

# MITOCHONDRIAL SULFIDE-SENSITIVITY IN COELOMOCYTES FROM THE SULFIDE-ADAPTED MARINE INVERTEBRATE *GLYCERA DIBRANCHIATA*

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Hydrogen sulfide occurs both naturally and from human industry in a number of marine and aquatic environments, including hydrothermal vents, cold seeps, sewer outfalls, marshes, mudflats, highly eutrophic freshwater lakes, and in benthic marine habitats worldwide. Hydrogen sulfide (the term “sulfide” will henceforth be used to refer to the sum of  $\text{H}_2\text{S}$ ,  $\text{HS}^-$  and  $\text{S}^{2-}$  in equilibrium) is a well-known toxin that poisons isolated mitochondria at low micromolar concentrations via reversible inhibition of cytochrome *c* oxidase. Despite the apparent toxicity of sulfide, in almost all sulfidic environments thus far studied, diverse communities of invertebrates have been found that are evolutionarily adapted to sulfide in concentrations ranging from 50  $\mu\text{M}$  for some mudflats to more than 12 mM for some salt marshes and cold seeps. Although sulfide-adapted invertebrates possess mechanisms for oxidizing, and thereby “detoxifying” sulfide, the very process of sulfide oxidation may produce free radicals, which have the potential to be even more damaging than sulfide itself. Chen and Morris (*Proc. Am. Soc. Civil Eng.* 98, 215-227, 1972) first proposed that sulfide oxidation produces free radicals, but this remained untested until Tapley *et al.* (*Biol. Bull.* 196, 52-56, 1999) demonstrated that sulfide oxidation in seawater and in animal tissues (the mollusk *Yoldia limatula*) forms oxygen- and sulfur-centered free radicals, including  $\text{O}_2^{\cdot-}$ ,  $\text{HO}^{\cdot}$  and  $\text{SO}_3^{\cdot-}$ . We hypothesize that sulfide exposure can injure mitochondria by at least two mechanisms, both of which result in a decrease in the mitochondrial transmembrane potential ( $\Delta\psi_m$ ). First, sulfide can directly inhibit cytochrome *c* oxidase and thereby inhibit electron transport, which can lead to a decrease in mitochondrial  $\Delta\psi_m$ . This would prevent ATP production, but more importantly it would also initiate a mitochondria-mediated pathway leading to cell death. Second, sulfide oxidation, which occurs in large part within mitochondria, may directly produce free radicals, causing increased oxidative damage to mitochondrial DNA, lipids and proteins. This could disrupt mitochondrial function and thereby decrease mitochondrial  $\Delta\psi_m$ .

We investigated the effect of sulfide exposure on cell survival and mitochondrial  $\Delta\psi_m$  using coelomocytes from the sulfide-adapted polychaete *Glycera dibranchiata*, which is endemic to coastal Maine. For cell survival studies, isolated coelomocytes were exposed to either air (control) or  $\text{H}_2\text{S}$  gas (0.001% to 10% in air) for 12 h followed by a 12 h recovery period, after which cell survival was measured using a neutral red assay. Survival of *G. dibranchiata* coelomocytes decreased with increasing sulfide concentration in a dose-dependent manner with a  $K_d$  of 1.7 mM (Fig. 1, filled circles). This was very similar to survival of coelomocytes from another sulfide-adapted annelid, *Urechis caupo* (Fig. 1, filled triangles). Surprisingly, coelomocytes from both sulfide-adapted animals appeared to have sulfide sensitivity that was similar to that of rat C6 glioma cells (Fig. 1, open circles). This suggests that the lethal mechanism is similar among these very different species.

To determine whether sulfide has an effect on the integrity of mitochondrial function, we measured the change in mitochondrial  $\Delta\psi_m$  during sulfide exposure utilizing the dye tetramethylrhodamine methyl ester (TMRM; Molecular Probes; P. Bernardi et al. *Eur. J. Biochem.* 264, 687-701, 1999). Isolated coelomocytes were loaded with TMRM and placed on a glass cover slip within a sealed, temperature-controlled micro-incubation chamber (Leiden chamber, Harvard Apparatus) on an inverted Olympus Fluoview confocal laser-scanning microscope. At the initiation of each experiment, air (control) or premixed  $H_2S$  gas (0.001% to 10% in air) was injected into the chamber, and fluorescence was measured at 595 nm (488 nm excitation) for the following hour. Under control conditions (air exposure), the fluorescence remained constant, indicating a stable  $\Delta\psi_m$ . In contrast, exposure to 1.9 mM  $H_2S$  caused a decrease in the fluorescence that was evident within 20 minutes and was complete within 1 hour (Fig. 2, left panel), suggesting a loss of mitochondrial  $\Delta\psi_m$ . The effect of  $H_2S$  on  $\Delta\psi_m$  showed a classic dose-response, with an  $EC_{50}$  of 460  $\mu M$   $H_2S$  (Fig. 2, right panel). This is the first evidence that  $H_2S$  causes mitochondrial depolarization even in cells of sulfide-adapted animals. It is not yet clear whether this mitochondrial depolarization leads to cell death, nor is it clear whether the depolarization is via opening of the mitochondrial permeability transition pore.

