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REDOX CHAIN AND ENERGY TRANSDUCTION IN CHROMATOPHORES FROM *RHODOPSEUDOMONAS CAPSULATA* CELLS GROWN ANAEROBICALLY IN THE DARK ON GLUCOSE AND DIMETHYL SULFOXIDE

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Membranes from cells of *Rhodopseudomonas capsulata* grown anaerobically in the dark on glucose plus dimethyl sulfoxide differ from those obtained from photoheterotrophically grown cells in several ways: (a) there are qualitative and quantitative variations in the cytochrome composition; (b) electron-transport rates are unusually low in the cytochrome *b* to cytochrome *c* region; (c) light-induced ATP synthesis is dependent on the ability of the alternate respiratory pathway to maintain the Q_{10}-cytochrome *b* complex in a partially oxidized state; (d) a non-energy-conserving NADH-dehydrogenase activity dominates the respiratory activity. In addition, data obtained with both wild-type and mutant cells that contain altered electron-transport systems tend to exclude a role of the redox chain as ATP-producing machinery during anaerobic/dark growth.

Introduction

It has been reported [1] that the facultative photosynthetic bacterium, *Rhodopseudomonas capsulata*, can grow anaerobically in the dark only when an accessory oxidant such as DMSO is added. It was demonstrated that the role of DMSO in this growth mode is to function as an oxidant and the authors suggested two possible mechanisms for ATP production during anaerobic/dark metabolism of *Rps. capsulata*: anaerobic respiration or a fermentation process that requires an accessory oxidant to proceed. Fermentative growth of other species of Rhodospirillaceae requires no accessory oxidant [2]. Further studies on the capacity of *Rps. capsulata* to grow anaerobically in the dark have demonstrated that trimethylamine-*N*-oxide can also serve as the accessory oxidant and typical fermentation end products accumulate in the medium [3,4]. Reduction of both DMSO and trimethylamine-*N*-oxide by a wide range of microorganisms has been observed, but very little is known about the mechanism involved [5,6]. Cox et al. [7] have compared the roles of trimethylamine-*N*-oxide in the anaerobic phosphorylation catalyzed by a membrane-bound system in *Escherichia coli* with the soluble trimethylamine-*N*-oxide reductase activity found in *Rps. capsulata*. Gest [8] has discussed the concept of accessory oxidant-dependent fermentation in an evolutionary context.

In this paper we present a detailed analysis of the bioenergetics associated with chromatophores obtained from *Rps. capsulata* cells grown anaerobically in the dark on glucose and DMSO. In addi-

Abbreviations: DMSO, dimethyl sulfoxide; Q_1, ubiquinone-1; Q_{10}, ubiquinone-10; DCIP, dichlorophenolindophenol; TMPD, trimethylphenylenediamine; PMS, *N*-methylphenazonium methosulfate; BChl, bacteriochlorophyll; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

tion, three mutant strains with lesions in respiratory electron-transport flow have been examined. Their capacity to grow on glucose and DMSO confirms and extends previously reported data [1] indicating that the redox chain, as a whole, does not appear to play a fundamental role during anaerobic growth in the dark, but the alternate oxidase branch may serve to poise the chain at a functional redox state in anaerobically dark-grown cells encountering photophosphorylation conditions.

Materials and Methods

The bacterial strains used in this study are described in Table I.

A model of the respiratory and photosynthetic electron-transport pathways in *Rps. capsulata*, indicating the features altered in each mutant strain employed, is presented in Fig. 1.

Anaerobic/dark growth was in a malate minimal medium with DMSO (80 mM) and glucose (0.5%, w/v) as described in Ref. 1.

