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Positive feedback during sulfide oxidation fine-tunes cellular affinity for oxygen

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ABSTRACT

Sulfide (H_2S in the gas form) is the third gaseous transmitter found in mammals. However, in contrast to nitric oxide (NO) or carbon monoxide (CO), sulfide is oxidized by a sulfide quinone reductase and generates electrons that enter the mitochondrial respiratory chain arriving ultimately at cytochrome oxidase, where they combine with oxygen to generate water. In addition, sulfide is also a strong inhibitor of cytochrome oxidase, similar to NO, CO and cyanide. The balance between the electron donor and the inhibitory role of sulfide is likely controlled by sulfide and oxygen availability. The present study aimed to evaluate if and how sulfide release and oxidation impacts on the cellular affinity for oxygen. *Results*: *i*) when sulfide delivery approaches the maximal sulfide oxidation rate cells become exquisitely dependent on oxygen; *ii*) a positive feedback makes the balance between sulfide-releasing and -oxidizing rates the relevant parameter rather than the absolute values of these rates, and; *iii*) this altered dependence on oxygen is detected with sulfide concentrations that remain in the low micromolar range. *Conclusions*: *i*) within the context of continuous release of sulfide stemming from cellular metabolism, alterations in the activity of the sulfide oxidation pathway fine-tunes the cell's affinity for oxygen, and; *ii*) a decrease in the expression of the sulfide oxidation pathway greatly enhances the cell's dependence on oxygen concentration.

1. Introduction

1.1. H_2S a gasotransmitter

Mounting evidence associates hydrogen sulfide (H_2S) to the control of various physiological functions. H_2S is a gas and, like nitric oxide (NO) and carbon monoxide (CO), it is considered as a gasotransmitter [1–4]. H_2S is produced from amino acid metabolism in mammalian tissues (Fig. 1A) reviewed in [3]. The enzymes involved are the cytosolic cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) that produce H_2S from cysteine, the mitochondrial 3-mercaptopyruvate sulfur transferase (3MST) that produces H_2S from 3-mercaptopyruvate in the presence of cysteine aminotransferase (CAT) and more recently 3MST associated to D-amino acid oxidase (DAO) that produces H_2S from D-cysteine [5]. A second important source of sulfide is the bacterial metabolism in the colonic lumen [6] where the free H_2S concentration was estimated to be around 60 μM [7]. At high concentration, hydrogen sulfide acts as a mitochondrial poison. This acute toxicity of H_2S is attributed to the inhibition of complex IV (cytochrome oxidase) of the mitochondrial respiratory chain in reversible and non-competitive manner [8]. In the case of CO and NO, the inhibition of complex IV is also reversible but competitive [8]. Interestingly, these gasotransmitters that impede mitochondrial respiration are also all involved in the control of the cardiovascular system, which supplies oxygen to tissues, for a recent review see [9].

1.2. Hypoxia, sulfide and mitochondrial affinity for oxygen

H_2S has been proposed as a key element of oxygen-sensing in the circulatory system of vertebrates, which is necessary to ensure correct oxygenation of tissues through circulatory and ventilatory reflexes [10,11]. Indeed mutual exclusion exists between oxygen and H_2S, the latter can then signal depletion of the former [10]. Addition of H_2S donors (sulfide salts) triggers responses similar to those induced by hypoxia [10]. The regulation of the cardiovascular system relies on

Abbreviations: ADP, Adenosine Diphosphate; ATP, Adenosine Triphosphate; CO, carbon monoxide; H_2S, hydrogen sulfide; NO, nitric oxide; RSF, relative sulfide flux (see Section 2.1); SOU, Sulfide Oxidation Unit; SQR, sulfide quinone reductase.

