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Iron: Life's primeval transition metal

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Modern life requires many different metal ions, which enable diverse biochemical functions. It is commonly assumed that metal ions' environmental availabilities controlled the evolution of early life. We argue that evolution can only explore the chemistry that life encounters, and fortuitous chemical interactions between metal ions and biological compounds can only be selected for if they first occur sufficiently frequently. We calculated maximal transition metal ion concentrations in the ancient ocean, determining that the amounts of biologically important transition metal ions were orders of magnitude lower than ferrous iron. Under such conditions, primitive bioligands would predominantly interact with Fe(II). While interactions with other metals in certain environments may have provided evolutionary opportunities, the biochemical capacities of Fe(II), Fe–S clusters, or the plentiful magnesium and calcium could have satisfied all functions needed by early life. Primitive organisms could have used Fe(II) exclusively for their transition metal ion requirements.

primitive enzymes | evolution of early life | Archean metal ion bioavailability | metalloenzyme functional analysis | ancient bioligand metal binding

The utilization of chemical elements in living organisms has coevolved with Earth's changing surface environment since the beginning of life. All known forms of life require 11 elements, commonly referred to as the bulk elements. The four most abundant are hydrogen, carbon, nitrogen, and oxygen; there are also substantial amounts of sodium, magnesium, phosphorus, sulfur, chlorine, potassium, and calcium. In addition to these bulk elements, modern organisms use trace quantities of multiple metallic elements, the most important of which are manganese, iron, cobalt, nickel, copper, zinc, and molybdenum. As integral components of metal ion-containing biological catalysts, these metal ions lend irreplaceable, highly tuned chemical faculties to essential biochemical processes like the extraction of usable energy from chemical reactions. Did life always harness this diversity of metal ions?

As primitive organisms emerged and evolved, Earth's surface chemistry must have been the foremost driver determining the biochemistry of early life (1). E.-I. Ochiai, R.J.P. Williams, J.J.R. Fraústo da Silva, and more recently R.E.M. Rickaby have led the way in appreciating how the unique chemical behaviors of metal ions in the changing environment would have inevitably controlled their biological use over time (2–6). Today, biology employs highly evolved metal acquisition strategies (7). Early life would have lacked this complex toolkit, and thus, the environment wielded a primary control over what metallic elements biology encountered and explored. We consequently propose that life could not have exploited a metal ion's chemical reactivity unless sufficient amounts existed to enable binding of that element to a biomolecule—in other words, metal availability must have set severe limits on the trial-and-error process that preceded evolutionary pressure to acquire a metal ion purposefully.

Here, we establish constraints on the availability of manganese, iron, cobalt, nickel, copper, and zinc from geologically and kinetically informed chemical equilibrium perspectives. We then hypothesize that early life used iron as the principal biologically required transition metal, and we show how ferrous ions would have been adequate to enable biochemical functions that fueled the vast majority of early anaerobic life.

Geology and History of Major Bioelements

Biological metal usage is ultimately limited by their abundances on the Earth's surface, which are dependent on the nature of the crustal rocks and the history of their formation. Cosmological and geologic processes govern the changing spatial mosaic of metallic element concentrations on Earth's surface, which life exploits and reshapes over time. The bulk Earth and its crustal surface are both iron-rich, with substantial oxygen, silicon, and magnesium (*SI Appendix*, Table S1) (8). Multiple lines of evidence indicate that Earth began as an anoxic planet and remained so until there was a profound biogeochemical transition after the end of the Archean Eon, called the Great Oxygenation Event (GOE) (9, 10).

Geological evidence suggests that the early oceans were rich in ferrous iron. Hydrothermal vents acted as a major source of Fe(II) for the Archean ocean (11–13). The absence of iron retention or accumulation in terrestrial soils and sediments suggests that Fe(II) was not oxidized to insoluble Fe(III) but instead dissolved from source rocks and concentrated in the ancient ocean (10). Widespread and often thick marine sedimentary deposits of iron minerals known as iron formations reveal that the ancient ocean was far richer in dissolved iron than the modern ocean's trace iron concentrations (14).

In addition, a lack of widespread sulfate salt deposits records how Archean oceans had much lower sulfate concentrations

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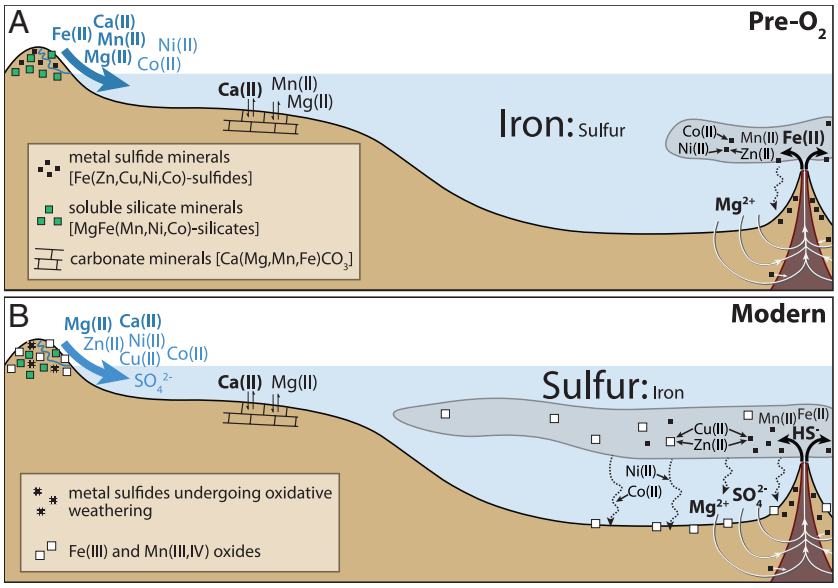

Fig. 1. Schematic of seawater elemental fluxes (*A*) prior to the GOE and (*B*) in the modern well-oxygenated ocean. Silicate-hosted metallic elements are released from hydrothermal vents and the weathering of exposed rocks. In the ocean, calcium and magnesium precipitate as carbonate minerals with trace divalent ions like Mn(II) substituting for Ca(II). Prior to the GOE (*A*), hydrothermal vents were large sources of Fe(II) and Mn(II) as well as Co(II), Ni(II), and Zn(II) (13, 25–28). In contrast, (*B*) modern oxidative weathering releases metal ions like Cu(II) from continents, hydrothermal Fe(II) and Mn(II) are oxidized to insoluble oxides, and hydrothermal vents are sulfidic due to high seawater sulfate concentrations. Oxide and sulfide minerals scavenge other metals from the ocean.

than the 29 mM in modern oceans (15). Low marine sulfate is consistent with the lack of dioxygen-dependent weathering of sulfides prior to the GOE (16). Isotopic studies of sedimentary sulfur minerals constrain ancient sulfate concentrations to less than 1 mM and potentially orders of magnitude lower (17), but sulfide concentrations are less constrained.

