How bacteria breathe in hydrogen sulfide-rich environments

Hydrogen sulfide (H₂S) is now universally recognized as an endogenous signalling molecule playing a central role in human physiology. This gas, although it controls a number of physiological processes at low (submicromolar) concentrations, is toxic at high concentrations as it blocks cell respiration by potently inhibiting cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain. In a recent study on the model micro-organism Escherichia coli, it was shown that the bacterial respiratory oxidase cytochrome bd is resistant to H₂S inhibition, thus enabling bacterial O₂ respiration and growth in the presence of sulfide. This may be relevant because many microbes are H₂S producers and some of them live in sulfide-rich environments, such as the human gut and other natural habitats. The potential impact of this finding in different areas (environment, life evolution and human health) is discussed.

Hydrogen sulfide, a Janus molecule

Hydrogen sulfide (H₂S), a colourless water-soluble gas with a characteristic odour of rotten eggs, has emerged over the last two decades as the third recognized 'gasotransmitter', in addition to nitric oxide (NO) and carbon monoxide (CO). Beyond playing essential roles in human and animal physiology and pathophysiology, this gaseous molecule is a known metabolite in plants and prokaryotes. Similarly to the other gasotransmitters, the effects of H₂S in mammals are largely concentration-dependent. Whereas it acts at low (submicromolar) concentrations as a signalling molecule and a bioenergetic fuel by directly stimulating the mitochondrial electron transport, at higher levels it displays deleterious effects. The high toxicity of H₂S to humans is evident from multiple industrial accidents that have occurred where people have been inadvertently exposed to the gas. Toxicity is mainly related to the ability of H₂S to inhibit oxidative phosphorylation, causing energy deficit in the cell. The gas is indeed a potent inhibitor of the mitochondrial cytochrome c oxidase (mtCcOX), the terminal enzymatic complex of the respiratory chain. By reducing O₂ to H₂O, this enzyme contributes to the generation of the transmembrane proton electrochemical gradient utilized by ATP synthase to synthesize ATP. Sulfide inhibition of mtCcOX is effective and leads to dissipation of the protonmotive force at the inner mitochondrial membrane, with consequent arrest of aerobic ATP production and eventually cell death.

In mammalian cells, H₂S is endogenously generated for signalling purposes mainly by three enzymes: cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopryruvate sulfurtransferase (3-MST). Equivalent enzymes are commonly found also in the microbial world, suggesting a fundamental role for H₂S from bacteria to humans. As an example, Escherichia coli harbours an orthologue of 3-MST that was shown to contribute to a significant extent to bacterial H₂S synthesis. The regulatory role of H₂S in bacterial physiology still needs to be elucidated. Yet, interestingly, in E. coli and other bacteria tested, the ability to produce H₂S was shown to enhance antibiotic resistance, thereby providing an adaptive advantage.

In the human intestinal lumen, H₂S is also produced by the resident microflora through bacterial amino acid metabolism, mainly via reduction of thiosulfate and dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB). Therefore H₂S levels in the gut are high. Direct measurements of the gas in the rat caecum and analysis of human faecal samples proved that the total concentration of sulfide-derived species in the colon is ~1 mM, whereas the concentration of free H₂S in the intestinal lumen is ~40–60 μM. As a result, the gut microbiota is routinely exposed to high levels of H₂S, similarly to many other bacteria living in sulfide-rich natural habitats, such as swamps, hot springs, hydrothermal vents and other volcanic areas. As H₂S is a potent inhibitor of cellular respiration, the following questions arose: can bacteria accomplish...
O₂-consumption in sulfide-rich environments? Are there sulfide-insensitive respiratory terminal oxidases in bacteria?