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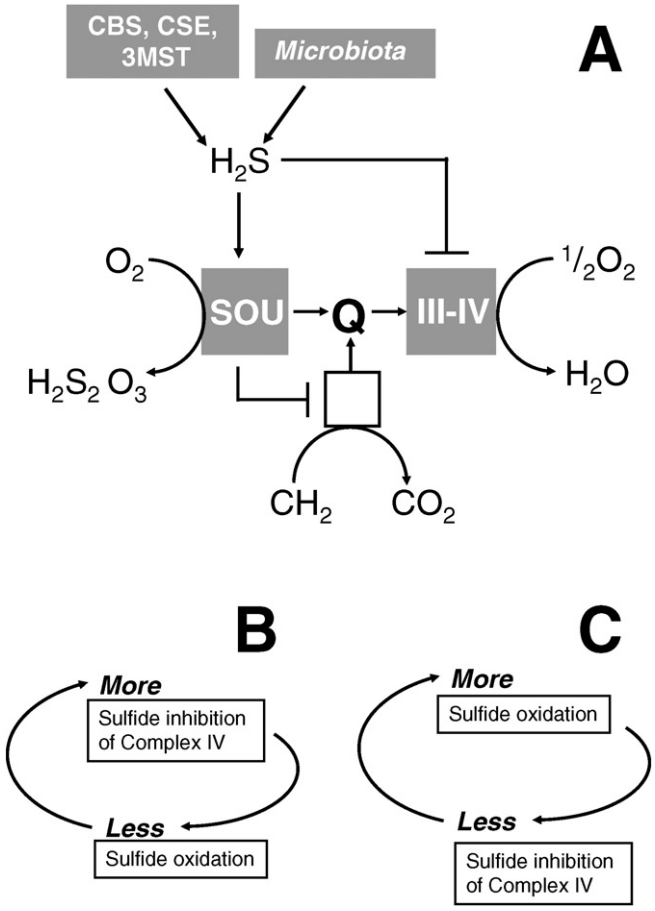

Fig. 1. A: Overall representation of sulfide metabolism in mammals: sulfide is produced in the eukaryotic cells by three enzymes: Cystathionine β-synthase (CBS), Cystathionine γ-lyase (CSE) and 3 mercaptopyruvate transferase (MST). Another source of sulfide is the microbiota present in the lumen of the digestive tract. The sulfide oxidation machinery involves a Sulfide Oxidation Unit (SOU) and the mitochondrial respiratory chain complexes III and IV (III–IV). SOU contains the sulfide quinone reductase that transfers electrons from sulfide (with protons) to the quinone (Q) present in the mitochondrial inner membrane, SOU also contains a sulfur transferase and a dioxygenase and consumes one molecule of oxygen to generate thiosulfate and transfer two electrons (and protons) to reduce quinone into QH_2. The catabolism of carbon containing mitochondrial substrates (white square) yields electrons that also converge to quinone. Therefore SOU and carbon metabolism are direct competitors. However, the high affinity of SOU for sulfide renders it extremely competitive against carbon metabolism. Sulfide is an inhibitor of mitochondrial complex IV and is thus able to inhibit its own oxidation. This is at the origin of positive feedback loops amplifying either mitochondrial inhibition (B) or sulfide disposal (C).

oxygen and carbon dioxide detection in the arterial blood by carotid bodies. Their response to low oxygen involves intracellular calcium fluxes resulting in the generation of action potentials in efferent nerves [12]. The firing rate of perfused carotid bodies increased abruptly when oxygen concentration was decreased between 60 and 30 mm Hg, which corresponds to 74–37 μM oxygen concentration in water at 37 °C [12]. Similar response was also obtained after exposure to 100 μM sodium sulfide [13], which is likely to cause immediate complete inhibition of cellular respiration [14–16]. Similarly, other authors attracted attention to the fact that the hypoxic response coincides with hallmarks of respiratory chain failure in oxygen-sensing cells [17–20], which could be reproduced by poisoning with cyanide, azide or carbon monoxide [17,19,21]. Consequently, oxygen shortage causing inhibition of mitochondrial respiration appears as a possible explanation for oxygen sensing in the carotid bodies. However, the apparent affinity constant of cellular respiration for oxygen (# 1 mm Hg) means that cellular respiration is hardly affected unless oxygen concentration is very low. Therefore, it remains to explain how in the presence of 30 mm Hg oxygen, the mitochondria in the oxygen-sensing cell can experience oxygen shortage [17]. Knocking down the activity of the sulfide-producing enzyme CSE, either by chemical inhibitors [10] or genetic ablation [12], deeply decreases the carotid bodies' response to hypoxia, while retaining their response to CO_2 accumulation [12]. This raises the issue of whether and how the presence of a sulfide releasing-rate would influence the dependence of cellular respiration on oxygen concentration. Of course this aspect of cellular response to sulfide is supplementary to the other pathways and possible consequences of sulfide signaling [1,2,4], which are not the subject of the present study.

