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Bacterial gasotransmitters: an innate defense against antibiotics

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In recent decades, there has been growing interest in the field of gasotransmitters, endogenous gaseous signaling molecules (NO, H_2S, and CO), as regulators of a multitude of biochemical pathways and physiological processes. Most of the concerted effort has been on eukaryotic gasotransmitters until the subsequent discovery of bacterial counterparts. While the fundamental aspects of bacterial gasotransmitters remain undefined and necessitate further research, we will discuss a known specific role they play in defense against antibiotics. Considering the current dilemma of multidrug-resistant bacteria we consider it particularly prudent to exploring novel targets and approaches, of which the bacterial gasotransmitters, nitric oxide and hydrogen sulfide represent.

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Introduction

The burgeoning rise in multidrug-resistant bacteria incidences has garnered keen attention recently. The World Health Organization (WHO) declared antibiotic resistance a global concern, one that extends beyond the obvious public health security to economic and societal welfare [1]. In its simplest terms, the fight against antibiotic resistance is essentially an armaments race between bacterial acquired resistance and our ability to continually produce new generations of antibiotics in response. Antimicrobial resistance is not a new concern; however, the precipitate rise in these superbugs concurrent with the decline in developing new antibiotics has created an imparity. Modification of existing classes of antibiotics has only been somewhat successful, if slow, and does not directly consider the eventual rise in new resistance. In the last decade, only two new classes of antibiotics were developed successfully for clinical applications [2]. Furthermore, the problem is compounded by the fact that the latest versions of the same classes of antibiotics will inevitably usher in a population of microbes that become impervious to the new drugs. Therefore, interest has turned to discovering different targets and approaches. Here, we will focus on novel potential therapeutic targets: endogenous bacterial hydrogen sulfide (H_2S) and nitric oxide (NO), gasotransmitters as a defense mechanism against antibiotics.

Bacterial gasotransmitters

Rui Wang first coined the term gasotransmitter in 2002. As defined, gasotransmitters denoted a family of endogenous gaseous signaling molecules of which comprised nitric oxide (NO), carbon monoxide (CO), and a third proposed member at the time, hydrogen sulfide (H_2S) [3]. As such, principal criteria for inclusion as a gasotransmitter in bacteria are as follows: small gaseous molecules that are freely diffusible and permeable to membranes that are generated either endogenously or encountered endogenously to include intercellular communication, functions at physiologically relevant concentrations and possess specific cellular targets. Gasotransmitters are best studied in eukaryotes and have well defined roles in mediating diverse physiological processes across multiple systems [4]. Comparatively, establishing functional attributes for these gaseous signaling molecules in bacteria is still in its infancy.

Nitric oxide is enzymatically generated by nitric oxide synthase (NOS) through the conversion of L-arginine to L-citrulline [5] and also by various nitrite reductases (reviewed in [6]). Adak *et al.* first established the production of NO by bacterial NOS (bNOS) *in vitro* [7]. Gusarov and Nudler demonstrated, *in vivo*, that bNOS does in fact generate NO endogenously under normal growth conditions [8]. Bioinformatics analysis predicted a small subset of gram-positive species contain putative bNOS orthologs [9]. In contrast, the majority of sequenced bacterial genomes possess putative orthologs of at least one of the three eukaryotic major H_2S generating enzymes: cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST) [10]. CBS and CSE are pyridoxal 5′-phosphate (PLP) dependent enzymes that form part of the trans-sulfuration pathway. Both can utilize L-cysteine and homocysteine as substrates to catalyze a series of catabolic reactions ending with H_2S as one of the products. 3MST is a PLP independent enzyme

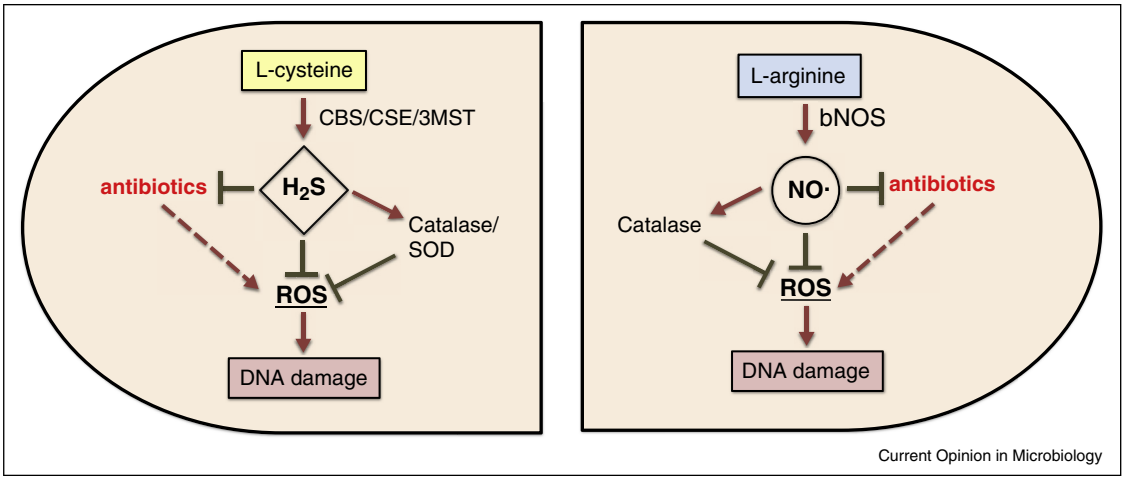
in the cysteine oxidation pathway that involves the intermediate synthesis of 3-mercaptopyruvate by cysteine aminotransferase [11]. Other types of cysteine desulfhydrases also contribute to bacterial H_2S production [12,13].

