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Chapter 2

The Life Evolution on the Sulfur Cycle: From Ancient Elemental Sulfur Reduction and Sulfide Oxidation to the Contemporary Thiol-Redox Challenges

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76749

Abstract

Organismal evolution led to innovations in metabolic pathways, many of which certainly modified the surface chemistry of the Earth. Volcanic activity introduced inorganic compounds (H_2, CO_2, CH_4, SO_2, and H_2S) driving the metabolism of early organisms of the domains archaea and bacteria. In the absence of light, H_2S and Fe^{2+} would have been the major electron donors and the electron acceptors could be either oxidized species such as the sulfurs, sulfate, and elemental sulfur, or carbon dioxide by the fermentation of acetate (forming methane). Elemental sulfur was produced by the reaction between H_2S and SO_2, while anoxygenic photosynthesis may have provided the sulfate which removed oceanic ferrous iron by its precipitation as sulfide into sediments. Hence, the sulfur cycle participation in life evolution comes from ancient anoxygenic elemental sulfur reduction generating environmental sulfide incorporated as mitochondrial Fe-S for the electron-transport chains. Anoxygenic photosynthesis may have provided the necessary sulfate to promote the evolution of sulfate-reducing bacteria. The evolution of oxygenic photosynthesis provided for diverse metabolic possibilities including non-photosynthetic sulfide oxidation, nitrification, and methanotrophy. An increase in oxygen levels would account for oxidative sulfur cycle, evolution of colorless sulfur bacteria, and emergence of large multicellular animals. Oxygen, initially a waste product of photosynthesis, first reacted with sulfur, iron or methane and latter accumulated in atmosphere resulting in more carbon production. Oxygenic photosynthesis becomes a positive feedback on the oxidation of the Earth-surface environment causing the growth and stabilization of continental platforms and carbon burial with more atmosphere oxidation. An increase in oxygen levels would account for oxidative sulfur cycle, evolution of colorless sulfur bacteria, and emergence of large multicellular animals. Oxygen enabled more efficient energy transformation from dietary food to ATP. However, evolution for mammals living on dry land has been closely linked to the adaptation of changes in O_2 concentration in the environment, which means mitochondrial aerobic respiration. By using ancestral geochemistry of iron-sulfur clusters at the protein complexes I and II, the respiratory chains become

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badly insulated wires in the presence of oxygen (with reduced respiratory complexes) and there is leakage of electrons on to molecular oxygen. The electron leakage results in the formation of superoxide anion (SO) that remains within the mitochondrial matrix. If not promptly detoxified by anti-oxidative defenses, SO and its derived-oxidative species can alter cell signaling or attack cell structures leading to cell apoptosis. Sulfur-containing compounds participate either in oxidative stress generation (at endoplasmatic reticulum) or in (thiol) antioxidant defenses (mainly glutathione), thus functioning as redox sensing for enzyme activity and gene expression. Sulfur compounds that contributed for electron leakage and oxidative stress have counteractions by thiol participation either as antioxidant defensors and/or as redox-modulators or cell functions, influencing life evolution and contemporary diseases.

Keywords: life evolution, evolutive sulfur cycle, evolutive oxygen, oxidative metabolism, thiol-redox

1. Introduction

1.1. The life evolution on the sulfur cycle

Organismal evolution led to innovations in metabolic pathways, many of which certainly modified the surface chemistry of the Earth. Such changes in surface chemistry provided new metabolic opportunities that promoted further evolutionary innovations (**Figure 1**). Life on Earth evolved under anaerobic conditions, with metabolic pathways centered on nitrogen, sulfur and carbon. There are two principal avenues of inquiry relevant to reconstructing the history of the sulfur cycle. One avenue relies on the comparison of molecular sequences derived from biologically essential proteins and genetic materials. Other is the geologic record that can provide direct evidence for the state of chemical oxidation of the Earth-surface, with possible indications of when specific bacterial metabolisms first occurred. However, the most complete understanding of the course of the chemical evolution of the surface environment will fully integrate organismal phylogenies with geochemical and geological evidence for surface change [1].

1.2. Evolutionary phylogenies

A deeper understanding of the evolutionary relationship among organisms is possible from phylogenies derived from comparisons of the small subunit (SSU) of the ribosomal RNA molecule (rRNA) comprising the 16S subunit for prokaryotic organisms and 18S subunit for eukaryotes [2]. Sequence analysis of small subunit of rRNA has revealed that all life can be divided into three principal domains; these are the bacteria, the archaea, and the eucarya [3].

The small subunit of rRNA-based tree of life bears little resemblance to the "classic" tree of life which divided the living world into five kingdoms [4]: Animalia, Plantae, Fungi, Protista, and Monera (prokaryotes). However, the new tree of life emphasizes the genetic diversity of prokaryotes (bacteria and archaea) and shows that the history of life on Earth is largely a history of prokaryotic evolution, not the evolution of macroscopic organisms as previous phylogenetic

