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Protocellular Heme and Iron−Sulfur Clusters

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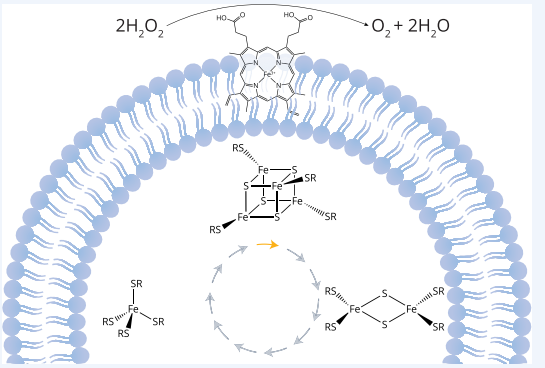
CONSPECTUS: Central to the quest of understanding the emergence of life is to uncover the role of metals, particularly iron, in shaping prebiotic chemistry. Iron, as the most abundant of the accessible transition metals on the prebiotic Earth, played a pivotal role in early biochemical processes and continues to be indispensable to modern biology. Here, we discuss our recent contributions to probing the plausibility of prebiotic complexes with iron, including heme and iron−sulfur clusters, in mediating chemistry beneficial to a protocell. Laboratory experiments and spectroscopic findings suggest plausible pathways, often facilitated by UV light, for the synthesis of heme and iron−sulfur clusters. Once formed, heme displays catalytic, peroxidase-like activity when complexed with amphiphiles. This activity could have been beneficial in two ways. First, heme could have catalytically removed a molecule (H_2O_2) that could have had degradative effects on a protocell. Second, heme could have helped in the synthesis of the building blocks of life by coupling the reduction of H_2O_2 with the oxidation of organic substrates. The necessity of amphiphiles to avoid the formation of inactive complexes of heme is telling, as the modern-day electron transport chain possesses heme embedded within a lipid membrane. Conversely, prebiotic iron−sulfur peptides have yet to be reported to partition into lipid membranes, nor have simple iron−sulfur peptides been found to be capable of participating in the synthesis of organic molecules. Instead, iron−sulfur peptides span a wide range of reduction potentials complementary to the reduction potentials of hemes. The reduction potential of iron−sulfur peptides can be tuned by the type of iron−sulfur cluster formed, e.g., [2Fe-2S] versus [4Fe-4S], or by the substitution of ligands to the metal center. Since iron−sulfur clusters easily form upon stochastic encounters between iron ions, hydrosulfide, and small organic molecules possessing a thiolate, including peptides, the likelihood of soluble iron−sulfur clusters seems to be high. What remains challenging to determine is if iron−sulfur peptides participated in early prebiotic chemistry or were recruited later when protocellular membranes evolved that were compatible with the exploitation of electron transfer for the storage of energy as a proton gradient. This problem mirrors in some ways the difficulty in deciphering the origins of metabolism as a whole. Chemistry that resembles some facets of extant metabolism must have transpired on the prebiotic Earth, but there are few clues as to how and when such chemistry was harnessed to support a (proto)cell. Ultimately, unraveling the roles of hemes and iron−sulfur clusters in prebiotic chemistry promises to deepen our understanding of the origins of life on Earth and aids the search for life elsewhere in the universe.



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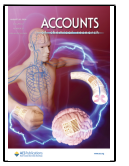

