GENERATION OF REDUCING POWER IN BACTERIAL PHOTOSYNTHESIS. RHODOPSEUDOMONAS PALUSTRIS

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SUMMARY. Cell-free extracts were prepared from Rps. palustris grown photosynthetically on thiosulfate. The extracts catalyzed an ATP-driven NAD^+ reduction in the dark with a concomitant cyt. c oxidation when thiosulfate served as the exogenous electron donor. A stoichiometry of 2/1 was obtained for cyt. c oxidized/NAD^+ reduced. The reversal of electrons was found to be sensitive to uncouplers of oxidative phosphorylation and to inhibitors of the respiratory electron transport chain.

Generation of assimilatory power in bacterial photosynthesis is considered by numerous workers to be ATP plus NADH produced by the primary photochemical reactions (1-3). However, opinions differ regarding the function of light in the bacterial photosynthetic apparatus. According to another view, assimilatory power generated in bacterial photosynthesis is solely ATP with reduction of pyridine nucleotides occurring in the dark by an energy-dependent reversal of electrons coupled to the oxidation of an external electron donor (4). Bacterial photosynthesis might be seen therefore as a process involving chemosynthetic steps to generate reduced pyridine nucleotide. The role of light energy in bacterial photosynthesis would then be limited to the production of ATP via cyclic photophosphorylation.

In support of this hypothesis, an ATP-dependent reduction of NAD^+ in the

Non-standard abbreviations: CCCP = carbonylcyanide-m-chlorophenyl-hydrazone, DNP = 2,4-dinitrophenol, GSH = reduced glutathione, NOQNO = 2-n-nonyl-4-hydroxyquinoline-N-oxide, PPi = inorganic pyrophosphate.

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dark has been reported for Rhodospirillum rubrum chromatophores during the oxidation of succinate or ascorbate (2,5,6). In addition, a similar ATP-dependent reaction in the dark coupled to the oxidation of succinate or hydrogen gas was described for a chromatophore system of Rhodopseudomonas capsulata (7).

The present contribution describes a dark ATP-dependent generation of reducing power by thiosulfate in cell-free extracts of the non-sulfur photosynthetic bacterium Rhodopseudomonas palustris.

MATERIALS AND METHODS

Rps. palustris (ATCC 11168) was grown initially photoheterotrophically on malate plus $S_2O_3^=$. The medium used was modified after Hutner (8) and Cohen-Bazire et al. (9). The organism was then transferred to a medium containing formate and $S_2O_3^=$. Finally, formate was omitted and photosynthetic growth was obtained with $S_2O_3^=$ as the sole electron donor. After washing twice, the cells were suspended in 0.05 M Tris (pH 8.0) containing 5.0 mM $MgCl_2$, 0.5 mM GSH and 0.5 mM EDTA, and ruptured by passing twice through the French Press. Crude cell-free extracts were obtained as the supernatants after 10,000 x g (S-10,000) and 144,000 x g (S-144,000) centrifugations, the latter being a chromatophore-deficient fraction.

RESULTS

Both cell-free supernatant fractions (S-10,000 and S-144,000) catalyzed an ATP-dependent NAD^+ reduction in the dark during $S_2O_3^=$ oxidation.

Spectrophotometric data revealed that the chromatophore deficient fraction contained predominantly cytochrome(s) of the c type and flavoproteins. Thiosulfate was an effective electron donor for cyt. c. Under anaerobic conditions and in the dark, cyt. c was reduced by thiosulfate, and in the presence of ATP, the reduced cytochrome c became oxidized with a simultaneous reduction of pyridine nucleotide (Fig. 1). The ATP could be replaced by ADP and also by PPi, the latter being at least as effective as ATP. The $S_2O_3^=$-linked ATP-driven reversal of electrons against the thermodynamic gradient

