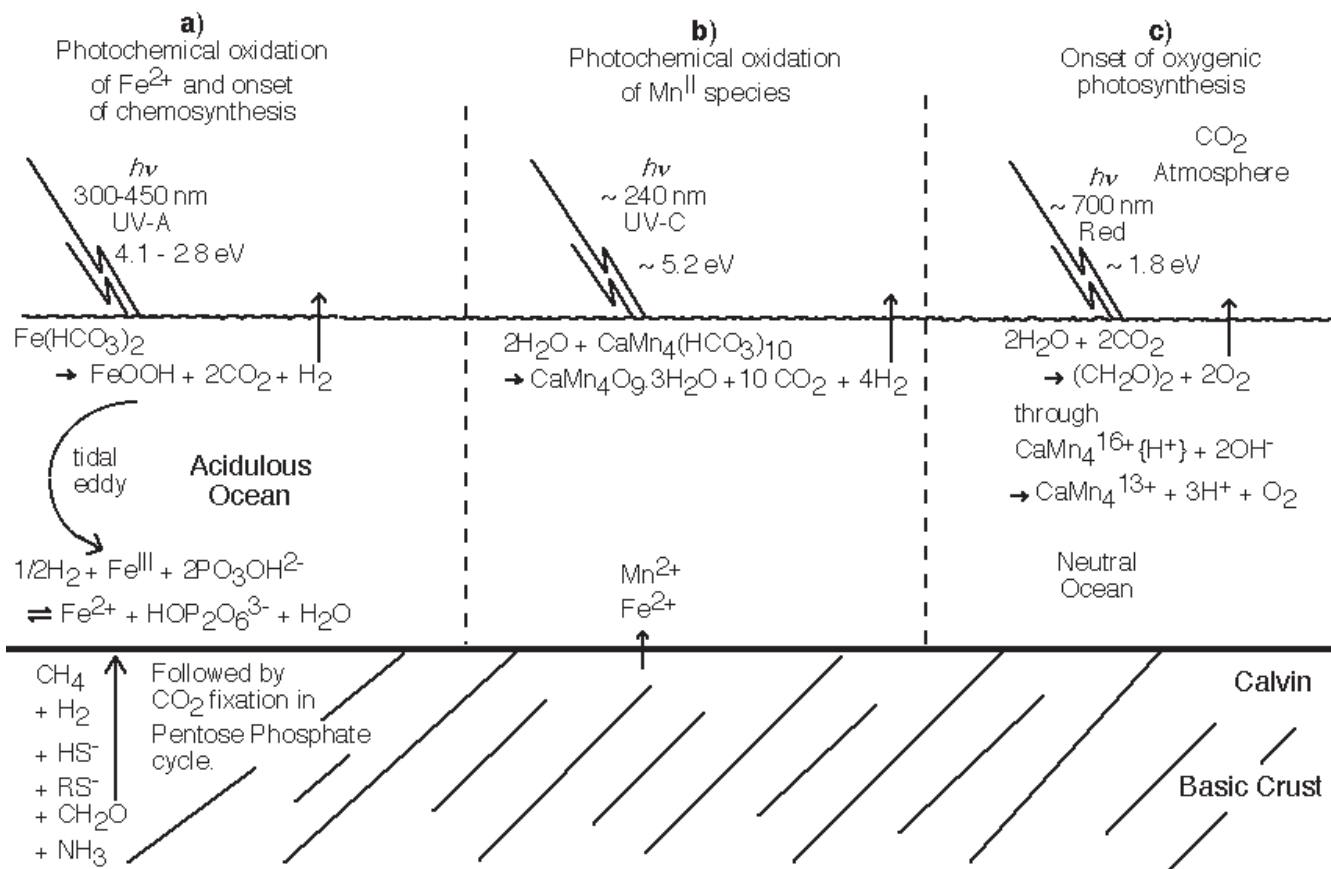
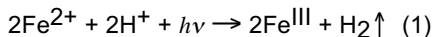


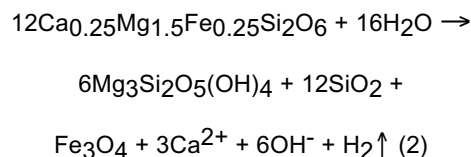
Fig. 2 Solar radiation produces **a)** photolytic Fe^{III} which potentiates chemosynthetic life; **b)** photolytic CaMn₄O₉·3H₂O clusters, precursor to the water oxidising complex (or the mineral ranciÉite); **c)** oxygenic photosynthesis through reduction of Mn₄^{IV}.

prevented immediate titration of an alkaline hydrothermal fluid with an ocean made acidulous (pH ~5.5) by an atmosphere rich in carbon dioxide. A particular advantage of alkaline hydrothermal solutions is that they would have the propensity to dissolve organic acids and bases, including the nucleic acid bases. Alkaline conditions also favour phosphate chemistry which must have been a feature of early metabolic cycles and genetic translation.