1.3. Sulfide oxidation/poisoning generates positive feedback loops

In contrast to other gasotransmitters (NO and CO), H_2S is a substrate avidly consumed by the mitochondrial respiratory chain [15,22–25]. This is due to a sulfide quinone reductase (SQR) that transfers electrons and protons from H_2S to coenzyme Q (Fig. 1). The apparent Km for sulfide oxidation by rat liver mitochondria is around 2 μM [25]. Cellular oxidation of sulfide is detected with concentrations in the 0.1–10 μM range [25], while higher concentrations inhibit respiration. Most of the mammalian cells/tissues are able to sustain elevated sulfide oxidation rates [15]. Therefore, the sulfide oxidation pathway appears in large excess with regard to the physiological sulfide-releasing rate. Therefore, in the absence of other limiting factor (see below) the physiological concentrations of sulfide are expected to be well below the apparent Km hence in the nanomolar range [26,27]. The exception is the gut epithelium challenged by the toxic micromolar concentrations of free sulfide generated by the microbiota [6,7,16]. Sulfide oxidation demands three times more oxygen for the same electron transfer in the mitochondrial respiratory chain than the oxidation of NADH or $FADH_2$, the other reductants of coenzyme Q [15]. This is due to the dioxygenase, which in addition to the SQR and sulfur transferase, constitutes the Sulfide Oxidation Unit (SOU in Fig. 1A) [28]. The obligatory consequence is that the ATP to oxygen ratio, *i.e.* the energetic efficiency of oxygen usage, is considerably lower for sulfide oxidation than it is for NADH or $FADH_2$ [29].

In addition to SOU, sulfide oxidation involves the mitochondrial respiratory chain from coenzyme Q to cytochrome oxidase [15], which is the target of sulfide inhibition. Consequently, sulfide has the potency to inhibit its own oxidation (Fig. 1A). This promotes a cascade of sulfide accumulation and further cytochrome oxidase inhibition (Fig. 1B). The opposite is true when sulfide oxidation releases inhibition (Fig. 1C).

These positive feedback loops drive the system towards two opposite endpoints: *i*) low (nanomolar) sulfide concentrations that preserve the respiratory rate because sulfide oxidation exceeds its delivery rate; or *ii*) high (micromolar) sulfide concentrations that inhibit respiration because sulfide oxidation is lower than its delivery rate. Matched sulfide delivery and oxidation rates render the system unstable and extremely sensitive to small variations in both of these rates. For example, a marginal decrease of mitochondrial respiration may initiate a vicious cycle of sulfide accumulation leading to a block in respiration. This scenario may be relevant to oxygen sensing, as it would explain how a moderate decrease in oxygen concentration, with little influence on cellular oxidation, eventually leads to bioenergetic consequences similar to hypoxia despite a significant availability of oxygen. Balancing the production and elimination of sulfide may be the means by which the oxygen dependence/sensitivity of mitochondrial respiration can be tuned over a large range of oxygen concentrations. We aimed to support this theoretical scheme by determining the apparent affinity constant of cellular respiration for oxygen under increasing rates of continuous sulfide infusion and by comparison of two different cell lines: a recombinant CHO cell line, CHO-J overexpressing the human sulfide quinone reductase, and its control counterpart, CHO-CO3 cells transfected with the empty vector [15].