RESPIRATION-DRIVEN PROTON TRANSLOCATION IN *THIOBACILLUS NEAPOLITANUS* C

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1. Introduction

Thiobacillus neapolitanus C is an aerobic chemoautotroph which can derive energy from the oxidation of a number of inorganic sulphur compounds including thiosulphate [1]. Thiobacilli contain high concentrations of *c*-type cytochromes which can be linked to the oxidation of thiosulphate [2,3]. Saxena and Aleem [4] found that once cytochrome *c* was reduced by thiosulphate oxidation, electrons could either pass to oxygen via the cytochrome oxidases with the associated production of ATP, or cytochrome *c* could be oxidised by pyridine nucleotides at the expense of ATP and this supply reducing power for biosynthetic reactions e.g. CO_2 fixation. In respiratory membrane particles from *T. neapolitanus* Ross et al. [5] found a P/O ratio of 1.0 for the oxidation of thiosulphate via cytochrome *c*, cytochrome oxidases of the *a* and *o* type, and oxygen.

I wish to report that intact cells of *T. neapolitanus* C can couple respiration to the outward translocation of protons in agreement with Mitchell's chemiosmotic hypothesis of oxidative phosphorylation [6]. For the oxidation of thiosulphate, ascorbate-TMPD, or endogenous substrate $\rightarrow H^+/O$ ratios approached a value of 2.0 which is consistent with the presence of one energy coupling site (i.e. P/O = 1.0, assuming $\rightarrow H^+/P$ = 2.0).

Abbreviations: $\rightarrow H^+/O$: g-equiv. H^+ translocated/g-atom O consumed; TMPD: NNN'N'-tetramethyl-*p*-phenylenediamine dihydrochloride; *m*-Cl CCCP: carbonyl cyanide-*m*-chlorophenylhydrazone.

2. Materials and methods

Thiobacillus neapolitanus C (from Dr D. P. Kelly, Queen Elizabeth College, London University) was grown at 30°C in the thiosulphate medium of Vishniac and Santer [7]. Solid Na_2CO_3 was added during growth to keep the pH above pH 6.5. The organisms were harvested at an A_{680} of approx. 0.1. The cells were washed twice in 1 mM Tris–140 mM KCl, pH 7.2 and re-suspended to a final density of approx. 7 mg dry wt/ml $\rightarrow H^+/O$ ratios were measured as previously described [8] with a final potassium thiocyanate concentration of 70 mM, and the routine addition of 30 μg carbonic anhydrase/ml to catalyse the $CO_2-H_2CO_3-HCO_3^-$ equilibrium [9]. The maximum pulse height was calculated by extrapolation of the semi-log plot of proton decay back to the zero time [10]. For cytochrome spectra the cell suspension was cooled in ice to stop metabolism and the spectrum was measured at room temperature with a Pye SP 1800 recording spectrophotometer. For *reduced* minus oxidised difference spectra the sample to be oxidised was shaken on a vortex mixer under O_2, sodium dithionite was added to the sample for reduction. For reduced +CO *minus* reduced difference spectra the sample was diluted, both sides reduced with dithionite, then CO was bubbled through the sample until a constant spectrum was obtained. An Aminco-Chance Dual Wavelength spectrophotometer was used for dual wavelength spectroscopy. O_2 uptake was measured with a Clark YSI 4004 O_2 electrode [8].

3. Results

Fig. 1A shows the reduced *minus* oxidised difference spectrum of intact cells of *T. neapolitanus* C. The spectrum indicates the presence of cytochromes of the *c* (523, 552 nm), *b* (shoulders at 530, 561 nm) and *a* types (588 nm). The shoulder at 517-nm is possibly due to a *c*-type cytochrome. The reduced +CO *minus* reduced spectrum (fig. 1B) shows a peak at 418 nm corresponding to cytochrome *o*, with a slight shoulder at higher wavelengths, perhaps corresponding to cytochrome a_1. These findings essentially agree with those of Ross et al. [5] for *T. neapolitanus*.

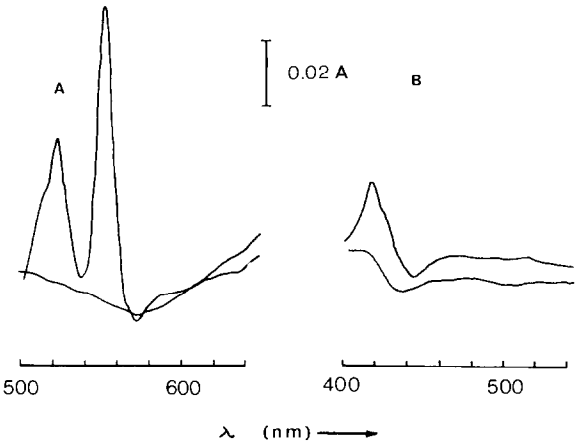

Fig. 1 Cytochrome spectra of intact thiosulphate grown *T. neapolitanus* C cells. (A). Reduced *minus*oxidised difference spectrum of cells at final A_{680} of 13.5. (B). Reduced + CO *minus* reduced difference spectrum of cells at final A_{680} of 3.0. For details see section Materials and methods.

