Oxidative Detoxification of Hydrogen Sulphide Detected by Mass Spectrometry in the Soil Amoeba *Acanthamoeba castellanii*

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Respiration of the soil amoeba *Acanthamoeba castellanii* is stimulated by low concentrations of H_2S (<17 Pa in the gas phase); consumption of H_2S accompanies respiratory stimulation. Inhibition of the main respiratory chain by 1 mM- NaN_3 prevents H_2S consumption. At higher concentrations, H_2S inhibits respiration incompletely; the inhibition is enhanced by 1 mM-salicylhydroxamic acid. Thus this organism has two means of protection against the potentially toxic gas: the ability to oxidize H_2S , and the presence of alternative pathways of electron transport which are not blocked by this respiratory inhibitor.

INTRODUCTION

The use of inhibitors that bind to the terminal oxidase of the mitochondrial respiratory chain, cytochrome $a + a_3$, has provided a key method for the elucidation of electron transport pathways (Degn *et al.*, 1978). Of these inhibitors, H_2S is perhaps the most commonly occurring under natural conditions (e.g. in freshwater and marine sediments where O_2 concentration is low, and especially in polluted rivers and lakes). Apart from its capacity for ligand binding to haem of cytochrome oxidase (Nicholls, 1975; Nicholls & Chance, 1974), H_2S can also serve as an electron donor at the flavoprotein level of the respiratory chains of some prokaryotes (e.g. the thiobacilli: Trudinger, 1969; Aleem, 1977).

This paper reports that the soil amoeba *Acanthamoeba castellanii* has at least two mechanisms for protection against the toxic effects of H_2S . At low concentrations the inhibitor is oxidized; higher concentrations cause only incomplete respiratory inhibition, as the organism has alternative pathways of electron transport (Lloyd *et al.*, 1979).

METHODS

Abbreviation. SHAM, salicylhydroxamic acid.

Maintenance and growth of organisms. *Acanthamoeba castellanii* was maintained, grown axenically and counted as described previously (Lloyd *et al.*, 1979).

Simultaneous measurements of H_2S and O_2 in solution. A quadrupole mass spectrometer fitted with a membrane-covered inlet was used to simultaneously and continuously measure concentrations of H_2S and O_2 in an open reaction system (Lundsgaard *et al.*, 1976; Degn *et al.*, 1980). The instrument used was a mass spectrometer type Q8 from VG Micromass (Nat Lane, Winsford, Cheshire CW7 3QH, U.K.) with a turbo-pump type TPH 100 from A. Pfeiffer Vakuumtechnik (D-6334 Asslar 1, F.R.G.).

The cell suspension (4.5 ml) was maintained at 30 °C in a cylindrical stainless-steel vessel of 7 ml total volume. Stirring was by a cross-shaped stirrer fixed to a stainless-steel shaft entering through a hole in the lid and driven by a synchronous motor. The membrane-covered inlet to the mass spectrometer was sealed into a port lying below the level of the vortex. The membrane (12 μm Teflon) served both to separate the cell suspension from the vacuum and as a gasket between the reaction vessel and mass spectrometer inlet. The response time of the system was limited

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by the rate of stirring; the half-time for equilibration with O_2 was slightly below 2 min, corresponding to an O_2 transfer constant of $7 \times 10^{-3} s^{-1}$. Concentration of dissolved O_2 (ordinates on Figures) under a constant partial pressure of O_2 in the gas phase is linearly and inversely related to steady-state respiration rate.

Inhibitors. Fixed concentrations of H_2S in the reaction mixtures were established by allowing H_2S/N_2 mixtures of known composition to enter the O_2/N_2 gas stream to the open reaction vessel (Petersen, 1977). The concentration of H_2S in an acetate buffer, pH 4.5, in equilibrium with the final gas mixture, was determined by the methylene blue method (Rand *et al.*, 1978). It was found that H_2S of 1 mmHg (133.3 Pa) partial pressure in the gas phase is in equilibrium with 0.12 mM undissociated H_2S in liquid at 30 °C. KCN and NaN_3 were used as aqueous solutions (pH 7.4); SHAM was dissolved in ethanol. The percentage inhibition is expressed relative to the respiration rate in the absence of any inhibitor.