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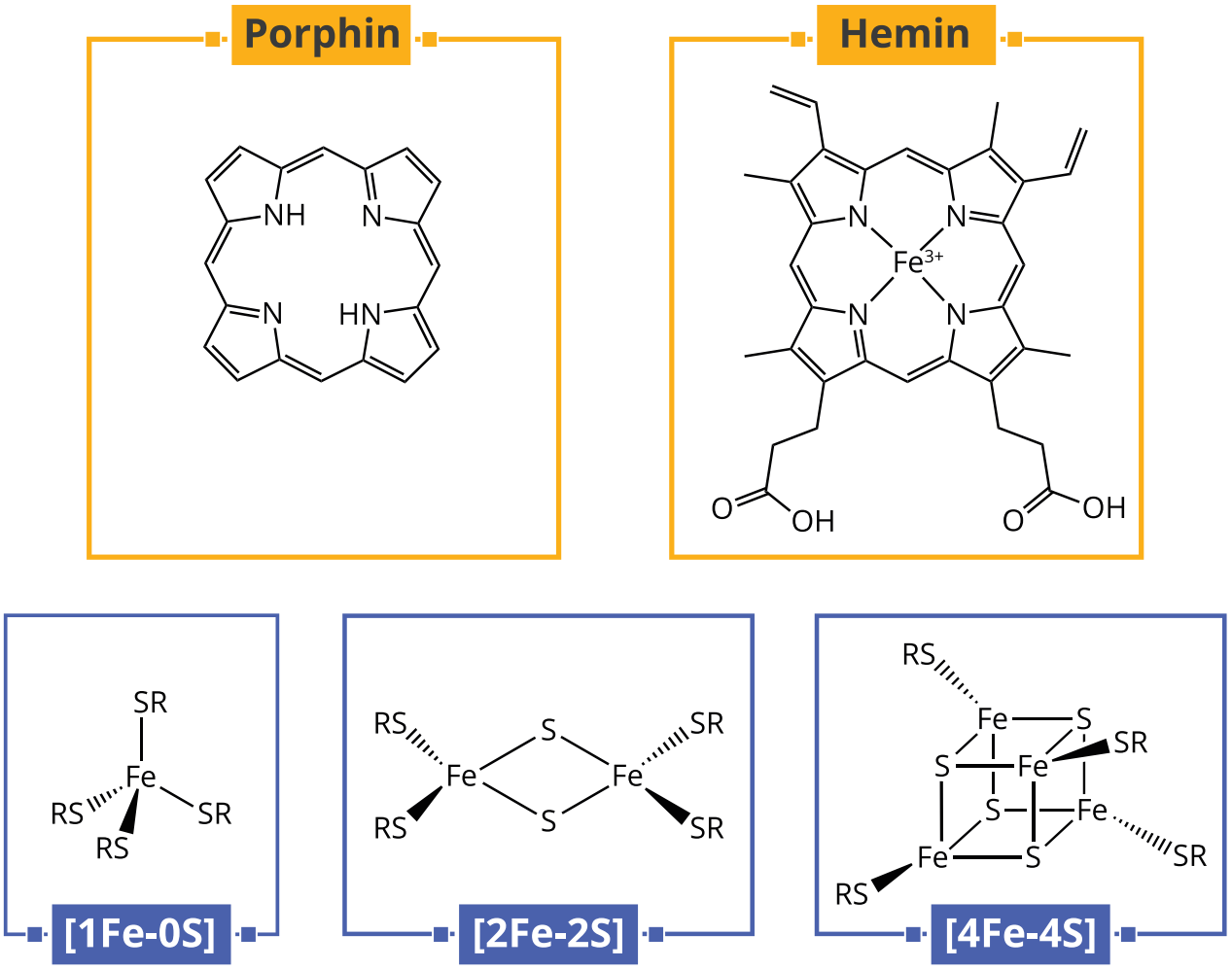

Figure 1. Representative structures of heme and iron−sulfur clusters.

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INTRODUCTION

The chemistry of life reflects a history of relentless selective pressures over billions of years of prebiotic chemistry and biological evolution. However, it remains a formidable challenge to confidently extrapolate back from extant life to a time prior to the last universal common ancestor. In the absence of well-preserved chemical relics related to the formation of the Earth's first cells, we are largely left to use the tools of chemistry and physics to gain insight into plausible pathways to the emergence of life. This has led to a series of insightful, but frequently debated, investigations on the prebiotic synthesis of the organic molecules of life. What is not debated is that our planet is and always has been rich in metals. Therefore, many prebiotic molecules would have been frequently found complexed with and impacted by the metal ions of the environment. Of these, iron was the most abundant transition metal that was accessible in water on the prebiotic Earth.

It is thus not surprising to find iron throughout biology. Iron occupies the active sites of many different types of proteins, ranging from enzymes involved in essential processes, such as the citric acid cycle and respiration, to enzymes responsible for DNA synthesis and repair. Since the concentration of Fe^{2+} on the Earth before the great oxidation event (GOE) was relatively high ($\sim 10^{-7}$ M),[5] early life capitalized on the inherent reactivity of this metal ion. The pervasive role of Fe^{2+} in metabolism during early evolution can be inferred from the continued reliance on iron ions after the environmental supply dwindled to $\sim 10^{-19}$ M after the GOE. That is, despite the much lower solubility of Fe^{3+}, the predominant oxidation state