But very high temperature springs did have a role to play in the emergence of life, that of supplying transition elements to the Priscoan ocean. The chemical energy dissipated by the first metabolist resulted from the tension between hydrothermal hydrogen and photolytic ferric iron in this acidulous ocean. The Earth was a giant photoelectrochemical cell — the ocean was the fluid matrix to a dispersed positive electrode, $\gamma\text{-Fe}^{\text{III}}\text{OOH}$, generated by UVC on Fe²⁺ supplied through 400° C submarine springs (Fig. 2a):



Hydrothermal hydrogen comprised the negative electrode at the warm seepage sites. The serpentinization of olivine and pyroxene in the crust during hydrothermal convection not only introduces hydrogen, it also has the effect of increasing the pH of 100° C hydrothermal solutions to about 10 (cf. Kelley et al. 2001; Marteinsson et al. 2001):



Life emerged in the mixing zone at ~40° C (Russell et al. 2003).

The First Membrane

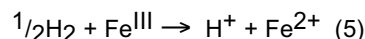
According to Williams (1961, p. 3) 'it may well be that the achievement of a separation of activated reagents in space plus restricted diffusion provides the fundamental distinction between biological chemistry and test-tube chemistry'. If so it follows that the membrane is the most conserved of all biological structures. But what was it made of? A semipermeable *inorganic* membrane can be made to precipitate at a pH front in the lab (or play room). The best known example is that of the silica gel comprising a chemical garden where growth is induced through *osmotic* pressure. In the conditions obtaining on the early Earth various membranes may have been generated at alkaline seeps (Russell et al. 2003). On occasions when bisulfide activities were high, reaction with ferrous iron in the ocean would have led to the precipitation of an iron sulfide membrane, predominantly of mackinawite [(Fe>>Ni)_{1+x}S], perhaps **diagenetically** within a hydrothermal clay matrix. Such a membrane would also have encouraged a different kind of feedback, viz. a *chemiosmotic*

pressure. Chemiosmotic pressure signals the change from mere mineral precipitation to informed, and thereby evolving encapsulated feedback cycles capable of exploiting commensurate chemical tensions elsewhere on the planet.

Osmosis and Chemiosmosis Compared

Osmosis works to equilibrate contrasting water activities across a semipermeable colloidal gel envelope or membrane. Water molecules permeate the membrane which is distended by osmotic

fundamental significance — redox. We suggest that hydrothermal hydrogen was the first fuel, split to electrons and protons on the interior of the FeS membrane, probably at a nickel site (Fig. 3):



Electrons would be conducted along the metallic-iron layer of mackinawite (Fe-Fe is 2.6Å), hopping from one crystallite to the next through the membrane toward the external photolytic Fe^{III}

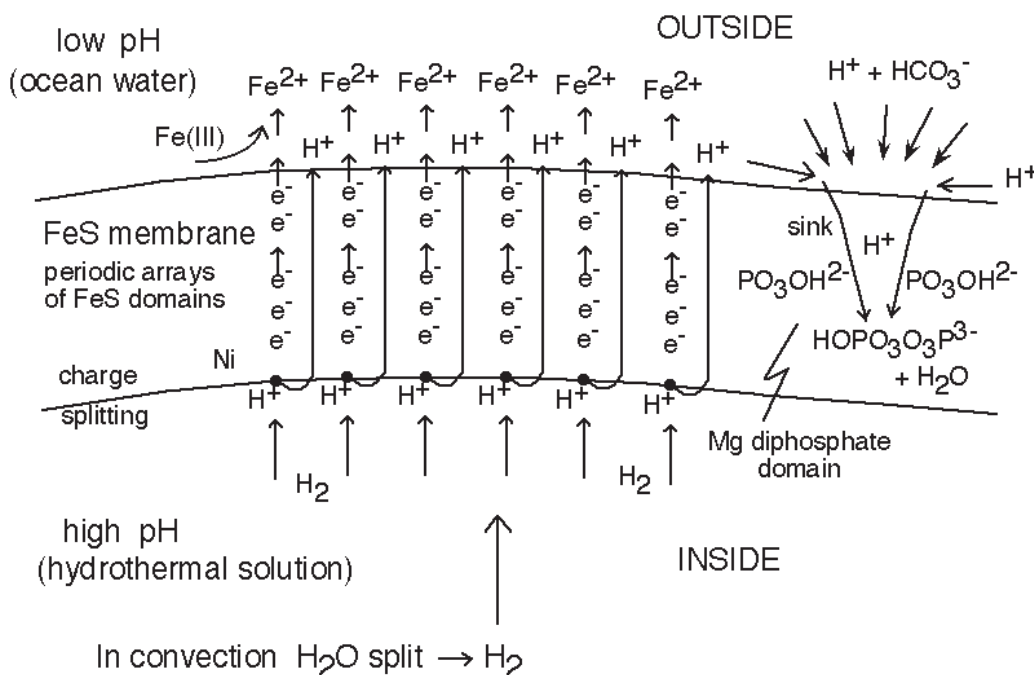
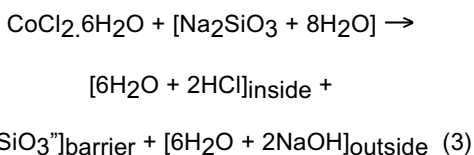
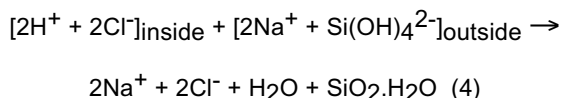


