Sulfur Chemistry May Have Paved the Way for Evolution of Antioxidants

Anna Neubeck¹ and Friedemann Freund^{2,3}

Abstract

The first organisms on the young Earth, just 1–1.5 billion years old, were likely chemolithoautotrophic anaerobes, thriving in an anoxic world rich in water, CO₂, and N₂. It is generally assumed that, until the accumulation of O₂ in the atmosphere, life was exempted from the oxidative stress that reactive oxygen species (ROS) impose on hydrocarbon-based life. Therefore, it is perplexing to note that life on the early Earth already carried antioxidants such as superoxide dismutase enzymes, catalase, and peroxiredoxins, the function of which is to counteract all forms of ROS, including H₂O₂. Phylogenetic investigations suggest that the presence of these enzymes in the last universal common ancestor, far predating the great oxygenation event (GOE) sometime between 2.3 and 2.7 billion years ago, is thought to be due to the appearance of oxygen-producing microorganisms and the subsequent need to respond to the appearance of ROS. Since the metabolic enzymes that counteract ROS have been found in all domains of life, they are considered of primitive origin. Two questions arise: (1) Could there be a nonbiological source of ROS that predates the oxygenic microbial activity? (2) Could sulfur, the homologue of oxygen, have played that role? Reactive sulfur species (RSS) may have triggered the evolution of antioxidants such that the ROS antioxidants started out as “antisulfur” enzymes developed to cope with, and take advantage of, various forms of RSS that were abundantly present on the early Earth. Key Words: Reactive sulfur species—Reactive oxygen species—Antioxidants—Great oxygenation event—Evolution. *Astrobiology* 20, 670–675.

1. Introduction

THE FIRST ORGANISMS to appear on the young Earth ~3.5 billion years ago were most likely chemolithoautotrophic anaerobes. It is widely assumed that, until the accumulation of O₂ in the atmosphere around the time of the great oxygenation event (GOE), life was exempted from the oxidative stress that reactive oxygen species (ROS) impose on hydrocarbon-based life. Therefore, it is perplexing to note that all life on the early Earth seems to have already carried antioxidants. Metabolic enzymes such as superoxide dismutase (SOD) enzymes, catalase (CAT), and peroxiredoxins (PRXR) whose only metabolic task is to remove all forms of ROS, including H₂O₂, have been found in all domains of life and are all considered to be of primitive origin (Olson *et al.*, 2017; Slesak *et al.*, 2016; Case, 2017 (and the references therein); Zelko *et al.*, 2002; Lenton, 2003; Zámocký *et al.*, 2012; Inupakutika *et al.*, 2016). Phylogenetic studies suggest that these enzymes were present in the last universal common ancestor (LUCA), far predating the appearance of O₂-producing microorganisms and the great oxygenation event

(GOE) sometime between 2.3 and 2.7 billion years ago. These enzymes are found in all domains of life, even in obligate anaerobes, which suggests that the need for protection against ROS prevails in nonaerobic environments (Runnegar, 1991; Castresana *et al.*, 1994; Castresana and Saraste, 1995; Lenton, 2003). ROS regulatory enzymes have been identified in organisms that originated between 3.5 and 4.1 billion years ago, indicating the antiquity of these enzymes (Inupakutika *et al.*, 2016). Inupakutika *et al.* (2016) showed, through phylogenetic studies, that the ROS metabolizing enzymes CAT, SOD (Fe-SOD), and PRXR could be found in organisms that evolved before GOE and that these enzymes may have coupled. Nonetheless, it is widely believed that early life was forced to develop a robust protection using antioxidant enzymes as soon as oxygenic biological activity produced harmful and damaging ROS. This raises the critical question: Why is it that, to the best of our knowledge, the earliest microorganisms must have already been forced to develop antioxidant enzymes against a reactive chemical species, ROS, that allegedly did not yet exist in their natural environment?

¹Department of Palaeobiology, Uppsala University, Uppsala, Sweden.

