Studies on an Energy-linked Pyridine Nucleotide Transhydrogenase in Photosynthetic Bacteria[1]

I. Demonstration of the Reaction in Rhodospirillum rubrum

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An energy-linked transhydrogenation between NADH and NADPH was first described in sub-mitochondrial particles by Danielson and Ernster (1963 a). The energy for the reaction could be supplied either by exogenous ATP or by the aerobic oxidation of NADH or succinate. Studies with uncouplers and inhibitors of oxidative phosphorylation indicated that the reaction was mediated by a high energy intermediate of oxidative phosphorylation. The following equation was proposed to describe the reaction:

$$NADH + NADP^{+} + \sim X \longrightarrow NAD^{+} + NADPH + X$$

The high energy intermediate ($\sim X$) could be generated from ATP or by the aerobic oxidation of substrates by the respiratory chain.

In this paper we will describe a similar reaction that occurs in chromat ophores of the photosynthetic non-sulfur purple bacterium, Rhodospirillum rubrum. The energy for the reaction in R. rubrum can be supplied by exogenous ATP, inorganic pyrophosphate (PPi) or light. A similar reaction has been found in chromatophores of Rhodopseudomonas spheriodes by Dr. J. A. Orlando[2] except that pyrophosphate cannot be utilized as an energy source.

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[1]Contribution number 242 of the Charles F. Kettering Laboratory
[2]Personal communication

Experimental. R. rubrum was grown for 48-64 hrs. on synthetic medium (Cohen-Bazire et al., 1957). After washing once, the cells were suspended in 0.1 M Tris-10% sucrose, pH 8, and ruptured by passage through the French Press. The chromatophore fraction consisted of the cell fraction sedimenting between 20,000g for 10 min and 105,000g for 90 min. This fraction was washed once and stored up to 3 days under argon at 4°C. Bacteriochlorophyll was determined using the in vivo extinction coefficient of 140 ($mM^{-1}cm^{-1}$) at 880 mμ (Clayton, 1963).

Results. Figure 1 illustrates the time course of the reaction. There is a slow endogenous reaction that is stimulated 4-5 fold by either ATP or PPi

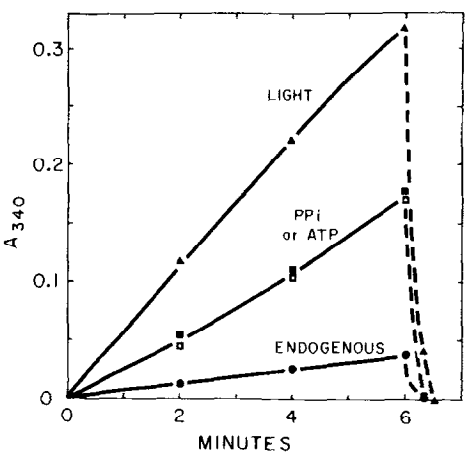


Fig. 1 Time course of $NADP^+$ reduction by NADH. The reaction mixture for the transhydrogenase contained in 3 ml the following: 50 mM Tris, pH 8; 1 mM $MgCl_2$; 0.067 mM NADH; 0.167 mM $NADP^+$; chromatophores equal to approximately 30 μgrams chlorophyll; and a NADH generating system, 0.18 M ethanol and 0.03 mg alcohol dehydrogenase. The mixture was preincubated 3 min at 25°C and initiated by addition of 0.33 mM ATP or pyrophosphate or by illumination. The increase in A_{340} is a direct measurement of $NADP^+$ reduction. The complete reaction mixture minus $NADP^+$ was the control. After 6 min, 5 μmoles of oxidized glutathione and 5 μg yeast glutathione reductase were added. This amount of reductase oxidized NADH very slowly.

and 8-10 fold by light. There was no reaction when either $NADP^+$ or NADH was omitted. GTP was as effective as ATP. ADP, 2'-AMP and 5'-AMP did not support the reaction but ADP inhibited the reaction when ATP was the energy source, but not when PPi or light was used. Magnesium was required for the utilization of PPi and ATP but not for light.

The effect of oligomycin on the reaction is demonstrated in Table I. Oligomycin is an antibiotic which has been demonstrated to inhibit energy transfer at one of the terminal stages in phosphorylation and to inhibit ATP utilization in mitochondrial reactions. In this experiment oligomycin inhibited the energy-linked transhydrogenase in the chromatophore when ATP was the energy source but not when the reaction was driven by light or PPi. This result indicated that ATP was being used as an energy source and not as an activator of the enzyme. It also eliminates free ATP as an intermediate in the utilization of PPi and light.

TABLE I

Effect of Oligomycin

Energy Source	A_{340}	
	Control	Oligomycin
		2.2 μg/ml
None	0.018	0.012
ATP	0.062	0.015
Light	0.110	0.109
		3.3μg/ml
PPi	0.066	0.055

Conditions are as in Fig. 1.