Fig. 3 Emergence of chemiosmosis driven by reduction of Fe^{III} on the exterior of the FeS membrane. Electrons are conducted through mackinawite nanocrystallites from H₂ on the interior. Protons track electrons through aqueous films to conserve charge balance. Elsewhere the double layers of sulfur atoms bestow an insulating capacity upon mackinawite. The membrane potential is augmented by protons in the acidulous ocean — an ambient pmf. Returning protons condense monophosphate.

pressure to form the colourful spires that constitute the gardens. The “fuel” is generally a crystal hydrate of a strong acid and weak base such as CoCl₂·6H₂O, introduced into a relatively dilute solution of sodium silicate. The crystal remains separated from the so-called “water glass” by a spontaneously precipitated silica membrane which bears a bloom of cobalt. The initial reaction, notionally equilibrated with respect to water activity is:

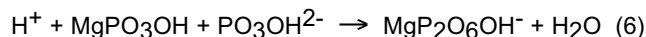


The membrane continues to develop by the reaction:

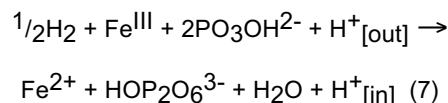


While osmosis works towards the equilibration of water activities across a *semipermeable* membrane, chemiosmosis works to equilibrate proton activities across a membrane that is also a *semiconductor*. A proton gradient would clearly result from the interfacing of the alkaline hydrothermal fluid (pH ~10) with the acidulous ocean (pH ~5.5). But there is another factor of

receptor (Fig. 2,3). At the same time the protons would be driven by rotational/translational diffusion of water molecules adhering to the mackinawite faces, through the membrane to maintain charge balance (Russell et al. 1994,2003). Once on the exterior the protons were abandoned by electrons, which satisfied the electrophilic ferric iron acceptor. Concentrated thus the protons exerted a further pressure across the membrane directed toward the still alkaline interior (Fig. 3). Characterized as proticity by Mitchell (1967) the hydrogen ions re-invaded the membrane where they dimerized inorganic phosphate, derived from the ocean, by a process of ‘hydrodehydration’:



These two separated reactions (5 and 6) are linked electrically through the membrane, simplified as:



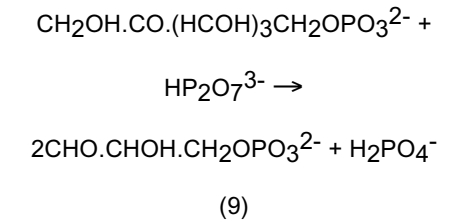
The total protonmotive force is considered thus:

$$\Delta_p = \Delta\psi + \Delta E_{\text{pH}} \quad (8)$$

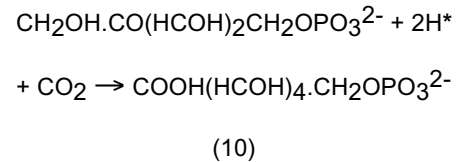
where Δ_p is the overall 'protonic' potential composed of the sum of the electric potential $\Delta\psi$ and that potential due to ambient pH, i.e., ΔE_{pH} . The overall electrochemical energy available in such a system is best appreciated in a Pourbaix diagram (Fig. 4). We caution that complications, leakage, and kinetic barriers in the far-from-equilibrium conditions pertaining across a membrane are such as to preclude exact calculation of the protonmotive force. Nevertheless we can gauge from Figure 4 that the potential available on the early Earth well exceeds the critical value for metabolism considered to be the ~250mV (or ~ -11.5 kcal per mole) required to generate pyrophosphate (PPi) from inorganic monophosphate (Pi) or adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and Pi. To borrow a phrase from Mitchell (1967), "oxidoreduction (is) coupled to hydrodehydration".

