



Oxidative Stress Response in Bacteria: A Review

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Abstract

Oxidative Stress Response (OSR) is a defense mechanism used to maintain redox homeostasis after an increase in levels of Reactive Oxygen Species (ROS). Due to ROS, cell components are vulnerable to damage including the membrane and DNA - which can impact essential functions and lead to cellular death. Without repair, damages caused by ROS have the potential to disrupt cell function in an irreparable manner. Bacterial cells respond to ROS using both endogenous and exogenous pathways depending on their method of metabolism and evolutionary ability. Bacteria have developed regulatory mechanisms to contain damage and are also known to use antioxidants as defense. In this review we will cover the damage induced by ROS to different cellular structures, and mechanisms of OSR used by bacterial cells to promote survival.

Introduction

The term Oxidative Stress (OS) was coined by Helmut Sies *et al.*, in 1985 [42], and is used to describe the stress an organism can experience when there is a disruption in its redox homeostasis due to reactive oxygen species (ROS). ROS are oxygen-containing chemicals that are highly reactive, such as hydrogen peroxide (H₂O₂), ozone (O₃) and their radicals. When they interact with cellular components, those components can become oxidized. Interactions with ROS can result in damaged DNA, RNA, lipid membranes, and proteins which may result in cellular death. In contrast, though counter-intuitive, this stress can also act as a cellular signal to encourage proliferation or survival [35]. OS affects all types of organisms, including bacteria. The presence of ROS and oxidative stress in bacteria is connected to the evolution of microbes in an aerobic environment millions of years ago [25]. Defenses against ROS and OS are complex, including genome regulation to reduce levels of ROS, and cellular damage repair. The mechanisms by which bacteria experience, defend against, and repair oxidative damage provides crucial conceptual insight into an organism and its metabolism. The purpose of this review article is to outline both endogenous and exogenous oxidative stress causes and responses to that stress in bacteria.

Causes of Oxidative Stress

The way different bacteria respond to oxygen is mostly defined by their method of metabolism and reflects how that microbe evolved to respond to oxygen and its potentially harmful byproducts. Oxidative stress is created by the production and accumulation of reactive oxygen species and the production of ROS and can be driven by numerous endogenous and exogenous factors [49]. Bacteria may be exposed to many environmental stressors, which may lead to the production of ROS and subsequent oxidative stress.

The production of endogenous ROS can result from a cell's own metabolic processes. ROS can be produced through the oxidation of respiratory enzymes during cellular respiration [40]. In the bacterium model *Escherichia coli*, ROS were confirmed to be produced as a byproduct of both aerobic and anaerobic cellular respiration with the simultaneous generation of both superoxide and hydrogen peroxide.

This occurs when molecular oxygen collides with redox enzymes and flavoenzymes. NADH dehydrogenase II, involved in bacterial respiratory metabolism, lipoamide dehydrogenase, involved in glycine catabolism, and fumarate reductase, involved in anaerobic bacterial respiration, are each known for the production of ROS due to their particular ability to transfer their electrons to molecular oxygen [29, 30]. While healthy aerobic cells have evolved to efficiently scavenge the produced ROS, obligate anaerobes may experience increased damage from ROS produced if exposed to oxygen.

Exogenous factors also have the potential to produce ROS within the cell. If bacteria are in an environment with a high concentration of oxygen, they will experience OS (discussed in depth in a review by Haugaard [14]). Other environmental factors can include toxic chemicals and substances introduced into the environment inhabited by the bacteria, such as herbicides, industrial additives, and medications. Paraquat, a lethal herbicide, as well as menadione and phenazines (industrial additives), are redox cycling compounds, molecules that can accept electrons as well as donate electrons to oxygen, producing ROS. They are exogenous causes of bacterial oxidative stress due to this ability [28]. Noticing how ROS are generated exogenously is important to understand why prokaryotes face OS. One of the first observations made by scientists when beginning to study the causes of ROS was realizing that radiation usually preceded the formation of ROS. Radiation has been observed to break water down to hydroxyl radicals, which may then externally damage the cell [45, 55].

Membrane damage

Reactive oxygen species can interact with structures found in the bacterial cell membrane. Cellular viability, membrane selective permeability, and proton motive force, rely on an intact membrane structure [26]. ROS-mediated stress can disrupt these by causing intracellular damage such as lipid peroxidation, which can be detrimental to the cell membrane structure [15]. Lipid peroxidation occurs when ROS takes away electrons from the lipids, disrupting associated functions of the membrane.