Table 1 indicates that a washed suspension of the thiosulphate grown *T. neapolitanus* C will only oxidise thiosulphate, and ascorbate-TMPD of the substrates tested. There is a little non-specific ascorbate-TMPD oxidation. The interaction of thiosulphate and ascorbate-TMPD with cytochrome *c* was determined by dual wavelength spectroscopy (552–540 nm). In the aerobic steady state thiosulphate caused a 50% reduction and ascorbate-TMPD nearly a 100% reduction of cytochrome *c*. In the anaerobic state thiosulphate caused a 77% reduction and ascorbate-TMPD caused a 100% reduction of *c*. Possibly there are several *c* species, of which only the major is reducible by thiosulphate, whereas all species are reduced by ascorbate-TMPD or dithionite.

Table 1

Substrate added	Relative rate of oxygen uptake (thiosulphate = 100)
Thiosulphate	100
Ascorbate-TMPD*	43
Succinate	3.0
Malate	5.7
Glycerol	4.6
Pyruvate	5.0
Lactate	5.3
–	5.3

0_2 uptake by a washed suspension of thiosulphate grown *T. neapolitanus* C. The cells were suspended in 1 mM Tris–140 mM KCl pH 7.2 to an A_{680} of 0.2. 0_2-uptake was measured with a Clark YS1 4004 oxygen electrode. Substrates were added to give a final concentration of 10 mM, except for ascorbate-TMPD where 100 µl of a solution containing 45 mg sodium ascorbate and TMPD/ml were added to 3.0 m of the cell suspension.
* Corrected for autoxidation of ascorbate-TMPD.

A typical proton pulse [6] was observed when a small pulse of air saturated 140 mM KCl was added to an anaerobic cell suspension of *T. neapolitanus* (see Materials and methods) to which no substrate had been added. Only a very small pulse was observed in

Table 2
→ H^+/O ratios for the oxidation of substrates by thiosulphate grown *T. neapolitanus* C.

Substrate	→ H^+/O ± S.E.M. with number of observations in brackets
Endogenous	1.86 ± 0.21 [9]
Endogenous + Rotenone	1.95 [2]
Thiosulphate	1.33 ± 0.15 [5]
Thiosulphate + Rotenone	1.92 ± 0.10 [4]
Ascorbate-TMPD	1.20 ± 0.13 [4]
Ascorbate-TMPD + Rotenone	1.50 [2]

Ethanolic Rotenone was added to give a final concentration of 350 µM. The substrates were added to give the same final concentrations as in table 1. The maximum extrapolated proton pulse was determined as in section Materials and methods

the absence of the thiocyanate anion. The decay rate of the respiration induced proton pulse was increased by the uncoupler *m*-Cl CCCP. Table 2 indicates that for the oxidation of endogenous substrate, ascorbate-TMPD or thiosulphate → H^+/O values approaching 2.0 were seen. The slightly lower ratios with ascorbate-TMPD might be due to the non specific oxidation of ascorbate. The addition of increasing concentrations of rotenone, which inhibits the energy dependant reduction of NAD^+ and flavins in *T. neapolitanus* [4] increased the → H^+/O values to a maximum near 2.0, without greatly affecting the value for endogenous substrate.

4. Discussion

Mitchell's chemiosmotic model of oxidative phosphorylation [6] postulates that the components of the respiratory chain are spatially arranged across the bacterial membrane so that 2 H^+ are translocated outwards per energy conservation site per 2 e^- transferred down the respiratory chain. The results presented indicate an → H^+/O ratio approaching 2.0 for the oxidation of endogenous substrate, thiosulphate, or ascorbate-TMPD. Both the latter substrates caused a high percentage steady state reduction of cytochrome *c*. Presumably ascorbate-TMPD can donate electrons directly to cytochrome *c* while thiosulphate donates electrons close to *c* on the respiratory chain. There is then one site of energy conservation (proton translocating loop) between cytochrome *c* and oxygen. This is consistent with previous findings suggesting one site [5] but disagrees with the presence of three sites of oxidative phosphorylation [11] associated with thiosulphate oxidation. Presumably rotenone maximises the proton pulses by inhibiting the energy-dependant reverse electron flow [4]. So far no direct evidence has been obtained that a proton gradient can drive this reverse electron flow in this organism.

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