²Space Biosciences Research (Code SCR), NASA Ames Research Center, Mountain View, California.

³SETI Institute, Carl Sagan Center, Mountain View, California.

Three scenarios may be considered:

1. ROS were available in early Earth environment but from other potentially nonbiological sources, predating oxygenic photosynthesis.
2. Different reactive species acting like ROS were available, forcing early anaerobic life to cope with them.
3. The antioxidants documented in early anaerobes possessed an affinity toward not only oxygen but also sulfur, which was abundantly available in different oxidation states.

The discovery of antioxidants in all domains of life has led to the suggestion that LUCA was not a strict anaerobe and that oxygenic photosynthesis might have appeared much earlier than previously thought (Slesak *et al.*, 2016). In their study, Slesak *et al.* (2016) suggested that, because of the assumed presence and even abundance of antioxidants in LUCA, early antioxidant systems may have developed as a response to pre-GOE abiotic and/or pre-GOE biological O₂ production. The argument in favor of a biological production of O₂ and ROS stems from the fact that some of the antioxidants themselves produce ROS (A). For example, an SOD-catalyzed reaction is known to defend living cells against the superoxide radical O₂^{•−} through its disproportionation to H₂O₂ and O₂ (McCord and Fridovich, 1969), producing intracellular O₂ and thereby functioning as an ROS producer. There are other antioxidant enzyme reactions also resulting in the production of intracellular O₂ (Case, 2017; Olson *et al.*, 2017). In addition, molecular phylogenetic analyses seem to confirm that Enzymatic Antioxidant Systems are primordial and that modern microorganisms lacking early enzymatic systems have lost them in progeny lineages (Slesak *et al.*, 2016).

A new aspect has been introduced by a recent series of studies, which demonstrate that an enzymatic interchangeability between sulfur and oxygen, indicating (1) that early enzymatic antioxidant systems could also have arisen in sulfur-rich environments and (2) that these systems were in reality not regulators of ROS but regulators of reactive sulfur species (RSS; Olson *et al.*, 2017). Thus, if the overall ROS metabolism was instead based on sulfur (sequence of one-electron oxidation of hydrogen sulfide, B), it could have had an entirely different biochemical impact, operating instead of or in addition to ROS removal through the conversion of O₂^{•−} into water through radical chemistry (sequence of one-electron reduction of oxygen, C) (Olson *et al.*, 2017).

In the article by Olson *et al.* (2017), the function of CAT in the presence of peroxides as well as of persulfides was analyzed and led to the unexpected result that CAT is able to remove both peroxides and persulfides from solution. Another additional observation of interest here is that, under certain conditions, CAT can generate H₂S from certain sulfur-bearing species, thus functioning as a sulfur oxidoreductase. These observations suggest that sulfur, instead of oxygen, might have played the role of the predominant re-

active and potentially toxic species on the early Earth and that antioxidants may have been in fact “antisulfidants” developed to protect cells from RSS with the beneficial side effect that they could also protect against ROSs.

As members of the same main group in the periodic table, oxygen and sulfur have similar chemical properties and their interchangeability suggests an intimate chemical relationship. Remarkably, several of the known antioxidants have a higher affinity for RSS than for ROS (Olson *et al.*, 2017), thus supporting the idea that, indeed, the early enzymatic evolution toward the defense against oxidizing species might have targeted RSS rather than ROS. This would be consistent with the fact that the synthesis of many prebiotic biomolecules requires anoxic conditions (Lazcano and Miller, 1996) and that primitive antioxidants are known to appear in strict anaerobes. The high affinity for H₂S and H₂S₂ in SOD and CAT also indicates an active sulfur metabolism on the early anoxic Earth before GOE.