3.8-4.0 Ga	Large continental land not yet formed Sub-aqueous and sub-aerial vigorous vulcanic activity generating inorganic compounds: H_2, CO_2, CH_3, SO_2, H_2S Virtually oxygen and far higher levels of CO_2 (acidic oceans) Ecosystem: thermophilic - hyperthermophilic Ergogenesis: anoxygenic phototrophs, fermentative, heterotrophs Metabolism: chemoheteroytophic (absence of light) and chemolithoantotrophic using O_2, NO_3 and metal oxide as electron acceptor Organisms: forms of life metabolizing sulfur compounds: procaryotic of Archea domains and bacteria
3.4 - 3.5 Ga	Anoxygenic photoseynthesis established Ecosystem: sulfur compounds accumulation, e.g. sulfur waste from H_2S elemental reduction and, sulfate waste from sulfite reduction Organism metabolism: a)anoxygenic phototrophic bacteria (oxidize) H_2S to elemental sulfur and sulfate, b)chemolithoautotrophic anoxygenic phototrophic (green sulfur bacteria), c)chemolithoautotrophic oxygenic phototrophic (cyanobacteria, purple bacteria, Gram + bacteria)
2.8 Ga	First oxygenic photosynthesis Ecosystem: increased carbon production and oxidation Metabolism: sunlight electrolyze of water and electron transferred to CO2 to form sugars
2.3 Ga	Ocean sulfate reached 1 mM
1.8 Ga	Sulfate reduction rates exceeded the deliver of iron and thus, sulfite-ferrous precipitation stopped in oceans
0.75 Ga	Ocean sulfate accumulation beyond 1 mM
0.54 Ga	Atmospheric oxygen achieved the present levels Second major burial episodes of organic matter

Figure 1. Timeline of life sulfur and oxygen cycle.

schemes would suggest [1]. Near the root of the tree of life are numerous bacteria metabolizing sulfur species including organisms living from dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, and anoxygenic photosynthesis. These metabolisms are likely very ancient (**Figure 1**).

Anoxygenic photosynthesis may have provided the necessary sulfate to promote the evolution of sulfate-reducing bacteria. Furthermore, the evolution of oxygenic photosynthesis provided for diverse metabolic possibilities including non-photosynthetic sulfide oxidation, nitrification and methanotrophy, Hence, it is likely that, in one way or another, some of the earliest organisms on Earth gained energy from the metabolism of sulfur compounds (**Figure 1**).

1.3. Evolutionary sulfur ecosystems

The geological record begins in the early Archean (3.8–3.9 Ga) (gyga annum = billion years). Large continental land masses had probably not yet formed and the rock record records vigorous volcanic activity in both sub-aerial and sub-aqueous settings [5–7]. A primitive early Earth terrestrial ecosystem was likely thermophilic to hyperthermophilic and housed around active hydrothermal areas with anoxygenic photosynthesis (when light was available) producing

organic matter and oxidized sulfur species (**Figure 1**). In the absence of light (chemoheterotrophic metabolisms), the organic matter production utilized H_2 as the electron donor and oxidized species as the electron acceptors. Hence, the oxidized sulfur species could have been used as electron acceptors in the mineralization of organic matter, completing the carbon cycle. In chemoheterotrophic metabolism, organic compounds were oxidized by the reduction of elemental sulfur and sulfate (forming H_2S) as well as by the carbon dioxide from fermentation of acetate (forming methane) [1].

In the anoxygenic photosynthesis, photo-oxidation of chlorophyll transforms it into an oxidant, which can strip electrons from many sources, passing them ultimately onto CO_2. If H_2S is the electron donor, the waste product is sulfur [1]. Anoxygenic photosynthesis by volcanogenic constituents includes sulfate reduction and elemental sulfur reduction. Elemental sulfur was produced by the reaction between H_2S and SO_2. A possible sulfate source might have been the hydrolysis of volcanogenic SO_2 originating from relatively oxidized magmas [8]. But seems, anoxygenic photosynthesis is the most important source of sulfate (**Figure 1**).

Early Earth had an anoxic atmosphere, with an anoxic ocean containing low concentrations of sulfate. By 3.5 Ga, anoxygenic photosynthesis was established and provided a weak source of sulfate to the global ocean. The origin of that sulfate was attributed to the anoxygenic phototrophic oxidation of primary mantle-derived sulfide to sulfate. However, in 3.4 Ga, there was the accumulation of minimally fractionated evaporitic sulfates in association with magmatically derived sulfides. The stable isotope record of sedimentary sulfides indicates that sulfate first accumulated into the global ocean to concentrations approx. 1 mM at around 2.3 Ga (**Figure 1**). At low concentrations, the rates of delivery of sulfate into sediments may be severely limited by diffusion of sulfate across the sediment-water interface.

Throughout the Archean and early Proterozoic the deep oceans contained appreciable concentrations of dissolved ferrous iron, and banded iron formations were a common form of chemical sediment. When the production rate of sulfide exceeded the delivery flux of iron, dissolved iron was removed from the oceans by reaction with sulfide. As a consequence, the oceans became sulfidic. At this point, sulfide accumulated and precipitated ferrous iron from the solution. It is suggested that banded iron formations (with sulfide) were stopped forming (about 1.8 Ga) when sulfate levels increased and, consequently, sulfate reduction rates rose to the point of exceeding the delivery flux of iron to the oceans. A rise in sulfate reduction rates would be promoted by an increase in the sulfate concentration beyond 1 mM providing a higher flux of sulfate into sediments [1]. It is proposed that the oceans remained sulfide-rich until the Neoproterozoic, where renewed deposition of banded iron formations occurred at around 0.75 Ga (**Figure 1**). The competing theory is that iron was removed from the oceans by oxidation with oxygen [5]. The increase in sulfate levels may have been promoted by a rise in the atmospheric oxygen concentration and an increase in the oxidized sulfur reservoir which is seen as a natural extension of the continued oxidation of the Earth-surface environment [1].