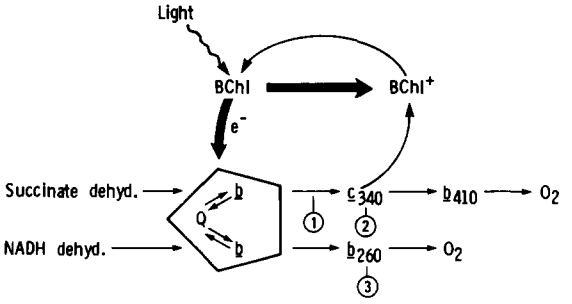

Fig. 1. A general model of the electron-transport pathways present in membranes of *Rps. capsulata*. The steps represented by arrows may include components in addition to those indicated. The Q-cytochrome *b* interactions, occurring within the pentagon in the diagram, await further clarification. Mutation *bps-11* causes a block in electron transport at point 1. Mutation *cytC113* blocks the synthesis of cytochrome c_{340}, point 2. Mutation *aer-512* blocks the synthesis of functional cytochrome b_{260}. Modified from Ref. 13. dehyd., dehydrogenase.

Photosynthetic cultures of MT1131 and ZM6 were grown in completely filled screw-capped bottles at 35°C and illuminated at a light intensity of about 30–40 W/m^2. The growth medium was the

TABLE I

RHODOPSEUDOMONAS CAPSULATA STRAINS

Aer$^+$, capable of respiratory growth; Aer$^-$, incapable of respiratory growth; Ars^r, arsenate resistant; green, accumulates nonaene carotenoids, like neurosporene; PS$^+$, capable of photosynthetic growth; PS$^-$, incapable of photosynthetic growth; Rif^r, rifampicin resistant.

Strain	Genotype	Phenotype	Comments and References
M50	*asr-1, aer-103*	Ars^r, Aer$^-$, PS$^+$	Derived from strain Z-1 [11] by NTG mutagenesis and penicillin selection against respiration
MT113	*rif-10, crtD121, cytC113*	Rif^r, green, PS$^-$, Aer$^+$	Cytochrome *c*-deficient; can respire [10].
MT1131	*rif-10, crtD121*	Rif^r, green, PS$^+$, Aer$^+$	Wild-type electron transport [10]
R126	*asr-1, crtD121, aer-103r11, bps-11*	Ars^r, green, PS$^-$, Aer$^+$	Blocked in electron transport between cytochrome *b* and cytochrome *c*; constructed by introducing the *crtD* marker from strain R121 [9] into strain Y11
SB1003	*rif-10*	Rif^r, PS$^+$	Wild-type electron transport [1]
Y11	*asr-1, aer-103r11 bps-11*	Ars^r, PS$^-$ Aer$^+$	Derived from strain M50 by NTG mutagenesis and selection for regain of respiration; wild-type carotenoids and bacteriochlorophyll
ZM6	*asr-1, crtD121, aer-412r20-512*	Asr^r, green PS$^+$	Alternate oxidase (cytochrome b_{260})-deficient; constructed by introducing the *crtD* marker from strain R121 [9] into strain M6 [13] via gene transfer agent

same as that described for anaerobic/dark cultures except that DMSO and glucose were omitted. Both anaerobically and photosynthetically grown cells were harvested in early stationary phase. Membrane fragments were prepared by disruption in a French pressure cell and differential centrifugation as previously described [11]. Redox titrations of cytochromes were performed at pH 7.0 in a medium containing a buffer system of Hepes, Mes, Tricine (30 mM each), according to the method of Dutton et al. [12]. Oxygen consumption, induced either by light or by respiratory substrates, was measured using a Yellow Springs model YSI 53 oxygen meter with a jacketed Gilson Oxygraph reaction chamber (modified to 1.6 ml volume). All measurements of light-induced oxygen uptake were performed as described by Zannoni et al. [13]. DCIP and horse-heart cytochrome *c* reduction were followed spectrophotometrically in the presence of 5 mM KCN [11].

Standard phosphorylation was assayed by measuring the incorporation of $^{32}P_i$ into glucose 6-phosphate according to Ref. 14. Photophosphorylation activities at controlled mid-point potential were carried out under strict anaerobic conditions following essentially the method described by Baccarini-Melandri et al. [15]. Measurements of fluorescence were made at 90° with a filter fluorimeter [16].

Proteins were assayed by using the method of Lowry et al. [17], and bacteriochlorophyll was measured spectrophotometrically at 775 nm in acetone/methanol (7 : 2, v/v) extracts using an extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ [18].