1.1. Antiquity of sulfur cycling microorganisms

Not only is the geological rock record suggestive of an early sulfur cycling metabolism (see Section 1.4; Johnston, 2011; Paris *et al.*, 2013; Knoll, 2016; Montinaro and Strauss, 2016; Ranjan *et al.*, 2018; Sim *et al.*, 2019) but it also provides information about the phylogenetic diversity of the sulfur redox enzyme DsrAB-type dissimilatory (bi)sulfite reductase, which produces sulfide as an end product (Müller *et al.*, 2015). This group of enzymes has prosthetic siroheme* (*e.g.*, nonprotein helper groups attached to proteins) as an active site for redox reactions of sulfur and nitrogen (Murphy *et al.*, 1974), and it has been phylogenetically determined to be very ancient, present in microorganisms even before the separation between bacteria and archaea. Interestingly, recent evidence also suggests that methane-oxidizing archaea rather than sulfate-reducing bacteria are involved in the intracellular reduction of sulfate to sulfide and that this process also is related to methane oxidation (Milucka *et al.*, 2012). This finding is especially interesting since the methanotrophic pathway has been suggested to be one of the oldest metabolic pathways due to the phylogenetic antiquity of the responsible sulfur-dependent metalloenzymes (Nitschke *et al.*, 2013). Evidently, life took advantage of the abundant availability of sulfur species in the early Earth surface and oceanic environments. Hence, due to the chemical similarities between oxygen and sulfur, when the concentration of atmospheric oxygen increased during GOE, life could adapt relatively easily.

1.2. Molybdenum and oxygenation event

Specific properties of the molybdenum geochemistry have been used for determining Precambrian redox conditions. Enrichment of molybdenum in euxinic paleosediments is commonly used as an indicator for reducing conditions (Erickson and Helz, 2000). Owing to its high sensitivity to oxygen, molybdenum mainly occurs in the form of hexavalent

*In molecular biology, sirohaem synthase (or siroheme synthase) (CysG) is a multifunctional enzyme with *S*-adenosyl-L-methionine (SAM)-dependent bismethyltransferase, dehydrogenase, and ferredoxin activities.

molybdate (MoO_4^{2-}) in natural oxic waters (Vorlicek *et al.*, 2004). The molybdate anion, however, exhibits a low absorptivity on clays and iron oxyhydroxides relative to other complex anions. Thus, lack of ready adsorption did not provide a facile path to enrich molybdenum in sediments on the early Earth. Other mechanisms have been suggested instead such as the biological accumulation of molybdenum in sediments driven by sulfate reducers (Dean and Gardner, 1997), depositional environments under conditions close to anoxic (Shaw *et al.*, 1990), or scavenging of molybdenum by manganese oxyhydroxides (Barling and Anbar, 2004). Great ambiguities still exist on the molybdenum speciation in euxinic environments and have led to detailed investigations of the molybdenum chemistry. Studies on the chemistry of molybdenum, sulfur, and oxygen (Prasilova and Burclova, 1972; Helz *et al.*, 1996) have shown that MoS_3 and MoS_2 are either too soluble or kinetically too unfavorable to be candidates for molybdenum sediment accumulation. On the other hand, MoS_2^{4-} is scavenged effectively by other particles. The conversion from MoO_4^{2-} to MoS_2^{4-} , however, is a slow process that proceeds through formation of other, intermediate, hexavalent thiomolybdates such as $\text{MoO}_3\text{S}^{2-}$, $\text{MoO}_2\text{S}_2^{2-}$, and MoOS_3^{2-} . These intermediate species may have been more abundant than MoO_4^{2-} and thereby served as a major reservoir of molybdenum in paleosediments (Erickson and Helz, 2000). Thus, at the time of early redox reactions, thiomolybdate intermediates may have become dominant, even when thermodynamically disfavored due to the slow conversion of MoO_4^{2-} to MoS_4^{2-} . In permanently sulfidic waters with H_2S concentrations of $\sim 11\ \mu\text{M}$ or higher, a sharp transition occurs from MoO_4^{2-} to MoS_4^{2-} , which, in turn, is susceptible to being scavenged by adsorption on mineral particles. Several studies on sediment traps in lakes have shown a strong increase in the downstream molybdenum flux below the sediment horizon where the transition to higher H_2S concentrations occurs (Balistreri *et al.*, 1994; Colodner *et al.*, 1995). Interestingly, once converted to thiomolybdates, these complex ions have been shown in laboratory experiments to resist oxidation for several weeks under oxic conditions. The presence of molybdenum in pyrites from pre-GOE rock samples can be explained by the same process, where molybdenum adsorption onto pyrites is promoted by the stepwise conversion of MoO_4^{2-} to MoS_4^{2-} through the intermediate species that are comparably more easily adsorbed. Pyrites, in turn, exhibit longtime stability over geological timescales (Vorlicek *et al.*, 2004) and are often the principal sulfide mineral preserved in sedimentary rocks.