The first evidence for oxygen production by oxygenic photosynthesis is found at around 2.8 Ga (**Figure 1**). Even so, the oxidation of the Earth-surface was quite protracted. As the waste of oxygenic photosynthesis, molecular oxygen initially reacted with sulfur, iron or methane but ultimately accumulated in the atmosphere. Sulfate did not accumulate in the

oceans to concentrations >around 1 mM until about 2.3 Ga, roughly contemporaneous with other indicators of Earth-surface oxidation. In fact, around 2.4 billion years ago, in the Great Oxidation Event, atmospheric oxygen perhaps precipitated the first global ice age—a "snowball earth" [9]. Despite an apparently major accumulation of oxygen into the atmosphere during the early Proterozoic, various lines of biological and geological evidence suggest that oxygen levels did not surpass approx. 10% of present-day levels until much later in Earth history [10].

The evolution of oxygenic photosynthesis provided for dramatically increased rates of carbon production, and a much wider range of ecosystems for both carbon production and carbon oxidation. In oxygenic photosynthesis, electrons are transferred from water—split by chlorophyll photo-oxidized by the sun—via an electron-transport chain ultimately onto CO_2 to form sugars. In principle, by freeing photosynthesis from the availability of reduced chemical substances, the global production of organic carbon could be greatly increased. In this way, the evolution of oxygenic photosynthesis becomes a positive feedback on the oxidation of the Earth-surface environment as the ability to produce more carbon should cause more carbon burial, and this should lead to Earth-surface oxidation. Also of possible significance in promoting Earth-surface oxidation would have been the growth and stabilization of continental platforms where carbon burial could occur [11]. High carbon burial rates increased levels of atmospheric oxygen to >10% present-day levels, promoting the widespread oxidation of marine surface sediments. Present-day levels of atmospheric oxygen may not have been reached until the Neoproterozoic (0.54–1.0 Ga) in association with a second major burial episode of organic matter [12].

1.4. Prokaryotic evolution

Associated with volcanic activity was the introduction of inorganic compounds (H_2, CO_2, CH_4, SO_2, and H_2S) driving the metabolism of early chemolithoautotrophic organisms, including organisms of the sulfur cycle. Prokaryotic organisms of the domains archaea and bacteria were probably the only forms of life because they have the ability to metabolize sulfur compounds. The deepest-branching lineages within the bacteria house hyperthermophilic organisms; these include chemolithoautotrophic group, sulfate reducers genus, and the dominantly fermentative organisms [13].

The deep-branching organisms within the domain bacteria are hyperthermophilic. Branching a little farther up tree from the hyperthermophilic groups are the green non-sulfur bacteria which includes several anoxygenic phototrophs. An evidence for sulfate formation by anoxygenic phototrophic bacteria was provided in 3.4 Ga (**Figure 1**). These phototrophs are the deepest-branching photosynthetic organisms, and oxidize, via a single photosystem, hydrogen sulfide to elemental sulfur, and sulfate. Farther up are found the green sulfur bacteria, and beyond that, the tree indicates a tremendous radiation of bacterial life. Some of the more conspicuous groups include the cyanobacteria (oxygenic phototrophs), the purple bacteria (an enormous variety of heterotrophic, chemolithoautotrophic, and anoxygenic phototrophic bacterial types), and the gram-positive bacteria (including a wide variety of anoxygenic phototrophs, fermentative bacteria, and heterotrophic bacteria, including many thermophilic

organisms). The domain bacteria house most of the prokaryotes with which we are most familiar. These organisms conduct an enormous range of metabolisms including fermentation, acetogenesis, sulfate reduction, elemental sulfur reduction, metal oxide reduction, denitrification, nitrification, aerobic respiration, oxygenic and anoxygenic photosynthesis, and the whole range of chemolithoautotrophic metabolisms using oxygen, nitrate, and possibly metal oxides as electron acceptors [1].

1.5. The role of oxygen

Oxygen was initially released as a by-product of photosynthesis following the emergence of blue-green algae. The evolution of oxygenic photosynthesis produced a dramatic increase in the primary production of organic material and promoted a profound expansion of the ecosystems available to prokaryotic life. Either associated with or following the evolution of oxygenic photosynthesis is the emergence of lineages housing most of the bacteria of which we are familiar, including most of the bacteria of the sulfur cycle. A dramatic evolution in bacterial life might have been made possible by: (1) the appearance of oxygen in sufficient quantities to fuel important metabolic pathways such as, for example, methanotrophy, and (2) an increase in available ecosystem space that global-ranging carbon production and subsequent carbon deposition onto sediments would provide [1]. An increase in oxygen levels would account for: (1) the initiation of large ^{34}S-depletions (>45 permil) in sedimentary sulfides, indicating the operation of the oxidative sulfur cycle; (2) the evolution of colorless sulfur bacteria; and (3) the emergence of large multicellular animals [10].

The evolution of oxygenic photosynthesis, like the evolution of life itself, was a singular event and not driven by any obvious environmental stimuli except for the opening of an enormous range of new environments that could support photosynthesis [1]. As atmospheric concentrations increased, it became possible to support more complex, multicellular life forms, including placental mammals [13]. Overall evolution for mammals living on dry land has been closely linked to adaptation to changes in the O_2 concentration in the environment [14, 15].