The evolution of oxygenic photosynthesis reshaped the planet, manifesting in the geologic record as the GOE at ~2.3 billion years ago (18). Earth's ocean ultimately transformed from a ferrous iron-rich, anoxic (ferruginous) sea to today's familiar, sulfur-rich, oxygenated body of water. This transformation was not immediate, and Earth's oceans may have spent over a billion years in intermediate, heterogeneous states (19, 20). While the timing and details of the chemistry of the post-GOE deep ocean are still debated (e.g., ref. 19), the predominance of Fe(II)-poor environments rapidly expanded as dioxygen oxidized and titrated dissolved Fe(II) from the surface ocean (e.g., ref. 21). The appearance of atmospheric dioxygen would have sequestered former iron sources as oxyhydroxide minerals in terrestrial environments and enabled the oxidative weathering of sulfide minerals on continents, introducing new large fluxes of dissolved sulfate and sulfide-bound metals to the ocean (22). This higher sulfate concentration in seawater, in turn, resulted in increasingly sulfidic hydrothermal vent fluids, curtailing another major input of marine iron (11–13). Thus, through multiple mechanisms working in concert, the rise of dioxygen drastically reduced iron's primary sources to the ocean.

Oxidative sulfide weathering on continents provided new sources of Cu(II), Zn(II), Co(II), and Ni(II). The increasing sulfate concentrations also enabled sulfidic environments, increasing the sedimentary removal of many metals as sulfide minerals (22, 23). Rising dioxygen in seawater oxidized Fe(II) and Mn(II), precipitating Fe-Mn oxides. Ni(II), Co(II), Cu(II), and Zn(II) readily adsorb to Fe-Mn oxides (24) and would have been scavenged from seawater. Combined, these O_2-triggered changing sources and sinks would be expected to result in a major increase in Cu(I, II), a net lowering of Ni(II), Mn(II), Co(II), and Zn(II), and a drastic reduction in Fe(II) as Earth's ocean was oxygenated (Fig. 1).

Trends in the composition and prevalence of chemical precipitates in the geological record generally support this understanding of how metal cycles reorganized. Post-GOE iron formations formed at shallower water depths than their older counterparts (29) and then no longer occurred as sustained deeper marine deposits after ~1.7 billion years ago (30, 31), indicative of a substantial decrease in dissolved iron around that time. The rising marine sulfur, in the form of sulfate, is conspicuous in post-GOE deposits of sulfate salts starting at ~2 billion years ago (32). Pre-GOE iron formations have low Cu contents compared to the average crust (33) and sediment-hosted copper deposits only form after the GOE (34). Mn(II) incorporated into precipitated carbonate minerals decreased by 100-fold or more from Archean to modern time (35), indicating a major drop in dissolved manganese. Archean iron formations contain more Ni than post-GOE iron formations (25). Similarly, the Co concentrations in ancient iron formations and iron sulfide minerals have higher maxima between ~2.8 and 1.8 Ga than in the last 1.7 Ga, attributed to higher Co in the early ocean (26). In contrast, shales and iron formations show no major changes in their Zn content, suggesting that the deep ocean did not substantially change its depositional flux of Zn over the past ~4 billion years and perhaps marine Zn concentration was similar to today's ~10 nM (27, 36).

Thermodynamic Constraints on Metal Ion Availability before the GOE

Biological usage of metals found on Earth's surface is modulated by environmental chemistry. Metal ions in solution are always attached to one or more other atoms or molecules, known as ligands. To acquire metal ions, life must compete

with other metal-binding organic and inorganic ligands in the aqueous environment or enable their extraction from minerals. This ability of an organism to acquire a metal ion from its environment so that it can interact with the cell's machinery is termed "bioavailability" (37). When all the ligands bound to the metal ion are water molecules, the metal ion is often referred to as a "free" metal ion, which is likely to be the most—but not necessarily the only—bioavailable form. In modern organisms, the ability to uptake metal ions—whether free, ligand-bound, or in a solid—is intricate, highly evolved, and varies widely from organism to organism. Presumably, the most ancient life lacked most of these sophisticated metal uptake mechanisms. In any case, constraining the concentration and aqueous speciation of the metal ions is a prerequisite to evaluating bioavailability.

Two approaches to estimating the concentrations of elements in ancient seawater are both rife with uncertainty. One approach considers the thermodynamic stability of minerals that may have buffered the concentrations of metal ions (e.g., ref. 38), and the other examines sedimentary rocks whose metal content may relate to metal ion concentrations in seawater when and where the rock formed (e.g., ref. 36). For most elements, dissolved concentrations are dependent on (bio)chemical and material fluxes into and out of the ocean, not set by mineral solubility equilibria (39), suggesting that, with sufficient understanding of how they form, sedimentary archives may provide a more accurate approximation. Broad trends in the composition and prevalence of these archives are reviewed above, but past sediments can be unfaithful records of the ocean for numerous reasons, including the risk of misinterpreting detrital particles or postdepositional alteration as primary signals in the geologic record (10, 40). Thus, the geologic record can provide insights into how depositional fluxes of elements have changed over time, but quantitative interpretation of ancient metal availability is complicated by incomplete mechanistic understanding of sedimentary and postdepositional processes.