Lipid peroxidation starts a cascade down the rest of the membrane until the entire membrane is affected. The created lipid radicals react with oxygen to form lipid peroxy radicals. They can react with other lipids to form hydroperoxide which can be broken down into lipid peroxide. These reactions can affect the structure of the lipids by shortening their tails. These chemical mechanisms are covered in detail in the review by Girotti [12]. It is thought that as the number of double bonds in the fatty acid increases, then the sensitivity to oxidation also increases [53].

The alterations of lipids in bacterial membranes cause morphological changes to bacteria. When *Campylobacter jejuni* interacts with ROS, the spiral-shaped bacteria change to a coccoid form. ROS increases the cell membrane permeability, which results in the structural change seen in *C. jejuni* [57]. *E. coli* also experiences morphological changes from the *bacillus* form to a coccoid form [18]. These morphological changes occur when the bacterium tries to heal itself from the damage caused by ROS by removing pieces of the membrane [58]. If this damage to the membrane is not repaired, it can lead to damage of the DNA and eventually the death of the cell [18].

Another damaging effect of oxidative stress is a decrease in proton motive force (PMF) from inhibition of transport across the cell membrane. The reduction of PMF due to oxidative damage of the membrane structure interferes with the ability of obligate aerobes to create ATP [11]. As a result, when their membranes are damaged this can lead to an inhibition of cell growth and eventually cell death [8]. Without a fully functional membrane, obligate aerobes cannot produce enough ATP to carry out necessary cellular functions.

Protein damage

Proteins are also affected by ROS due to their structure. Amino acids can be modified by ROS which can damage proteins. Proteins containing cysteine and methionine are particularly susceptible to being oxidized. Both can become oxidized to form sulfenic acids, while further reduction can become an irreversible modification [17, 56]. These modifications can affect the functionality of the associated protein. For example, when methionine is oxidized, proteins become denatured and the hydrophilic properties of

methionine are lost, which results in structural alterations [2]. It is important to note that although methionine and cysteine are more readily oxidized, other amino acids can also become damaged by ROS. Amino acids such as arginine, lysine, proline, histidine, and threonine can be carbonylated [39, 50]. When amino acids are carbonylated, this is an irreversible sign of aging and oxidative damage as shown in *E. coli* [8]. These damages, unless repaired, ultimately lead to loss of function of proteins and cause protein misfolding.

The degradation of proteins can be repaired by enzymes and chaperone proteins. Heat shock proteins (Hsp), a group of chaperone proteins, can aid in the refolding, repair, and recycling of damaged proteins. A redox regulated chaperone protein, Hsp33, is specific to prokaryotes and is inactive under reduced conditions. This chaperone protein is activated when the environment becomes oxidized. Upon exposure to protein oxidation, Hsp33 also unfolds but instead of losing activity and aggregating, it uses the structural rearrangements to activate its chaperone function [16]. Additional heat shock chaperone proteins that are not redox regulated can also refold proteins that have been affected by oxidative stress, thus helping restore protein function.

DNA damage

Overproduction of ROS can lead to modification of nucleotides or the sugar phosphate backbone of the DNA helix. Damage to the DNA can potentially result in mutations, or changes in the genetic sequence. When ROS interacts with DNA, it will oxidize the structure, generating damage to the DNA in the form of strand breaks and base modifications [10]. Similar to other endogenously caused OS, oxidative DNA damage is an inevitable consequence of aerobic cellular metabolism (discussed in reviews by Storz & Imlay and Sigler *et al.* [43, 46]), and threatens the survival and growth of bacterial cells.

ROS can interact with both purine and pyrimidine bases, and the deoxyribose sugar backbone of the DNA molecule. The likelihood of the oxidation of DNA bases depends on the redox potential of the individual nucleotide. It is shown that purines are more likely to oxidize because of their low redox potential. When there is a single oxygen from ROS reacting with a purine, it results in the addition of a carbonyl

group - this is the most common damage to purines. For example, guanine has a low oxidation potential and that makes it a prime target for ROS, resulting in the formation of 8-oxo-7, 8-dihydroguanine (8-oxoG) [22]. After hydroxyl radicals react with DNA, the lesions are going to result in a damaged site that, when replicated, causes mutations. The oxidation product of guanine, 8-oxo-G, can base pair with either adenine or cytosine. The base pairing of 8-oxo-G with adenine in bacteria is seen to cause G:C to T:A base transversion mutations [31, 32]. If these transversion mutations happen in sites responsible for protein function, the mutations may decrease the bacterial cell's survival.