1.6. Phosphagenic energy

On Earth 4 billion years ago, there was virtually no oxygen and far higher levels of CO_2 (anything up to a thousand-fold more). When dissolved in water, CO_2 forms carbonic acid that is the difference CO_2 made and acidified the oceans. Back then, the pH of the oceans was likely to have been in the range of 5–6 [16]. Polyphosphates, such as ATP and pyrophosphate, form under acidic conditions at low water activity (in hydrophobic membranes) whereas their hydrolysis is favored under alkaline aqueous conditions [17]. As the alkaline fluids percolated into acidic oceans, through a labyrinth of interconnected micropores lined with hydrophobic iron-sulfur membranes, the vent system would have developed a natural proton gradient. It is therefore plausible that natural proton gradients could have driven the cycling between pyrophosphate and phosphate, or ATP and ADP, in the vent environment [18]. Whatever the mechanism, the first cells could not have left the vents without chemiosmotic coupling—nothing else could have provided the necessary energy. The vents also equipped the first cells with all the necessary tools—proton gradients, electron-conducting iron-sulfur clusters, and

charged membranes. When the first prokaryotic cells did emerge, these were the tools of their trade. With them, they were set for photosynthesis. Thus, as a general rule, it is fair to say that prokaryotes can be classified not by their morphology but by their metabolic capabilities, and the most significant of those was photosynthesis [18]. Photosynthesis reverses respiration. Drawing on water as a fuel, rather than reduced chemicals derived from volcanic and hydrothermal processes, probably increased global biomass 10-fold. Photosynthetic water-splitting transformed the planet. Additionally, photosynthesis in its oxygenic form changed the environmental O_2 concentration [9, 19].

The ancient methanogens (archaea) and acetogens (bacteria) presented probably the most ancient chemolithotrophic pathway in life, the direct reaction of hydrogen with carbon dioxide, known as the acetyl CoA pathway. This pathway provides both the carbon and energy metabolism of life—there is no need for solar power, primordial soup, ATP or any other accouterments [13]. In oxygenic photosynthesis, electrons are transferred from water—split by chlorophyll photo-oxidized by the sun—via an electron-transport chain that is exactly analogous to the respiratory chain, ultimately onto CO_2 to form sugars. The flow of electrons drives the transfer of protons across the thylakoid membranes to generate a proton gradient, which in turn drives ATP synthesis. Therefore, oxygenic photosynthesis is limited only by nutrient and light availability (water is nearly ubiquitous) and not by the availability of electron donors [20]. Oxygenic photosynthesis provided for diverse metabolic possibilities including nitrification, methanotrophy, and non-photosynthetic sulfide oxidation.

There are only six known pathways of carbon assimilation across all life, including the Calvin cycle (used in oxygenic photosynthesis), the reverse Krebs cycle (found in many vent bacteria), and the acetyl CoA pathway. All but the acetyl CoA pathway require an input of energy, in the form of ATP or some equivalent, which is provided by sunlight in photosynthesis and oxygen in the case of chemosynthesis in vents. In the case of the acetyl CoA pathway, 1 ATP must be spent to overcome the kinetic energy "hump"; but instead of reclaiming just one ATP, chemiosmotic coupling makes it possible to gain about 1.5 ATPs per CO_2. Therefore, only the acetyl CoA pathway, the direct reaction of H_2 with CO_2 can provide the energy required for growth in the absence of light or oxygen, and even this pathway can only do so by the way of chemiosmotic coupling. In chemiosmotic, the energy released by an exergonic reaction is used to transfer one or more protons across a membrane. So, as long as the energy released is sufficient to transfer a single proton at least part of the way across the membrane, the reaction can be repeated indefinitely to generate, in the end, a proton gradient. Gradient can be used independently to power ATP synthesis. In all forms of oxidative phosphorylation, the passage of electrons from the donor to the acceptor is coupled to ATP synthesis by the way of an intermediary proton gradient across a membrane—chemiosmotic coupling [13].

1.7. Respiration

Respiration, by necessity, evolved early. Respiration is typically divided into aerobic and anaerobic. Aerobic respiration obviously requires oxygen. Anaerobic respiration is typically taken to mean anaerobic glycolysis, or fermentation. The distinction is between substrate-level phosphorylations. In fermentation, the phosphate groups are transferred directly by

chemistry. In oxidative phosphorylation, electrons are transferred from an electron donor such as glucose (but which could be other organic or inorganic donors such as Fe^{2+}) via a series of redox centers to a terminal acceptor. In aerobic respiration, this acceptor is oxygen. In anaerobic respiration, the electrons donated by anaerobic glycolysis or fermentation (probably hydrogen) is taken by a range of other electron acceptors (initially CO_2), from NO to Fe^{3+} to protons. On the early Earth, H_2S and Fe^{2+} would have been major electron donors and Fe-S clusters have the important intrinsic factor of transferring single electrons. Iron-sulfur clusters (Fe-S) are yet found at respiratory complexes: notably in I and II [13].