Nitric oxide is protective against oxidative stress and antibiotics

In eukaryotes, one of the isoforms of nitric oxide synthase is inducible NOS (iNOS). It is generated by macrophages and other innate immune cells and controls intracellular bacterial infections in a multitude of ways by inducing nitrosative and oxidative stress [14]. How then does bNOS produced NO paradoxically protect the microbes? NO's effects are leveraged as a function of time, concentration, targets and compartmentalization. As elucidated in the case of *Bacillus anthracis*, bNOS is required for survival in macrophages during the initial hours post infection and in a murine model of infection [15]. The bacterial derived NO dominates at the outset, to protect the germinating cells from oxidative stress imposed by macrophages and to establish infection. iNOS is triggered by the host much later [15]. In addition, *B. anthracis* generated NO can be a virulence factor by S-nitrosylating host proteins leading to macrophage death [16]. The elimination of endogenous NO in methicillin-resistant *Staphylococcus aureus* (MRSA) sensitized the cells to oxidative stress killing, and vancomycin and daptomycin. In an *in vivo* murine model of *S. aureus* skin infection, bNOS promoted larger abscess and increased survival [17]. Addition of NO to *Bacillus subtilis* cells before exposure to a lethal dose of H_2O_2 significantly and rapidly increased resistance. The same protective effect was not seen with pretreatment with low dosage H_2O_2, or if NO was added concurrently or after H_2O_2. Addition of oxidized NO or nitrite did not increase survival, suggesting that the process is highly specific to the actions mediated by NO. This effect was partly determined to be catalase (KatA) dependent, the major antioxidant enzyme in *B. subtilis*. NO increased the cells' capability to degrade H_2O_2 [8]. This augmented scavenging activity was not seen in Δ*katA* cells, nor did NO increase resistance against H_2O_2 to the same extent. What is the exact mechanism by which NO targets KatA? Free cysteine can partly suppress KatA activity by way of a KatA–Cys complex. The authors proposed that NO can derepress KatA *via* S-nitrosylation, which disrupts the complex. Moreover, NO also prevents cystine reduction to cysteine [8], which drives the damaging Fenton reaction by effectively re-reducing Fe [18]. Thus, bNOS in *B. subtilis* can alleviate oxidative stress in two ways: boosting KatA antioxidant activity and interrupting Fenton reaction by depleting cells of free Fe^{2+} (Figure 1).

Gusarov *et al.* investigated the role of bNOS upon exposing cells to a wide spectrum of antimicrobials. They discovered that bNOS consistently provided protection against different classes of antibiotics. First, NO can interfere with the efficacy of some antimicrobials through direct modification [19]. Second, bNOS helped alleviate

Figure 1



Bacterial gasotransmitters protect the cell from oxidative stress and antibiotics. Enzymatic production of H_2S and NO from cysteine and arginine, respectively, is markedly enhanced in response to oxidative stress and antibiotics in bacteria, including *E. coli*, *S. aureus*, and *Bacilli* [9,10] H_2S protects various bacterial species from both oxidative stress and antibiotics exposure [10]. The anti-ROS mechanism include: (i) depletion of free cysteine (a substrate for H_2S-producing enzymes) that fuels the Fenton reaction, (ii) direct reaction with H_2O_2 and Fe^{2+}, and (iii) augmentation of catalase and SOD activities. Mechanisms of H_2S-mediated defense against antibiotics that do not involve the oxidative stress may also exist and require further investigation. In *B. subtilis*, bNOS-generated NO can directly detoxify some antimicrobials *via* chemical modification [9]. NO also activates catalase (KatA) and inhibits thioredoxine/thioredoxine reductase, which leads to rapid depletion of reduced free cysteine and interruption of the Fenton cycle [9]. The role of ROS in antibiotics-mediated cellular death has been questioned ([24,25,26•] hence the dotted arrows). However, the strong correlation between gas-mediated protection against oxidative stress and different classes of bactericidal antibiotics supports a causal link between ROS and antibiotics toxicity.