Respiratory Electron Flow and ATPase System in Photosynthetically Grown *Rhodopseudomonas palustris*

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Rhodopseudomonas palustris is grown photosynthetically on thiosulfate. As pointed out earlier, the chromatophore deficient cell-free fraction S-144,000 catalyzes the thiosulfate-linked ATP-dependent reversal of electron transfer in anaerobiosis, thus providing reducing equivalents in the form of NADH. Under aerobic conditions, this fraction also catalyzes the oxidation of NADH, ferro-cytochrome c, or ascorbate. ATP, ADP, and PP_i are active in retarding the aerobic electron flow. The electron retardation is stimulated by the addition of Mg^{2+} due to a Mg^{2+}-stimulated ATPase present. The ATPase system in S-144,000 hydrolyzes ATP, ADP, and PP_i. Similarly, ATP or ADP or even PP_i can function as energy sources in order to achieve the reduction of pyridine nucleotide. The ATPase turnover is diminished by NADH or NAD^+. Admixture of ascorbate results in an increased ATPase activity. Exactly the enhanced amount of adenine nucleotide hydrolysis caused by the addition of ascorbate is inhibited by cyanide.

Introduction

Respiratory activity by whole cells of non-sulfur purple bacteria was shown in earlier studies to be inhibited by illumination [1]. Later, similar results were obtained with *Rhodospirillum rubrum*, *Rhodopseudomonas spheroides* and *Rps. capsulata* cells [2]. Studies with *R. rubrum* revealed that light absorbed by the photosynthetic pigments caused the inhibition of the oxidation of respiratory substrates [3–5]. Suggestions were made that the inhibition is created by energy-rich compounds and/or electron carriers common to both the photosynthetic and the respiratory system [5, 6]. This view was supported by the fact that ATP is efficient in replacing light energy while performing an energy-dependent reduction of pyridine nucleotide linked to the oxidation of succinate by *R. rubrum* chromatophores [7]. Also, it has been demonstrated by use of cell-free extracts from *Rhodopseudomonas palustris* that the reduction of nicotinamide adenine dinucleotide was achieved by a thiosulfate-linked, energy-dependent reversed electron transfer in the dark, where ATP served as the energy source [8]. Therefore, it was examined whether in the dark ATP would influence the respiratory downhill electron flow in *Rps. palustris*.

The reversal of electron transfer is performed by the ATPase reaction which functions as the driving force with ATP being the substrate [9]. Recent reports, however, on *Chromatium* strain D chromatophores [10] as well as on purified cell-free systems from *Bacillus stearothermophilus* [11] do suggest that ADP also may be the substrate for "ATPase"-linked functions. ADP will cause, for example, energy-dependent conformational changes in *B. stearothermophilus* which were attributed previously to ATP. For this reason, the ATPase system in *Rps. palustris* cell-free extracts was studied and was investigated especially concerning energy-rich phosphates other than ATP to suit as substrates for the ATPase reaction.

Since the light supernatant fraction S-144,000 $\times g$ from the bacterium also catalyzes the energy-dependent reversed electron flow [8], this cell-free fraction was used mainly in the experiments. Furthermore, the existence of closed vesicles in S-144,000 could not be demonstrated by electron microscopic studies [12, 13] and failed to be proved by biochemical methods including the action of ionophore antibiotics (see discussion). Nevertheless, respiratory electron flow could be demonstrated in S-144,000. An active NADH oxidase as well as a cytochrome

Abbreviations: BAL, British anti-lewisite (2,3-dimercaptopropanol); DSPD, N,N′-disalicylidene-1,3-diaminopropane; EDTA, ethylenediamine tetraacetate, disodium salt; GSH, reduced glutathione; PP_i, inorganic pyrophosphate.

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c-oxygen oxidoreductase were found to be operative in the light cell-free fraction [14]. Therefore, using NADH and reduced cytochrome c as the substrates, studies were undertaken to determine the respiratory activities catalyzed by S-144,000. While this fraction practically is devoid of the photosynthetic apparatus [8], the possible interference of ATP, and eventually other energy-rich phosphates substituting light energy, with the aerobic electron transport was investigated.