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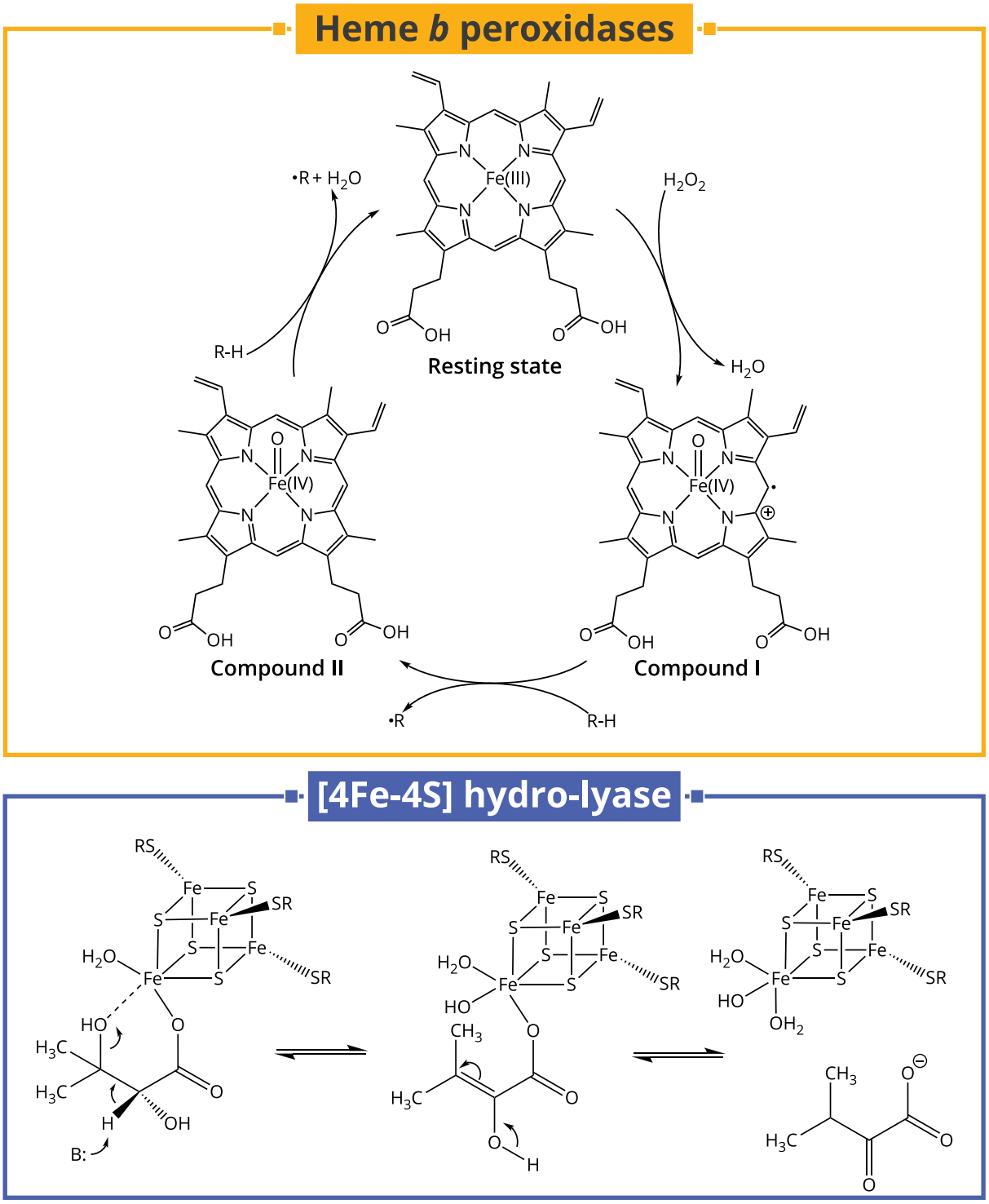

Figure 2. Examples of chemical reactions catalyzed by hemes and iron−sulfur clusters. The reaction catalyzed by heme *b* involves H_2O_2 as oxidant and R-H as reducing substrate (R being an organic moiety). The iron−sulfur cluster reaction is of the conversion of 2,3-dihydroxy-3-methylbutanoate to 3-methyl-2-oxobutanoate by dihydroxy-acid dehydratase.

in the presence of O_2, iron ions remained indispensable cofactors for numerous proteins. Approximately 2% of all human genes encode iron-containing proteins.[6] The continued reliance on iron chemistry has driven the development of sophisticated biological mechanisms to acquire, store, and efficiently deliver iron to target proteins.

While the significance of iron in early evolution is well-established, understanding the impact of iron on prebiotic chemistry remains a challenge when relying solely on biological analyses. Laboratory investigations have predominantly focused on free iron ions[7] or Fe^{2+} complexed with long strands of RNA.[8] Although these studies provide valuable insights, the probed complexes only represent a subset of the prebiotic possibilities of iron. Biological systems utilize a diverse array of iron-containing cofactors that are often interpreted to reflect relics from a prebiotic era.[9−11] These

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cofactors include iron coordinated to porphyrin (heme) and iron complexed with inorganic sulfides and organic thiolates (iron−sulfur clusters). In 1959, M. Calvin proposed a potential pathway from prebiotic chemistry to more modern-like heme proteins.[12] According to his scenario, the breakdown of hydrogen peroxide, for example, occurred sequentially: first catalyzed by free Fe^{3+}, then by free heme, and finally by heme coordinated to a polypeptide. Each step led to a substantial increase in catalytic rate, highlighting how an evolving iron-binding site can enhance the intrinsic reactivity of environmentally available iron.[13] Recent work has shown how *in vitro* evolving polymers can recruit cofactors from the environment,[14] consistent with the last step in this hypothetical evolution of a heme peptide. Similar pathways have been suggested for iron−sulfur clusters.[15−17] This review critically examines the relevance of such iron-containing compounds to prebiotic systems and protocellular chemistry.[18,19]

Prebiotic Heme

Hemes (iron porphyrins) are widespread across the domains of life (Bacteria, Archaea, and Eukarya)[20] and are believed to have been integral to the function of the last universal common ancestor (LUCA), where these metallocofactors facilitated crucial electron transfer and catalytic reactions.[21−23] However, other tetrapyrroles more ancient than heme, such as corrin-based molecules, may have been exploited, as found in acetogens and methanogens.[24,25] Structurally, hemes comprise an iron ion in either a +2 or +3 oxidation state, bound to a methine-bridged aromatic cyclic tetrapyrrole ring, known as a porphyrin. The simplest methine-bridged cyclic tetrapyrrole is the unsubstituted, metal-free parent porphyrin, often referred to as porphin.[26] In this Account, 'porphin' denotes this parent structure, while 'porphyrin' includes derivatives of porphin. Amphiphilic protoporphyrin IX (or simply "protoporphyrin" or PPIX)[26] is one such derivative of porphin (Figure 1). When Fe^{3+} coordinates to PPIX, the resulting complex is termed ferric heme *b* or 'hemin.'[21,27] It has been proposed that LUCA contained a catalase.[28] If this catalase were a heme protein, the enzyme likely facilitated the disproportionation of hydrogen peroxide to dioxygen and water ($2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$),[29] potentially shielding early cells from the deleterious impacts of encountered H_2O_2 (or possibly H_2S_2).[30] H_2O_2 may have been produced from water photochemically,[31] at defect sites of pyrite (FeS_2),[32] on the surface of silicates,[33] or in aqueous microdroplets.[34]