The First Metabolic Cycle and Pathway

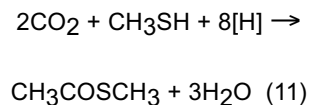
At the alkaline seepage just prior to the onset of metabolism we can imagine the nucleation of mineral clusters (many of them hydrates) and their polymerization to form inorganic crystallites. In contrast, partially reduced carbon molecules such as formaldehyde generated in the Priscoan ocean crust by reaction of hydrogen and carbon monoxide and entrained in the alkaline fluid till they stuck to mackinawite (Rickard et al. 2001), could only polymerize to unstable "carbohydrates". Phosphorylated, carbohydrates such as ribulose biphosphate would also adhere to (be selected by) the FeS crystallites. Uncooperative molecules would remain in the solution and be passed to the ocean as waste. Reacting to fix further formaldehyde the still more unstable fructose monophosphate is formed. If polyphosphate is available this molecule cleaves to glyceraldehyde phosphate in stage one of a precursor to the Pentose Phosphate Cycle before repolymerizing (Quayle and Ferenci 1978, fig. 3):



In the neutral to exergonic evolved metabolic cycle ribulose monophosphate can also assimilate carbon dioxide in the presence of activated hydrogen (Quayle and Ferenci 1978):



Carbon may also have been fixed in the iron>>nickel sulfide membrane from the oxides by a precursor to the Acetyl-CoA Pathway involving activated hydrogen and a thiol (Huber and Wächtershäuser 1997; Martin and Russell 2003):



We suggest that the chaos of these autocatalytic feedback cycles can be ordered both in terms of product and stereochemistry by the active surfaces of clays in the hydrothermal mound as well as of the mackinawite comprising the membrane. Thus a controlled "formose" reaction was transmuted in the presence of inorganic pyrophosphate derived from