Base excision repair (BER) is the most common DNA repair mechanism to address oxidative damage. Glycosylases are a ubiquitous family of enzymes that catalyze the removal of damaged bases from the DNA strand in the BER pathway. DNA repair mechanisms however, often overlap. Oxidized bases can be excised through both the BER pathway, which removes a single lesion using glycosylase, and the nucleotide excision repair (NER) pathway, which removes a lesion-containing segment of the DNA strand [7]. The state of the bacteria, however, may influence the use of these repair pathways. For example, in starving *P. putida*, 8-oxo-G repair is limited, increasing mutations for the purpose of adaptation [37].

Regulatory modifications

Oxidative stress influences how much mRNA from genes not required for stress response is transcribed. A study by Muthukrishnan *et al.* performed in *E. coli* cells noted a decrease in the transcription rates specifically for genes not related to stress when the cells were experiencing OS. The researchers noted that under oxidative stress, there was a 76% decrease in the cells ability to transcribe compared to cells under normal conditions. This is likely caused by a decrease in the number of available inducers, regulators, and transcriptional components, extending the time frame in which it takes transcription to occur and decreasing growth rate [33, 60].

Translational control under oxidative stress conditions is focused on producing proteins necessary to negate OS [3, 51]. Oxidative stress negatively impacts the ability of *E. coli* cells to translate proteins in a timely manner.

As concentrations of H₂O₂ increase, the rate of ribosome translational elongation decreases and the time it takes for the translation elongation rate to recover increases.

The cause of this reduced translational elongation rate is the downregulation of tRNAs by degradation within *E. coli* under oxidative stress [60, 61]. Demonstrated by Zhong *et al.*, translation elongation rates under continual experimental OS conditions recovered by an increase in tRNA species 75 minutes after the initial drop [60].

In *E. coli*, small RNAs (sRNAs) are shown to help cells adjust to environmental changes by controlling expression of key proteins, causing a competition for binding which can result in repressed translation. Genome regulation under oxidative stress is also affected by the number of functional and available small RNAs (as discussed in a review by Van Assche *et al.* [51]). For example, in *Salmonella enterica*, small RNAs RyhB-1 and RyhB-2 participate in the oxidative stress response and deletion of these sRNA lead to an increase of ROS in the cell. These sRNAs are upregulated by OxyR, an important regulon later discussed, a result of OxyR interacting with the sRNA promoters [3]. Small RNAs help the cell adjust in response to oxidative stress.

Another example of sRNA involved in managing oxidative stress is found in the bacterium *Deinococcus radiodurans* R1, where a specific sRNA, OsiA, is required for helping the bacterium handle varying levels of oxidative stress. When the sRNA is knocked out, the mutant bacteria are more sensitive to H₂O₂ and produce less catalase, an enzyme that breaks down H₂O₂. Chen *et al.* show that in the OsiA mutant, the mRNA of a catalase gene, *katA*, has a decreased half-life. The regulation of *katA* by OsiA helps *D. radiodurans* R1 to cope with oxidative stress, induced by H₂O₂ [4].

Removal of ROS

In order to neutralize ROS, bacteria are equipped with antioxidant molecules (AOX) [1]. AOX are responsible for protecting bacteria from and fighting against ROS and include enzymatic and non-enzymatic antioxidant defenses. Enzymatic defenses directly target ROS molecules, inactivating them and converting them into molecules that are significantly less reactive [44]. Non-enzymatic defenses such as vitamins C and E and thiols are molecules

that naturally behave as reducing agents. Non-enzymatic antioxidants become oxidized in place of sensitive cellular components [38]. (Discussed in reviews by J.G. Scandalios and Staerck *et al.* [38, 44].) Bacteria use both enzymatic and non-enzymatic methods jointly to address ROS and OS [1].

Enzymatic inactivation of ROS includes enzymes such as dismutases, catalases, peroxidases, and reductases, commonly found in aerotolerant bacteria [44]. These enzymes, such as superoxide dismutase (SOD), an enzymatic inactivator of superoxide, help to prevent varying types of damage from OS. Loss of these enzymes have been shown to coincide with increased oxidative damage [19, 47]. One example function of SOD is in the periplasm of *E. coli*. SodC is thought to be a dismutase that detoxifies the superoxide anions that are released from the oxidative phosphorylation process [21]. This process allows for ROS damage to be prevented in the membrane. Another enzyme involved in ROS inactivation is catalase which deactivates hydrogen peroxide into oxygen and water. Catalase has been shown to increase survivability of bacteria in the presence of hydrogen peroxide [36]. These enzymatic defenses continue to evolve to help bacteria survive in the presence of ROS.

