Energy Conservation in *Thiobacillus neapolitanus* C: Sulphide and Sulphite Oxidation

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INTRODUCTION

The aerobic chemoautotroph *Thiobacillus neapolitanus* C, when grown on thiosulphate, can couple thiosulphate oxidation to cytochrome *c* reduction so there is one functional proton translocating loop (loop 3; Drozd, 1974) which indicates a maximal P/O ratio of 1 ($\rightarrow\text{H}^+/\text{O}$ ratio of 2.0, i.e. 2 g-equiv. H^+ translocated outwards per g-atom oxygen consumed; Mitchell, 1966*a, b*) on the assumption that the $\rightarrow\text{H}^+/\text{ATP}$ ratio is 2. Aleem (1975) reasoned from inhibitor studies that aerobic sulphide oxidation in *T. neapolitanus* was associated with two sites of oxidative phosphorylation, whereas sulphite oxidation, like that of thiosulphate, was probably only associated with one site. Both thiosulphate and sulphide oxidation can be used to drive pyridine nucleotide reduction in *T. neapolitanus* (Roth *et al.*, 1973). This study uses the measurement of respiration-driven proton translocation (Mitchell, 1966*a, b*) to indicate the P/O ratio association with sulphide and sulphite oxidation.

METHODS

Batch growth of *T. neapolitanus* C, and measurements of $\rightarrow\text{H}^+/\text{O}$ ratios and of respiration (in 50 mM-Tris/HCl, pH 7.0) have been described previously (Drozd, 1974). The apparent K_m and maximum oxidation velocity for substrate were measured from the oxygen uptake rates of the bacteria (corrected for endogenous respiration) with various concentrations of added substrate (Harrison, 1973). Valinomycin and carbonylcyanide-*m*-chlorophenylhydrazone (both from Sigma) were prepared as 0.5 mg ml⁻¹ and 5 mM solutions in ethanol respectively. Sodium sulphide (Hopkin and Williams, Swansea; recrystallized) and sodium sulphite (BDH, Analar grade) solutions were freshly prepared every 2 h, adjusted to approx. pH 7.0, and stored on ice under nitrogen. Cytochrome *c* reduction was measured at room temperature in a Pye Unicam SP 1800 spectrophotometer at 552 nm. The prepared cell suspension (Drozd, 1974) was aerated vigorously on a vortex mixer. The substrate was added to cell suspension in the sample cuvette and the steady-state increase in absorbance was noted. When all the oxygen had been utilized there was a further increase in absorbance to the anaerobic steady-state level. The addition of a few crystals of sodium dithionite to the sample cuvette gave a further increase in absorbance due to complete cytochrome(s) *c* reduction. As a check, dithionite was then added to the reference (oxidized) cuvette to see if the original (oxidized/oxidized) absorbance reading was obtained. Cell biomass was estimated by measuring total carbon (Beckman 915 Total Organic Carbon Analyser) and converting to dry weight on the assumption that the dried cells were 46 % carbon.

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Table 1. *Respiration-driven proton translocation and substrate oxidation in intact cells of T. neapolitanus C*

The K_m for substrate oxidation and the maximum velocity of oxidation were determined on washed, intact, unstarved cells of *T. neapolitanus C* in 50 mM-Tris/HCl buffer, pH 7.0, at 30 °C by measuring O_2 uptake in a Rank oxygen electrode (Rank, Bottisham, Cambridgeshire) as described in Methods. Extrapolated $\rightarrow H^+/O$ ratios were measured on the unstarved cells at pH 6.8 to 7.2 in the presence of 70 mM-potassium thiocyanate as described in the Methods. (Values are given $\pm$ S.E.M., with the number of observations in parentheses.)

Substrate	Maximum oxidation velocity [mmol O_2 (g dry wt) $^{-1}$ h $^{-1}$]	K_m (μ M)	Final concn (mM) of substrate in $\rightarrow H^+/O$ assay	$\rightarrow H^+/O$ (g-equiv. H^+ per g-atom O)
Sodium sulphite	6	47 ± 1.2 (4)	0.5	1.7 ± 0.1 (6)
			1.0	1.4 ± 0.1 (4)
Sodium sulphide	15	60.4 ± 9.9 (4)	0.5	1.5 ± 0.1 (6)
			1.0	1.6 ± 0.1 (4)
Sodium thiosulphate	9	13.5 ± 1.8 (6)	—	*

* Values are given by Drozd (1974).

RESULTS

Intact, washed cells of thiosulphate-grown *T. neapolitanus C* oxidized sodium thiosulphate, sulphite and sulphide (Table 1) at the given maximum rates and apparent K_m values for the substrate. Sodium sulphite or sulphide did not cause any oxygen uptake in the absence of bacteria.