1.8. Evolutionary oxidative metabolism

Reactivity allows oxygen to participate in high-energy electron transfers, and hence support the generation of large amounts of adenosine-5-triphosphate (ATP) through oxidative phosphorylation. This is necessary to permit the evolution of complex multicellular organisms once O_2 enables more efficient energy transformation from dietary proteins, carbohydrates, and fats to ATP. ATP molecules provide the chemical energy required to conduct the biochemical reactions essential to cellular life including protein biosynthesis, active transport of molecules across cellular membranes, and muscular contraction [15].

1.9. Oxygen reactivity

Oxygen is hardly toxic if left to itself; but it is readily activated in the presence of the every respiratory chain that is necessary for life. The reactivity of oxygen, of course, is limited by kinetics. The kinetic limitation on the reactivity of oxygen relates to its unusual electron outer orbital structure, giving molecular oxygen two electrons in parallel spin. Most of the O_2 used during the oxidation of dietary organic molecules is converted into water via the combined action of the enzymes of the respiratory chain. Around 1–2% of the O_2 consumed escapes this process and is diverted into highly reactive O_2 free radicals and other reactive O_2 species (ROS) at a rate dependent on the prevailing O_2 tension. The term "reactive oxygen species" is applied to both free radicals and their non-radical intermediates. Free radicals are defined as species containing one or more unpaired electrons, and it is this incomplete electron shell that confers their high reactivity [13]. Free radicals can be generated from many elements, but in biological systems it is those involving oxygen and nitrogen that are the most important [21].

1.10. Superoxide production

Under normal conditions, 2% of oxygen consumed is converted to superoxide (SO) in the mitochondria rather than being reduced to water. Because of its charge, SO is membrane impermeable and so remains within the mitochondrial matrix [20]. Hence, under physiological conditions, the most common oxygen-free radical is the superoxide anion, and mitochondria are considered the principal source. Because the roots of respiratory chains are in geochemistry composition, notably the Fe-S clusters, all these respiratory chains become badly insulated wires in the presence of oxygen. Consequently, the transfer of electrons along the enzymes of the respiratory chain is not totally efficient, and the leakage of electrons on to molecular oxygen, in particular from complexes I and III, results in the formation of SO. ROS

leak has more to do with the speed of electron flow down electron-transport chains than it does with the concentration of oxygen itself. In general, ROS leak is lower in state III respiration (when ATP consumption is fast) than it is in state IV respiration, when electron flow is limited by ADP deficiency [22]. If the respiratory complexes become highly reduced, they become more reactive with oxygen; and the higher membrane potential can drive electrons in reverse back into complex I, again increasing the rate of ROS leak.

The rate of superoxide formation is determined by the number of electrons present on the chain, and so is elevated under conditions of hyperoxia and of raised glucose. Paradoxically, it is also increased under conditions of hypoxia, when the reduced availability of oxygen that acts as the final electron acceptor for complex IV causes electrons to accumulate. Superoxide can also be generated through the leakage of electrons from the shorter electron-transport chain within the ER [23, 24]. About 25% of SO within cells is generated within the ER mostly by the formation of disulfide bonds during protein folding. This can increase in cells with a high secretory output, and also under conditions of ER stress when repeated attempts to refold misfolded proteins may take place. Other sources of superoxide under physiological conditions include the enzymes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cytochrome P450, and other oxidoreductases. Hence, various growth factors, drugs, and toxins cause increased generation of ROS. Additionally, under pathological conditions, the enzyme xanthine dehydrogenase becomes an important contributor. This enzyme degrades purines, xanthine, and hypoxanthine to uric acid and, under normal conditions, uses NAD as the electron recipient. However, under hypoxic conditions, it is proteolytically cleaved to the oxidase form, which donates electrons to molecular oxygen. This enzyme plays a key role in the reperfusion phase of ischemia-reperfusion injury, when its action is augmented by the buildup of hypoxanthine as a result of ATP breakdown during the hypoxic period [25].

Superoxide is detoxified by the superoxide dismutase enzymes, which convert it to hydrogen peroxide. Two isoforms of superoxide dismutase convert SO to hydrogen peroxide, the manganese form that is restricted to the mitochondria and the copper and zinc form that is located in the cytosol [21].

1.11. Oxidant/anti-oxidant balance

Aerobic reactions lead to the accumulation of reactive oxygen species, which can be toxic to the cells. Therefore, oxygen has both positive benefits and potentially damaging side effects for biological systems. In fact, biotic and abiotic stresses can trigger a dramatic increase in the generation of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide in the intracellular environment. Consequently, our body is under constant oxidative attack from reactive oxygen species (ROS). Oxygen reactivity renders it liable to attack any biological molecule, be it a protein, lipid or DNA [21]. Hence, a complex system of antioxidant defenses has evolved that generally holds this attack in balance. In this context, aerobic organisms have developed several non-enzymatic and enzymatic systems to neutralize these compounds. The enzymatic systems include a set of gene products such as superoxide dismutases, catalases, ascorbate peroxidases and glutathione peroxidases (GPx). Enzymatic and non-enzymatic defenses inhibit oxidant attack. The enzymatic defenses all

have a transition metal at their core, capable of taking on different valences as they transfer electrons during the detoxification process [26].

