OXIDATIVE PHOSPHORYLATION IN PSEUDOMONAS SACCHAROPHILA UNDER AUTOTROPHIC AND HETEROTROPHIC GROWTH CONDITIONS

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SUMMARY

Cell-free extracts obtained from heterotrophically or autotrophically grown Pseudomonas saccharophila catalyzed phosphorylation coupled to the oxidation of generated-NADH yielding P/O ratios of 0.9 - 1.2. Succinate oxidation was also coupled to phosphorylation but the P/O ratios were poor in the autotrophic system. Moreover, in the latter system the oxidation of exogensously added NADH was not coupled to ATP synthesis. The autotrophic system in addition appears to lack the terminal energy-coupling site. Although the oxidation of H_2 yielded P/O ratio of 0.5 - 0.7, the entry of electrons from H_2 occurred predominantly at the level of cytochrome b. The phosphorylation with H_2 or succinate was unaffected by rotenone; the latter compound however, markedly inhibited the NADH-linked phosphorylation. The oxidative phosphorylation with H_2, NADH, and succinate was sensitive to antimycin A, cyanide, and to various uncoupling agents.

It has been reported that cell-free extracts obtained from autotrophically grown Hydrogenomonas H-20 exhibited oxidative phosphorylation with H_2 involving only the segment of the respiratory chain between H_2 and cytochrome b (1). Since the oxidation of H_2 and the coupled phosphorylation was not substantially inhibited by the added NADH-trap and in the presence of amytal or rotenone, it was suggested that NAD^+ was not involved in H_2 oxidation. Recent studies in this laboratory have shown, however, that all of the energy-coupling sites were functional in the electron transport chain of autotrophically grown Hydrogenomonas eutropha and that NAD^+ was involved not only in H_2 oxidation but also in the coupled phosphorylation (2). Moreover, we have also demon-

strated the involvement of all of the energy-coupling sites in NADH or H_2 oxidation in the case of autotrophically grown Micrococcus denitrificans (3). The experiments reported in this report reveal that all the three energy-conservation sites are operative in the electron transport chain of heterotrophically grown Ps. saccharophila while only the first two phosphorylation sites are functional in autotrophically grown cells; however, H_2 oxidation and coupled phosphorylation appears to involve only the coupling site II.

MATERIALS AND METHODS

Pseudomonas saccharophila (wild type sucrose III) kindly supplied by Dr. Duodroff was grown autotrophically in a liquid medium similar to the one described earlier for Hydrogenomonas eutropha (2). The heterotrophic growth was obtained with forced aeration in the same medium except the H_2 and CO_2 were replaced by 1.35% succinate. The cells were harvested in the early exponential growth phase and after washing twice the cell-free extracts were prepared as described earlier (2). The 20,000 x g cell-free supernatant was used as the enzyme source.

Oxidation was measured polarographically at 30°C using a gold-silver electrode for H_2 and a platinum electrode for added or generated NADH, succinate or ascorbate. The reaction mixture contained sucrose 250 mM, $MgCl_2.6\ H_2O$ 10 mM; K_2HPO 12 mM; ADP 2 mM; KF 10 mM; and Tris-Hcl buffer 50 mM, final pH 7.2. For H_2 oxidation the gas phase was 50% hydrogen and 50% air. The NADH was generated as described by Imai et al. (4) and the ATP was measured as described previously (2).

RESULTS AND DISCUSSION

The data in Table I show that the cell-free preparations from

TABLE I

Phosphorylation coupled to the oxidation of various substrates in Ps. saccharophila under heterotrophic and autotrophic growth conditions.

Substrate	Autotrophic		Heterotrophic	
	O_2 Consumed (natoms)	P/O Ratios	O_2 Consumed (natoms)	P/O Ratios
H_2	456	0.73		
NADH generated	303	1.22	352	0.92
NADH added	404	0.00	379	0.29
Succinate	208	0.15	263	0.49
Ascorbate + Cyt. c	324	0.00	380	0.25
Ascorbate + TMPD	221	0.00	360	0.00

The experimental conditions were similar as described in Materials and Methods. The final concentration of substrates was as follows: 17 mM succinate, 17 mM ascorbate and 3 mM NADH. Gas phase for H_2 oxidation was 50% hydrogen and 50% air. When ascorbate was used, Cyt. c 5×10^{-5}M or TMPD 2×10^{-5}M was included in the reaction mixture.

autotrophic or heterotrophic cells could catalyze an efficient coupling of phosphorylation with the oxidation of generated NADH. There were some important differences, however, with respect to energy-coupling in the autotrophic and heterotrophic systems. Thus the H_2 oxidation was adequately coupled to phosphorylation in the former system while the latter system failed to catalyze either the oxidation of H_2 or the coupled ATP synthesis. Although added NADH was oxidized in both the systems, there was no phosphorylation in the case of autotrophic cell-free extracts and succinate oxidation in the latter case was poorly coupled. In addition, the oxidation of ascorbate in the presence of

added cytochrome c or TMPD did not result in phosphate esterification suggesting thereby that the autotrophic system lacked the coupling site III in the ferrocytochrome c:O_2 oxidoreductase region of the electron transport chain. The differences in the energy conversion processes in autotrophic and heterotrophic systems are rather important since they indicate that the nature and efficiency of the electron transport chains under both conditions of growth are not the same. Unfortunately assumptions have been made that similar electron transport chains are involved in hydrogenomonads under autotrophic and heterotrophic growth conditions in the interpretation of the molar growth studies with organic substrates (5).