Materials and Methods

Rps. palustris (ATCC 11168, identical to ATCC 17001, neo type, 1974) was grown photosynthetically with thiosulfate as the electron donor [8]. The cells were harvested after two days of growth and ruptured by passing twice through the French pressure cell at 18,000 PSI in a medium containing 0.05 M Tris-HCl (pH 8.0), 5.0 mM $MgCl_2$, 0.5 mM GSH and 0.5 mM EDTA. The light cell-free fraction was obtained as the supernatant after centrifugation at $144{,}000 \times g$ for 1 h (S-144,000) and was dialyzed subsequently for 12 h. More detailed information on the methods used is given in ref. [8, 15] and also in the legends to the Figures and Tables.

Oxygen uptake was measured polarographically in Tris-HCl buffer, pH 8.0, at 30 °C (see Table I). ATPase activity was determined using the method given by Fiske and Subbarow [16]. An ADP-trapping system [17] was not applied. Protein determination was performed by the biuret method of Gornall *et al.* [18]. Biochemicals were obtained from Boehringer Mannheim. Corp., Germany. DSPD was purchased from Fluka AG, Buchs, Switzerland.

Results

Oxidation chain and aerobic electron transport

As far as electron transport carriers are concerned, it can be demonstrated that S-144,000 from the anaerobically grown photosynthetic bacterium contains pyridine nucleotide, a flavoprotein system, and cytochromes of type c, a, and cytochrome o [8, 14]. The latter actually may be identical with cytochrome cc′. Although in very small amounts, also a cytochrome of type b could be found to be in S-144,000 *. The possible involvement of copper in the terminal section of the oxidation chain has been shown indirectly [14]. Its presence could be proved now by means of atomic absorption spectrophotometry. 20 ng Cu were found to be present per 1 mg S-144,000 cell-free protein.

Active electron donors for the aerobic oxidation chain operating in S-144,000 are NADH, ferro-cytochrome c, and ascorbate (Table I). The S-144,000 mediated oxidation of exogenous substrates is sensitive to rather high concentrations of cyanide with the inhibition effect remaining in-

Table I. Oxidation of different substrates by S-144,000 cell-free extract from *Rps. palustris* grown photosynthetically with thiosulfate. Preparation of the cell-free extract was similar to that described under Materials and Methods. The oxygen uptake was measured by a Y.S.I. oxygen polarograph (Yelow Springs Instruments Co., Ohio, U.S.). Temperature was 30 °C and the reactions were terminated after 3 min. The reaction mixture in a total volume of 3.0 ml contained 140 μmol Tris-HCl buffer (pH 8.0). 12 mg of enzyme protein were applied. The amounts of substrates used were 2 μmol NADH, 0.04 ml of a 4% solution of reduced mammalian cytochrome c, and 20 μmol of ascorbate. Where indicated, cyanide was added to the reaction mixture prior to the injection of the substrates. The values were corrected for the endogenous amounts of oxygen taken up.

Substrate	Cyanide addition [mM]	$\left[\frac{\text{natoms } O_2 \text{ consumed}}{\text{mg protein} \times \text{min}}\right]$	[%]
NADH	—	12.5	100
	0.8	11.5	92
	1.6	4.5	36
Cyt c · Fe^{2+}	—	2.65	100
	1.6	0.70	26
Ascorbate	—	21.3	100
	1.6	16.4	**77**

complete (Table I). The aerobic electron flow occurring with NADH or ferro-cytochrome c also can be inhibited by copper complexing agents, *e. g.*, salicylaldoxime or DSPD. Oxidation of NADH likewise reveals sensitivity towards antimycin A when 1.5 μg per 1 mg S-144,000 cell-free protein are applied. A 50% inhibition is observed in case 4 μg/mg protein are inserted. A 0.3 mM concentration of rotenone decreases the NADH oxidation by about 50% in S-144,000.