1.3. Metallothiols

According to the Irving–Williams series of transition metal stabilities, the solubility and bioavailability of transition metals in aqueous solutions are affected by redox conditions and sulfur contents (Irving and Williams, 1948, 1953; Saito *et al.*, 2003; Case, 2017). In an early Earth ocean in which oxygen was absent or scarce and sulfide more abundant than in later oceans, the solubility of iron, nickel, and manganese was high, whereas zinc and copper were insoluble and thus bioavailable. The availability of zinc and copper ions were low in an early ocean where the sulfide concentration slightly exceeds the concentrations of zinc

and copper. In the same ocean, however, the iron, nickel and manganese ions remained available (Saito *et al.*, 2003). An increase in aqueous sulfides by two orders of magnitude (corresponding to $\sim 1\ \text{mM}$) in the same ocean would have been required to precipitate out iron, nickel, and manganese. Thus, the concentration of bioavailable iron, nickel, and manganese must have been considerably higher in the early euxinic ocean than in later oxygenated oceans. Furthermore, complexation of many metal ions by sulfides begins at much lower concentrations, long before the formation of solid precipitates, leading to an inaccessibility of certain trace metals even at relatively low sulfide concentrations. It has been shown that the bioavailability of metal–sulfide complexes is significantly lower than free ions or other inorganic complexes (Edgcomb *et al.*, 2004), resulting in pronounced differences between the free cations of cobalt, manganese, nickel, and iron and the sulfide-complexed cations of zinc and copper. This means that an euxinic ocean provides an iron-, nickel-, and manganese-rich habitable environment for life to exploit. The high concentration of iron estimated in the Precambrian ocean may have, in the view of some researchers (Canfield, 1998), removed sulfide through pyrite precipitation, resulting in a sulfide-depleted ocean, which would be counter indicative to the scenarios already suggested. This reduction of sulfide will, however, not deplete the total amount of sulfides to concentrations lower than $\sim 15\ \mu\text{M}$, due to the formation of soluble $\text{FeSH}^+(\text{aq})$, which will be the dominant sulfide species in such an environment (Saito *et al.*, 2003). Thus, because of the presence of FeSH^+ , sulfides will always be biologically available.

1.4. Sulfur isotope mass-independent fractionation

If the early oceans were high in enzymatically and biologically available sulfidic iron, nickel, cobalt, and manganese, this should be expressed in the geological rock record by a mass-dependent fractionation (MDF) of sulfur. Indeed, in the early 2000s, Farquhar *et al.* (2000) measured the stable sulfur isotope fractionation signatures in Archean sediments and found a remarkable difference between those MDF values and sulfur isotopic signatures in more recent strata. Before Earth became oxygenated during the GOE, the sulfur isotope record shows a mass-independent fractionation (MIF) of sulfur isotopes, whereas after the GOE, all sulfur isotope signatures are of the mass-dependent type, MDF. The observed MIF has thus been argued to be evidence for a low oxygen early Earth environment (Pavlov and Kasting, 2002; Farquhar *et al.*, 2011; Johnston, 2011) and also to be evidence against the presence of sulfur oxidizing bacteria before the GOE. Low atmospheric oxygen partial pressures lead to low ozone levels in the upper atmosphere, which cause less absorption of the UV light and MIF of SO_2 . To preserve such a MIF signature in the rock record, the partial pressure of oxygen needs to be 10^{-5} or lower, which strongly supports a low oxygen level during the early Archean (Pavlov and Kasting, 2002). However, despite a MIF-dominated Archean sulfur isotope record, there are several examples of preserved evidence of MDF in Paleoarchean sedimentary pyrites, barites, and stromatolites (Philippot *et al.*, 2007; Ueno *et al.*, 2008; Bontognali *et al.*, 2012; Roerdink *et al.*, 2012). Microscale *in situ* analyses of individual fossils allow for independent