In contrast, thermodynamic approaches consider chemical equilibria among observed or likely contemporaneous minerals to provide broad but robust constraints on elemental concentrations. For example, many studies have attempted to use the solubility of Fe(II) minerals that accumulated in Archean sediments to estimate ancient marine Fe(II) concentrations. The saturation of iron carbonate (siderite, $FeCO_3$), a common mineral in iron formations, provided an original estimate of maximum dissolved Fe(II) of 40 to 120 μM (9). However, the textures and isotopes of this siderite suggest that it formed postdepositionally (e.g., ref. 41) and recent experimental studies investigating the precipitation of iron carbonate have revealed the strong kinetic inhibition of siderite and its precursor phases (42). These results indicate that seawater iron concentrations may have been much higher than the limit of siderite solubility. Instead, the solubility of an Fe(II)-silicate phase—recently shown to be an early precipitate in many Archean iron formations and found to form readily from simulated seawater (43–45)—was suggested to limit the maximal ferrous iron concentrations in early seawater to 1 to 2 mM (42).

This abundant ferrous iron would have influenced the chemistry of many other elements, particularly sulfur. Dissolved ferrous iron and sulfide species bind to each other in aqueous complexes and clusters. Rickard (46) showed that the solubility of FeS minerals is controlled by $FeS_{(aq)}$ clusters, which are polynuclear dissolved species with variable iron and sulfur stoichiometries. These clusters would have been ubiquitous in Archean seawater, forming and growing along known interaction pathways between ferrous iron and sulfide species and eventually dehydrating and crystallizing as iron sulfide nanoparticles upon reaching approximately $(FeS)_{150}$ (47).

Saito et al. (38) recognized the importance of these aqueous iron-sulfide species in Fe(II)-rich environments and developed a thermodynamic approach to investigate the geochemical changes in ocean chemistry across the GOE. They showed that the abundance of aqueous Fe(II) controlled the concentration of aqueous sulfide, and in turn, aqueous sulfide concentrations set the solubility, speciation, and hence, bioavailability of other transition metals (38). Here, we applied Saito et al.'s approach to an updated thermodynamic database that incorporates recent understanding of metal sulfide solubility and aqueous speciation (*SI Appendix*, Table S2). We then determined maximum concentrations of transition metal ions using plausible ranges of Fe(II) and sulfide in the Archean ocean.

We estimated the upper limits of possible sulfide concentrations in Archean seawater using three constraints: 1) the range of ferrous iron concentrations, encompassing iron carbonate and silicate solubilities as discussed above, was assumed to be 40 μM to 3 mM Fe(II); 2) iron concentrations were assumed to be greater than that of sulfide, consistent with the presence of iron formations; and 3) the solubility of FeS nanoparticles was assumed to provide a maximal limit on sulfide concentrations because they are more soluble than disordered FeS or mackinawite (crystalline FeS) precipitates (46). We used the programs PHREEQC and PhreePlot (48, 49) and the MINTEQA2 v. 4 natural waters database (50) appended by sulfide complex stability constants and mineral solubilities (*SI Appendix*, Table S2). Equilibria among carbonate, bicarbonate, chloride, sulfide, hydrosulfide, polysulfide, and hydroxide species were considered. The calculations, described in detail in *SI Appendix*, showed that at pH 7, seawater could not have contained more than 30 μM total aqueous sulfide species. As Archean oceans contained more iron than sulfur, the sulfide was predominately speciated as aqueous $(FeS)_n$ clusters. This calculation resulted in similar sulfide concentrations as those observed in modern ferruginous environments (4 to 30 μM sulfide; (e.g., refs. 17, and 51).

As we sought upper limits on transition metal concentrations from thermodynamic equilibria, we endeavored to represent the kinetic inhibitions on crystalline mineral precipitation and dissolution. We assumed that anoxic seawater could not have been more concentrated in any metal ion than the solubility of readily precipitated phases observed in laboratory experiments or natural waters. For many transition metals in anoxic solutions, these are sulfide phases, and, in the case of Mn, carbonate phases (*SI Appendix*, Table S2). Our calculations reveal the metal concentrations in solutions saturated with respect to these minerals and equilibrated with Fe(II), sulfide species, and aqueous complexes or clusters at pH 7. By considering sulfide concentrations well below FeS nanoparticle saturation, we obtained conservatively high estimates of maximum metal concentrations. The range of the upper limits of

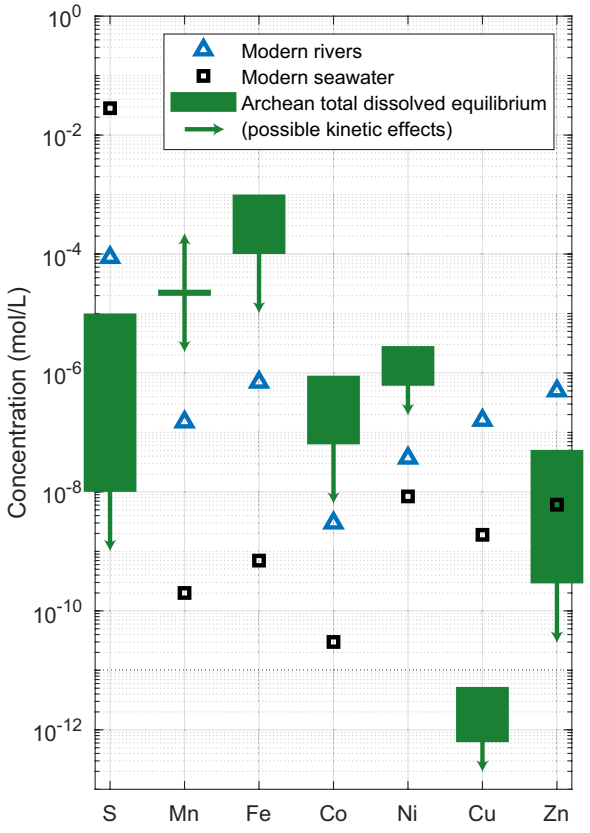

Fig. 2. Estimates of uppermost Archean metal ion concentrations (green bars). Note that these ranges represent interdependent suites of model outputs (see red boxes in *SI Appendix*, Fig. S1). With the possible exception of Mn, Archean seawater likely contained lower metal concentrations than these solubilities (as indicated by arrows; see *SI Appendix*). Modern values derive from refs. 52 and 53.

dissolved metal ions in Archean seawater is compared to their modern seawater and river concentrations in Fig. 2.