TABLE II

Effect of respiratory chain inhibitors and uncouplers on oxidative phosphorylation in heterotrophically grown Ps. saccharophila.

Substrate	Inhibitor	Conc.	P/O
NADH generated	-	-	0.90
	Rotenone	0.1 µM	0.11
	Antimycin A	0.07 µg/mg prot.	0.01
	Cyanide	50 µM	0.00
	DNP	10 µM	0.02
	CCCP	10 µM	0.04
Succinate	-	-	0.51
	Rotenone	10 µM	0.45
	Antimycin A	0.02 µg/mg prot.	0.08
	Cyanide	0.03 µM	0.00
	DNP	10 µM	0.03
	CCCP	10 µM	0.04

Experimental conditions were similar to those described in Table I, except various inhibitors and uncouplers were added where indicated.

The heterotrophic cell under aerobic growth conditions should normally possess all of the energy conservation sites. This is supported not only by the data in Table I but also by the effect of the respiratory chain inhibitors; for example, low concentrations of rotenone, antimycin A, and cyanide caused a potent inhibition of the oxidative phosphorylation when generated-NADH served as the electron donor (Table II). In this system the succinate-linked phosphorylation was insensitive to rotenone but was inhibited by antimycin A and cyanide.

Interesting are the characteristics of the electron transport chain from the autotrophic cell. The oxidation of H_2 as well as the associated ATP synthesis was not inhibited by 100 μM rotenone whereas about 90% inhibition of oxidative phosphorylation occurred in the presence of 1 μM rotenone with generated-NADH as the electron donor. The phosphorylation was inhibited in both cases, however, by low concentrations of antimycin A. Since the electron transport chain becomes deficient in the site III of energy-coupling, and since the phosphorylation with H_2 is insensitive to rotenone but sensitive to antimycin A, it might be concluded that H_2 oxidation and coupled ATP synthesis involves predominantly the participation of coupling site II (Table III). The pyridine nucleotide, therefore, does not appear to be involved in H_2 metabolism of Ps. saccharophila, a conclusion which is in contrast to the one obtained in the case of H. eutropha (2). This is further supported by our recent observations (Unpublished data) that H_2 oxidation as well as the coupled ATP synthesis in Ps. saccharophila were unaffected by the NADH-trapping system.

The ATP generation in both autotrophic and heterotrophic systems was in fact due to the process of oxidative phosphorylation since the phosphate esterification was completely uncoupled by low concentrations of DNP (2, 4-dinitrophenol) and CCCP (carbonyl cyanide m-chlorophenyl-

TABLE III

Effect of various inhibitors and uncouplers on oxidative phosphorylation in autotrophically grown Ps. saccharophila.

Substrate	Inhibitor	Conc.	P/O
H_2	-	-	0.45
	Rotenone	100 μM	0.40
	Antimycin A	0.07 μg/mg prot.	0.05
	Cyanide	50 μM	0.00
	DNP	20 μM	0.02
	CCCP	20 μM	0.00
NADH generated	-	-	1.23
	Rotenone	1 μM	0.06
	Antimycin A	0.01 μg/mg prot.	0.13
	Cyanide	0.1 μM	0.00
	DNP	20 μM	0.00
	CCCP	50 μM	0.06

Experimental conditions were the same as described in Table I, except various inhibitors and uncouplers were added as indicated.

hydrazone). In addition, extremely low concentrations of cyanide appear to exhibit an uncoupling effect in both the systems.

One possible explanation for the loss of phosphorylation site III in autotrophic cells might lie in the fact that these cells or the cell-free extracts therefrom appear to lack cytochrome a, although an o-type cytochrome acts as the terminal oxidase whereas in the heterotrophic system both cytochrome o and a have been observed to be present (Unpublished data).

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Volume 42, No.6 (1971), in the Communications "Stimulation of Cell Free Polyphenylalanine Synthesis by 30S Ribosomal Subunits in Escherichia coli"; by Yoshifumi Takeda and Kyoko Miyazaki, pp. 1134-1141:

Page 1139, last line of the legend of Figure 5 should read:
-- without 70S; o---o, with 70S... instead of
-- without 50S; o---o, with 50S.

Page 1140, line nine from bottom should read:
-- nine synthesis by 30S ... instead of
-- nine by 30S.