If prebiotic hemes acted as catalysts, then there must have existed prebiotic pathways for the production of these metallocomplexes. Spectrophotometric findings of hemes in meteorites[35] suggest either a mode of delivery to early Earth or evidence for plausible prebiotic synthesis. Additionally, numerous studies have documented prebiotic routes to the synthesis of porphyrins and hemes. Small quantities of heme can be generated through electric discharge of samples containing CH_4, NH_3, and water vapor.[36] Porphin-like molecules, such as $\alpha,\beta,\gamma,\delta$-tetraphenylporphine, can be synthesized by irradiating pyrrole and benzaldehyde with UV light.[37] Lindsey and colleagues demonstrated the abiotic production of porphin from reactions involving δ-amino-levulinic acid and 1,3-dicarbonyl compounds,[38] which could then undergo photooxidation to yield porphyrins.[39] Metalation of the resulting porphyrin occurs readily in neutral aqueous environments at 60 °C in the presence of dissolved salts, including $(NH_4)_2Fe(II)(SO_4)_2$.[40] When incorporated from the outset, metal ions facilitate various pathways, such as the synthesis of porphyrins from pyrrole and formaldehyde.[41] Notably, synthesis can be enhanced further by the inclusion of sodium dodecyl sulfate (SDS) micelles or phosphatidylcholine (egg PC) lipid vesicles.[42,43] These aggregates of amphiphiles, acting as "reaction promoters," help bring reactants, such as formaldehyde and derivatives of pyrrole, together within a microenvironment distinct from bulk aqueous solutions. Though SDS is an artificial, human-made amphiphile, analysis of the Murchison meteorite[44] suggests an abundance of amphiphilic compounds similar to SDS, including alkylsulfonates and alkylbenzenesulfonates, that possess sufficiently long alkyl chains to aggregate in aqueous solution.

Given the probable presence of amphiphiles on the early Earth, the impact of amphiphiles on the synthesis of porphyrins is suggestive. It is conceivable that in addition to forming protocellular membranes resembling modern cellular membranes, which host numerous heme proteins, prebiotic lipids could have also created local environments akin to the active sites of protein enzymes. To probe this possibility, Cvjetan et al. examined the activity of hemin in the presence of hydrogen peroxide. Hemin could have reacted with H_2O_2 in two different ways, either producing O_2 and water, as seen with catalases, or generating oxidized organics, as seen with heme peroxidases (Figure 2). The latter possibility was explored using UV absorbing substrates, such as *p*-aminodiphenylamine (PADPA or N^1-phenylbenzene-1,4-diamine) and 3,3′,5,5′-tetramethylbenzidine (TMB), that are commonly employed to assess the peroxidase activity of protein enzymes.[21] The anionic, micelle-forming amphiphiles sodium dodecylbenzenesulfonate (SDBS) and sodium dodecyl sulfate (SDS) were employed to disrupt the tendency of free hemin to form various aggregates that are catalytically inactive.[21] In both cases, the goal of the work was not to run reactions with hemin under prebiotic conditions, but rather to determine if complexes of hemin and simple amphiphiles could show peroxidase-like activity in the absence of a protein scaffold with H_2O_2 as an oxidant.

Peroxidase activity is evident with both substrates.[1,2] Although the reactants and products are the same for surfactant stabilized hemin as for horseradish peroxidase, it remains uncertain whether the underlying reaction mechanisms are identical. Peroxidases couple the reduction of H_2O_2 to the oxidation of an organic substrate. The catalytic cycle begins with the heterolytic cleavage of H_2O_2, leading to the donation of two electrons from the Fe^{3+}-porphyrin to generate water and an intermediate state known as Compound I with a Fe^{4+}-porphyrin pi-cation radical. The subsequent one-electron oxidation of the organic substrate gives rise to a second intermediate state (Compound II) with a Fe^{4+}-porphyrin. A final transfer of a single electron from the substrate restores the enzyme to the resting state. Reactions with TMB halt after a single electron transfer to hemin due to the stability of an intermediate state, called the "charge transfer complex" (CTC).[2] Conversely, reactions with PADPA progress further.[1] However, it is unclear whether hemin provides the second electron. In both reactions, anionic rather than cationic or nonionic amphiphiles support optimal activity, with maximal activity with TMB observed at the critical micellization concentration (CMC).[2] For reactions with PADPA, the anionic amphiphile also acted as a counterion, interacting with the half-oxidized product that resembles the green emeraldine salt form of polyaniline.[1] Nevertheless, further