Our calculations of maximum Archean metal ion concentrations indicate that Fe(II) was orders of magnitude more abundant than Co(II), Ni(II), Cu(I), and Zn(II). Thus, it is apparent that the decrease in aqueous Fe(II) concentrations that occurred between the anoxic Archean and oxic modern ocean far exceeded any change that occurred with other metal ions. The decrease in aqueous Fe(II) ultimately led to the nanomolar concentrations of dissolved iron that we observe in the ocean today—six orders of magnitude lower than in Archean seawater. In contrast, Co(II), Ni(II), and Zn(II) levels changed by less than four orders of magnitude between the Archean and modern ocean, and Cu(I, II) concentrations increased by two or more orders of magnitude.

Metallobiochemistry of the Major Bioelements

The biochemistry of modern life requires the action of diverse biomolecular catalysts, termed enzymes. Today, enzymes are large, elaborate, and complex structures consisting primarily of lengthy chains of linked amino acids that are termed polypeptides or proteins. For their catalytic activity, an estimated 40% or more of enzymes require bound metal ions, which generally attach to groups within certain amino acid side chains in the polypeptide (54). In addition to metalloenzymes that bind metal ions directly to the polypeptide, there are also those that bind small metal-containing nonprotein molecules called metal cofactors. The most common examples of metal cofactors are iron-sulfur clusters, comprised solely of iron and sulfide ions, and metallotetrapyrroles, e.g., hemes, chlorophyll, and corrins (*SI Appendix*, Fig. S2) (55).

The metal ion binding properties and chemical reactivities of modern metalloenzymes have been fine-tuned by evolutionary processes, and modern cells frequently contain metalloenzymes that are activated by a metal ion other than that which would bind most tightly to them. Modern cells have therefore evolved elaborate mechanisms to scavenge and concentrate desirable metal ions from the environment and direct them to their target metalloproteins (7).

The evolutionary precursors of modern metalloenzymes are widely believed to have been much simpler, catalytically active metal ion complexes of small peptides (56). Therefore, the metal ion selectivity of early biomolecules—i.e., the binding preference for particular metal ions among those in the environment—primarily depended upon the chemical properties of metal ions and of the other competing inorganic or biomolecular ligands. These interactions follow a well-established series of preferences for ligands binding to divalent metal ions called the Irving–Williams Series: Mg(II)<Mn(II)<Fe(II)<Co(II)<Ni(II)<Cu(II)>Zn(II). (See additional discussion in *SI Appendix*).

Metal Preferences of Archean Model Ligands. If the playing field were level—i.e., equal concentrations of Mn(II), Fe(II), Co(II), Ni(II), and Cu(II)—the latter three metal ions would be expected to outcompete Fe(II) in binding to primitive biological ligands due to their positions in the Irving–Williams series. However, the huge concentration advantage of aqueous Fe(II) species in the pre-GOE ocean would have rendered the other first-row transition metal ions largely imperceptible to these organisms, unless they could produce special metal-selective ligands that could outcompete Fe(II) in binding.

We examined whether the binding affinities for different metals from some representative simple ligands supported significant binding of another metal in the presence of overwhelming Fe(II) concentrations. Table 1 shows the binding affinities of some divalent metal ions with a simple organic acid (acetate), two commonly used organic ligands (EDTA and TPEN), the naturally occurring small tripeptide glutathione, and two cysteine-containing model peptides synthesized by Valer et al. (57). We chose these ligands for our analysis because they attach to the metal ions using combinations of O, N, or S atoms, thus resembling small metal ion-binding peptides predicted for early life and, most importantly, because reliable values for their binding affinities are available for several of the metal ions of interest. The overwhelming Fe(II) concentration in the early oceans (Fig. 2) is predominantly a consequence of the solubilities of each metal sulfide, which are also shown in Table 1. (Cu(II) is omitted because of its scarcity prior to the GOE). The solubility of each metal sulfide varies more than ligand affinities for most transition metals. For example, ZnS is $10^{5.5}$ less soluble than FeS nanoparticles, but early bioligand types resembling EDTA, TPEN, or glutathione would bind Zn^{2+} only 10^{2}, 10^{1}, and $10^{3.7}$ better than Fe^{2+}, respectively. Intriguingly, NiS is $10^{2.1}$ less soluble than FeS, but Ni(EDTA) is $10^{4.1}$ times more stable than Fe(EDTA) and Ni(TPEN) is 10^{7} more stable, suggesting the possibility that relatively simple early bioligands binding to

Table 1. Selectivity of simple ligands for divalent metal ions relative to Fe(II), compared to metal sulfide insolubility

	α-$MS_{(s)}$ insolubility (-log $K_{sp}K_{a,HS}$)	Acetate[c] (log β_{110})	EDTA[c] (log β_{110})	TPEN (log β_{110})	GSH (-log K_d)	GCG[h] (-log K_d)	S-rich peptide CCCC[h] (-log K_d)
Mg^{2+}		1.3	10.6	1.7[d]			
Mn^{2+}	-3.3[a]	1.4	15.6	10.3[e]			
Fe^{2+}	3.5[b]	1.4	16.0	14.6[e]	5.1[f]	2.6	4.2
Co^{2+}	7.4[a]	1.4	18.2	16.6[e]		2.9	4.6
Ni^{2+}	5.6[a]	1.4	20.1	21.6[e]			
Zn^{2+}	9.0[a]	1.6	18.0	15.6[e]	8.8[g]	4.5	9.9
Insolubility Difference and Binding Preference Relative to Iron							
Mg/Fe		-0.1	-5.4	-12.9			
Mn/Fe	n.a.	0.0	-0.4	-4.3			
Co/Fe	3.9	0.0	2.2	2.0		0.3	0.4
Ni/Fe	2.1	0.0	**4.1**	**7.0**			
Zn/Fe	5.5	0.2	2.0	1.0	3.7	1.9	**5.7**

