ELECTRON TRANSPORT AND COUPLED PHOSPHORYLATION IN THE CHEMOAUTOTROPH THIOBACILLUS NEAPOLITANUS*

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Received June 19, 1968

Adenosine triphosphate (ATP) and reduced pyridine nucleotides (NADH) are the driving forces for the carbon reduction cycle and other cellular biosynthetic reactions in chemosynthetic bacteria. It was shown previously that the cell-free extracts of the obligate chemoautotroph T. neapolitanus required ATP and NADH for the active reduction of carbon dioxide (Aleem and Huang, 1965). We have also reported earlier that the generation of reduced pyridine nucleotides by thiosulfate in this organism is an energy-linked process which is driven by ATP (Aleem, 1966). Attempts to achieve phosphorylation coupled to the oxidation of thiosulfate in T. neapolitanus have so far yielded negative results (Hempfling and Vishniac, 1965). However, investigations by Hempfling (1964) concerning the molar growth yields of T. neapolitanus indicate that the organism produces more ATP than is accounted for by the substrate-level phosphorylation alone. The experiments reported here reveal that the cell-free extracts of the chemoautotroph catalyze thiosulfate oxidation involving various cytochromes of c-type reported earlier by Trudinger (1961), and that the electron transport from thiosulfate to molecular oxygen is

* Supported by National Science Foundation Grant GB 6649

coupled to ATP formation yielding P/O ratios close to 1.0. The terminal oxidase in T. neapolitanus appears to be cytochromes of a and o type.

METHODS

T. neapolitanus was grown in a modified inorganic medium as described previously by Vishniac and Santer (1957). After an incubation period of 68-72 hours, the cells were chilled with ice and harvested by a DeLaval continuous flow centrifuge. The cell paste was washed once with cold distilled water and subsequently with 0.03 M Tris-HCl buffer (pH 8.0). A 2.0 g of wet packed cells were suspended in 4.0 ml of 0.05 M Tris-HCl (pH7.0) containing 3×10^{-1}M sucrose, 5×10^{-3}M $MgCl_2$, 5×10^{-4}M Na_4-EDTA, and 5×10^{-4}M GSH. The cells were disrupted by a Gifford Mini-Mill or a Sorvall Omni Mixer for 15 min using one volume of cells and 4 volumes of 75-100 micron glass beads. The cell-free supernatent fraction 10,000 xg 30 min was used as the source of enzyme for electron transport and coupled phosphorylation studies.

Thiosulfate oxidation was measured polarographically and ATP production was measured by the glucose-6-phosphate dehydrogenase system employed by Gibson and Morita (1967). The difference absorption spectra were obtained in a Cary Model 14 recording spectrophotometer. Experimental details are provided in the legend to the figures or table.

RESULTS AND DISCUSSION

Intact cells or cell-free extracts from T. neapolitanus upon treatment with 10^{-2}M thiosulfate under anaerobic conditions revealed absorption bands characteristic of cytochromes of b, c, c_1, and c_{557} (Fig. 1). The alpha bands of the a-type cytochromes

were not observed, however, the shoulders appearing at 440 and 445 mμ indicative of the gamma peaks of cyt. $\underline{a}_1$ and $\underline{a}_3$ respectively prevent one from unequivically denying their presence. Upon exposing the reaction mixture to air, rapid oxidation of all of the cytochrome types occurred followed by a steady-state reduction similar to the one observed under anaerobic conditions. Thus thiosulfate oxidation in T. neapolitanus appears to be mediated by the cytochrome systems and support the inference that the organism can derive energy from the cytochrome-linked phosphorylation.

The presence of Cytochrome O was confirmed by reducing the enzyme preparations with ascorbate or dithionite followed by a spectrophotometric recording of the reduced plus CO minus reduced difference absorption spectra (Fig. 2). Absorption bands in the region of 560-565, 540 and 417 mμ are indicative of the α , β and γ peaks of an o type cytochrome; the γ/α peak ratio being

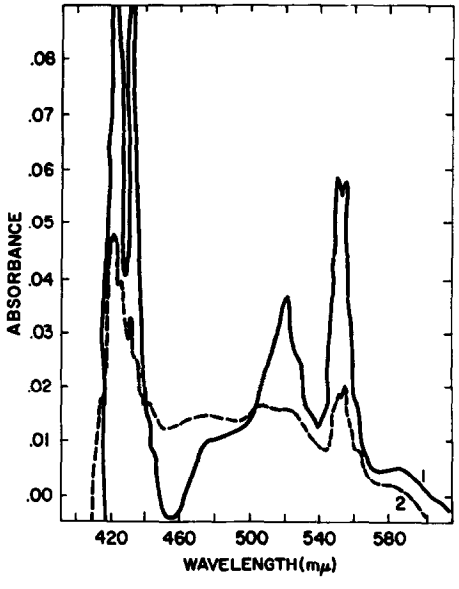

Figure 1. Steady-state difference absorption spectra of T. neapolitanus intact cells or cell-free extracts upon treatment with thiosulfate under anaerobic conditions. Reaction mixture in a total volume of 2.0 ml contained intact cell-suspension or cell-free extracts containing 2.0 mg protein and 85 μmoles of Tris-HCl (pH 8.0). The treatment cuvette in addition was supplied with 20 μmoles of $S_2O_3^=$ (trace 1); trace 2 shows change in absorption after the cuvettes were exposed to air.