Bacteria were prepared for electron microscopy by glutaraldehyde fixation, osmium tetroxide post-fixation, embedding in Epon Araldite and sectioning.

Results

Membrane morphology

Earlier studies showed that normal photosynthetic pigments accumulate in cells grown anaerobically in darkness [1,2]. Thin sections of *Rps. capsulata* cells grown anaerobically in darkness in the presence of DMSO show an extensive intracytoplasmic membrane system like that of photosynthetically grown cells (data not shown). The only morphological difference noted is a marked thickening of the cell envelope in cells grown anaerobically in the dark compared to photosynthetically grown cells.

Cytochrome content

Oxidoreduction titrations performed on membrane vesicles from *Rps. capsulata* cells grown anaerobically in the light, i.e., photosynthetically, have resolved at least four cytochromes: b_{60}, b_{25}, c_{340} and c_{120} [19,20], plus cytochrome b_{150}, which has been detected in both photosynthetic and aerobic chromatophores of *Rps. capsulata* [21,22]. Cytochromes c_{340} and b_{60} have been shown to be involved in cyclic electron flow induced by light [19,20], while the characteristics and the roles of cytochromes c_{120}, b_{150} and b_{25} are still not understood (for a discussion see Ref. 20). Two *b* type cytochromes of high mid-point potential ($E_{m,7.0} = 410$ and 270 mV) have also been detected in membrane vesicles from aerobic cells. The latter are difficult to detect in photosynthetically grown cells because of their low concentrations [23]. Fig. 2 shows the redox titration of chromatophores from anaerobically/dark-grown cells of *Rps. capsulata* MT1131 monitored at 561 – 570 nm (pH 7.0). It is evident that only two main components are present, one with an $E_{m,7.0}$ value of about +144 ± 10 and the other of about 0 ± 10 mV. The first one may be analogous to cytochrome b_{150} of photosynthetic and aerobic membranes while the redox carrier at 0 mV is not clearly analogous to any of the *b* type cytochromes so far detected in membrane vesicles from *Rps. capsulata.* In addition, the relative amount of cytochrome b_0 (0.92 ± 0.05 nmol/mg protein) is much higher than the total *b* type complement contained

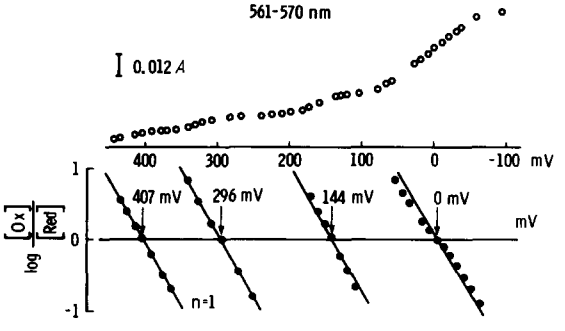

Fig. 2. Oxidoreduction titration of cytochromes *b* (561–570 nm) in membranes from anaerobically/dark-grown cells of *Rps. capsulata* MT1131. The measurement was performed as detailed in Materials and Methods. The protein concentration in the assay was 3.2 mg/ml.

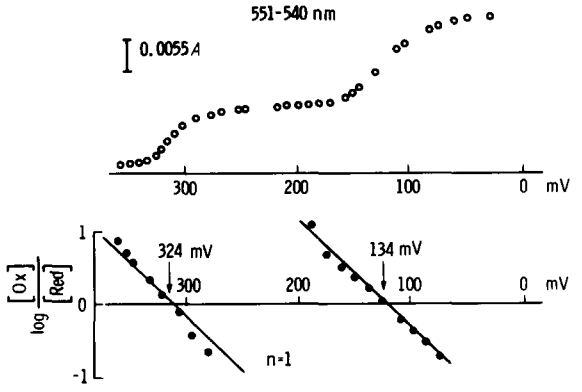

Fig. 3. Oxidoreduction titration of cytochromes *c* (551–540 nm) in membranes from anaerobically/dark-grown cells of *Rps. capsulata* MT1131. The assay contained 3.5 mg of proteins per ml.

in photosynthetic membranes (0.35 ± 0.06 nmol/mg protein).