(from abiotic signatures) sulfur isotope signatures that are linked only to biology (Bontognali *et al.*, 2012) and can thus reveal if sulfur metabolism was present even in a world dominated by abiotic mass-independent fractionation. Owing to recent technical development, new much more detailed data on sulfur isotope fractionation in the Archean are constantly increasing (Ueno *et al.*, 2008; Bontognali *et al.*, 2012; Paris *et al.*, 2013; Eickmann *et al.*, 2018). As an example, Eickmann *et al.* (2018) presented negative $\delta^{34}\text{S}$ signatures (indicative of microbial sulfate reduction) on microdrilled subsamples of pyrite nodules from the Mesoarchean, suggesting an oxygenated formation environment of the samples, despite the overall reduced atmosphere. Bulk samples of sulfur isotopes have also been measured to show the existence of microbial sulfur fractionation. Ueno *et al.* (2008) suggested that the 3.5 Ga barite and pyrite sulfur isotope patterns, which they obtained, were the result of bacterial sulfate reduction. This result was subsequently supported by Roerdink *et al.* (2012). Other studies also suggested the influence of microbial sulfur fractionation in the Paleoarchean (Wacey *et al.*, 2011). However, due to the lack of largescale records (amount of data as well as spatially distributed) on microbial sulfur data from the Archean, sulfate reduction must have been limited (Strauss, 2003).

Negative $\delta^{34}\text{S}$ isotopic sulfur data are regularly used as an argument on the presence of oxygen (Slesak *et al.*, 2016; Eickmann *et al.*, 2018). Large isotopic fractionations have been thought to be indicative of a high concentration of sulfate to create a large fractionation when reduced to sulfide. However, if Olson *et al.* (2017) were correct in their ideas on RSS metabolism, negative $\delta^{34}\text{S}$ values could also be an indication that the sulfur isotope chemistry is not so dependent on oxygen as previously thought (Crowe *et al.*, 2014). In their article, Crowe *et al.* (2014) described large negative $\delta^{34}\text{S}$ values from a lake that has been considered an analog for the Archean oceans due to its extremely low sulfate concentrations. Both electron donors supply rate and microbial growth rate seem to influence the fractionation of sulfur to a large extent (Sim *et al.*, 2011) so that a lower sulfate reduction rate creates a stronger fractionation. This scarcity of sulfate in the Archean oceans would possibly, therefore, even support a strong fractionation and would explain why negative $\delta^{34}\text{S}$ data can be found in early Earth Archean sediments and in some current anoxic environments. Microbial sulfate reduction seems to require far less sulfate than previously thought and is coupled to strongly negative $\delta^{34}\text{S}$ values found in anoxic environments today or in the Archean (Sim *et al.*, 2011; Crowe *et al.*, 2014). An experiment, wherein $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ (and perhaps also $\delta^{18}\text{O}$) are monitored in batch cultures of Sulfur Reducing Bacteria (SRB) as a function of starvation and electron donor availability (*i.e.*, acetate; van den Brand *et al.*, 2014), would be helpful to understand the metabolic pathways of SRBs more in detail. Other more exotic pathways, such as the sulfur metabolism in methanogens (Liu *et al.*, 2012), could be investigated as well, in both syntrophic cultures (Ozuolmez *et al.*, 2015) and pure cultures. All these culturing experiments would, perhaps, give isotopic signatures of both sulfur and carbon that may be visible also in the geological rock record. Another approach could be to measure the $\delta^{34}\text{S}$ by replicating the experiments by Olson *et al.* (2017) but adding sulfur isotopic measurements to test the fractionation factor of an “antisulfur” metabolism. If the