Respiratory electron transport retarded by energy-rich phosphates

The light fraction S-144,000 from the photosynthetically grown bacterium, when catalyzing the oxidation of respiratory substrates, does not reveal respiratory control after the addition of ADP plus

* The efforts of Dr. A. R. Crofts and his research group, Dept. of Biochemistry, University of Bristol, England, to support investigations in this system during my stay there are appreciated.

P_i. An unexpected property is observed when ADP, as well as ATP or even PP_i, is added to the cell-free extract oxidizing NADH, ferro-cytochrome c, or ascorbate. The electron flow actually is retarded by mM concentrations of the energy-rich phosphates (Table II, Fig. 1). AMP does not cause the electron retardation.

Table II. Influence of energy-rich phosphates on the oxidation of NADH, ferro-cytochrome c, and ascorbate by S-144,000. The experimental conditions were the same as given in Table I. Adenosine phosphates as well as PP_i were preincubated with the cell-free extract for about 1 min prior to the addition of the substrates.

Treatment	Conc. [mM]	[natoms O_2 consumed/mg protein × min] NADH	[%]	Cyt c $\cdot Fe^{2+}$	[%]	Ascorbate	[%]
—	—	12.5	100	2.65	100	21.3	100
ATP	4	0.6	5	1.26	48	3.7	17
	8	—		0.29	11	0	0
ADP	4	9.1	73	1.63	62	6.7	31
	8	6.9	55	1.06	40	7.1	33
AMP	4	12.5	100	2.61	99	19.8	93
	8	12.7	102	—		—	
PP_i	4	6.3	50	0.99	38	4.6	22
	8	4.8	38	0.37	14	0.5	2

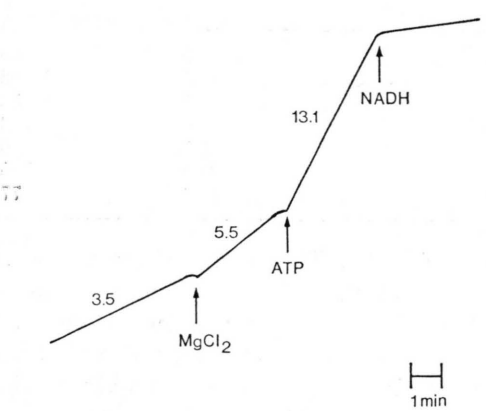

Fig. 1. Aerobic oxidation of NADH by S-144,000. The experimental conditions were similar to those given under Materials and Methods. The reaction mixture in a total volume of 2.8 ml contained 0.05 M Tris-HCl buffer (pH =8.0) and 10 mg of cell-free protein. Where indicated by arrows, 1 μmol NADH, 5 μmol ATP, and 20 μmol $MgCl_2$ were added. The numbers represent natoms of oxygen consumed per 1 mg protein and 1 min. — The oxygen uptake traces from the upper right to the lower left of the chart.

Substrate oxidation decreased by Mg^{2+}-ions

The above mentioned effect of ATP and homologues on the oxygen uptake also can be observed simply by the addition of Mg^{2+}-ions to S-144,000. The decreased rate of oxygen consumed caused by Mg^{2+} (Fig. 1) apparently is correlated with the Mg^{2+}-stimulated ATPase active in S-144,000. Obviously, even after dialysis the ATPase protein keeps its substrate closely bound so that an addition of Mg^{2+} stimulates the ATPase system present. The effect of ATP and Mg^{2+} is viewed to inhibit the proceeding oxidation of reduced pyridine nucleotide (Fig. 1). Also, the oxidation of ferro-cytochrome c or ascorbate is diminished by Mg^{2+}-ions.

Energy-rich phosphates as substrates for the ATPase-system in S-144,000

The ATPase-system present in the light cell-free fraction uses as its substrate, besides ATP, also the ADP or PPi compound. ATP, however, remains as the most effective energy-rich substrate (Table III).