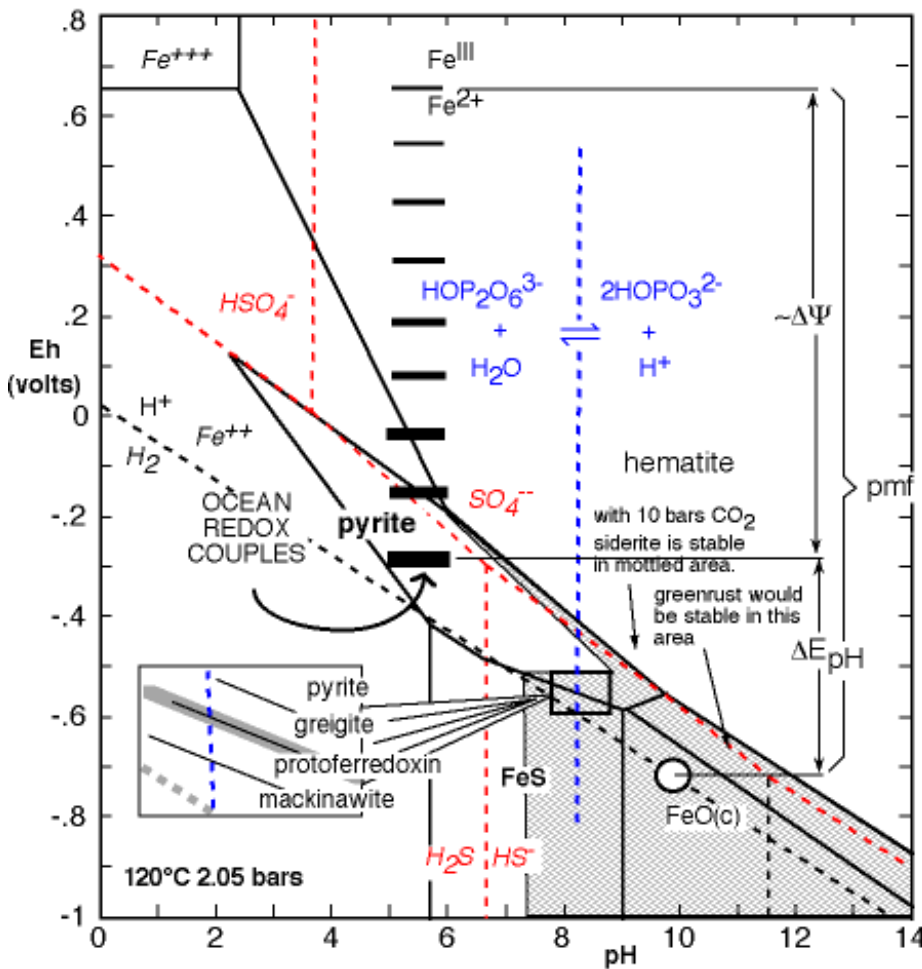


Fig. 4 Pourbaix diagram to illustrate the mixing of the alkaline hydrothermal solution (circle) and acidulous seawater with a sequence (bars) of notional redox couples extending to Fe3+/Fe2+. Stabilities of relevant minerals at activities of H2S(aq) = 10⁻³, and Fe2+ = 10⁻⁶ (using Geochemists Workbench) are given and, inset, notional phase relations of protoferredoxin. The protonmotive force (pmf) of ~700mV theoretically exceeds the critical value (250mV) required to generate pyrophosphate (PPi) (cf. Zachara et al. 2002, fig. 2). Note that the pH boundary of Pi/PPi intersects the region (square box) of the membranous precipitate.

the ocean to the Pentose Phosphate Cycle, precursor to the Calvin Cycle of ribose synthesis. HCN condensates by contrast are relatively stable at C₅ (e.g., adenine [HCN]₅) or may be hydrolysed to C₄ (e.g., uracil C₄N₂H₂O₂). Nevertheless, apart from adenine, the nucleic acid bases are difficult to synthesise in hydrothermal conditions, a fact that runs counter to the RNA World hypothesis. Phosphorylated bases will react with nucleosides to make nucleotides. Organophosphates are strongly adsorbed on sulfides. We assume that phosphorylations could generate limited amounts of RNA from the ribose and the nucleosides. RNA could achieve lengths of up to ten bases on the surface of the mackinawite. Distortion at greater lengths renders the polymer unstable. Adsorbed thus RNA would conform to three triplets, i.e., three potential codons, on a surface (Mellersh 1993; Mellersh and Wilkinson 2000).

Clusters, Catalysts and Enzymes

The amino acids glycine, alanine, aspartate, serine, glutamate, isoleucine, lysine, proline, valine and leucine are produced at 150° C in the presence of hydrothermal hydrogen, though in sharply descending order of yield [glycine yield ~2% with respect to KCN + NH₄Cl + HCHO with excess CO₂ + H₂; conditions comparable to those obtaining at the seepage (Hennet et al. 1992)]. It so happens that the most stable RNA triplets form the codons for just these simple abiotic amino acids (Trifonov 2000). We suggest that once these amino acids nestle into the RNA triplet clefts they polymerize in the oscillating pH conditions in the membrane by acid-base catalysis (Russell et al. 2003). Released from their RNA moulds, the regulated amino acid trimers can cast around for other ligands. The most common will be [Fe₄S₄]⁺²⁺ cubane clusters that otherwise would have been interred in the mineral greigite (as [SNiS][Fe₄S₄][SFeS]) (Vaughan and Craig 1978) (Fig. 5). Chelated thus, e.g., (gly)₃[Fe₄S₄](ala)₃, the complex would have the capacity to store and transfer electrons. Alternatively ser₂cys[SNiS][Fe₄S₄](ala)₃ would have the power to hydrogenate and dehydrogenate organic acids and alcohols (Russell et al. 2003). Perhaps greigite, as well as mackinawite, acted as an abiotic hydrogenase in the inorganic membrane. These same “abiotic” amino acids — glycine, alanine, aspartate and valine — also exclusively comprise the active site motifs of enzymes that generate pyrophosphate (PPi), the likely first central energy carrier (Baltscheffsky et al. 1999).

The chelation of “ready made” [Fe₄S₄]²⁺ cubane clusters with amino acid trimers helps explain a central problem in the emergence of life, that of the organic takeover of the membrane.