13. In addition, the γ peak of the CO bound cytochrome of a-type was also consistantly observed at 432 mμ; which of the two cytochrome oxidases (o or a_3) is the final electron acceptor in the respiratory chain of T. neapolitanus is not yet clear.

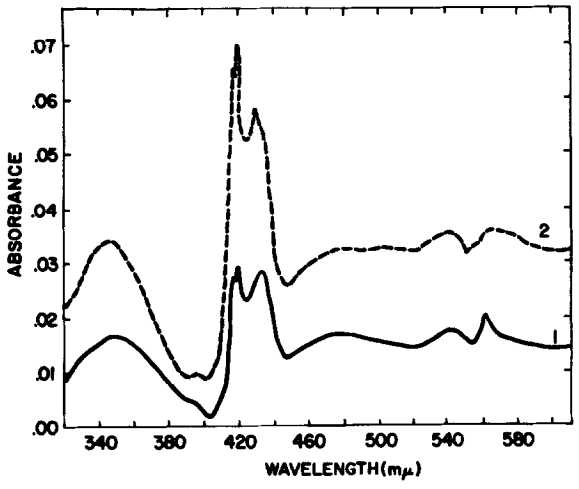

Figure 2. Reduced plus CO minus reduced absorption spectra of T. neapolitanus cell-free extracts. Reaction mixture in a total volume of 3.0 ml contained T. neapolitanus cell-free extract containing 4.0 mg protein and 85 μmoles of Tris-HCl (pH 8.0). Trace 1 represents difference absorption spectra of the enzyme suspension in the presence of 10 μmoles of ascorbate ± CO; trace 2, 0.4 μmole dithionite ± CO.

The data in Table I show that thiosulfate oxidation by the T. neapolitanus cell free extracts is coupled to the generation of ATP when bovine serum albumin (BSA) is present in the reaction mixture. No phosphorylation was observed in the absence of added BSA when $S_2O_3^=$ served as the electron donor. The contribution of substrate-level phosphorylation was insignificant as catalytic amounts of ADP were used along with the glucose-hexokinase trapping system; and moreover, the observed phosphate esterification was markedly sensitive to 2,4-dinitrophenol (DNP). In the case of cells disrupted by the Sorvall Omni Mixer, thiosulfate oxidation by the

TABLE I

Phosphorylation coupled to thiosulfate oxidation catalyzed by *T. neapolitanus* cell-free extracts.

Conditions	ATP formed μmoles	O_2 consumed μatoms	P/O
Exp. I Complete	0.873	2.16	0.40
" minus BSA	0.00	3.30	0.00
" minus $S_2O_3^=$	0.00	0.00	0.00
Exp. II Complete	1.35	1.62	0.83
" + 10^{-4}M DNP	0.24	1.50	0.16
" minus $S_2O_3^=$	0.00	0.00	0.00

Exp. I, cells broken by Mini-Mill, 3 mg enzyme protein used. Exp. II, cells broken by Omni mixer, 2 mg enzyme protein used. Complete reaction mixture in a total volume of 3.0 ml contained cell-free supernatent fraction 10,000xg, 10 μmoles $MgCl_2$, 6 μmoles K_2HPO_4, 250 ¥ BSA, 1 μmole ADP, 40 μmoles glucose, 0.5 mg hexokinase, 20 μmoles KF, 6 μmoles $Na_2S_2O_3$, and 300 μmoles Tris-HCl (pH 7.0). Reaction vessel was shaken at 28° and the experiment was terminated after 30 min. Oxygen uptake was measured polarographically in identical reaction mixtures.

cell-free extracts yielded P/O ratios close to 1.0 (0.83) compared to P/O of 0.4 when the cells were broken by a Gifford Mini-Mill. The phosphorylating activity of the enzyme preparations obtained by sonic treatment was markedly reduced although the rate of electron transport catalyzed by such preparations was not affected at all. It appears that the structural integrity of the phosphorylating particles can easily be broken during cell disruption procedures and added BSA perhaps helps restore the proper orientation of the phosphorylating enzymes with respect to the electron transport enzyme systems.

Although cytochrome b was reduced in intact cells or cell free preparations in the presence of $S_2O_3^=$, neither its oxidation

nor the electron transport was inhibited by antimycin A or HOQNO when used at a concentration of 10 γ /mg enzyme protein. Moreover, the reduction of flavins in T. neapolitanus have been shown to require ATP (Aleem, unpublished data). It therefore appears that thiosulfate enters at the cytochrome c-level as proposed by Hempfling (1964) and thus, the observed phosphorylation occurs in the cytochrome oxidase region of the electron transport chain of T. neapolitanus.

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