Table III. Comparison of different substrates for the ATPase system in the cell-free extract S-144,000 from *Rps. palustris*. ATPase activity was performed in centrifuge tubes that were prepared at ice temperature. The reactions were carried out in a shaking water bath at 30 °C and were terminated after 20 min by adding 0.2 ml of TCA (20%). The reaction mixture in a total volume of 2.8 ml contained 125 μmol Tris-HCl buffer (pH 8.0), 10 μmol $MgCl_2$, 1 μmol of substrate, and the 144,000 × *g* supernatant equivalent to 7.4 mg of protein. Inorganic phosphate was determinated in the deproteinized samples after Fiske and Subbarow. The numbers for P_i released were corrected for the endogenous amount of P_i present.

Substrate	conc. [mM]	Total amount of P_i formed [nmol]	$\left[\frac{\text{nmol } P_i \text{ formed}}{\text{mg protein} \times \text{min}}\right]$	[%]
ATP	0.36	1820	12.3	100
ADP	0.36	1140	7.7	63
AMP	0.36	35	0.0	—
PP_i	0.36	2 × 575	2 × 6.8	50

Likewise, not only ATP functions as the energy source in S-144,000 in order to achieve reversed electron transport in anaerobiosis [8]. NAD^+ reduction also can be observed when energy is applied as the ADP and even PPi molecule (Fig. 2).

ATPase mediated hydrolysis of ATP and ADP

It can be observed by chromatographic analysis that ATP becomes hydrolyzed through the mediation of S-144,000 whereby, as a function of time, ADP-spots become visible and, while these are getting lighter, the spots representing AMP will increase in distinctness. Also, the S-144,000-catalyzed hydro-

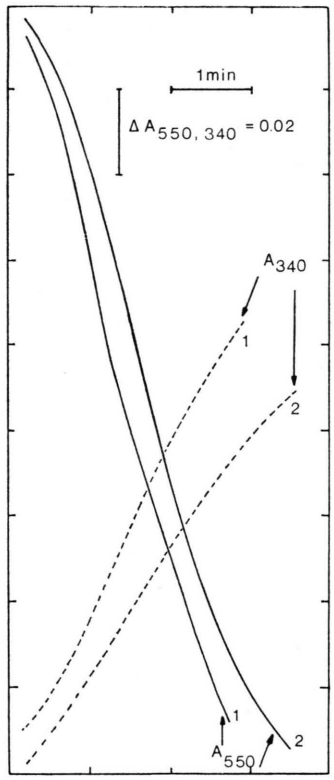

Fig. 2. ATP-dependent (traces 1) and ADP-dependent (traces 2) reduction of NAD^+ by sulfite coupled to the oxidation of cytochrome c, catalyzed by the light cell-free fraction S-144,000 from *Rps. palustris*. Similar results on the energy-dependent reduction of pyridine nucleotide were obtained with thiosulfate or ferro-cytochrome c as the electron donors. Likewise, inorganic pyrophosphate (PP_i) could serve as the energy source which results in similar absorption changes under the same experimental conditions. — The reactions were carried out in anaerobic Thunberg-type cuvettes. The Tris-buffered (0.05 M, pH 8.0) reaction mixture in a total volume of 3.0 ml contained 6.0 mg of S-144,000 cell-free protein. In addition, the main compartment of the sample cuvette contained 2 μmol NAD^+ plus 5 μmol ATP (or ADP, respectively). The control cuvette contained all the compounds except ATP (or ADP). The side arm of both cuvettes contained 20 μmol $SO_3^=$, 0.165 μmol mammalian cytochrome c, and 5 μmol $MgCl_2$. After evacuation, the contents of the side arm were tipped in to start the reaction. The absorption changes at 340 and 550 nm were measured simultaneously in a dual-wavelength split-beam recording spectrophotometer capable of recording absorption changes at two separate wavelengths at the same time in the same reaction mixture. — Experiments conducted with the same spectrophotometer were publishes previously [8, 15].

lysis of ADP can be viewed by chromatographic separation of the product AMP. Minor but distinct spots of ATP appear under similar conditions after 0.5 to 2.0 min following the start of ADP hydrolysis (Fig. 3). Further studies have to clarify whether or not adenylate kinase activities are involved.