In environments where metal concentrations are limited by metal sulfide insolubilities, only the metal ion–ligand complexes that are substantially more selective than the solubility difference between the metal sulfide and iron sulfide could have formed. The bottom section of the table compares insolubility and selectivity of each ligand for divalent metals compared to Fe. Bolded ratios indicate ligands that could obtain a transition metal other than Fe (see text). The availability of Mg(II) and Mn(II) is not controlled by sulfide mineral solubility. Acetate (blue) is a nonchelating carboxylic acid that binds via hard O atoms and is not selective for transition metal ions; ligands like acetate would bind metals according to their environmental availability. Chelating ligands with borderline and soft binding atoms like N and S more strongly select for softer divalent metals. EDTA (green) is ethylenediaminetetraacetic acid, a synthetic chelating carboxylic acid ligand that binds via hard O and N atoms. TPEN (yellow) is N, N, N′, N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine, a synthetic chelating pyridine ligand that binds via hard and borderline N atoms. Glutathione (GSH), a naturally occurring cysteine-containing tripeptide, and the simple tripeptide glycine–cysteine–glycine (GCG) chelate metal ions via S and other atoms and are shown in orange. We also show a more complex 25-residue peptide with four cysteine residues synthesized by Valer et al. (57). This S-rich peptide (red) was designed to be similar to biological metal-binding sites in Rieske iron-sulfur electron transfer proteins. Sources: [a] (58); [b] (46); [c] (50); [d] (59); [e](60); [f] (61); [g] (62); [h] (57).

metal ions via some combination of O- and N-atoms might have evolved to enable early life to explore Ni chemistry, even in the presence of very high Fe(II) concentrations. To select other metal ions like zinc, life would have needed to evolve highly specialized ligands optimized to outcompete Fe(II) at these high concentrations.

We imagine that primitive cells evolved to improve the uptake and binding of desired metal ions from their environment first by optimizing polypeptide ligands using relatively simple strategies to enhance metal ion binding ability (*SI Appendix*, Fig. S3). In one strategy, if a polypeptide can evolve to attach to a single metal ion via multiple amino acid side chains, then the binding strength can be greatly enhanced by a mechanism referred to as the chelate effect. This effect is illustrated by the four-cysteine peptide designed by Valer et al. (57), called here "S-rich peptide CCCC," which has an affinity for Zn(II) that is much greater than that for Fe(II) compared to the one-cysteine peptides glutathione and glycine–cysteine–glycine (Table 1). This relative affinity for Zn(II) is also greater than the solubility difference between $FeS_{(s)}$ and $ZnS_{(s)}$, suggesting that if such a peptide had evolved, it could select for Zn(II) in a high-Fe(II) environment like the Archean ocean. None of the simpler ligands—including simple peptides and nonpeptides—could have overcome environmental dominance of Fe(II) to achieve significant Zn(II) binding in the early ocean.

Critically, our results demonstrate that Fe(II) availability likely vastly surpassed that of other transition metal ions (with the possible exception of Ni), making it highly challenging or even impossible for early life to uptake the other metal ions. If transition metal ions other than Fe(II) were essentially invisible to early life, there would have been no way for those organisms to experience their unique chemical properties that might have prompted the evolution of such metal-selective ligands.

Accidental and Evolved Swaps in Metal Binding in Metalloenzymes. Insights into which metals were used by early life can come from looking at instances when modern metalloenzymes bind metals other than their presumed usual target ions, as such metal swaps show when it is possible to exchange a metal center and retain enzymatic activity. Importantly, the metal ion used in a particular metalloenzyme is not coded in the gene for that protein, which only encodes a series of amino acids that form a binding site for a metal ion. Quite often, different metal ions are capable of binding to these same positions. A dramatic example of misidentification of the binding metal has become apparent in the "zinc finger" proteins that bind Zn(II) and act as interaction modules in Archaea, Bacteria, and Eukaryotes (55). Recently, some protein sequences that were misidentified in sequence databases as zinc-binding sites in zinc finger proteins were shown in fact to be iron-sulfur cluster binding sites of unknown function (63, 64).

Binding of alternative metal ions occurs either accidentally ("mismetalation") or purposefully because of an evolved response to cope with changing environmental conditions (65). Such alternate metalation can entirely inactivate the metalloenzyme, but sometimes partial or full catalytic activity may be retained. An evolved alternative metalation is exemplified when diverse species of Bacteria are subjected to oxidative stress. Because the Fe(II) in iron enzymes is highly susceptible to reactions with oxidizing species, many Bacteria under this stress switch to using Mn(II), which is able to provide enzyme activity in multiple iron proteins and yet is much less sensitive to oxidative damage (66). Another intriguing result of mismetalation is the emergence of a new chemical reactivity, such as the hypothesized accidental binding of vanadate to a phosphatase enzyme to switch the enzyme function from breaking phosphate bonds to generating chlorinated organic molecules (67).

With widespread potential metal swaps, the genetic code or amino acid sequence for a metalloenzyme binding site cannot be depended upon to determine which metal is binding to the enzyme. Thus, it is highly challenging to use phylogenies to infer with any certainty the historical usage of specific metals over the course of evolution.

A Biochemical Function-Based Analysis of Metals in Enzymes. We hypothesize that without sufficient interaction with different metal ions, ancient organisms would not have evolved to rely on scarcer metal ions. Only magnesium, calcium, and iron were likely available in high (~millimolar) abundance before the GOE (Fig. 2 and *SI Appendix*, Table S3). Given these environmental constraints, could ancient life access all its needed catalytic abilities? It is difficult to deduce how metals were used in early metalloenzymes because enzymes have surely evolved over the history of life and alternative metalations obfuscate phylogenetic inferences of enzyme metal usage. However, a metal ion's ability to perform a specific function should represent inherent chemical reactivity, so we can approach this tricky task by considering the chemical functions that metals perform in modern metalloenzymes.

We analyzed how alkaline earth and first-row transition metal ions function in many of the modern metalloenzymes (*SI Appendix*, Fig. S3). In a comprehensive analysis of the diverse metal ion reactivities in modern metalloenzymes, Andreini et al. (68, 69) divide metalloenzymes into two categories: nonredox and redox metalloenzymes. Nonredox metalloenzymes have redox-inactive metal ions that do not change oxidation state during catalysis. Instead, they act by binding substrates, stabilizing negative charges, and/or modifying the chemical reactivity of bound water molecules or substrates. The metal ions used in nonredox metalloenzymes are relatively unreactive toward substrates when not bound to the enzyme. In the mildly reducing atmosphere encountered by life prior to the GOE, more metal ions would have functioned as "redox-inactive" than today: Without O_2, the lower oxidation state transition metal ions, i.e., Mn(II), Fe(II), Co(II), Ni(II), and Cu(I), would have been operationally redox-inactive.