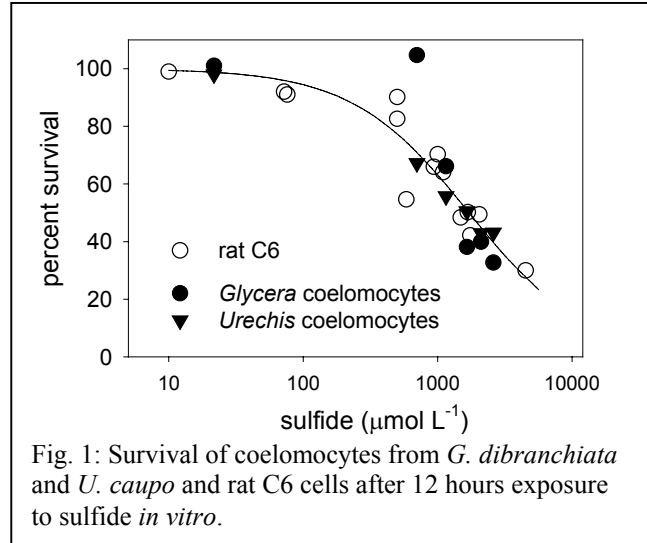


Fig. 1: Survival of coelomocytes from *G. dibranchiata* and *U. caupo* and rat C6 cells after 12 hours exposure to sulfide *in vitro*.

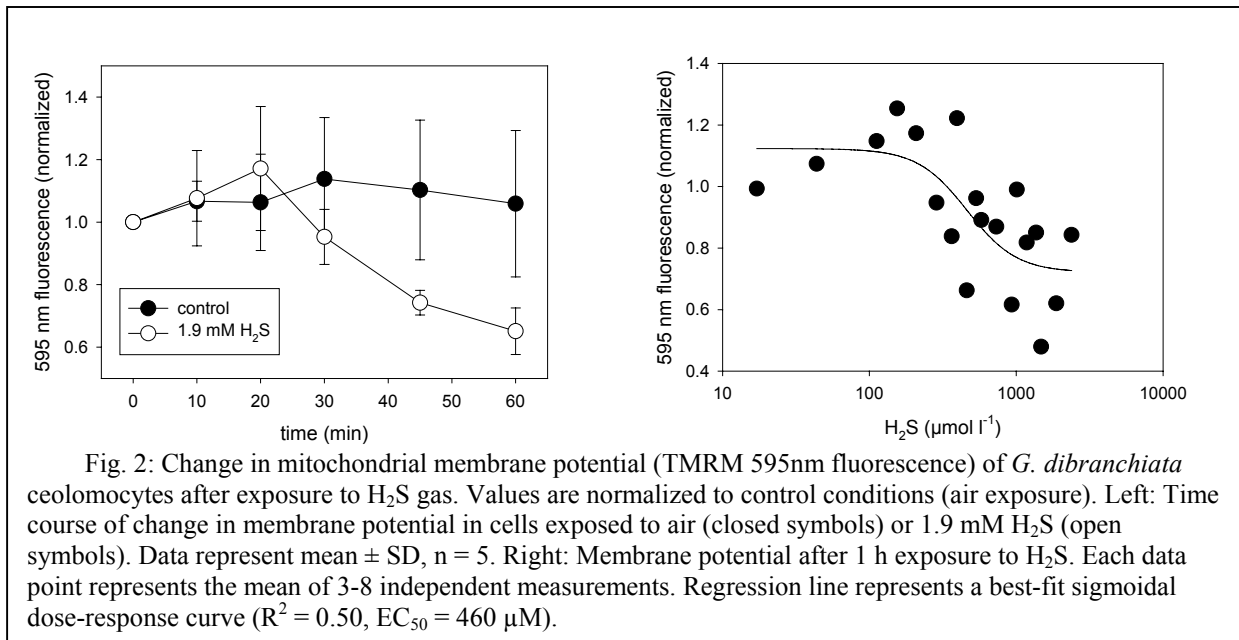


Fig. 2: Change in mitochondrial membrane potential (TMRM 595nm fluorescence) of *G. dibranchiata* coelomocytes after exposure to  $H_2S$  gas. Values are normalized to control conditions (air exposure). Left: Time course of change in membrane potential in cells exposed to air (closed symbols) or 1.9 mM  $H_2S$  (open symbols). Data represent mean  $\pm$  SD,  $n = 5$ . Right: Membrane potential after 1 h exposure to  $H_2S$ . Each data point represents the mean of 3-8 independent measurements. Regression line represents a best-fit sigmoidal dose-response curve ( $R^2 = 0.50$ ,  $EC_{50} = 460 \mu M$ ).

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