outcome would show that RSS could be switched with oxygen with a conserved isotopic signature, the negative signatures of $\delta^{34}\text{S}$ found in 3.8 billion year-old sediments could result in an oxygen OR sulfur-dependent metabolism. Another way of testing the idea of “antisulfur” enzymes in the Archean is to experimentally investigate the precipitation differences between RSS and ROS in metal-rich environments (such as an Fe-rich anoxic Archean ocean). Are there differences in precipitation rates, fractionation factors, or temperature?

These insights into the behavior of oxidation-sensitive indicators in the oceans do not rule out, however, that ROS may have existed locally in the rocks and produced constantly at the rock–water interface during water–rock interactions (Freund and Freund, 2015), but also scavenged by oxidation reaction such as ferrous to ferric, Fe^{2+} to Fe^{3+} , as long as the minerals being weathered were rich in Fe^{2+} .

1.5. Sulfur metabolism enables local oxygen resistance

Even when the bulk of the oceanic water exists in an anoxic sulfide-containing environment with high precipitation rates of sulfides, on the microscale, at the rock–water interface, hydrogen peroxides will form abiotically during aqueous weathering through interaction between water with mineral surfaces (Balk *et al.*, 2009). This suggests that, when mineral surfaces reacting with water in an anoxic sulfidic aqueous environment, peroxides will provide oxidizing conditions at the local scale, thereby subjecting microorganisms in contact with mineral surfaces to peroxy hydrolysis and to the effects this has on sulfur compounds and organic matter. If there is a process by which ROS become available during water–rock interactions in natural environments, it would be not surprising that early antioxidant enzymes have evolved a high affinity to both sulfur and oxygen. There are, however, important differences that may have played an essential role in the primitive metabolism. Although peroxide bonds (RO–OR, bond enthalpy of 204 kJ/mol) are unstable and thus reactive, their sulfur counterparts are more stable and, therefore, incorporated into proteins as disulfide bonds (RS–SR, bond enthalpy of 266 kJ/mol) as an essential component in the structural properties in proteins. Biochemically and physiologically, the RSS is comparable to ROS and several regulatory systems have shown to be almost identical when it comes to peroxidation and persulfidation. It has been shown that only ROS in excess are pathophysiologically harmful. Thus, through the similarity of the sulfur and oxygen chemistry and the presence of RSS-removing enzymes, the earliest microorganisms might have already been “trained” for high ROS at the time of GOE.

2. Conclusions

Owing to the chemical similarities, RSS and ROS, respectively, may have been equally challenging, even harmful to early life on Earth. However, because of the absence of free oxygen in the early Archean, the earliest life-forms may have evolved the enzymatic capability to minimize the toxic effects of RSS before facing higher concentrations of ROS. This would explain why antioxidants became available to early life long before the GOE. It would also explain the antiquity of many sulfur-bearing enzymes and sulfur cycling

microorganisms. Experimental evidence also concludes that many antioxidants are more efficient in removing harmful RSS than removing ROS. We, therefore, suggest that, despite—or because of—their intrinsic arsenal of antioxidants, the earliest life-forms could be obligate anaerobes.

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Address correspondence to:
 Anna Neubeck
 Department of Palaeobiology
 Uppsala University
 Geocentrum
 Villavägen 16
 SE-752 36 Uppsala
 Sweden

E-mail: anna.neubeck@geo.uu.se

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Abbreviations Used

CAT = catalase
 GOE = great oxygenation event
 LUCA = last universal common ancestor
 MDF = mass-dependent fractionation
 MIF = mass-independent fractionation
 ROS = reactive oxygen species
 RSS = reactive sulfur species
 SOD = superoxide dismutase