In the aerobic steady-state, added sodium sulphite and sodium sulphide (both at 500 μ M) gave an apparent 55 % and 73 % reduction of cytochrome *c*. In the anaerobic steady-state the values were 80 % and 85 %. The measurement of respiration-driven proton translocation indicated that with endogenous substrate, sulphite or sulphide the maximum $\rightarrow H^+/O$ value never exceeded 2.0, which corresponds to a maximum P/O ratio of 1 (on the assumption that the $\rightarrow H^+/P$ ratio is 2.0). Addition of sodium sulphide to above 2.5 mM caused no pulses to be formed, although in the oxygen electrode respiration was unaffected by this concentration. Likewise, experiments in which bacteria were starved of endogenous substrate (Jones *et al.*, 1975) and then loaded with 0.1 to 1.0 mM sodium sulphite or sodium sulphide did not give $\rightarrow H^+/O$ ratios in excess of 2. The addition of valinomycin to a final concentration of 2.0 μ g ml $^{-1}$ did not markedly affect the extrapolated $\rightarrow H^+/O$ ratios but the addition of the uncoupling agent carbonylcyanide-*m*-chlorophenylhydrazine to a final concentration of 5 μ M caused a rapid collapse of the pH gradient. Rotenone (350 μ M) caused no increase in the $\rightarrow H^+/O$ ratios in the presence of sulphide or sulphite.

DISCUSSION

The results indicate that, like thiosulphate oxidation, both sulphite and sulphide oxidation are associated with a maximal $\rightarrow H^+/O$ ratio slightly under 2.0, i.e. a maximal P/O ratio of 0.8 to 0.9. This low P/O ratio associated with sulphide oxidation is surprising because sulphide is a relatively strong reductant and thermodynamically one might expect a P/O ratio of at least 2 (Aleem, 1975). Sodium sulphide is slowly oxidized under aerobic conditions; the rate of oxidation is increased in the presence of iron-containing compounds (Trudinger 1969) so that the identity of the substrates with sulphide oxidation is unclear. Steady-state cytochrome *c* reduction is greater with sulphide than with sulphite or thio-

sulphate but this might indicate that sulphide has caused some non-specific reduction of iron-containing redox couples within the cell or that there is merely a greater rate of electron flux through the respiratory chain (Chance, 1957). The effect of rotenone on electron transport is unknown because with thiosulphate as substrate it slightly increased the $\rightarrow\text{H}^+/\text{O}$ ratios (Drozd, 1974). An inhibition of reverse electron flow by rotenone should not increase the extrapolated $\rightarrow\text{H}^+/\text{O}$ ratios and only the rate of decay of the proton gradient might be affected by an energy-dependent reverse electron flow.

From the above results and the continuous-culture growth yield data for thiosulphate-limited cultures of *T. neapolitanus* (Hempfling & Vishniac, 1967), the calculated maximum Y^{ATP} , corrected for maintenance, is 5.0 on the assumption that there is maximally one site of oxidative phosphorylation associated with substrate oxidation. With one additional phosphorylation (substrate level) per mol thiosulphate oxidized via the formation of adenosine 5'-phosphosulphate (Peck, 1962), the maximum Y^{ATP} is 3.7. This is similar to the value of 3.0 calculated by Rittenberg (1972) from the same data, but is somewhat less than the theoretical maximum values of the Y^{ATP} for chemoautotrophic growth of 7.9 calculated by Hempfling & Vishniac (1973) and of 4.85 by Stouthamer & Bettenhausen (1973). There is little data available for the yields of other chemoautotrophs in continuous culture. For *Hydrogenomonas eutropha* H16 (now *Alcaligenes eutrophus* H16) grown autotrophically in continuous culture under oxygen limitation with hydrogen as the energy source, Schuster & Schlegel (1967) calculated a maximum Y^{ATP} (corrected for maintenance) of 1.7 when the P/O ratio was taken to be 3. This P/O ratio was partially confirmed by the $\rightarrow\text{H}^+/\text{O}$ ratio of 7.88 obtained by Beatrice & Chappell (1974), which indicated three sites of oxidative phosphorylation and an energy-linked, proton-translocating NADPH $\rightarrow$ NAD⁺ transhydrogenase.

Finally, it is unlikely that energy can be generated between cytochrome *c* and the respiratory nitrate reductase in *Thiobacillus denitrificans* grown with NO₃⁻ as the terminal electron acceptor, as is also the case with the cytochrome *c* containing aerobic bacteria *Pseudomonas denitrificans* (Koike & Hattori, 1975*a, b*) and *Alcaligenes eutrophus* H16 (Drozd & Jones, unpublished). Either all the ATP is generated by substrate level phosphorylation (via the adenosine 5'-phosphosulphate system) or, as suggested by Aleem (1975), there is a split of thiosulphate to sulphite and sulphide with the oxidation of sulphide via a flavin $\rightarrow$ cytochrome *c* system such that one ATP is generated between the flavin and cytochrome *c*. Thus, under anaerobic conditions, sulphide oxidation may be associated with energy coupling before cytochrome(s) *c* as suggested by Aminuddin & Nicholas (1973, 1974) and Aleem (1975).

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