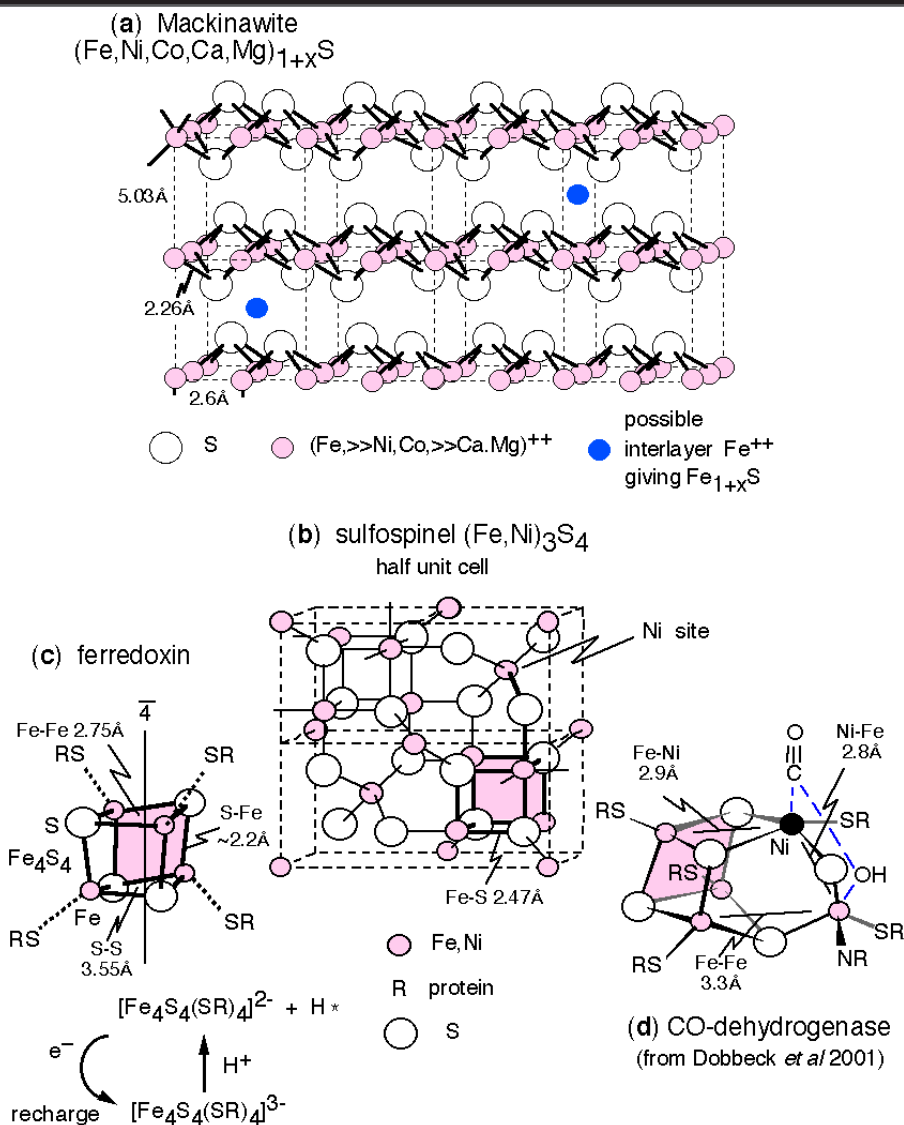


Fig. 5 Structural relatedness of **a**) mackinawite Fe_{1+x}S ; **b**) greigite Fe_5NiS_8 ; **c**) the thiocubane $[\text{Fe}_4\text{S}_4]$ unit in protoferredoxins and ferredoxins; **d**) the $[\text{Fe}_4\text{NiS}_5]$ open cuboidal complex in CO-dehydrogenase. Affine sulfur sublattices, cubic close-packed in **a** & **b**, are distorted in **c** & **d**. The presence or absence of $\text{Fe}^{\text{III/3+}}$ and organic ligands dictates which of these entities form.

The first microbes were probably encapsulated in a proteinaceous envelope (Wächtershäuser 1998). Lipids were later (next section). Not only would protoferredoxins afford a greater control to electron transfer across the proteinaceous membrane, but, as ties from one protopeptide to another, they would serve also to stabilize it, a role significant in heterodimeric proteins to this day.

