



Introduction

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Organohalide respiration: microbes breathing chlorinated molecules

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Bacterial respiration has taken advantage of almost every redox couple present in the environment. The reduction of organohalide compounds to release the reduced halide ion drives energy production in organohalide respiring bacteria. This process is centred around the reductive dehalogenases, an iron–sulfur and corrinoid containing family of enzymes. These enzymes, transcriptional regulators and the bacteria themselves have potential to contribute to future bioremediation solutions that address the pollution of the environment by halogenated organic compounds.

1. Introduction

With the discovery of molecular oxygen, depicted as *dephlogisticated* air, around 1775, Joseph Priestley ignited further research in understanding the molecular basis of respiration. His famous experiments demonstrated that mice survived happily in this gas and convinced him to take a deep breath of it himself: 'The feeling of it to my lungs was not sensibly different from that of common air; but I fancied that my breast felt particularly light and easy for some time afterwards. Who can tell but that, in time, this pure air may become a fashionable article in luxury? Hitherto only two mice and myself have had the privilege of breathing it.' [1, vol. 2, p. 102]. We now understand that higher organisms, including human, require oxygen to generate life-sustaining energy from their food. This respiration has some similarities to combustion of organic matter: carbon dioxide and water are generated by oxidation of the reduced organic matter (the electron donor) with molecular oxygen (the electron acceptor). The biochemistry underlying the respiratory process is complex, with electrons liberated through oxidation of reduced organic matter not directly linked to reduction of oxygen (with liberation of large quantities of heat as occurs in combustion), but guided along a series of electron transfer molecules, ultimately culminating in reduction of oxygen as the terminal electron acceptor. This step-wise process of reduction/oxidation of a series of individual compounds is linked to a remarkable energy conversion process, first proposed by Mitchell [2] and termed chemiosmosis.

In terms of energy released, molecular oxygen is highly efficient as a terminal electron acceptor, but respiratory processes are not necessarily linked to oxygen reduction. Indeed, prior to the advent of oxygenic photosynthesis, which ultimately led to the great oxygenation event, Earth presented an anaerobic environment [3]. Respiration is thought to have preceded oxygenic photosynthesis, with a range of oxidizing molecules (i.e. sulfate, nitrate, Fe^{3+}) used as terminal electron acceptors [4]. Indeed, bacterial respiration today presents us with a wide array of 'alien' lifestyles including respiration on insoluble minerals [5]. Many of these processes occur on a global scale and form part of the biogeochemical cycles that have a profound effect on our environment [6]. Among those elements that are cycled through various redox states (such as carbon, nitrogen and sulfur) through both biochemical and geological processes are the halogens (predominantly chloride, but also fluoride, bromide and iodide) which cycle predominantly between the free ion (reduced) and the oxidized organohalide state (i.e. as part of a C–X bond) [7]. Indeed, a surprising array of halogenated organic components can be found in the environment [8], some of which have a geogenic origin (forest fires, volcanoes),

while others are produced biogenically. The latter was until recently considered a rare process in biochemistry, limited to a few examples such as the production of the iodinated thyroxine hormone [9]. However, recent discovery and characterization of several distinct classes of halogenases demonstrate biosynthesis of organohalides occurs at a significant scale [10].

Another, very recent contribution to the chloride cycle is the anthropogenic production and release of large quantities of chlorinated compounds into the environment. The industrial synthesis and use of organohalides has brought with it many benefits, and organohalides are used as solvents, pesticides, plastics, pharmaceuticals, but also as key intermediates in chemical synthesis [11]. However, their uncontrolled release into the environment has caused environmental damage, as many (xenobiotic) organohalides are not only toxic, but highly recalcitrant to biodegradation, and they readily accumulate in lipids leading to bioaccumulation. Infamous examples include the presence of dioxins and polychlorinated biphenyls (PCBs) in the environment as a consequence on waste incineration [12]. In search of treatment options for this damage, the (bio)degradation of anthropogenic organohalides has been the object of intense study in recent years [13]. Whereas organic molecules with few halogen substituents can often be mineralized under aerobic conditions, highly chlorinated compounds such as tetrachloroethene, hexachlorobenzene, chlorinated dioxins or PCBs are often persistent. The only documented microbial process leading to a transformation of such highly halogenated compounds is the reductive dehalogenation under anaerobic conditions, for example in aquifers, sediments, submerged soil or waste water sludge. Under these conditions, the reduction of organohalide molecules is a favourable process leading to a less halogenated product. In view of the remarkable ability of microbes to use nearly every redox couple to drive respiratory energy generating processes, it should come as no surprise that bacteria have been found that couple reduction of organohalides to generation of ATP, which was conclusively demonstrated in the early 1990s [14]. This process is not only interesting from a microbial physiology and biochemistry viewpoint, but it also has obvious potential in the application of such organisms in bioremediation as illustrated by the reduction of chlorinated dioxins by *Dehalococcoides* strain CBDB1 [15,16].