Modern nonredox metalloenzymes most frequently bind Mg(II). Mg(II) would have been similarly accessible to serve in Archean nonredox metalloenzymes that did not strongly select for transition metals (Table 1), although the likely somewhat lower amount of Mg(II) in early seawater [perhaps ~10 mM instead of ~50 mM today (70)] might have affected its binding to metalloenzymes with interesting implications. Notably, some simple ligands prefer divalent transition metal ions such as Fe(II) and Mn(II) to Mg(II) by over five orders of magnitude (Table 1). An interesting case of mismetalation occurs in nucleic acid processing enzymes that require divalent metal ions for activation. Mg(II) is used to activate most of these enzymes in modern biology, but their enzymatic activity is often retained when other divalent metal ions are substituted for Mg(II). For example, DNA polymerase, the enzyme that synthesizes DNA molecules, usually uses Mg(II), but can also use Mn(II), Co(II), or Fe(II) (71, 72). Ribosomes also typically use Mg(II) but recently were found to function partially with Mn(II) or Fe(II) (73).

Another important nonredox enzyme group, the metallohydrolases, is unique in that these enzymes often rely on Zn(II) to promote the ionization of a bound water molecule, producing a metal-bound hydroxide that then attacks a substrate. This chemistry catalyzes several critical reactions, such as the breakdown of proteins (peptidases) and the reversable hydration of carbon dioxide (the carbonic anhydrases). These enzymes more often bind Zn(II) than Mg(II) because zinc is more effective at activating a bound water molecule. However, the pK_a of an Fe(II)-bound water molecule (9.4) is similar to the pK_a of a Zn(II)-bound water molecule (9.0) (74), indicating that Fe(II) should be similarly effective in activating a bound water molecule. Indeed, as discussed by Pandelia et al., Fe(II) can achieve the same functionality in some modern metallohydrolases as Zn(II), and metal ion substitution experiments using isolated and purified enzymes demonstrate that Zn(II) in a variety of zinc enzymes can be functionally replaced by other divalent metal ions, including Fe(II) (64, 75).

The redox group comprises metalloenzymes that catalyze redox reactions by using metal ions to relay electrons between donors and acceptors (the oxidoreductases), to bind and activate O_2, and/or to stabilize radical intermediates. In contrast to nonredox metalloenzymes, redox metalloenzymes take advantage of a potentially highly reactive metal ion, usually with multiple oxidation states, that is capable of indiscriminate reactions with a wide variety of substrates when existing freely in water or even when immobilized in a mineral. The enzyme in effect tames the metal ion by binding it within a protein active site that limits access to desired substrates and tunes it to the desired redox potential and chemical reactivity.

Among the "redox-active" metal ions, iron is undoubtedly the most important to modern life. Iron is an essential element for all known free-living organisms and iron enzymes make up the majority of the redox metallo-catalysts (68). It is highly abundant in the Earth's crust (*SI Appendix*, Table S1), but today iron is not easily accessible to modern organisms because it commonly forms insoluble Fe(III) oxides. The chemical versatility of iron is well illustrated by the wide range of observed different chemical reactivities today. Heme-containing proteins and enzymes are intimately involved in nearly every aspect of dioxygen metabolism as dioxygen carriers and activators of dioxygen and peroxide (55). In addition, iron is a critical component in iron-sulfur cluster cofactors. The proteins that rely on these iron-sulfur clusters are present in virtually all living organisms. As redox catalysts and in other roles, iron-sulfur proteins play diverse, important, and often essential roles in numerous cellular processes such as respiration, photosynthesis, and many other metabolic processes (76). Their biosynthesis requires assembly of the iron-sulfur cluster cofactor, but, unlike heme proteins, these cofactors are entirely inorganic.

Manganese is used today in nonredox catalysts as well as oxidoreductase metalloenzymes, many of which are involved in dioxygen reactions (28). Similarly, copper is predominantly used by metalloenzymes to catalyze reactions of dioxygen and species derived from dioxygen (55). Importantly, other Cu-utilizing functions that do not handle dioxygen or its metabolites and products, including electron transfer reactions, can be performed by other metal ions, including iron [*SI Appendix*, (55)].

Cobalt is only irreplaceable in modern biology when in the cobalamin (vitamin B12) cofactor, where it helps to catalyze

many critical biological reactions in aerobic and anaerobic life, including radical-initiated rearrangements and transfers of methyl groups. However, a more recently discovered superfamily of proteins, the iron-dependent radical SAM (S-adenosyl-l-methionine) family, performs the same and additional functions as these cobalamin-dependent proteins (77). Radical SAM proteins use the dioxygen-sensitive FeS cluster to enable the cleavage of an organic molecule, SAM, into a highly oxidizing organic radical that can mediate at least 80 different reactions (78).

Nickel is only absolutely required by four modern metalloenzymes (*SI Appendix*), but these few enzymes have critical roles in anaerobic metabolism. Three of these catalysts employ clusters of nickel, iron, and sulfur. The hydrogenases catalyze the reversible oxidation of H_2 to give protons. Because this family includes solely Fe-utilizing hydrogenases (55), the group's common ancestor did not need to contain nickel. CO dehydrogenase performs the reversible oxidation of CO to yield CO_2, and acetyl-CoA synthetase converts CO_2 and a methyl group into acetyl-CoA; together, these NiFeS enzymes form the core of the reductive acetyl CoA (Wood–Ljungdahl) carbon fixation pathway. However, iron-containing species including FeS clusters can act as catalysts to reduce CO_2 and CO into organic hydrocarbons under mild conditions, mimicking the activity of the reductive acetyl CoA pathway (79, 80). The fourth nickel enzyme, methyl-coenzyme M reductase (MCR), is used in methane production and relies on an unusual Ni cofactor called F430. This enzyme performs a type of reaction that an iron-containing enzyme could conceivably catalyze. However, such chemical reactivity for iron has never been experimentally demonstrated, and so the intriguing possibility exists that methanogenesis, if it strictly requires nickel, might be the only metabolic pathway of early life that requires a transition metal other than iron.