**The sulfide-insensitivity of the bacterial terminal oxidase cytochrome bd**

Bacteria have a highly flexible metabolism that increases their chance to survive and thrive in a changing environment. Typically, they have branched respiratory chains ending with multiple terminal oxidases. The majority of respiratory oxidases identified to date, including mitCcOX, belong to the superfamily of haem–copper oxidases, having a characteristic bimetallic active site comprising a haem and a copper ion (Cu₃). The *bd*-type terminal oxidases, widely distributed among prokaryotes, but absent from eukaryotes, are phylogenetically unrelated to the haem–copper oxidases. They do not have a copper co-factor, but have a distinctive haem composition consisting of two haems *b* and one haem *d*. Characterized by a remarkable affinity for O₂, the cyanide-resistant *bd*-type oxidases accomplish the complete reduction of O₂ to H₂O using quinols as reducing substrates. The catalysed reaction is electrogenic, but is not associated with proton pumping, thus leading to protonmotive force generation with a lower energy yield compared with haem–copper oxidases. Besides its role in bacterial energy metabolism, cytochrome *bd* was suggested to play other physiological functions, being implicated in the bacterial response to oxidative and nitrosative stress. This respiratory oxidase was identified in a number of pathogens, such as *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Listeria monocytogenes*, *Streptococcus*, *Brucella* and *Salmonella*, and, in many of them, it was intriguingly shown to promote virulence. In this regard, it is worth noting that, compared with haem–copper oxidases, cytochrome *bd* is more resistant to NO inhibition, owing to a faster dissociation of this gaseous ligand from haem *d* in the active site. An interesting finding in the light of the information that NO is produced as part of the host immune response to counteract microbial infections.

Since Cu₃ in the active site of mitCcOX has been proposed to take part in the mechanism of sulfide inhibition, the Cu-free cytochrome *bd* was suggested to be sulfide-insensitive. The hypothesis was tested in *E. coli* ³⁰. This model micro-organism is a ubiquitous member of the human gut microbiota that possesses three respiratory terminal oxidases expressed under different O₂ conditions: the haem–copper cytochrome *bo*, preferentially expressed at higher O₂ concentrations, and two *bd*-type oxidases (*bd*-I and *bd*-II), induced under O₂-limiting conditions, such as those found in the human gut. The ability of these three enzymes to sustain bacterial O₂ consumption and growth in the presence of sulfide was tested ³⁰. Interestingly, by measuring the O₂ reductase activity of the purified terminal oxidases, both *bd* oxidases were found to be insensitive to sulfide up to 58 μM, whereas under identical experimental conditions the *bo* oxidase was potently inhibited by sulfide (Figure 1). In *E. coli* respiratory mutants expressing only a single terminal oxidase, both O₂ consumption and aerobic growth were severely impaired by sulfide when respiration was sustained by the *bo* oxidase alone, but completely unaffected by less than or equal to 200 μM sulfide when either *bd* enzyme acted as the only terminal oxidase. Consistently, in the wild-type parental strain, H₂S affected cell growth and respiration only in the early phase of the culture, when O₂ availability was sufficiently high to favour the expression of the *bo* oxidase, but it caused no effect in the late phase of the culture, when O₂ limitation is expected to stimulate the expression of the *bd* oxidases. The results demonstrate that, in contrast with the haem–copper *bo* enzyme,
E. coli bd oxidases can enable respiration and growth in the presence of high levels of sulfide (Figure 2). In this light, it will be important to assess whether the reported H$_2$S insensitivity represents a common feature among bd-type oxidases.

**Life in sulfide-rich environments and evolution**

Thanks to their tremendous metabolic plasticity, prokaryotes are able to colonize very diverse ecological niches on our planet, including sulfide-rich extreme environments that are prohibitive for most living organisms. Naturally produced through geochemical or biological processes, H$_2$S can be found in large amounts in several habitats, such as swamps, hot springs, deep-sea hydrothermal vents and other volcanic areas (Figure 3). In these habitats, microbes have to cope with H$_2$S toxicity and it is therefore conceivable that the expression of bd-type terminal oxidases represents an adaptive advantage, enabling bacterial O$_2$ consumption for bioenergetic or detoxification purposes, despite the presence of H$_2$S. Accordingly, bd type oxidases have been identified in several prokaryotes adapted to these unique environments. For example, in the sulfide-rich waters of hydrothermal vents and hot springs, this enzyme has been found in aerobic bacteria, such as *Salinisphaera hydrothermalis*, *Thiobacillus prosperus* and *Halothiobacillus neapolitanus*, but also in anaerobic bacteria, such as the SRB *Desulfococcus multivorans*, in which cytochrome bd possibly acts as a detoxifier of environmental O$_2$ to protect from oxidative damage.