The First Lipid Membranes

Lipids comprise the impermeable and insulating spacing between membrane bound enzymes in all prokaryotes. But Archaea and Bacteria possess completely different lipids (Kandler 1998). Archaeobacterial lipids consist of isoprenoids and various derivatives connected to stereospecifically-numbered-glycerol-1-phosphate by an ether bond. Eubacterial lipids consist of fatty

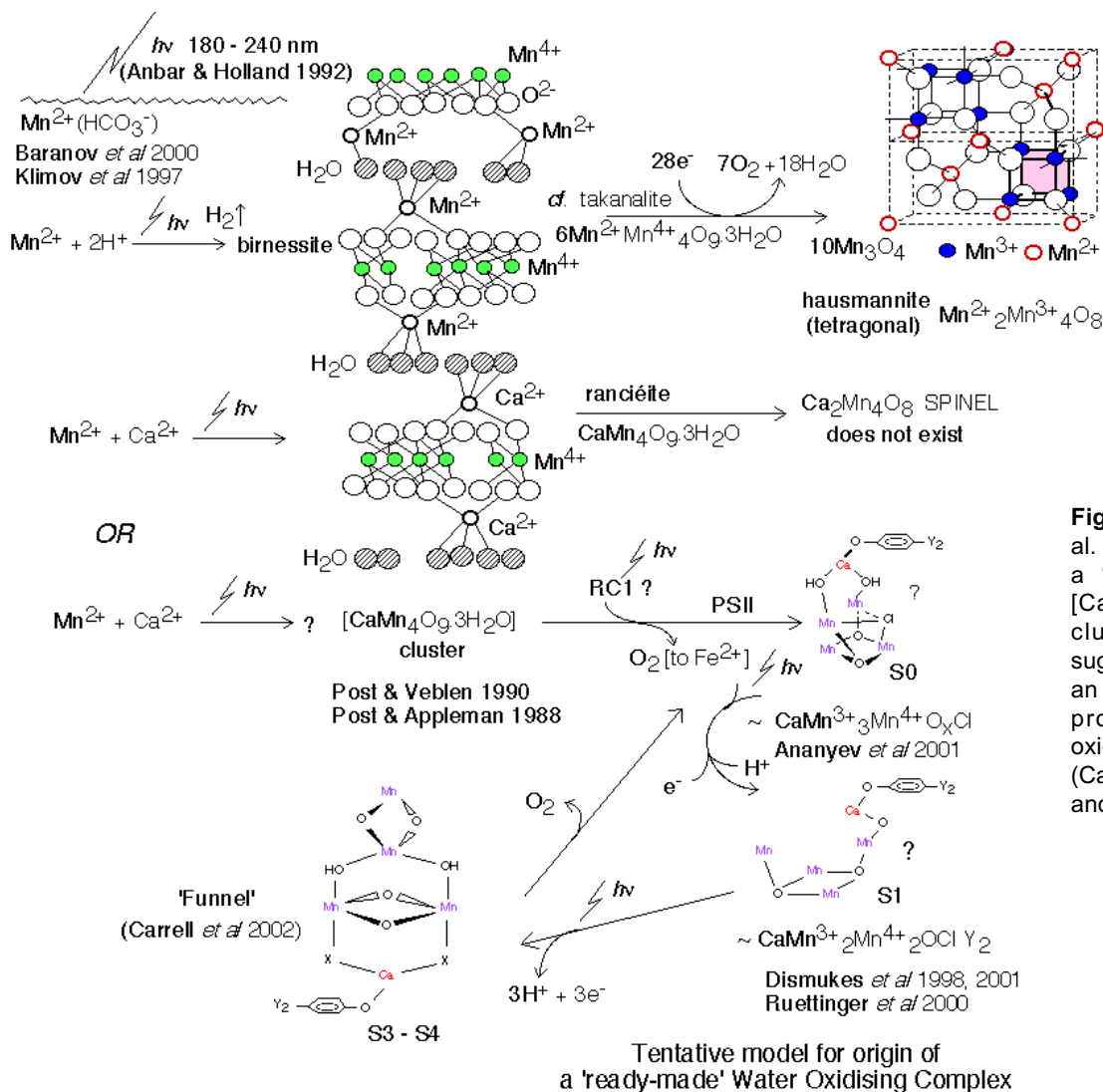


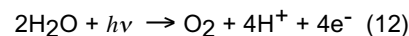
Fig. 6 Model (Russell et al. 2003) for the origin of a 'preformed' abiotic [CaMn₄O₉.3H₂O] core cluster which, we suggest, was enrolled in an antecedent of RCII to produce the water-oxidising complex in PSII (Carrell et al. 2002; Sauer and Yachandra 2002).

acids connected to *sn*-glycerol-3-phosphate by an ester bond. The biochemical pathways to their respective syntheses also differ markedly. Moreover the Bacterial cell wall consists of murine whereas that of the Archaea is pseudomurine. Although there is overlap in the redox reactions involving inorganic donors and acceptors that both domains exploit in order to sustain ATP synthesis, those used by the hardier Archaea are the more restricted (Amend and Shock 2001). These differences have been interpreted as relicts of a phase of differentiation driven by stereochemical "immiscibility", perhaps as higher temperature regions of the hydrothermal mound were accessed after divergence from the universal ancestor, though prior to the emergence of free-living chemoautotrophs (Martin and Russell 2003).

