Thiosulfate-Linked ATP-Dependent NAD$^+$ Reduction in *Rhodopseudomonas palustris*

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Summary. A cytochrome containing fraction virtually devoid of the photosynthetic apparatus (bacteriochlorophyll and/or chromatophores) was isolated from *Rps. palustris* grown photolithotrophically with $S_2O_3^-$ as the exogenous electron donor. This fraction contained predominantly cytochromes of c, a and o type and exhibited thiosulfate: cytochrome c oxidoreductase and ferrocytochrome c:O$_2$ oxidoreductase activities. Under anaerobic conditions the enzyme preparation catalyzed an ATP-dependent NAD$^+$ reduction by $S_2O_3^-$ in the dark involving a reversal of electron transfer from cytochrome c and yielding a molar stoichiometry of approximately 2:1 for the ferrocytochrome c oxidized and NAD$^+$ reduced. In this process approximately 4 to 7 molar equivalents of ATP were utilized/equivalent of NAD$^+$ reduced. The optimal reaction occurred at pH 8.0 and in the presence of 55 $\mu$M added mammalian cyt. c, 1.7 mM Mg$^{++}$, 1.7 mM ATP and 7.0 mM $S_2O_3^-$. The $S_2O_3^-$-linked ATP-driven reduction of NAD$^+$ as well as the coupled oxidation of cyt. c were inhibited completely by 5 $\mu$m CCCP or 10 $\mu$M DNP and the reaction was also markedly sensitive to other uncouplers of the energy transfer reactions. The pathway of electron transfer from $S_2O_3^-$ to NAD$^+$ appears to involve cyt. c, b, and flavoprotein systems as evidenced by the complete inhibition of the process by low concentrations of antimycin A, NOQNO, rotenone and amytal.

Among members of the Athiorhodaccae, *Rhodopseudomonas palustris* is unique in its ability to assimilate CO$_2$ or formate photosynthetically with thiosulfate as the electron donor (van Niel, 1944). Even under photoheterotrophic growth conditions the presence of thiosulfate results in the increased cell yield, indicating that the organic electron donors not do compete with or suppress the photoautotrophic metabolism of the bacterium (Rolls and Lindstrom, 1967a, 1967b). Quite obviously the thiosulfate-linked photosynthetic metabolism in *Rps. palustris* must

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Non-standard abbreviations: BAL = British Anti-Lewisite (2,3-Dimercaptopropanol), CCCP = Carbonyl-cyanide-m-chlorophenylhydrazone, DBP = 2,6-Dibromophenol, DNP = 2,4-Dinitrophenol, EDTA = Ethylenediamine tetraacetic acid, GSH = reduced glutathione, NOQNO = 2-n-Nonyl-4-hydroxyquinoline-N-oxide, PCP = Pentachlorophenol, PABA = p-aminobenzoic acid.

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generate ATP as well as reducing power in the form of either reduced pyridine nucleotide or some other reductant such as reduced ferredoxin. Unfortunately little is known at present concerning the nature and the mechanism by which the reducing power is generated in *Rps. palustris* under photoautotrophic growth conditions.

Experimental observations concerned with the mechanism of pyridine nucleotide reduction in photosynthetic bacteria have led to controversies among various investigators. One group favors the conclusion that the photoreduction of pyridine nucleotides is achieved by the direct electron transfer from an excited chlorophyll molecule with the mediation of some cofactors such as ferredoxin and a flavoprotein involving the so-called non-cyclic electron flow process (Arnon *et al.*, 1961; Arnon, 1963; Amesz, 1963; Nozaki *et al.*, 1963; Yamanaka and Kamen, 1965). According to a second school of thought, however, the function of the bacterial light-catalyzed cyclic electron flow lies mainly in the generation of ATP, while the pyridine nucleotide reduction is considered to involve an energy-linked reversal of electron transfer (Bose and Gest, 1963; Gest, 1966). This concept appears to be supported by the initial observations of Frenkel (1958) that the photoreduction of NAD$^+$ in *Rhodospirillum rubrum* chromatophores was linked with the oxidation of exogenous FMNH$_2$ or succinate. Chance and Olson (1960) and Chance and Nishimura (1960) suggested that the light-stimulated NAD$^+$ reduction in intact cells of purple sulfur bacteria would involve an energy-linked reversal of electron transfer similar to the one observed in animal mitochondria with succinate as the electron donor (Chance and Hollunger, 1961). The observations of Bose and Gest (1962) revealed subsequently that photoreduction of NAD$^+$ by succinate and photoevolution of hydrogen in *R. rubrum* was very likely promoted by the “dark” oxidoreduction reactions which could proceed only at the expense of energy-rich compounds generated in the primary light reaction. As a matter of fact succinate-linked ATP-dependent NAD$^+$ reduction in the dark has been demonstrated in chromatophores from *R. rubrum* (Löw and Alm, 1964; Keister and Yike, 1966, 1967), and *Rhodopseudomonas capsulata* (Klemme 1969). It has also been shown that the photoreduction of NAD$^+$ in intact cells from *R. rubrum* (Jackson and Crofts, 1968) and *Rhodopseudomonas sphaeroides* (Jones and Whale, 1970) was driven by an energy-linked reverse electron flow process which was inhibited by uncouplers of the energy-transfer reactions. The latter workers reported, however, that in the obligately photoautotrophic green sulfur bacterium *Chlorobium thiosulfatophilum* NAD$^+$ reduction did not involve an energy-linked reversal of electron transfer and the pyridine nucleotide was reduced directly by the reduced ferredoxin-NAD reductase. The situation has been complicated further by a recent report (Govindjee and Sybesma,