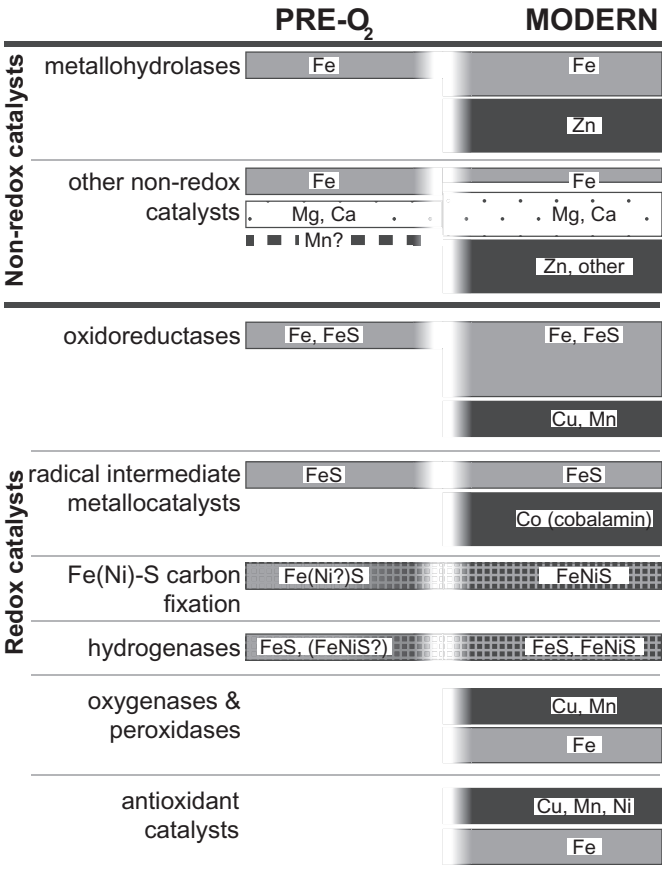

Fig. 3. Metal ion usage in metallo-catalysts in pre-GOE and modern life. The evolutionary transition between these states may have occurred over a billion years or more, in increasingly widespread environments where decreased Fe(II) challenged life to find replacements, and the increased bioavailability of other metals provided life with new opportunities.

Discussion

Dioxygen radically altered the geochemistry and biochemistry of Earth. To conjecture how early life evolved metallocatalysts in the primordial ocean, we must carefully consider each of the chemical changes that dioxygen has rendered and envision a world without its ubiquitous influence. First, the Earth's crust is Fe(II), Mg(II), and Ca(II)-rich (*SI Appendix*, Table S1), setting the major element composition of the early ocean (Fig. 1). A related consideration is that only the more reduced species of the various redox-active transition metal ions would have been abundant in the environment before O_2. Mg(II) and Ca(II) would not be highly impacted by the presence or absence of dioxygen, and thus, these metal ions would largely have remained available—likely at tens of millimolar levels—for life's use prior to and after the GOE. We modeled the maximum concentrations of other metal ions and found that the estimated hundreds of micromolar to a millimolar Fe(II) was substantially more abundant than other transition metal ions (Fig. 2). Depending on Fe(II) and sulfide abundance, Mn(II) and Ni(II) were likely at concentrations less than tens of micromolar, while Co(II) and Zn(II) would have been less than tens to hundreds of nanomolar. Copper was predominantly locked away in sulfide minerals and at picomolar levels of Cu(I) at most, thus rendered inaccessible for ancient life. These abundances would have formed a system poised for Fe(II)-based life (Fig. 3), where the use of other metal ions would have required targeting by biological ligands and selective binding sites, features that were unlikely in early, simple bioligands (Table 1). Is it possible for primitive organisms to have only used the abundant metal ions—predominantly Fe(II), Mg(II), or Ca(II)—in their metalloenzymes?

Mn(II) would have offered life nothing unique from Mg(II) except as an electron donor in photosynthesis. In nonredox metalloenzymes, Mn(II) is usually interchangeable with Mg(II) (81). Prior to the GOE, the relatively abundant Mn(II) could have been harnessed by a newly evolved high-potential photosynthetic reaction center as a new electron source (82). If it existed, this manganese-oxidizing photosystem would be a logical precursor to the development of oxygenic photosynthesis (82). In addition, given the right binding atoms, the somewhat abundant Mn(II) could have accidentally mismetalated Mg catalysts (Table 1). Interestingly, in DNA polymerase, the replacement of Mg(II) by other metal ions leads to increased numbers of errors in the newly synthesized DNA (71). Mn(II) substitution in DNA polymerase is the probable explanation for the high observed mutagenicity of Mn(II) in living organisms (83). Prior to the GOE, when the ratios of Fe(II) and Mn(II) concentrations to Mg(II) concentrations were higher, such metal ion substitutions in early nucleic acid processing enzymes could have promoted rapid evolution.

Zn(II) is required for life today, but redox-inactive Fe(II) could have served the same biochemical purpose as Zn(II) in ancient metallo-catalysts. In an anoxic ocean where iron was far more abundant than zinc, it is highly likely that Fe(II) could have activated numerous nonredox metalloenzymes that now typically rely on Zn(II) (Fig. 3). Indeed, life may not have started to select for zinc until dioxygen made Fe(II) a scarce commodity, relegated to increasingly fringe niches, and therefore best to conserve for functions where iron was irreplaceable.

Many redox-active metal ions serve functions in modern metalloenzymes that were unnecessary prior to dioxygen's central role in Earth's environment. The earliest role for heme-containing biomolecules was presumably as electron-transfer agents (e.g., cytochromes) rather than as catalysts. Copper's absence in ancient biology is largely well-accepted (2, 5, 84). The post-GOE release of dissolved Cu(II) likely spurred the development of Cu-utilizing enzymes that catalyze reactions of substrates with dioxygen and peroxides, as well as electron transfer Cu proteins and other copper metalloenzymes, as previously hypothesized by Ochiai as well as by Fraústo da Silva and Williams (Fig. 3) (2, 5). Similarly, Mn(II) abundance would have decreased after the GOE, especially in oxic portions of the ocean (35, 85), but the expanded set of higher oxidation states of Mn would likely have promoted the evolution of manganese-based oxidoreductase enzymes and dioxygen-associated enzymes (Fig. 3) (28).

Redox functions were highly limited in Earth's early reducing environment. Potential areas of redox activity could have been the surface ocean, exposed to highly oxidizing ultraviolet radiation. We hypothesize that most nonphotochemical redox reactions involving metals were enabled by FeS clusters (Fig. 3). FeS clusters encompass a large range of redox potentials and can accept and donate electrons (55). These simple inorganic cluster complexes form spontaneously when Fe(II) and sulfide ions are present (47) and would have been accessible in the ancient ocean, with most marine sulfide already in aqueous complexes or clusters with Fe(II), although the abundance of such clusters would be limited by the low sulfide concentrations.