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investigation is essential to unravel the catalytic mechanism and the different roles played by amphiphiles. While PPIX is known to partition into micelles, the polarity of hemin, i.e. ferric PPIX, is quite different due to the presence of the iron ion. Therefore, effort should be focused on determining whether hemin localizes at the surface of micelles or vesicles, or if hemin penetrates deeper into the hydrophobic core of the lipid phase.[2]

We also observed an increase in peroxidase activity upon the introduction of His, indicating potential axial coordination to the Fe^{3+} of hemin similar to that observed with protein peroxidases.[2] As there are two potential coordination sites, referred to as proximal and distal positions in heme proteins, it is important to maintain one site accessible for the binding of substrate.[2] This discovery of increased peroxidase activity with His suggests that peptides could have served as an alternative scaffold for hemin in place of amphiphiles. While heme coordination to proteins tends to involve predominantly noncovalent interactions, direct coordination to the Fe^{3+} center by an electron-donating side chain, such as by His, Cys, or Tyr, is often observed. Therefore, shorter peptide fragments with potential ligands to the iron center of hemin could be envisaged. For example, a His-containing 30 amino acid fragment of a prion protein[45] and nanofibers consisting of Fmoc protected His[46] exhibit elevated peroxidase activity compared to free hemin. Beyond peroxidase activity, a heptaptide[47] and tetrapeptide[48] were shown to catalyze the cyclopropanation of styrene derivatives and the oxidation of DOPA to dopachrome, respectively, when complexed with hemin.

Alternatively, the interaction between hemin and nucleic acids could have significantly influenced prebiotic chemistry. Hemin binds to guanine (G)-rich DNA and RNA sequences, forming complexes that exhibit peroxidase-like activity.[49] Notably, hemin-G-quadruplexes demonstrate a range of catalytic abilities, including oxygen transfer reactions,[50] thioether oxidation,[51] and the decomposition of H_2O_2 (catalase-like activity).[52] From a physicochemical perspective, the binding of hemin to RNA prevents aggregation. Such a mechanism is conceptually similar to the role played by amphiphiles and peptides. Additionally, the coordination of hemin may have expanded the capability of functionally poor primordial ribozymes.[50]

Prebiotic Iron–Sulfur Clusters

The prevalence of iron and hydrosulfide (HS^-, the deprotonated form of hydrogen sulfide, H_2S) on the prebiotic Earth, the ease with which iron–sulfur clusters form *in vitro*, and the importance of iron–sulfur clusters in central metabolism have all been interpreted to indicate an ancient role of iron–sulfur clusters in biology and perhaps prebiotic chemical transformations. In biology, iron–sulfur proteins at least partially coordinate iron through the thiolates of cysteinyl side chains. Polynuclear iron–sulfur clusters additionally possess acid-labile, bridging inorganic sulfides. The most prevalent forms in nature include mononuclear, rubredoxin-like centers ([1Fe-0S]), [2Fe-2S] clusters, and [4Fe-4S] clusters (Figure 1). Because of the impact of the surrounding environment, including ligating and nonligating residues, iron–sulfur clusters span a wide range of reduction potentials. These cofactors are, therefore, robust and malleable mediators of electron transfer, which is why iron–sulfur clusters are indispensable components of cellular respiration and photosynthesis.[53] For example, within Complex I of the respiratory chain, electrons traverse a series of iron–sulfur clusters with varying reduction potentials, including two [2Fe-2S] clusters and seven [4Fe-4S] clusters in the organism *T. thermophilus*.[54] While evolutionary studies affirm the antiquity of cellular respiration,[55,56] pinpointing the age of processes reliant on the exploitation of proton gradients remains difficult.

Initial explorations into the origins of metabolism have often centered on minerals that mirror the iron–sulfur clusters found in contemporary biological proteins. Notably, the resemblance of pyrite (FeS_2) and greigite (Fe_3S_4) to the [2Fe-2S] and [4Fe-4S] clusters in proteins has prompted theories that these minerals were the catalysts of early metabolic processes before the advent of iron–sulfur proteins.[57,58] Laboratory experiments have demonstrated the ability to synthesize small organic molecules from prebiotic precursors, such as CO_2 and formaldehyde, lending some credence to these theories. However, the efficiency of many of these reported pathways is low,[57,58] and with a few exceptions,[59,60] the experimental conditions often do not align with those of the invoked geological settings, i.e. hydrothermal vents.

Perspectives began to broaden when Cowan and colleagues demonstrated that the tripeptide glutathione (Glu-Cys-Gly or EγCG) could coordinate a [2Fe-2S] cluster by forming a tetrameric complex with one iron–sulfur cluster per tetramer.[61] As amino acids,[62−64] including Cys[65] and small peptides,[66] are believed to be prebiotically plausible, this discovery suggests that soluble iron–sulfur clusters much more similar to those found in biology than insoluble rock-like minerals provide a more reasonable path toward metabolic catalysts.[15] In fact, we subsequently found that a wide-range (>40) of peptides and thiolate-containing small molecules can coordinate an iron–sulfur cluster in aqueous solution,[67] meaning that there could have been many opportunities for soluble iron–sulfur clusters to form on the prebiotic Earth. One compelling aspect of such a scenario is that, as Eck and Dayhoff suggested,[17] modern-day iron–sulfur proteins may have arisen from the fusion of such ancient peptides. Similar ideas of accretion have been put forward for a variety of (metallo)proteins.[9,10] Computational models of [2Fe-2S] glutathione support this possibility by showing that coordination to a common iron–sulfur cluster places functional groups close enough to form peptide bonds.[15] In other words, an iron–sulfur cluster could potentially facilitate the condensation of short peptides into longer metal-binding motifs. Nevertheless, experimental demonstration of metallocofactor-templated peptide bond formation, e.g. through aminonitrile coupling[68] or dehydration-driven condensation,[66] is still lacking.