Oxygenic Photosynthesis

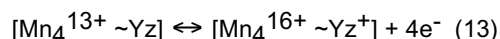
That the early active centres to enzymes were pre-existing entities which required no genetic design is echoed in one other extraordinary evolutionary jump, that to oxygenic photosynthesis. While the onset of life changed the nature of our planet, the invention of oxygenic photosynthesis changed its face (Dismukes et al. 2001). Oxygenic photosynthesis is the most demanding of

macro-evolutionary developments, requiring the processing of four protons and four electrons in order to photo-oxidize water (Fig. 2,6):



The active water oxidizing complex (WOC) in what is known as photosystem II (PSII) catalyzing this reaction is invariably a CaMn₄ centre in all cyanobacteria and plants (Blankenship 2002). We suggest that the introduction of the CaMn₄ catalytic core is such an extraordinary biological innovation that a "ready-made" cluster must have been co-opted whole by a (mutant?) protein (Russell et al. 2003). This suggestion recalls our idea that an [4Fe-4S] cubane cluster could have been sequestered by peptides into a ferredoxin where it provided a "preformed" electron transfer site for the first metabolist. Both the [4Fe-4S] cubane and the CaMn₄ complex allow for loss and, in the case of the iron sulfide, gain of (delocalized) electrons, from Fe^{2.5+} and Mn^{2.75+} respectively. Sequestering of the [Fe₄S₄] cubane with peptide both protects and allows the integrity of the structure to survive, with minor distortion, the addition or subtraction of a single electron.

During the extraction and accumulation of the four electrons and protons from water, the conformational changes of the CaMn₄ structure (ligated to the tyrosine Yz in PSII) are much more extreme than the minor flexions of [Fe₄S₄]⁺²⁺, and are not yet fully characterized (Fig. 5,6):



The original sequestering site of the CaMn₄ complex may have been where tyrosine and amines were already present in an ancestral reaction centre. We now revisit the experimental and geochemical evidence that favours such a speculation.

Hot springs would have discharged Fe and Mn to the ocean in the early Archaean just as they do today. Because Fe is less soluble at higher pH, it would have precipitated around the springs, partly as iron oxyhydroxides and partly as iron sulfides. The more soluble Mn²⁺, on gaining the ocean surface, would be photo-oxidized at extremely short wavelength beyond UV-C (Anbar and Holland 1992) to colloidal clusters of [CaMn₄O₉.3H₂O] (Fig. 6). This putative cluster also represents a nucleus for the mineral ranciéite (Russell et al. 2003). Thus a CaMn₄ species may have been the free cluster sequestered by a mutant reaction centre (cf. Sauer and Yachandra 2002). A comparison between the mineral and the oxygen-evolving polymorphic complex is instructive. Calcium is ligated to three water molecules in ranciéite (Fig. 6) and the atom may also act as the water donor site in the water oxidizing complex, releasing it to the fully-accumulated positive charge state four of the tetramanganese cluster. It is as a Lewis acid that calcium activates water oxidation.

Calcium has several other possible roles: that of conducting electrons to tyrosine during oxidation of the complex; preventing dissolution on reduction to state zero, or, because of its low charge concentration, in preventing the formation and locking of [Mn₄O₄]⁴⁺ as a cubane (as in the spinel hausmannite [(Mn²⁺)₂(Mn³⁺)₄O₈], Fig. 6) during the reduction of the oxidised phase in the precursor to PSII-WOC.

Proposal

How can the alkaline hydrothermal hypothesis be tested? It must be said that experimental work on the origin of life has not fulfilled early expectations. Experiential, rather than experimental knowledge has been the more useful, i.e., that gleaned from pertinent geochemical, geological, geophysical and biochemical discoveries. Experimentalists have tended to design experiments that, even if successful in themselves, have ignored likely initial conditions. And interpretations and extrapolations of reactions from experiments with few reactants, usually run to equilibrium, fail to address the effects of the electrical and protonic potentials that drove early life to emerge, perhaps through a semiconducting, semipermeable inorganic membrane. We suggest that experiments using a reactor nexus which takes account of chemiosmosis at the final stages of moderate temperature aqueous geochemical interaction are required, experiments that may have to resort to the oft disparaged "cook and look" approach (Russell et al. 2003).

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