Typically, both Fe(II) and Fe(III) are required to form stable clusters, but on early Earth, Fe(III) was scarce in much of the ocean. Synthetic iron-sulfur cluster complexes self-assemble in the laboratory using Fe(III) and sulfide ions and a variety of ligands, including cysteine (86–88). If we hypothesize that iron-sulfur clusters self-assembled under early Earth's conditions, we must explain the oxidation of at least one Fe(II) to Fe(III) to stabilize the clusters. Bonfio et al. suggested that Fe(II) could be oxidized to Fe(III) by ultraviolet radiation during self-assembly of early iron-sulfur clusters (89). We propose another possibility: that protons can act as an oxidant of at least one Fe(II) per cluster during cluster formation. Such a reaction would be analogous to the serpentinization reaction in which Fe(II) is oxidized by protons, forming H_2 (90), or the known oxidation of Fe(II)-sulfide nanoparticles to ferric iron-bearing sulfides by protons in water or from hydrogen sulfide (e.g., ref. 91). Thus, iron-sulfur clusters were undoubtedly the first metal-bearing cofactors available to early life, justifiably called the primordial catalysts (1, 92–94).

An earlier assumption that cobalamin was a required cofactor for ancient organisms was recently questioned based on the complexity of its biosynthetic pathway, which requires ~30 enzymes for biosynthesis, some of which are themselves dependent on other complex cofactors (95, 96). The FeS cluster-utilizing radical SAM enzyme family could have fulfilled the functional roles of the modern cobalamin-dependent enzymes, enabling early life to thrive without any dependence on cobalt. The simplicity of the SAM molecule compared to cobalamin, its reliance on FeS clusters that would have been more abundant in the early ocean than dissolved cobalt, and the expanded functional roles of radical SAM proteins suggest these proteins were present much earlier than cobalamin (77, 97). FeS clusters are highly sensitive to dioxygen (98) and were consequently much more difficult to manage after the rise of environmental dioxygen. However, Co(II) was likely similar in concentration to other transition metal ions after the GOE (Fig. 2), and therefore we suggest that life in oxic environments developed the cobalamin cofactor and cobalamin-reliant enzymes to deliver many of the functions of radical SAM enzymes (Fig. 3).

The question of whether early life harnessed Ni(II) requires nuance. Biologically, with the possible exception of methanogenesis (see above), there is no compelling biochemical reason that Ni(II) would be required by primordial life. No Ni(II)-utilizing chemistry appears uniquely to require Ni(II), such as the FeS-only version of hydrogenases (55) and the ability of FeS clusters to fix carbon without Ni(II) (79, 80). An FeS cluster-containing enzyme might easily evolve into a NiFeS enzyme (Fig. 3), as shown by how FeS cluster proteins and synthetic FeS clusters can be reacted with Ni(II) to obtain their NiFeS cluster analogues (99, 100). The accidental addition of Ni into an earlier-evolved nickel-free FeS cluster-containing enzyme would have been analogous to the occurrence of a highly beneficial mutation, resulting in a greatly improved enzyme. Given nickel's potential comparative abundance (Fig. 2), it is possible that ancient life could have used some simple bioligands to access Ni(II) in seawater (Table 1) and/or Ni-utilizing metalloproteins evolved in high-Ni niches. For example, if MCR absolutely requires nickel and cannot be achieved by iron, we must conclude that methanogenesis arose at a time or in a location (e.g., ref. 101) where sufficient nickel ions were available to outcompete iron ions in binding to the initial biological ligand. Prior to earth's widespread oxygenation, these niches or time intervals may have been associated with weathering ultramafic rocks containing high Ni concentrations, or in very shallow ocean waters where ultraviolet photooxidation of Fe(II) may have depleted the surface water of much of its ferrous iron (102). Organisms that could concentrate Ni from the environment (e.g., Table 1) and place this metal into the FeS cofactor could then have outcompeted organisms that could not obtain Ni, thereby replacing earlier versions of the enzymes.

Adoption of Other Metal Ions with Iron's Decline

The accumulation of dioxygen in the atmosphere triggered transformative biological evolution and innovation. To organisms experiencing extreme oxidative stress for the first time, the rise of dioxygen was also a collapse of iron. The oxidation and precipitation of iron minerals in terrestrial and marine

environments suppressed iron availability, and oxidative weathering unleashed sulfide-bound metal ions such as copper and zinc (Fig. 1). As increasingly oxidized environments grew in number and expanded in size, the other metallic trace elements could compete with iron to bind to biomolecules. Evolutionary pressure would have developed for cells to use—and even select for —these other trace elements with increasingly sophisticated acquisition and management strategies. Life also began developing new functions to regulate intracellular concentrations and mechanisms to avoid or mitigate new critical toxicities (6).

The scarcity of iron is central to modern life in our oxygenated world. While iron can be an excellent redox-active metal in oxidizing environments, its post-GOE shortage likely required life to expand to other metal ions to complete various functions (Fig. 3). Copper replaced Fe as the metal cofactor in some electron carriers, and Cu and Mn began to be used along with Fe for oxygen-processing functions such as superoxide dismutase and cytochrome c oxidase, the final enzyme in the aerobic respiratory electron transport chain responsible for passing electrons to dioxygen. The lowering availability of Fe(II) hindered its use as a redox-inactive divalent ion in hydrolase enzymes, but Zn(II) could substitute in and continue enabling the critical functions of this enzyme family. Finally, the rise of dioxygen challenged life to find a way to manage FeS clusters. The post-GOE relative availability of Co(II) provided a solution, sending evolution toward cobalamin-reliant enzymes in oxic environments.

We propose that iron was the sole transition metal needed by primeval life. The widespread decline of iron and expanded availability of other transition metal ions as Earth's environments oxygenated must have forced dramatic adaptation and evolution, which ultimately enabled the development of an array of new biochemical functions and a major increase in biological complexity that led to modern life.

Data, Materials, and Software Availability. No new data were produced in this study. Database files and scripts for metal ion solubility and speciation calculations are available at ref. 103 and the *SI Appendix*.

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