Generally, experimental evidence in support of the hypothesis that extant metal-binding motifs reflect ancient peptides are few. Peptide analogues of motifs found in proteins that contain a complete iron–sulfur-binding site with four ligating residues bind [4Fe-4S][69,70] and [2Fe-2S][71,72] clusters in aqueous solution, demonstrating that extant motifs can function outside of the full protein. These motifs typically possess a Pro residue immediately following one of the ligating Cys.[73] Since the terminally protected dipeptide Cys-Pro binds Fe^{2+} ca. 3-fold more strongly than Cys-Gly,[74] this Cys-Pro sequence may serve as an anchor point for the coordination of one of the iron ions of an iron–sulfur cluster. The addition of a properly spaced second Cys would then have increased affinity even further through chelation. For example, a hexapeptide

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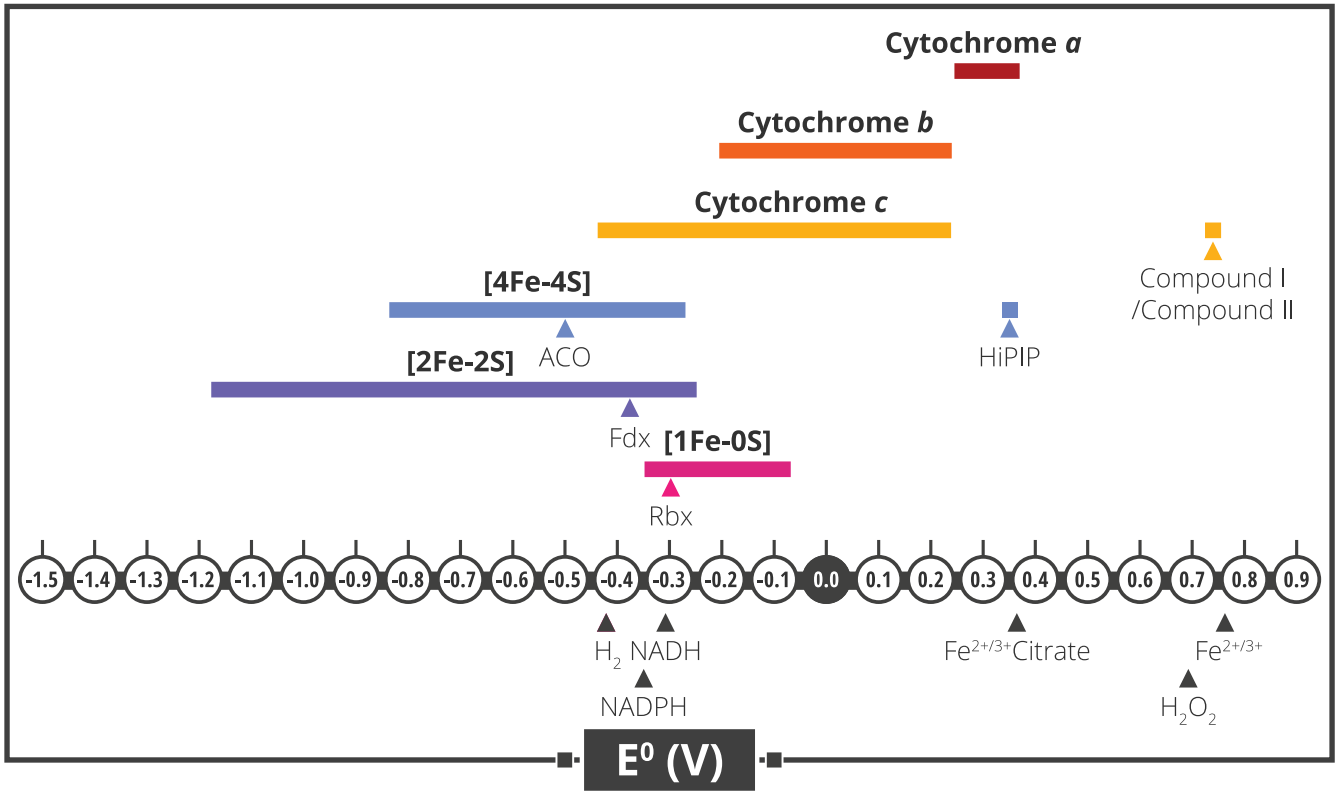

Figure 3. Reduction potentials of iron−sulfur and heme cofactors. Data compiled from refs 83 and 85. Data for Compound I/II of Cytochrome *c* peroxidase is from ref 94. E° is reported vs SHE.

containing a Cys-Pro-Leu-Cys sequence binds Fe^{2+} 10-fold more strongly than a peptide with a single Cys.[4] These small motifs are found throughout biology and are particularly adept at coordinating iron−sulfur clusters. However, it should be noted that Cys-X_2-Cys (where X is any amino acid) motifs are also capable of binding Zn^{2+} in a cell and *in vitro*.[73] In fact, as expected from the Irving-Williams series, Zn^{2+} binds these motifs 50−100-fold more strongly than Fe^{2+}. It is, therefore, important to take into consideration the impact of environmental conditions on metal-binding. For comparison, seawater concentrations are thought to have been 10^5 times greater for Fe^{2+} than Zn^{2+},[5] thereby favoring the binding of Fe^{2+}. However, niche environments could have been quite different.