The concept of a pro-oxidant-antioxidant balance is central to an understanding of oxidative stress for several reasons. Firstly, it emphasizes that the disturbance may be caused through changes on either side of the equilibrium (e.g. abnormally high generation of ROS or deficiencies in the antioxidant defenses). Secondly, it highlights the homeostatic concentrations of ROS. The concept of a balance draws attention to the fact that there will be a graded response to oxidative stress. Hence, minor disturbances in the balance are likely to lead to homeostatic adaptations in response to changes in the immediate environment, whereas more major perturbations may lead to irreparable damage and cell death. The boundary between normal physiological changes and pathological insults is therefore inevitably indistinct. The definition of oxidative stress is necessarily broad because the outcome depends in part on the cellular compartment in which the ROS are generated. There are many potential sources of ROS, and the relative contributions of these will depend on the environmental circumstances prevailing. As the reactions of ROS are often diffusion-limited, the effects on cell function depend to a large extent on the biomolecules in the immediate vicinity. Different insults will therefore generate different outcomes [21].

1.12. Thiol redox

The term thiol refers to compounds containing sulfur. Sulfur-containing compounds are found in all body cells and are indispensable for life. Among plasma thiols, total Cys is the most abundant, followed by Hcy and GSH. Sulfur atoms are also important in the iron-containing flavoenzymes, such as, succinate dehydrogenase and NADH dehydrogenase [27]. The thiols are in a dynamic relationship through thiol-dissulfide exchanges and redox reactions. Cys residues are susceptible to a variety of modifications by reactive oxygen and nitrogen oxide species (ROS and RNS). Oxidation by ROS or RNS can result in a disulfide bridge forming between two thiols, either within a protein chain or between protein chains [28]. The introduction of potential disulfide-forming thiol pairs may be facilitated by the fact that both Cys do not need to be introduced into the protein chain simultaneously. Acquisition of structural disulfides in proteins can potentially occur via transition through a redox-active disulfide state. Reaction of protein thiols with low-molecular weight thiols such as glutathione (GSH) can yield mixed disulfides. However, the formation of the disulfide-bonded form will only occur under conditions of oxidative stress. Hence, the incorporation of a single Cys may make the protein immediately responsive to a range of oxidative modifications. Cys can be nitrosated, glutathionylated, and can form covalent bonds with other Cys. RNS such as nitric oxide (•NO) can mediate S-nitrosation to yield an S-nitrosothiol (RSNO). Other RNS, such as peroxynitrite (ONOO—), can also mediate S-nitration to yield S-nitrothiols ($RSNO_2$). Sequential oxidation of Cys thiols yields sulfenic (—SOH), sulfinic ($—SO_2$ H), or sulfonic ($—SO_3$ H) acid derivatives. Introduction of a second Cys at a later stage may then enable disulfide formation subject to further constraints [29].

1.13. Sulfur-containing antioxidants

Some of sulfur-containing antioxidant compounds are cysteine (Cys), methionine (Met), taurine (Tau), glutathione (GSH), lipoic acid, and mercaptopropionyl glycine. Glutathione

(L-gamma-glutamyl-L-cysteinylglycine) is the principal tripeptide thiol involved in the antioxidant cellular defense and a major hydro-soluble component of the cellular antioxidant system [30]. GSH is highly reactive and instills several vital roles within a cell including antioxidation, maintenance of the redox state, modulation of the immune response, and detoxification of xenobiotics. These reactions can be divided into those involved with the sulfhydryl moiety or with the gamma-glutamyl portion of the tripeptide [30]. In the former are included the oxidation-reduction reactions and the nucleophilic reactions in which the reduced sulfhydryl reacts with electrophiles to form a thioester [31].

In its antioxidant performance, the oxidation of the reduced form of glutathione (GSH) to form GSSG is carried out either by direct interaction with free radicals or, more often, when GSH acts as a cofactor for antioxidant enzymes such as GSH peroxidases. The activity of glutathione peroxidase depends on the presence of reduced glutathione (GSH) as a hydrogen donor [27]. Cytosolic GSH peroxidase reacts in peroxisomes with the hydrogen peroxide produced during the aerobic metabolism. In this reaction, GSH is oxidized to GSSG. In order to prevent oxidative damage, the GSSG is reduced to GSH by (riboflavin-dependent) GSSG reductase at the expense of NADPH (generated by pentose-shunt pathway), forming a redox cycle. Glucose-6-phosphate dehydrogenase is the first enzyme of the pentose-shunt pathway and this enzyme is subject to common polymorphisms, and decreased activity may compromise GSH concentrations. Other thiol compounds, such a thioredoxin, are capable of detoxifying hydrogen peroxide, but in turn require converting back to the reduced form by thioredoxin reductase [21, 27].

A function of GSH is the maintenance of the intracellular redox balance and the essential thiol status of proteins. In the reaction, the oxidized protein (protein-SSG) is reduced (protein-SH) and the reduced glutathione (GSH) is oxidized (GSSG). The equilibrium of this reaction depends on the concentrations of GSH and GSSG [25]. In extreme conditions of oxidative stress, the ability of the cell to reduce GSSG to GSH may be less, inducing the accumulation of GSSG within the cytosol. To avoid a shift in the redox equilibrium, the GSSG can be actively transported out of the cell or react with protein sulfhydryl groups and form mixed disulfides [32].

