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Review

Reducing systems protecting the bacterial cell envelope from oxidative damage

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ABSTRACT

Exposure of cells to elevated levels of reactive oxygen species (ROS) damages DNA, membrane lipids and proteins, which can potentially lead to cell death. In proteins, the sulfur-containing residues cysteine and methionine are particularly sensitive to oxidation, forming sulfenic acids and methionine sulfoxides, respectively. The presence of protection mechanisms to scavenge ROS and repair damaged cellular components is therefore essential for cell survival. The bacterial cell envelope, which constitutes the first protection barrier from the extracellular environment, is particularly exposed to the oxidizing molecules generated by the host cells to kill invading microorganisms. Therefore, the presence of oxidative stress defense mechanisms in that compartment is crucial for cell survival. Here, we review recent findings that led to the identification of several reducing pathways protecting the cell envelope from oxidative damage. We focus in particular on the mechanisms that repair envelope proteins with oxidized cysteine and methionine residues and we discuss the major questions that remain to be solved.

1. Introduction

Inadvertent one-electron transfer reactions between intracellular molecular oxygen and redox enzymes such as those of the respiratory chain generate partially reduced, reactive oxygen species (ROS). These include the superoxide anion ($O_2^{\cdot-}$, +1 e^-), hydrogen peroxide (H_2O_2, +2 e^-), and the hydroxyl radical ($HO^{\cdot}$, +3 e^-) [1,2]. In addition to this production as by-products of aerobic metabolism, ROS can also be actively generated by enzymes such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [3,4], cytochrome P450 [4] or Ero1, a protein involved in oxidative protein folding in the endoplasmic reticulum [5]. In the case of bacteria, these microorganisms also encounter high levels of ROS generated, for instance, in metal-rich environments or released by competing microbes and host cells [2].

Elevated levels of ROS can damage DNA, proteins and membrane lipids, which can potentially lead to cell death. Therefore, most living organisms contain enzymatic systems that help them to cope with oxidative stress. Proteins like catalases, peroxiredoxins and superoxide dismutases (SODs) are on the front lines and directly react with harmful ROS to convert them to innocuous products: catalases and peroxiredoxins reduce peroxides while SODs catalyze the dismutation of the superoxide anion. In addition, oxidoreductases such as thioredoxins and glutaredoxins not only supply certain of these ROS scavengers with the reducing equivalents they need to fight the redox battle but are also actively involved in the repair of oxidatively damaged proteins. The bacterial defense mechanisms against oxidative stress have been extensively studied in the cytoplasm. In contrast, how the bacterial cell envelope is protected from oxidative damage is much less understood. In this review, we summarize the recent findings that led

Abbreviations: BCP, bacterioferritin-comigratory protein; e^-, electron; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; IM, inner membrane; LPS, lipopolysaccharide; M, membrane; Met, methionine; Met-O, methionine sulfoxide; Met-O_2, methionine sulfone; Msr, methionine sulfoxide reductase; NADPH, nicotinamide adenine dinucleotide phosphate; O_2, molecular oxygen; O_2^-, superoxide anion; OM, outer membrane; PG, peptidoglycan; Prx, peroxiredoxin; ROS, reactive oxygen species; RNS, reactive nitrogen species; RCS, reactive chlorine species; SOD, superoxide dismutase; SOH, sulfenic acid; SO_2H, sulfinic acid; SO_3H, sulfonic acid; SRP, signal recognition particle; Tpx, thiol peroxidase; TR, thioredoxin reductase; Trx, thioredoxin

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to the identification of a multi-layered cellular network scavenging ROS and rescuing proteins from oxidation in bacterial extracytoplasmic compartments. Although we mostly focus here on the Gram-negative bacterium *Escherichia coli*, we also discuss recent data on the protection of proteins targeted to the envelope of other microorganisms.

2. The periplasm is an oxidizing compartment where disulfide bond formation occurs

The envelope of *E. coli* and of other Gram-negative bacteria is a structural and permeability barrier, which is essential for cell shape and growth. It is composed of two membranes: the inner membrane (IM), which is in direct contact with the cytoplasm, and the outer membrane (OM), which constitutes the interface between the cell and the external environment [6]. The IM and the OM differ in terms of structure and composition [6]. While the IM is a classical phospholipid bilayer, the OM is asymmetric and composed of phospholipids and lipopolysaccharides (LPS) in the inner and outer leaflet, respectively [6]. The IM and the OM are separated by the periplasm, a viscous compartment that contains a thin layer of peptidoglycan and represents 10–20% of the total cell volume [7,8]. In *E. coli*, about 400 proteins are targeted to the periplasm [9], where they perform a variety of important physiological functions, such as assisting the folding of nascent proteins, mediating the uptake and transport of nutrients or detoxifying toxic compounds.