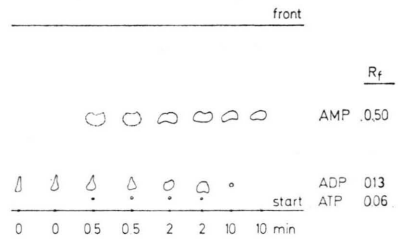

Fig. 3. Chromatographic analysis (qualitative) of the products of ATPase activity in the cell-free fraction S-144,000 from *Rps. palustris*. — Prior to the preparation of the chromatogram, the cell-free extract was allowed to perform ATPase activity at 30 °C for different time periods, as indicated. The reaction mixture in a total volume of 2.8 ml contained 6.0 mg of enzyme protein, 140 μmol Tris-HCl (pH 8.0), 30 μmol $MgCl_2$, and 10 μmol ADP. The samples were prepared in the ice and the reactions were terminated by adding 0.2 ml of TCA [20%]. After centrifugation, aliquots of 0.025 ml were taken from the clear supernatants and spotted on pre-coated TLC plates of PEI-cellulose F (Merck AG, Darmstadt, W.-Germany). The products of ATPase reactions were separated by chromatography in 0.8 M NaCl solution. Identification of the products was performed by R_F comparison to standards under UV-irradiation (254 nm).

Influence of electron donors on ATPase activity

The NADH-linked, aerobic electron flow is shown to be retarded in S-144,000 by the addition of energy-rich phosphates (Fig. 1; Table II). On the other hand, the ATPase reaction can be diminished by the admixture of NADH (Table IV). The addition of ferro-cytochrome c does not have an effect

Table IV. Effect of electron donors on ATPase activity in S-144,000. Experimental conditions were similar to those described in Table III. Where indicated above, 0.01 to 0.04 ml of a 4% solution of reduced mammalian cytochrome c were added. In these experiments, the numbers for nmoles P_i released have been corrected for the amount of P_i absorbed by the mammalian cytochrome.

Substrate	Electron donor added		$\left[\frac{\text{nmol } P_i \text{ formed}}{\text{mg protein} \times \text{min}}\right]$	[%]
ATP, 0.36 mM	— —		12.3	100
	NADH	(1 μmol)	7.0	57
	Cyt c·Fe^{2+}	(0.01 ml)	11.4	93
	Cyt c·Fe^{2+}	(0.02 ml)	10.9	89
	Cyt c·Fe^{2+}	(0.03 ml)	11.0	89
	Cyt c·Fe^{2+}	(0.04 ml)	10.7	87
	Ascorbate	(20 μmol)	16.3	133
ADP, 0.36 mM	— —		7.7	100
	NADH	(1 μmol)	3.4	44
	Cyt c·Fe^{2+}	(0.01 ml)	8.4	109
	Cyt c·Fe^{2+}	(0.02 ml)	7.7	100
	Cyt c·Fe^{2+}	(0.03 ml)	7.6	99
	Cyt c·Fe^{2+}	(0.04 ml)	7.7	100
	Ascorbate	(20 μmol)	11.4	148

on the ATPase turnover in S-144,000, whereas the ascorbate-treated enzymatic system reveals clearly an increased ATPase reaction rate (Table IV).

Cyanide, even in very high concentrations (9 mM), does not affect the ATPase reaction itself and also does not cause a significant effect on the NADH-inhibited as well as on the ferro-cytochrome c-treated (yet unaffected) ATPase turnover. However, precisely the amount of the ATPase reaction rate which is increased by the addition of ascorbate will be inhibited by cyanide (Table V).

Table V. Effect of cyanide on ATPase activity treated with respiratory substrates. The experimental conditions were the same as described in Table IV. The amount of electron donors used was 1 μmol NADH, 0.04 ml of 4% reduced mammalian cytochrome c, and 20 μmol of ascorbate. The protein content was 3.0 mg per reaction vessel.