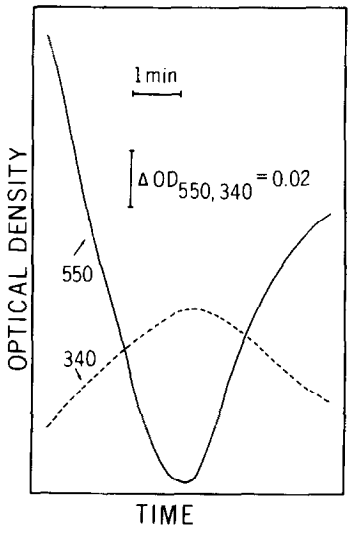

Fig. 1. Reversal of electron transfer from cytochrome c to NAD^+ coupled to thiosulfate oxidation. The reaction was carried out in anaerobic Thunberg-type cuvettes of 1 cm light path. Cell-free extract from the S-144,000 was used as the enzyme source and was dialyzed for 12 hours against 0.05 M Tris (pH 8.0) containing 1 x 10^{-3} M $MgCl_2$, 1 x 10^{-4} M reduced glutathione and 1 x 10^{-4} M EDTA. Reaction mixture in a total volume of 3.0 ml contained 0.45 ml of cell-free extract containing 13.5 mg protein, 0.165 µmoles mammalian cytochrome c, 5 µmoles $MgCl_2$, and 20 µmoles thiosulfate in the side arm. The main compartment of the cuvette contained 2 µmoles NAD^+, 5 µmoles ATP, and 0.05 M Tris buffer (pH 8.0). The control cuvette contained all the reagents except ATP. After incubating the contents in the side arm at 25°C for 3 min, the cuvettes were evacuated and the contents of the side arm were tipped in to start the reaction. The change in optical density at 340 and 550 nm was measured simultaneously in a dual wavelength double beam recording spectrophotometer.

occurred at an optimal rate at pH 8.0 (Tris-HCl buffer, 0.05 M) with 6.7 mM $S_2O_3^=$, 55 µM mammalian cyt. c, 0.7 mM NAD^+, 1.7 mM $MgCl_2$ and 1.7 mM ATP.

Figure 1 illustrates ATP-linked difference absorbance changes occurring simultaneously at 550 and at 340 nm in the dark under anaerobic conditions. The change in optical densities represents 32 nmoles of cyt. c oxidized and 18 nmoles of NAD^+ reduced within 150 secs, showing a stoichiometry of 2/1. That an active NADH oxidase (NADH:cyt. c oxidoreductase) is present in the enzymatic preparation is shown by the subsequent oxidation of NADH and coupled reduction of cyt. c.

TABLE 1

FACTORS AFFECTING THE ATP-DEPENDENT NAD^+ REDUCTION COUPLED TO CYTOCHROME c OXIDATION

Reaction mixture	Cyt. c oxidized	NAD^+ reduced
	(nmoles)	
complete	19.0	10.3
- cell-free extract	0	0
- $S_2O_3^=$	0	0
- cyt. c	0	0
- $MgCl_2$	12.3	5.5
- NAD^+	14.2	7.5

Experimental conditions were the same as described in Figure 1. The reaction mixture contained 2.6 mg of protein.

Data in Table 1 again show a stoichiometry of 2/1 for cyt. c oxidized/ NAD^+ reduced. The ATP-dependent NAD^+ reduction via cyt. c oxidation did not occur without the addition of the cell-free extracts or when the external electron donor i.e. $S_2O_3^=$ was omitted. It was also essential to add catalytic amount of (mammalian) cyt. c. The reaction occurred without added $MgCl_2$ or NAD^+ but the rate was enhanced by their addition.

The reversed electron flow was studied further by the use of uncouplers and inhibitors. It was observed that 1 μM CCCP suppressed NAD^+ reduction and coupled cyt. c oxidation by about 65%, a conc. of 5 μM CCCP was completely inhibitory; likewise 10 μM DNP caused total inhibition. Arsenate also was found to be a potent inhibitor and its action could be overcome by an excess of added PO_4^{3-} ions. The flavoprotein inhibitors such as rotenone and amytal also caused a total inhibition at a conc. of 50 μM and 50 nM, respectively. Antimycin A and NOQNO when used at a conc. of 5 μg/mg protein likewise suppressed the reversal of electrons almost completely.

DISCUSSION

The data presented on bacterial photosynthesis demonstrate for the first time that energy-dependent NAD^+ reduction in the dark can be coupled to the oxidation of the inorganic electron donor thiosulfate. Similar to the results found for the chemosynthetic bacterium Thiobacillus (10), electrons from thiosulfate enter the electron transport chain in cell-free extracts of Rps. palustris at the level of cyt. c and the energy-linked reverse electron flow to NAD^+ appears to be mediated by cyt. b and the flavoprotein system.

From the sensitivity of the reversed electron transfer to flavoprotein inhibitors like rotenone and amytal as well as to inhibitors such as antimycin A and NOQNO, we suggest that the electron transport pathway involved in the reversal of electrons may be different from the pathway followed in the primary photochemical reactions of bacterial photosynthesis. The pathway of reversal of electrons thus again seems to be similar to the one observed in chemosynthetic bacteria (11-13), and in mammalian mitochondria (14). The inhibitory action of uncouplers indicates the ATP-dependency of the reaction.

The fact that the energy-linked reduction of pyridine nucleotide in the dark was catalyzed without chromatophores indicates that the reduction of NAD^+ in Rps. palustris is not dependent on the primary photochemical steps of bacterial photosynthesis. Thus NAD^+ reduction in this organism can take place as a secondary energy-dependent process in the dark and does not hinge on an integrated chromatophore structure. The "assimilatory power" in Rps. palustris can therefore be understood as being ATP exclusively.

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