RESULTS AND DISCUSSION

Low concentrations of H_2S stimulated the respiration of washed late-exponential phase *A. castellanii*: 17 Pa H_2S in the gas phase gave 38% stimulation of respiration and the steady-state concentration in the liquid phase remained below a detectable level (Fig. 1a). Increasing the inhibitor to 25 Pa resulted in 20% inhibition of respiration accompanied by a transition to a detectable steady-state level of dissolved H_2S . When 1 mM-SHAM preceded H_2S addition (Fig. 1b), slight inhibition (<10%) of respiration was observed when the gas phase contained H_2S in the range 2 to 8 Pa; H_2S consumption still occurred as shown by the lack of detectable H_2S in solution. However, virtually complete inhibition of respiration was observed when H_2S was raised to 42 Pa, and free H_2S then became detectable in the liquid phase. Exposure of the organisms to this level of H_2S caused some irreversible inactivation of the respiratory system, as shown by incomplete recovery on switching from H_2S to N_2 in the gas stream. Microscopical examination did not reveal any obvious damage to the organisms. Exposure to lower partial pressures of H_2S (<17 Pa) for up to 40 min gave completely reversible effects.

Fig. 1. Effects of H_2S on the respiration of a washed suspension of *Acanthamoeba castellanii* in an open system, measured by mass spectrometry. A late-exponential phase culture (4.2×10^6 organisms ml^{-1}) was harvested and the organisms were washed twice and resuspended in 0.9% NaCl at a density of 3.4×10^5 organisms ml^{-1} . The gas phase contained 2.5% O_2 in N_2 throughout the experiments; H_2S was added as indicated into the gas stream entering the open system. —, Dissolved O_2 concentration; ----, dissolved H_2S concentration (arbitrary scale: H_2S undetectable at the minimum level shown).

Fig. 2. Effects of H_2S on the respiration of a washed suspension of *Acanthamoeba castellanii* in an open system, measured by mass spectrometry. Conditions were as in Fig. 1, except that the culture was harvested at 4.6×10^6 organisms ml^{-1} and the cell densities in the experiments were in (a) 3×10^5 organisms ml^{-1} and in (b) 6×10^5 organisms ml^{-1} . —, Dissolved O_2 concentration; ----, dissolved H_2S concentration (arbitrary scale: H_2S undetectable at minimum level shown).

When 30 mM- NaN_3 was added to late-exponential phase organisms, almost 70% inhibition of respiration was observed (Fig. 2a), and even low partial pressures (<4 Pa) of H_2S in the gas phase resulted in the accumulation of detectable H_2S in solution. Further inhibition of respiration was not observed until the partial pressure of H_2S was increased to 17 Pa. When 1 mM-SHAM was added and then 30 mM- NaN_3 , eventually 85% of the respiratory activity was inhibited (Fig. 2b). Increasing the partial pressure of H_2S from 4 to 33 Pa then gave complete inhibition whilst accumulating in solution. Similar results were obtained when 1 mM- NaN_3 replaced 30 mM- NaN_3 . Addition of 1 mM-SHAM or 1 mM- NaN_3 after adjusting the partial pressure of H_2S to 42 Pa gave further inhibition of respiration. These results were typical of twelve experiments with five different batches of organisms. Similar observations were also made in nine further experiments with three separate batches of cells harvested in the early-exponential growth phase. In all cases respiratory stimulation was accompanied by H_2S consumption, and conditions which inhibited respiration via the main respiratory chain always led to the accumulation of H_2S in solution. The residual respiration observed in the presence of 30 mM- NaN_3 , 1 mM-SHAM and 33 Pa H_2S was not inhibited by 2 mM-KCN.

Thus this soil amoeba can dispose of H_2S when this potentially toxic agent is encountered at low concentrations. At higher concentrations it can survive extended exposure times by using electron transport pathways alternative to the main respiratory chain. The former mechanism has not previously been reported for any eukaryote; oxidation of H_2S has been regarded as a prokaryotic prerogative. The latter mechanism provides a clear function for the simultaneous operation of alternative electron transport chains.

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