With a redox potential higher than that of the cytoplasm (−165 mV *vs* −260/−280 mV in *E. coli*, respectively) [10–13], the periplasmic space is considered as an oxidizing compartment. Consistently, the majority of cysteine residues present in periplasmic proteins are oxidized to disulfides. These disulfides, which are important for protein stability, are introduced in periplasmic proteins by the soluble oxidoreductase DsbA (Fig. 1), a thioredoxin-fold protein [14] with a CXXC catalytic site [15]. The cysteine residues of this conserved motif form a very unstable disulfide, which is transferred to newly synthesized proteins as they enter the periplasm, releasing DsbA in the reduced state [16,17]. DsbA is then recycled back to the oxidized state by the IM protein DsbB (Fig. 1), which generates disulfide bonds *de novo* from quinone reduction [18–20]. DsbA preferentially introduces disulfides into proteins entering the periplasm by oxidizing cysteine residues that are consecutive in the protein sequence [21]. Therefore, when proteins require disulfides to be formed between non-consecutive cysteines, DsbA often introduces incorrect disulfides. These non-native disulfides are corrected by DsbC (Fig. 1), a periplasmic V-shaped homodimeric protein [22–24]. Each subunit of DsbC contains a catalytic CXXC motif, located within a thioredoxin-fold. In contrast to DsbA, the catalytic cysteines of DsbC are kept reduced in the periplasm, allowing this protein to function as an isomerase or a reductase [23]. While dozens of proteins have been identified as DsbA substrates [9,25–28], only a handful of proteins with multiple cysteine residues depend on DsbC for folding. They include the periplasmic enzymes MepA, AppA, RNase I and End1 [25,28,29] as well as the OM-localized ß-barrel protein LptD [30] and the stress sensor lipoprotein RcsF [31,32]. The protein that maintains the CXXC motif of DsbC in the reduced state is DsbD (Fig. 1), an IM protein that transfers reducing power across the membrane by passing electrons received from cytoplasmic thioredoxin (Trx) to a variety of reducing pathways that function in the cell envelope [23,33–36]. Thioredoxin is a ubiquitous oxidoreductase that is kept reduced by thioredoxin reductase (TR) at the expense of NADPH (Fig. 1) [33–35].

3. ROS scavengers in the bacterial periplasm

Scavenging enzymes constitute the first line of defense against ROS. The spontaneous dismutation of O_2^- to O_2 and H_2O_2 not being sufficient to maintain low intracellular concentrations, Gram-negative bacteria, like most living organisms, commonly synthesize SODs to catalyze this reaction. As superoxide cannot easily cross biological membranes [37,38], it is not surprising that SODs are commonly targeted to the different compartments of the cell that are exposed to superoxide anions to reduce them in situ. In *E. coli* for instance, SodA and SodB function in the cytoplasm [39,40], while SodC, whose folding and activity require the formation of a disulfide bond [41,42], is targeted to the periplasm (Fig. 2) [43]. The physiological importance of SodC, a protein synthesized by cells in stationary phase [43], remains unclear, even though the enzyme most likely serves to detoxify the superoxide anions released in the periplasm by the respiratory complexes (Fig. 2) [44]. In pathogenic bacteria, periplasmic SODs have been implicated in virulence, probably by scavenging the superoxide anions released by the host macrophages during the oxidative burst [45].

In most organisms, H_2O_2 is reduced by peroxidases and catalases [2]. In *E. coli*, the primary scavenger is the cytoplasmic peroxiredoxin (Prx) AhpCF (Fig. 2), a two-component thiol-based peroxidase that transfers electrons from NADH to H_2O_2, thereby reducing it to water [46]. AhpCF is very efficient in reducing H_2O_2 generated at physiological concentration (μM) [46]. At least two additional Prxs, the thiol peroxidase (Tpx) and the bacterioferritin-comigratory protein (BCP) (Fig. 2), are active in the *E. coli* cytoplasm: Tpx is involved in the reduction of bulkier hydroperoxides [47], while BCP is able to reduce a broader range of substrates, but with lowered catalytic efficiencies [48]. In addition to Prxs, *E. coli* also encodes two cytoplasmic catalases, KatG and KatE (Fig. 2), which mostly serve to reduce peroxides when they accumulate at higher concentration (mM) [46,49,50]. KatG, which is only weakly expressed in cells growing in exponential phase, is induced under oxidative stress conditions [51], while KatE is strongly expressed in stationary-phase cells [52].

While peroxide scavengers are commonly found in the bacterial cytoplasm, they seem to be absent from the cell envelope (*E. coli* Tpx, which was initially described as a periplasmic Prx [53], is actually expressed in the cytoplasm [54]). This probably correlates with the fact that H_2O_2 is able to diffuse through biological membranes and suggests that the detoxification systems expressed in the cytoplasm are enough to protect the cell from peroxide-induced damages. However, a recent search for the substrates of the IM protein ScsB, a DsbD homolog present in the alpha-proteobacterium *Caulobacter crescentus*, led to the identification of the first peroxide reduction pathway active in the bacterial periplasm [55]. In this bacterium, ScsB was shown to deliver electrons to TlpA, a thioredoxin-like protein present in the periplasm, which in turn reduces a periplasmic Prx, PprX (Fig. 2) [55]. Characterization of PprX revealed that this enzyme is active against H_2O_2 and cumene hydroperoxide. Although the physiological importance of PprX in *C. crescentus* remains to be determined, this discovery indicates that, at least for certain bacteria, it is important to directly scavenge peroxides in the cell envelope before they reach the cytoplasm.

4. Repair of oxidatively damaged envelope proteins

Within proteins, the sulfur-containing amino acids cysteine and methionine are particularly vulnerable to oxidation by ROS. The first oxidation product of cysteine residues exposed to ROS is the sulfenic acid derivative (-SOH). Sulfenic acids are highly reactive

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