Interestingly, H$_2$S-rich extreme environments like those found near the deep-sea vents or other volcanic areas are thought to resemble the primordial Earth milieu where life originated. Gases have always played a key role in life evolution on Earth. Before the onset of oxygenic photosynthetic activity of cyanobacteria, due to active volcanism, H$_2$S is thought to have been present at high concentrations in the O$_2$-poor atmosphere of Earth and to be used as an energy source$^{11}$. H$_2$S metabolism probably played a major role in those primordial times, as it still does in many living beings nowadays. When O$_2$ levels started to rise due to the oxygenic photosynthesis, H$_2$S levels decreased dramatically. These notable changes in O$_2$ and H$_2$S availability probably resulted in a strong selective pressure, requiring profound adaptive mechanisms in living beings. In this scenario, it is tempting to speculate that bd-type oxidases with their ability to metabolize O$_2$ in the presence of high H$_2$S levels have played a major role throughout evolution, representing an adaptive advantage for those bacteria living in habitats with coexistent O$_2$ and H$_2$S.

**The human gut ecosystem**

Microbes are an integral part of the human gastrointestinal system. They control many aspects of human physiology and play vital functions such as facilitating digestion, supplying vitamins and providing resistance to invading pathogens. An imbalance in the number or composition of the gut microbial communities can therefore trigger pathological
consequences. As reported above, \( \text{H}_2\text{S} \) is present at a high concentration in the intestinal lumen. This gas, derived mainly from bacterial amino acid metabolism and SBR-mediated sulfate reduction, was suggested to play a role in shaping the human gut microbiota, based on bacterial tolerance to sulfide. It is becoming evident that bacteria-derived \( \text{H}_2\text{S} \) has a broad impact on human health and disease, and controls several physiological functions, well beyond the intestinal ones\(^5\). In studies on healthy rats, inhibition of \( \text{H}_2\text{S} \) synthesis resulted in inflammation of the small intestine and colon, whereas in a study on germ-free mice the host microbiota was shown to control systemic \( \text{H}_2\text{S} \) bioavailability and metabolism in tissues. However, the beneficial versus toxic effects of \( \text{H}_2\text{S} \) require further clarification.

The \( \text{bd} \)-type oxidases have been identified in numerous enterobacteria. Expression of these oxidases is probably stimulated in the microaerobic conditions found in the human colon. Not only commensal, but also several pathogenic enterobacteria, such as \( \text{S. flexneri} \), \( \text{Enterococcus faecalis} \), \( \text{E. coli} \) or \( \text{Salmonella enterica} \), harbour cytochrome \( \text{bd} \), presumably sustaining growth in the \( \text{H}_2\text{S} \)-rich and \( \text{O}_2 \)-poor environment of the gut. Several bacterial pathogens are known to make use of cytochrome \( \text{bd} \) to colonize \( \text{O}_2 \)-poor niches of the host and, in some micro-organisms, such as \( \text{M. tuberculosis} \), this terminal oxidase was shown to enhance antibiotic resistance. Cytochrome \( \text{bd} \) has therefore recently emerged as an attractive pharmacological target for the development of new antimicrobial drugs, also because the enzyme is absent from humans. Only a few cytochrome \( \text{bd} \) inhibitors are known to date, with aurachin \( \text{D} \) being the only selective example. The identification of novel selective inhibitors of cytochrome \( \text{bd} \) would certainly represent an important step towards the development of new antibacterials, a research field which has been recently boosted by the resolution of the first crystallographic structure of a \( \text{bd} \)-type oxidase, the enzyme from \textit{Geobacillus thermodenitrificans}\(^12\).

In conclusion, the newly discovered insensitivity of \( \text{E. coli} \) cytochrome \( \text{bd} \) to \( \text{H}_2\text{S} \) has opened new perspectives and will hopefully prompt future studies on these bacterial enzymes with interesting implications in different research areas.

\**References**