Conditions at or near the surface of the early Earth are frequently invoked when investigating the synthesis of the building blocks of life, because laboratory approximations of such conditions have been shown to be compatible with the prebiotic synthesis of amino acids, nucleotides, sugars, and lipids.[75−79] For this reason, Bonfio et al.[67] probed whether the synthesis of iron−sulfur clusters would also be compatible with surface conditions. As iron−sulfur clusters are composed of iron and sulfide ions, the behavior of these components under UV light was explored. Sunlight is by far the most abundant energy source on the Earth.[80] Although the luminosity of the sun was lower, the lack of an ozone layer before the GOE meant that the total flux of photons was greater. Nevertheless, shielding was provided by CO_2 and water, below 204 and 168 nm, respectively.[81] We found that 254 nm UV light led to the photooxidation of Fe^{2+} to Fe^{3+}.[67] This is important, because polynuclear iron−sulfur clusters almost universally contain at least one oxidized Fe^{3+}. Since the prebiotic Earth was anaerobic, iron ions would have existed as Fe^{2+} in the absence of a mechanism for oxidation. Therefore, the surface of the early Earth would have been capable of providing the ferric ions needed for the assembly of iron−sulfur clusters and hemes.

Iron−sulfur clusters also possess bridging inorganic sulfides. Because of the equilibrium between hydrosulfide and the volatile hydrogen sulfide, a key ingredient of iron−sulfur clusters is continuously lost to the atmosphere, unless trapped in some way. HS^- can be captured within organic molecules, as HS^- is a frequent participant in prebiotic reactions that produce sulfur-containing molecules.[65,82] HS^- can then be later released by photolysis.[67] Therefore, a mixture of organic thiolates and Fe^{2+} that alone cannot form an iron−sulfur cluster, spontaneously assemble into [2Fe-2S] and [4Fe-4S] clusters when irradiated with UV light.[67] That is, few ingredients would have been needed for iron−sulfur clusters to form at or near the surface of the early Earth.

Once formed, iron−sulfur peptides could have engaged in electron transfer reactions. Tri- and hexa-peptides form mononuclear centers[4] and [2Fe-2S] clusters[15] that are redox active. Although $[4Fe\text{-}4S]^{2+}$ glutathione is not redox active, longer [4Fe-4S] peptides are capable of repeated cycling between reduced and oxidized states.[69] Because of the various ways that iron ions can be coordinated, their associated reduction potentials span a wide range (Figure 3) from 0 mV to −400 mV vs SHE (standard hydrogen electrode).[3,4,69] For example, substitution of a ligating Cys with a His increases the reduction potential of a [2Fe-2S] cluster by ca. 150 mV.[4] It should also be noted that small peptides form dynamic complexes with iron and sulfide so that a single peptide can often coordinate all three types of iron−sulfur centers.[83] Therefore, iron can coordinate to cysteinyl peptides, even a

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single cysteinyl peptide, in a variety of ways that give a broad range of reduction potentials that are far from that of free, fully aquated iron ions ($E°$ = 770 mV vs SHE)[84] or iron bound to simple chelates, such as citrate (+372 mV vs SHE).[85] In other words, an electron transport chain could conceivably be built with a single peptide that forms different types of iron−sulfur clusters, each with a different reduction potential.

The exploration of prebiotic iron−sulfur peptides has predominantly emphasized their potential roles in electron transfer reactions. However, iron−sulfur clusters also serve as vital catalysts in biology (Figure 2). Despite reports of metals facilitating model protometabolic reactions,[7,86] it is surprising that clear examples of prebiotically plausible metallopeptides mediating similar reactions are lacking. To achieve such reactivity, the iron−sulfur cluster of the peptide would need to possess an easily accessible iron similar to the scenario described for hemin earlier. Such an iron center could arise from a peptide scaffold containing three ligating residues, or potentially from multiple shorter peptides, each carrying a single ligating residue with lower affinity. Octa- and nona-peptides designed to mimic S-adenosylmethionine (SAM) enzymes are capable of binding a [4Fe-4S] cluster;[87] however, no catalytic activity has yet been reported for these SAM mimics or for iron−sulfur clusters stabilized by shorter, more dynamic peptides. If conditions were identified that allowed for catalytic activity, then iron−sulfur dependent protometabolic reactions could potentially encompass processes involved in heme[88] and lipid[89] synthesis, as well as participating in a citric acid-like cycle.