Substrate	Electron donor added	Cyanide added [mM]	μmol P_i formed	[%]
ATP	— —	—	0.760	100
0.36 mM	NADH	—	0.384	51
	NADH	9.0	0.440	58
	Cyt c·Fe^{2+}	—	0.810	106
	Cyt c·Fe^{2+}	9.0	0.735	97
	Ascorbate	—	1.400	184
	Ascorbate	9.0	0.760	100

Furthermore, it is observed that the ATPase activity is not only inhibited by NADH but can be diminished equally well by NAD^+ (Table VI).

Table VI. Effect of pyridine nucleotide (NADH and NAD^+) on ATPase activity in S-144,000. Experimental conditions were similar to those given in Table V. 4.3 mg protein were inserted per preparation.

Experiment	Treatment	Conc. [μmol]	$\left[\frac{\text{nmol } P_i \text{ formed}}{\text{mg protein} \times \text{min}}\right]$	[%]
1	ATP	1		
	$MgCl_2$	10	7.3	100
2	ATP	1		
	$MgCl_2$	10		
	NADH	1	3.3	45
3	ATP	1		
	$MgCl_2$	10		
	NAD^+	1	3.8	52

Discussion

Rps. palustris develops an intracytoplasmic continuous membrane system that originates from and even remains connected to the cytoplasma membrane [6]. Electron micrographs reveal that after breaking the cells the crude cell-free extract contains particles of different size and form which are not closed completely [13]. Attempts to obtain photophosphorylation rates with chromatophore preparations from the bacterium by use of proton uptake experiments [19] were not successful. Likewise, experiments to study the atebrin fluorescence for measuring pH gradients [20] across chromatophore membranes reveal very low activities compared, *e.g.*, to preparations obtained from *Rps. spheroides*. These findings also support the view that membrane particles in cell-free extracts from *Rps. palustris* do not fold up perfectly to form closed vesicles. If the supernatant fraction S-144,000 again is centrifuged at 144,000 × *g* for about 12 h, a small residue is obtained. Using this portion, an alteration in the degree of 9-aminoacridine fluorescence by an induced pH gradient [20] results in small values that decline rapidly. Likewise, this observation indicates that, if vesicles are still present in S-144,000, then they must be leaking *, **. — Besides membrane fragments [12, 13], the supernatant fraction S-144,000 contains electron transport carriers.

However, the aerobic oxidation of all suitable substrates by *Rps. palustris* cell-free extracts (Table I) exhibits sensitivity towards ATP and other energy-rich phosphates. The electron flow is observed to be retarded by the concomitant hydrolysis of ATP, ADP, or PPi (Tables II, III; Fig. 1). It appears that cell-free preparations from the anaerobically grown photosynthetic bacterium catalyze preferentially the ATPase dependent reverse electron transport under anaerobic conditions against the thermodynamic gradient. The hydrolysis of the energy-rich phosphates that can not be avoided in the *in vitro* experiments with the supernatant fraction explains the failure to observe oxidative phosphorylation coupled to the oxidation of respiratory substrates in S-144,000.

The ATPase activities observable in cell-free extracts from the genus *Rhodopseudomonas* (Table III) are found to be low in comparison with reaction rates determined in other genera of photosynthetic bacteria [22]. Throughout the experiments reported here, a nonsaturating amount of ATP was supplied (0.36 mM ATP). It has been observed in other bacterial systems that ATPase reactions at

** Thanks are due to Eli Lilly GmbH, Bad Homburg, W.-Germany, for providing samples of Nigericin and Dianemycin in spite of extreme difficulties.

low ATP concentrations reveal interesting properties of the enzyme which would not have been noticed at high ATP levels [23].

The effectiveness of ADP and PP_i in addition to ATP as substrates for the ATPase system in S-144,000 (Table III) supports the observation that also the reduction of pyridinnucleotide catalyzed by the supernatant fraction in anaerobiosis is achieved by the addition of ATP, ADP, or PP_i as energy sources (Fig. 2).

Similar to ATP which is hydrolyzed to ADP and is completely hydrolyzed further to AMP, the action of ADP also appears to be due to its hydrolyzation (Fig. 3). However, the appearance of ATP besides AMP during the ADP reaction indicates the influence of an adenylate kinase. In studies with chloroplasts it has been observed that 1 ATPase binds 2 ADP which will be transphosphorylated into ATP and AMP [24].