Introduction of a single Cys into a protein may allow reversible GSH conjugation to occur. Glutathione-cysteine adducts may be removed from proteins by glutaredoxin, whereas disulfides may be reduced by thioredoxin. Thiol compounds, such a thioredoxin, are capable of detoxifying hydrogen peroxide, but in turn require converting back to the reduced form by thioredoxin reductase [21]. Storage of Cys is another important function of GSH because Cys is extremely unstable extracellularly and rapidly auto oxidizes to cystine in a process that produces potentially toxic oxygen-free radicals [33]. The gamma-glutamyl cycle allows GSH to be the main source of Cys. In this cycle, GSH is released from the cell and the enzyme gamma-glutamyl transferase (yGT) transfers the y-glutamyl moiety of GSH to an amino acid (the best acceptor being Cys), forming y-glutamyl-amino acid and cysteinylglycine [32]. Cysteinylglycine is broken down by dipeptidase to generate Cys and Gly. Once inside the cell, the majority of Cys is incorporated into GSH, some being incorporated into protein and some degraded into sulfate and Tau. Similarly, the y-glutamyl-amino acid (Gln) can be transported back into the cell and once inside can be converted to Glu and used for GSH synthesis [32, 33].

2. Discussion

2.1. The role of oxygen and oxidative stress in prokaryotes evolution

Oxygen produces only about an order of magnitude more power than fermentation; and the difference between aerobic and true anaerobic respiration is somewhat less than that. While this is substantial, it is orders of magnitude less than the difference made by mitochondria, and probably differences in nutrient availability or concentration gradients outweighed any metabolic advantages of oxygen, at least among bacteria. Oxygen hardly wrought a global revolution in prokaryotic physiology. Even in the presence of oxygen, no prokaryote ever came close to evolving the morphological complexity of eukaryotes. In this context, the evolution of aerobic respiration may have made a difference, but the most immediate impact of the rising tide of oxygen was its juxtaposition with the electron-transport chains of bacteria, all of which transfer single electron [13]. The basic problem, which is central to eukaryotic evolution too, is that the rates of photooxidation and electron transfer, being essentially quantum events, differ from the rates of chemical reduction and carbon assimilation. This means that conditions such as high light intensity (which rapidly photo-oxidizes chlorophyll), low temperatures (electron transfers are barely slowed, but chemical reactions are much slower), and iron deficiency (leading to poor respiratory stoichiometry) all cause high ROS leak. Without compensation, then, ROS leak is largely defined by poor growth: by a low demand for ATP and highly reduced respiratory complexes. There are various ways out of this "high-voltage" situation, from mild uncoupling to complete depolarization of the membrane, or the use of alternative oxidases, which pass electrons directly on to oxygen, without coupling to proton translocation. All of them, in effect, short circuit the membrane potential, enabling faster electron flow, less reduced respiratory complexes and lower ROS leak. If this high ROS leak is not brought under control quickly, the caspase enzymes are activated, significantly by the loss of the respiratory carrier cytochrome c, in plants as well as animals, and the cell is eliminated. Much the same problems affect the respiratory chains of non-photosynthetic aerobic bacteria, among them the free-living ancestors of mitochondria, which likewise are capable of controlled cell death using metacaspase enzymes. The later development of apoptosis in metazoans makes use of enzymes that are bacterial in ancestry, notably the caspases, but also the Bcl-2 family and other mitochondrial apoptotic proteins [34]. Controlled cell death offers the advantage of recycling scarce nutrients, and so can be beneficial to the larger grouping, whether an organism, a colony, or selfish genes [13].

The point is that the evolution of metazoan cell death that require apoptosis was built on a system that evolved in relatively complex clonal bacteria capable of an apoptotic-style of cell death in response to oxidative stress. Consequently, the single greatest danger is the failure to pass electrons on swiftly down respiratory chains, resulting in highly reduced complexes in an aerobic atmosphere. The way in which these factors played out in the respiratory chains of eukaryotes may have been one of the most significant selective forces in eukaryote evolution [13]. However, there must be an adjustable threshold, above which ROS leak stimulates apoptosis and developmental failure, and below which ROS leak is tolerated (hormesis), or might even be beneficial as a redox signal [35]. A variable apoptotic threshold has profound implications

for fertility, fecundity, adaptability, fitness, aging, and age-related disease. Setting the apoptotic threshold high, meaning a high tolerance of ROS leak before apoptosis is triggered, enables high fertility and fecundity. However, the offspring is less fit, and more likely to suffer from mitochondrial diseases. They will have lower aerobic capacity. Worst of all, they will leak ROS from their mitochondria at a faster rate, without triggering apoptosis. The outcome is a shorter lifespan, and a greater tendency to oxidative stress and chronic inflammatory conditions linked with aging, such as diabetes, cardiovascular disease, and cancer. In short, there is a trade-off between fertility, fecundity, and adaptability, on the one hand, and aerobic capacity, lifespan, and susceptibility to age-related disease on the other. The trade-off is mediated by sensitivity to oxidative stress [35]. Thus, oxygen introduces a new penalty for failure, controlled cell death, that later played a central role in the evolution of true multicellular organisms [13].