Protocelluar Activity

The broad range of reduction potentials exhibited by iron when complexed with porphyrin and sulfide hints at the ease of constructing an electron transport system. Moreover, the inherent affinity between porphyrin and amphiphiles aligns with the spatial organization of heme proteins within the electron transport chain of extant biology. Nevertheless, thus far, prebiotically plausible iron−sulfur peptides have only been localized to the surface of lipid membranes.[3] To penetrate the hydrophobic core, a more intricate peptide scaffold might be necessary to counterbalance the energy loss associated with the immersion of an iron−sulfur cluster within the interior of the membrane. Perhaps more importantly, none of the currently known prebiotic lipids possess the capacity to maintain a pH gradient.[90,91] This deficiency poses a significant challenge, as membranes permeable to protons impede the coupling of the energetically favorable flux of electrons with the unfavorable pumping of protons. Consequently, the integration of catabolic and anabolic processes via an electron transport chain may have emerged at a later stage of (proto)cellular evolution when less dynamic membranes were available.

Alternatively, hemes and iron−sulfur clusters may have played a direct role in facilitating the synthesis of the building blocks of life. The capacity of hemin to form complexes with various molecules, such as amphiphiles, peptides, and nucleic acids, that are capable of oxidizing organic compounds suggests an early role for hemes. Given that evolution tends to take the most expedient path to function, it is reasonable to speculate that early polymers exploited the intrinsic catalytic properties of what was available in the environment rather than to generate activity *de novo*.[13] Because of progress in our understanding of prebiotic mechanisms for the nonenzymatic and enzymatic copying of RNA, heme complexes with RNA are particularly attractive as early, heritable catalysts. Since there are no documented instances of iron−sulfur clusters coordinated to RNA, the potential catalytic role of (metallo)-peptides in a protocell is worth examining. Considering peptides lack the inherent ability to self-replicate and the unlikelihood of genetically encoded peptide synthesis at early stages, then alternative mechanisms must have existed if (metallo)peptides conferred a selective advantage to protocells. One conceivable scenario is if heterotrophic protocells inhabited environments rich in catalytic peptides. This pool of peptides might have been less diverse than expected from prebiotic synthesis, as only a fraction may have been able to withstand environmental conditions.[92] For instance, it has been proposed that exposure to UV-light selected for biological nucleotides that are photostable over alternatives.[93] If similar selective pressures were applied to metallopeptides, then protocells would have been fed with a steady supply of a few metallopeptides capable of surviving the environment. If abiotic mechanisms of synthesis and selection were sufficiently robust, then the uptake of these potential catalysts could have been exploited until the advent of genetically encoded protein synthesis. Furthermore, the identification of lipid compositions capable of growth and division in the presence of metal ions[91] suggests the potential emergence of metabolically driven Darwinian evolution under such conditions. Ultimately, the plausibility of this or any other premise will need to be established by experimental investigation, as we and others are currently attempting.

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Author Contributions

[⊥](D.R. and N.C.) These authors contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Daniele Rossetto** conceptualization, writing-original draft, writing-review & editing; **Nemanja Cvjetan** conceptualization, writing-original draft, writing-review & editing; **Peter Walde** conceptualization, funding acquisition, supervision, writing-original draft, writing-review & editing; **Sheref S Mansy** conceptualization, funding

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Notes

The authors declare no competing financial interest.

Biographies

Daniele Rossetto earned his PhD in Biomolecular Sciences at the University of Trento with Sheref Mansy as his advisor. He investigated the prebiotic chemistry of metallopeptides with a focus on the origin and evolution of metal-binding motifs. He then joined Dora Tang's research group at the MPI-CBG in Dresden as a postdoctoral Fellow, where he probes the emergence of early compartments.

Nemanja Cvjetan obtained a doctoral degree from ETH Zürich, under the supervision of Prof. em. Dr. Peter Walde. He then joined the group of Prof. Dr. Sheref Mansy at the University of Alberta as a postdoctoral researcher for one year. His interests include polymolecular self-assemblies and porphyrins.

Peter Walde has been Professor Emeritus at the Swiss Federal Institute of Technology (ETH) in Zurich (Switzerland) since 2022. He studied chemistry at ETH and obtained his doctorate at ETH in 1983. After postdoctoral research stays at the University of Auckland (New Zealand) and the University of Nagasaki (Japan), he taught and researched at ETH from 1986 on, obtained his habilitation in 1992 and received the title of professor in 1997. His research topics included the investigation of lipid vesicles as protocell models and the use of enzymes for the synthesis of conductive polymers and for bioanalytical applications.

Sheref S. Mansy is a professor of biochemistry at the University of Trento. He studied at Ohio State University and was a postdoc at Harvard/Massachusetts General Hospital before starting a laboratory at the University of Trento as an assistant and then associate professor of biochemistry. He then moved to the University of Alberta as a professor of chemistry before returning to the University of Trento. He works on protocells, artificial cells, and iron−sulfur peptides.

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■ ABBREVIATIONS

GOE, great oxidation event; LUCA, last universal common ancestor; PPIX, protoporphyrin IX; PADPA, *p*-aminodiphenylamine; TMB, 3,3′,5,5′-tetramethylbenzidine; CTC, charge transfer complex; CMC, critical micellization concentration; ACO, Aconitase; Fdx, Ferredoxin; Rbx, Rubredoxin; HiPIP, High Potential Iron−sulfur Protein

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