The effect of some uncoupling agents is shown in Table II. Arsenate had no effect on the reaction with either of the energy sources. This result indicated that free Pi was not involved in the reaction and that a phosphorylat

ed high energy intermediate, ($X \sim P$) probably was not the intermediate used to drive the reaction. Dinitrophenol inhibited the reaction approximately 50% at 2×10^{-4} M irrespective of the energy source used. This is in agreement with the previously reported low sensitivity of bacterial photophos phorylation to this uncoupling agent (Baltscheffsky and Baltscheffsky, 1960). Quinacrine, which inhibited the reaction 50% at 3.3×10^{-5}M, has been demonstrated to be an uncoupler of photophosphorylation in chloroplasts (Avron and Shavit, 1963), however, it is not certain whether it is an uncoupling agent or an inhibitor in chromatophores. Gramicidin is an uncoupling agent in mitochondrial reactions (Lehninger, 1950) and it has been shown to be a potent inhibitor of phosphorylation in chromatophores (Baltscheffsky and Baltscheffsky, 1960). It is a potent inhibitor of the transhydrogenase reaction.

In preliminary attempts to determine the stoichiometry of ATP and

TABLE II

Effect of Uncoupling Agents

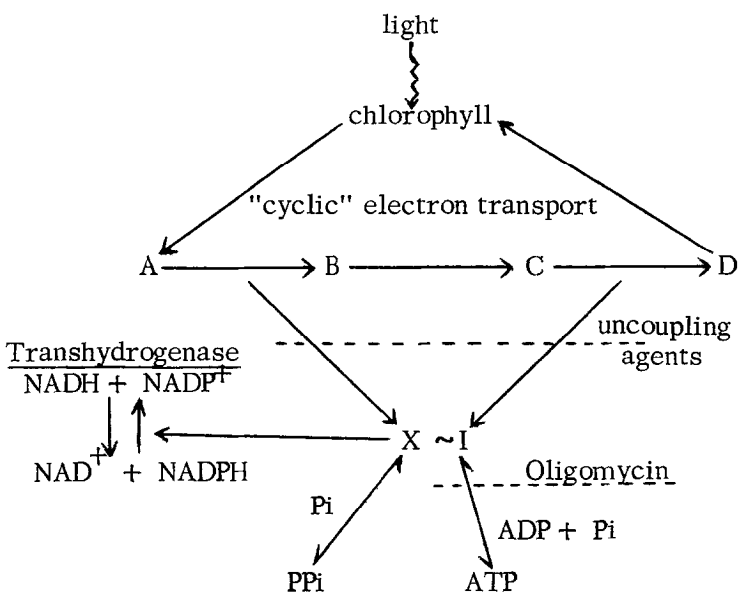
Compound	Energy Source		
	Light	ATP	PPi
	% Inhibition		
Dinitrophenol, 2×10^{-4}M	50	42	53
Gramicidin, 3.3×10^{-8}M	68	65	67
Quinacrine, 3.3×10^{-5}M	46	50	56
m-Cl-CCP, 1×10^{-6}M	14	33	81
Arsenate, 10^{-2}M	2	6	5

NADPH, we have obtained ratios of ATP/NADPH as low as 1.2. This is in agreement with the postulation of Danielson and Ernster (1963 a) that one high energy bond is utilized for each mole of $NADP^+$ reduced.

Determining the stoichiometry of PPi utilization has thus far been unsuccessful due to the high pyrophosphatase activity (200 μmoles/mg Bchl/hr) of the chromatophores which was several fold higher than the ATPase. (Baltscheffsky, 1964). Oligomycin had no effect on the pyrophosphatase activity whereas it completely inhibited the ATPase reaction.

Discussion. The energy-linked transhydrogenase of R. rubrum chromatophores is apparently very similar to that previously reported in mitochondria (Danielson and Ernster, 1963 a, b; Lee and Ernster, 1965); except that light rather than the oxidation of substrate is used to generate the high energy intermediate that apparently drives the reaction.

Our interpretation of the data may be summarized in the following scheme which was adapted from Danielson and Ernster (1963 a) to fit the reactions in the chromatophore.



This scheme makes no attempt to describe the sequence of electron transport or the number of phosphorylation sites but is purely a schematic representation of the utilization of a high energy intermediate(s) generated

by ATP, PPi or by light induced electron transport. The enzyme(s) catalyzing the transhydrogenase is tightly bound to the chromatophore but we have no evidence which would indicate that it is part of the electron transfer chain.

Horio et al. (1966) have recently reported a disappearance of Pi by illumination of R. rubrum chromatophores in the absence of added ADP. The product was later shown to be in PPi (Baltscheffsky and von Stedingk, 1966). The formation of PPi was not inhibited by oligomycin but was abolished by gramicidin or by ADP. Baltscheffsky and von Stedingk concluded that PPi was formed from an intermediate of energy transfer by the chromatophore, presumably X~P.

The results reported in this paper demonstrate that PPi can be utilized, presumably as a source of energy by chromatophores of R. rubrum and supports the postulation of Baltscheffsky and von Stedingk (1966) that the formation of PPi is a reversible process. Siu and Wood (1962) have demonstrated previously that PPi can serve as a source of Pi in the reversible formation of a high energy compound (phosphoenolpyruvate) and Cole and Hughes (1965) have recently demonstrated that polyphosphate can be used as a source of energy by serving as a Pi donor in the reversible formation of ATP by extracts of Chlorobium.

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