This special issue 'Organohalide respiration: using organohalides as the terminal electron acceptor' emerged from a Royal Society meeting held at the Kavli centre in July 2011. Since the initial discovery of organohalide respiring bacteria (terms that occurred in earlier literature include halo-respiration and dehalorespiration) much has been discovered regarding the fundamentals of this process [17,18]. Bacteria have been identified in diverse phyla and range from metabolically versatile and non-obligate to obligate organohalide respiring species. In this volume, Hug *et al.* [19] provide an overview of the organohalide respiring bacteria, whereas Villemur [20] is the author of a contribution reviewing an important model organism capable of degrading the toxic wood preservative pentachlorophenol.

Central to the organohalide respiration process is the reductive dehalogenase enzyme, a membrane associated iron-sulfur and corrinoid-containing protein that catalyses the reduction of the organohalide [21]. The reductive dehalogenase enzyme family forms a distinct class of B₁₂-containing enzymes that is comparatively ill-understood owing to the lack of detailed structural and mechanistic insight. The unique *rdhA* genes encoding for these enzymes are often found in multiple copies in the

genomes of organohalide respiring bacteria (up to 36 have been found in a single genome [19]) and their presence in the environment proves a useful molecular marker for organohalide respiring bacteria. Futagami *et al.* [22] demonstrate the presence and activity of organohalide bacteria in sub-sea floor sediments using detection of *rdhA* as a marker. The presence of *rdhA*-like genes in a bacterial genome also pinpoints to organohalide respiring potential, and the contribution from Lohner & Spormann [23] demonstrates the presence of reductive dehalogenases in *Shewanella sediminis*, belonging to a genus more famously associated with mineral Fe(III) respiration [5].

In this post-genomic era, the increasing availability of genomic information for organohalide respiring bacteria drives functional genomics and proteomics approaches aimed at unravelling the metabolic network that underpin growth in these species. Rupakula *et al.* [24] illustrate this approach for the obligate organohalide respiring *Dehalobacter restrictus*. The latter has no less than 25 distinct *rdhA* genes, despite the reported restricted range of organohalides this bacterium can use. Unfortunately, a conclusive link between *rdhA* gene sequence and substrate specificity has yet to be found, making prediction of the substrate range that will support growth in these bacteria difficult [19]. Tang & Edwards [25] used blue native polyacrylamide gel electrophoresis to isolate and study the activity of dehalogenase enzymes. They report the presence of two highly similar dehalogenases from *Dehalobacter* sp. that have distinct substrate preferences, pinpointing to those amino acids likely involved in substrate specificity.

Many organohalide respiring bacteria require corrinoid in the media to support incorporation in the reductive dehalogenase. As yet, the exact nature of the corrinoid cofactor for many RdhA enzymes has not been conclusively established, and, as one of the most complex cofactors known to date, a wide range of B₁₂-derivatives in terms of upper and lower axial ligand to the cobalt atom occur in nature [26]. Yan *et al.* [27] and Schipp *et al.* [28] both contribute to this issue with reports studying the exact nature of the corrinoid requirement and other vitamins of *Dehalococcoides mccartyi*, a corrinoid-auxotrophic species that can dechlorinate a variety of pollutants including PCBs, chlorobenzenes and chlorinated solvents. Understanding the exact growth requirements of these bacteria is crucial to support future *in situ* applications.

In line with the large metabolic costs of producing reductive dehalogenases and associated molecular components, the expression of *rdhA* genes mainly seems to be under transcriptional control. A wide range of distinct transcriptional regulators have been implicated in this process, and it has been reported that the regulators can have a more restricted specificity than the corresponding enzyme [17]. Wagner *et al.* [29] describe the response of *D. mccartyi rdhA* genes to the presence of dioxins, and show that a particular regulator belonging to the MarR family likely acts as a repressor. Kemp *et al.* [30] provide an update on the function of CprK, one of the best studied transcriptional regulators that senses chlorophenolic compounds. They reveal that both the presence of the halogen atom as well as the phenolic alcohol group are required for effective transcriptional activation [30].

Finally, Nikel *et al.* [31] ask the question why chlorinated pollutants are so difficult to degrade aerobically. Their contribution suggests the oxidative stress associated with biodegradation presents an additional barrier to the development of efficient aerobic degradation processes, favouring evolution of anaerobic metabolisms such as organohalide respiration [31].

Much remains to be discovered in this area, which has seen a significant growth in the literature since the early 1990s. As with all emerging fields, a clear and generally accepted nomenclature is required for efficient communication. The use of the terms 'dehalorespiration' and 'halorespiration' as terms to describe this respiratory process is now commonly discouraged, and the terms should be replaced by the term 'organohalide

respiration'. Furthermore, a clear classification system is needed for the key reductive dehalogenases, and a proposal for this is described in this issue [19]. The structure and mechanism of the reductive dehalogenase is a key to understanding the basis of organohalide respiration, and recent advances in producing this enzyme heterologously will hopefully lead to progress [32].

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