Enzymatic antioxidants are often regulated by systems called regulons. Regulons are groups of bacterial genes that are regulated together to control specific responses. A prominently known regulon, seen in many Gram-negative and some Gram-positive bacteria is the OxyR regulon. An influx of hydrogen peroxide (H₂O₂) in the cell will initiate a response from the OxyR transcription factor, causing it to convert to its oxidized form. Once oxidized, the transcription factor will positively regulate the genes associated with the OxyR regulon [54]. The RpoS and PerR regulons also respond to H₂O₂. RpoS behaves as a sigma factor, recruiting RNA polymerase, while PerR is similar to OxyR and is more often found in Gram-positive cells [52, 9]. While OxyR, RpoS, and PerR regulons tend to respond to H₂O₂, the SoxRS regulon functions to counteract an increase in superoxide radical anions. The OhrR regulon is another antioxidant regulon which functions to identify and destroy organic peroxides during oxidative stress [9]. Although every antioxidant regulon is not present in all types of bacteria, more than one regulon can contribute to bacterial stress responses [9, 52].

Non-enzymatic antioxidants can be acquired from the environment, synthesized biologically, or both. Vitamin C, or ascorbic acid, is a water soluble, non-enzymatic scavenger of free radicals. Ascorbic acid is taken from the environment, though some bacterium, such as *Streptomyces antibioticus* and *Acetobacter suboxydans* are able to produce it [59, 48]. Additionally, vitamin C is capable of assisting in the regeneration of oxidized vitamin E, another important antioxidant [34]. Vitamin E or alpha tocopherol is lipid-soluble and resides in the hydrophobic region of the cell membrane; it works to defend the membrane from oxidative stress injuries. Vitamin E acts to reduce lipid peroxy radicals and will eliminate the chances of lipid peroxidation of the cell [13, 20]. Non-enzymatic defenses can also work jointly with enzymes. Glutathione (GSH) is a thiol that can detoxify hydrogen peroxide and lipid peroxides in conjunction with glutathione peroxidase (GSH-Px) and will reduce hydrogen peroxide into water and oxygen by donating an electron to hydrogen peroxide. This antioxidant will also protect the cell from lipid peroxidation and convert vitamins C and E back into their active forms [1, 6, 27].

Thiols, such as glutathione, thioredoxin, and glutaredoxin, become oxidized to decrease OS within the cell. Gram-negative bacteria both synthesize and import active thiols like glutathione from the environment using dedicated transporter systems. In contrast, most Gram-positive bacteria do not synthesize glutathione, but can import it [5]. Once cellular thiols are inactivated through oxidation, they must be returned to their active, reduced form. This maintenance is performed by enzymes such as glutathione reductase, thioredoxin reductase, and glutaredoxin [5]. Glutathione reductase helps to keep glutathione in its reduced form to respond to an increase of ROS when the cell is under oxidative stress [5]. Thioredoxin is reduced by thioredoxin reductase (discussed in this review by Lu & Holmgren [23]). The importance of thioredoxin reductase can be noted in a study by Serrano *et al.* of *Lactobacillus plantarum* where they found that overexpression of *trxB1*, a thioredoxin reductase, results in a high resistance to ROS [41]. The thioredoxin system also plays a role in DNA repair and is highly conserved among various bacteria including, *E. coli*, *Helicobacter pylori*, *Bacillus subtilis*, and *Mycobacterium tuberculosis* [23].

Glutaredoxin is important in that it will deactivate OxyR when H₂O₂ levels are reduced to a normal range [54].

Thiol maintenance is vital for the survival of bacteria in oxidative stress environments.

Conclusion

While oxidative stress can be an important signaling strategy for bacteria, it can also be a dangerous source of cellular damage [35]. ROS of both endogenous and exogenous creation threaten to oxidize key components of the cell [49]. This review has discussed the range of damage that can be caused. ROS can oxidize proteins resulting in both reversible and irreversible damage, lipid peroxidation can threaten the integrity of the cell membrane, and DNA damage can induce mutations within the genome [17, 56, 15, 10]. Fortunately, microbial evolution has provided bacteria with mechanisms to counteract OS and its damage.

Although more abundant in aerobic bacteria, all bacteria have some mechanisms for addressing ROS. Regulatory modifications can be made to both transcription and translation to modulate the use of resources and the production of antioxidants [3, 33, 51, 60]. ROS can then be neutralized using enzymatic and non-enzymatic mechanisms and the damage repaired [38, 44]. Bacterial genomes have entire regulons dedicated to these responses. Antioxidants and their maintenance enzymes are closely regulated to both evaluate the oxidative state of the cell and ensure bacterial survival. While bacteria have the capability to respond to oxidative damage, the process is not infallible if the stress outweighs the capacity of the cell to respond.

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