On one hand ATP causes the retardation of NADH oxidation (Fig. 1), while on the other hand the ATPase turnover is decreased by the addition of NADH or even NAD^+ (Table VI). In this connection, it may be of interest to note that a NADH dehydrogenase from beef heart mitochondria is inhibited competitively by ATP [25].

Besides NADH (Tables IV – VI), the ATPase system can be influenced differently by other electron donors in S-144,000 which use oxygen as the acceptor, such as ascorbate or even dithiothreitol (Tables IV, V). Ascorbate stimulates the ATPase reaction rate as does dithiothreitol which also is known to stimulate the ATPase of spinach chloroplast CF_1 [26]. It is noticed that exactly the amount of ATPase turnover that is stimulated by ascorbate or dithiothreitol will be inhibited by the addition of cyanide in S-144,000 (Table V). Reducing agents which do not cause an electron flow accompanied by oxygen consumption in S-144,000, *e. g.*, dithionite, GSH, or BAL, will not induce an increased rate of the ATPase reaction, indicating that aerobic electron flow is involved in the stimulation of an ATPase activity.

Ferro-cytochrome c, which is a rather weak electron donor to couple with the aerobic oxidation chain present in S-144,000 (Table I), does not reveal an influence on the ATPase system (Tables IV, V). The primary electron donor under anaerobic conditions, *i. e.*, thiosulfate, reduces endogenous cytochrome of type c [8] in cell-free extracts from the bacterium. Thus, in anaerobiosis, the ATPase dependent reduction of pyridine nucleotide is initiated. It can be viewed to proceed with the concomitant oxidation of reduced cytochrome c [8] (Fig. 2).

In *Rps. palustris*, the generation of reducing power *via* reversal of electron transfer may be controlled by the diphosphopyridinnucleotide as indicated by the observation that added NAD^+ or NADH decreases the ATPase reaction rate. However, NADPH or $NADP^+$ causes a much less diminishing effect on the ATPase turnover. Likewise, the aerobic oxidation of NADPH is catalyzed by S-144,000 at about half the rate compared to NADH oxidation. Since transhydrogenase activity can be proved in *Rps. palustris* after photosynthetic growth on thiosulfate (unpublished), one can view rather the NADPH molecule to represent a trap for the reducing power generated in the bacterium.

The well-known and often described observation that respiration in photosynthetically grown Rhodospirillaceae is decreased in the light can be explained by the action of energy-rich phosphates. Since compounds such as ATP, ADP, or PP_i represent suitable energy sources for the reverse electron transport, the light-dependent decrease in the respiratory electron flow can be seen as an action of ATP, or even PP_i [27, 28], or of a common precursor of the energy-rich phosphates generated during the bacterial photophosphorylation.

The data presented in this communication on

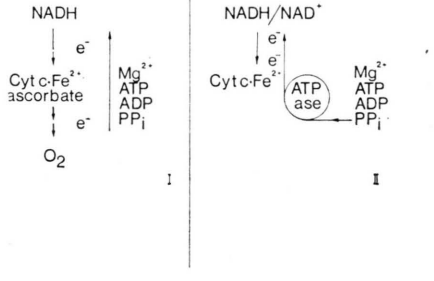

Fig. 4. Directions of electrons and energy transfer in S-144,000. The respiratory substrate initiated aerobic electron transport is retarded by the addition of energy-rich phosphates due to Mg^{2+}-stimulated ATPase efficiency. From the opposite point of view, the ATPase activity is inhibited by the admixture of NADH or NAD^+ (I). — The energy dependent reduction of NAD^+ occurs *via* reversed electron transport coupled to the oxidation of cytochrome c under anaerobic conditions. The ATPase system brings about the reversal of electron transfer involving reduced cytochrome c as the electron donor (II).

electron and energy transfer in S-144,000 are summarized in the scheme given in Fig. 4.

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