Stress induced Eh jumps are caused by sulfide efflux from Escherichia coli cells

It was previously established that in aerobic cultures of a number of bacteria subjected to stress, there is a sharp change in the redox potential (Eh) of the medium to reductive (negative) values. Eh jumps were detected by a platinum electrode when growth ceased due to depletion of carbon or nitrogen, heat shock, and also in cultures treated with certain antibiotics. It is known that in aerobically growing cultures of microorganisms, the main factor determining the value of Eh is oxygen concentration. Being a strong oxidant, oxygen maintains a positive redox potential in the aerated culture. During growth oxygen is consumed for respiration and, accordingly, Eh gradually decreases to reductive values. When growth stops, oxygen consumption decreases sharply, the concentration of dissolved oxygen increases, and, according to theory, we should expect a change in Eh towards oxidative values. However, in the stress situations described above, Eh quickly decreased to reductive values. In some cases the amplitude of the Eh jump reached 150 mV. It was suggested that, under stressful conditions, growth arrest is accompanied by release of the redox substance (s) from the cells, which, despite the presence of oxygen, shift Eh to reductive values.

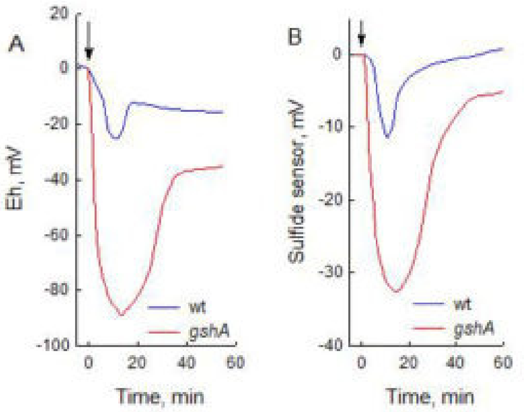

Fig. 1. Changes in Eh (A) and sulfide (B) in *E. coli* cultures treated with valine (isoleucine starvation).

Our preliminary work has shown that low molecular weight thiols may be the most likely compounds capable of reducing Eh in response to stress. This is indicated by the ability of thiol inhibitors to prevent stress-induced Eh jumps. In *Escherichia coli*, such thiols can be glutathione (GSH), cysteine (Cys) and hydrogen sulfide (H_2S). In our recent study, published in Bioelectrochemistry, we examined the role of these sulfur-containing substances in the generation of Eh jumps during starvation and antibiotic-induced stresses.

Redox potential (Eh) and extracellular sulfide levels in *E. coli* cultures were continuously measured directly in the flasks using platinum electrode and sulfide-specific ion-selective electrode, respectively. Extracellular L-cysteine and glutathione were determined by chemical methods in samples taken by rapid filtration through membrane filters. We found that Eh jumps, occurring during transition of *E. coli* from exponential growth to starvation and under antibiotic-induced stresses, are the result of sulfide excretion from the cells. Under isoleucine starvation, glucose depletion and ciprofloxacin exposure, the level of extracellular sulfide reached

43 ± 3, 96 ± 5 and 140 ± 1 nM, respectively. In the glutathione-deficientmutant (Δ*gshA*), the sulfide concentration increased 1.5-4 times compared to the parent. A close relationship was found between the transmembrane fluxes of sulfide, cysteine and glutathione.

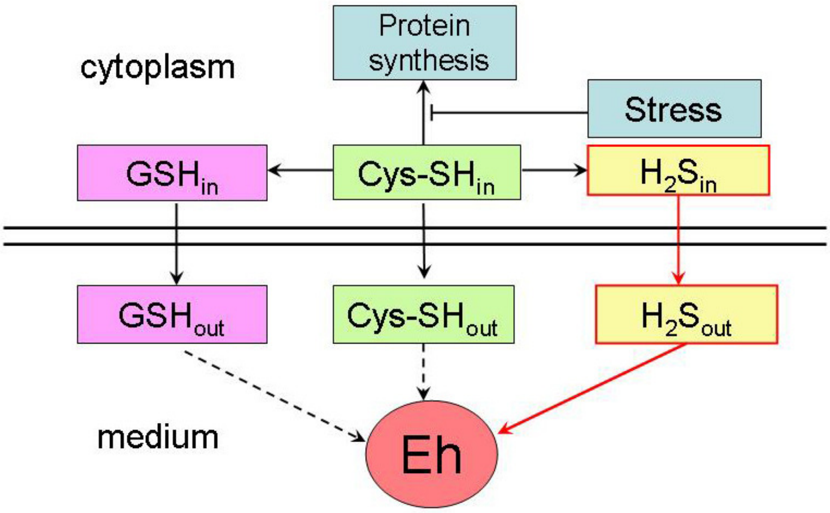

Fig. 2. Proposed scheme of the relation between sulfide excretion and changes in the redox potential (Eh) in *E. coli* cultures under stress conditions.

A model was presentedto explain the role of sulfides in stress-induced changes of Eh. Consistent with this model, inhibition of protein synthesis under stresses results in a transient increase in the intracellular level of cysteine that in the presence of free iron creates a danger of oxidative stress. To maintain the cysteine homeostasis, the flux of cysteine is redirected from protein synthesis to glutathione that is followed by an efflux of excess GSH into the medium. Another part of cysteine is exported to the medium in free form. Finally, one more part of cysteine undergoes desulfurization, and the resulting sulfide diffuses into the medium where it interacts with the platinum electrode, reducing its potential. Under certain conditions, continuous recording of sulfide changes using Eh and sulfide sensors can be used to control bacterial cultures in a scientific experiment and biotechnology. However, it should be noted that the effects described above are observed in aerobic cultures growing on minimal medium that do not contain organic sources of sulfur.

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