2.2. Redox signaling

Although ROS first came to the attention of biologists as potentially harmful by-products of aerobic metabolism, it is now recognized that they play important roles as secondary messengers in many intracellular signaling pathways [25]. This is because as hydrogen peroxide is nonpolar, it is able to diffuse through cell and organelle membranes, and hence acts widely as a second messenger in signal transduction pathways [21]. The redox system can modify functions of proteins through regulating their expression, post-translational modifications, and stabilities. Intracellular redox homeostasis regulates the expression of multiple gene-encoded proteins affecting cell death and survival. In response to alterations in oxidative status, the transcription of those genes can be modulated in part through a redox control of transcription factors such as NF-kB, AP-1, Nrf2, and HIF [36]. Upon exposure of cells to oxidative stress, signaling pathways such as protein kinase C, phosphatidylinositol-3 kinase, and MAP kinase, phosphorylate the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2). After phosphorylation, Nrf2 translocates to the nucleus and binds to the antioxidant response element (ARE) within the promoters of genes encoding antioxidant enzymes and detoxifying enzymes. Key Nrf2 target genes include glutathione peroxidases (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD), cytochrome P450, NAD(P)H quinone oxidoreductase, and heme oxygenase (HO) [27].

Thiol-based redox signaling is the collective name for biochemical pathways that regulate cellular processes by post-translational modification of sulfur moieties in cysteine (Cys) and methionine (Met) residues of proteins. A single Cys residue can form a redox-sensitive site on a protein. Thus, a redox-active disulfide may be introduced into a protein structure by stepwise mutation of two residues in the native sequence to Cys. By extension, evolutionary acquisition of structural disulfides in proteins can potentially occur via transition through a redox-active disulfide state. However, oxidation of a cytosolic molecule, promoting formation of the disulfide-bonded form, will only occur under conditions of oxidative stress [29].

When a single Cys is present in a protein, conjugation of the redox buffer glutathione may induce conformational changes, resulting in a simple redox switch that effects a signaling cascade. In its role as a redox buffer, GSH is conjugated to reactive Cys of endogenous proteins, inducing conformational changes in the substrate proteins, and effecting a signaling cascade that evokes biological responses [28].

A single Cys residue can form a potential redox-sensitive site on a protein because a second cysteine can be introduced into the sequence with a disulfide formation and oxidation of the cytosolic molecule. The formation of disulfide bridges between two Cys molecules is important in stabilizing protein conformation; therefore sulfur atoms in Cys are responsible for the major covalent cross-links in protein structures. Disulfide bonds between Cys residues are generally thought to confer extra rigidity and stability to their resident protein, forming a type of proteinaceous spot weld. Conformational changes are generally small, involving protein backbone, and are often accompanied by a local increase in protein disorder [28, 37]. Surface modification of proteins by GSH results in significant disorder of the GSH distal to the covalent bond [37].

GSH plays important roles in nutrient metabolism and regulation of cellular processes, including cell differentiation, proliferation, and apoptosis [38]. DNA-binding activity of transcription factors often involves critical Cys residues, and the maintenance of these residues in a reduced form, at least in the nuclear compartment, is necessary. The reversible thiolation of proteins is known to regulate several metabolic processes including enzyme activity, signal transduction, and gene expression through redox-sensitive nuclear transcription factors such as AP-1, NF-kB, and p53 protein [39]. GSH is involved in a variety of cell functions such as DNA repair, cell cycle, regulation of cell signaling, and transcription factors, GSH therefore can modulate the genes of cell proliferation, differentiation, and apoptosis. The molecular mechanism of how GSH modulates cell proliferation remains largely speculative. A key mechanism for GSH's role in DNA synthesis relates to the maintenance of reduced glutaredoxin or thioredoxin, which is required for the activity of ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis [32].

Due to different roles of ROS in cell signaling and many human pathological processes, imbalance of GSH is observed in a wide range of pathologies including cancer, neurodegenerative disorders, cystic fibrosis, HIV, and aging [30, 40].

2.3. Modular redox switches in life evolution

It is generally believed that before multiple genome sequences were complete, the increased complexity of organisms correlated with the gene number. Hence, after completion of the first genomes, the small differences in gene number between simple unicellular eukaryotes and mammals forced revision of how complexity is encoded. Additional complexity at the organismal level is likely encoded at the molecular level by noncoding DNA [41]. Hence, increased complexity may also be encoded at the protein level. It is recognized that concatenation of existing domains through gene fusion, also known as protein domain mosaicism, encodes new functions in more complex organisms [42]. Studies on the changing amino acid content of proteins show that domains are also not static structures. Additional complexity added to protein domains in the form of redox and other switches likely increases the signaling capabilities of individual domains. In other words, nature is continually tinkering with these independent folding units: a domain from archaea may not have the same sophisticated set of switches as the homologous domain from a mammalian protein. Thus, two modes of acquisition of increased protein complexity have been demonstrated to date: protein domain mosaicism [42] and acquisition of allosteric control sites. Babu et al. [43] showed that Zn finger allosteric control sites are added to protein sequences via retrotransposons. Both are Cys-based sites and are known to be redox regulated.

3. Conclusion

The sulfur cycle participation in life evolution comes from ancient anoxygenic elemental sulfur reduction generating environmental sulfide incorporated as mitochondrial Fe-S for the electron-transport chains. Sulfur compounds that contributed for electron leakage and oxidative stress have counteractions by thiol participation either as antioxidant defensors and/or as redox-modulators or cell functions, influencing life evolution and contemporary diseases.

Acknowledgements

Special thanks to the Brazilian Research Funding CNPq (RCB researcher fellowship) and CAPES (HTK graduate fellowship).

Conflict of interest

The authors declare no conflict of interest.

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