The two high-potential cytochromes with $E_{m,7.0}$ values of 407 ± 5 and 296 ± 5 mV represent the two oxidases characteristic of the respiratory electron-transport chain of *Rps. capsulata*. As expected for chromatophores obtained from anaerobically grown cells, their absorption signal is very low.

The redox analysis performed at 551 – 540 nm shows the presence of two *c* type cytochromes ($E_{m,7.0}$ of 324 ± 10 and 134 ± 10 mV) with concentrations of 0.12 ± 0.05 and 0.15 ± 0.05 nmol/mg protein, respectively (Fig. 3). These values indicate that in DMSO chromatophores, cytochrome c_{324} represents less than 50% of the total *c* type content, in contrast with photosynthetic membranes which generally contain about 0.36 nmol/mg protein cytochrome c_{340} and 0.06 nmol/mg protein cytochrome c_{150}.

Respiratory activities

Functional differences between membranes from cells grown anaerobically in the dark on glucose and DMSO and those grown photosynthetically are represented by measurements of electron-transport rates coupled to substrate oxidation (Table II). As previously demonstrated [24,25], both aerobic and photosynthetic membrane particles from *Rps. capsulata* cells contain a branched respiratory apparatus with two oxidases differing in their redox potentials

TABLE II

RESPIRATORY ACTIVITIES IN CHROMATOPHORES FROM PHOTOSYNTHETIC AND ANAEROBICALLY DARK-GROWN CELLS OF *RPS. CAPSULATA* MT1131

The measurements were performed as described in Materials and Methods. The concentrations of the various electron acceptors used were: equine cytochrome *c*, 0.03 mM; DCIP, 0.2 mM. Activities expressed as μequiv. of electron acceptor reduced/h per mg of protein. L.D.O.U., light-driven oxygen uptake. cyt. *c*, cytochrome *c*; ant A, antimycin A.

Substrates (inhibitors)	Electron acceptor	Membrane activities	
		Photosynthetically grown	Anaerobically/dark-grown
NADH	O_2	3.81	1.60
NADH (5 μM ant A)	O_2	1.95	1.41
NADH (50 μM KCN)	O_2	2.1	1.50
Succinate	O_2	0.45	0.65
Succinate (5 μM ant A)	O_2	0.27	0.52
Succinate (50 μM KCN)	O_2	0.39	0.62
NADH	DCIP	2.10 *	2.0 *
Succinate	DCIP	0.80 *	0.7 *
NADH	cyt. *c*	1.50 *	0.58 *
NADH (5 μM ant A)	cyt. *c*.	0.30 *	0.24 *
Succinate	cyt. *c*	1.10 *	0.35 *
Succinate (5 μM ant A)	cyt. *c*	0.13 *	0.14 *
Ascorbate-TMPD	O_2	4.70	4.70
Ascorbate-TMPD (50 μM KCN)	O_2	0.2	0.2
L.D.O.U.	O_2	1.80	1.60
L.D.O.U. (5 μM ant A)	O_2	2.50	1.60

* Activities measured in the presence of 5 mM KCN.

(cytochromes b_{410} and b_{260}) and their sensitivities to KCN and CO. Each of these two *b* type oxidases belongs to one of the two branches which diverge from the Q-low potential-cytochrome *b* region of the chain. Since cytochrome b_{410} is responsible for cytochrome *c* oxidase activity, which is sensitive to low KCN levels (50 μM), and cytochrome b_{260} functions in the oxygen-consumption pathway inhibited only by high concentrations of cyanide

(5 mM), NADH oxidation insensitive to low KCN levels is a measure of the respiration going through the cytochrome b_{260}-containing pathway. NADH oxidase activity is 60–70% lower in the membranes from anaerobically/dark-grown MT1131 cells than from those grown photosynthetically (Table II). On the other hand, NADH oxidation in anaerobically/dark-grown membranes is practically insensitive to low KCN (50 μM) or antimycin A (5 μM) concentrations. NADH dehydrogenase-dependent electron flow, measured as DCIP reduction with 5 mM KCN added, is present at the same values in both types of chromatophores. Therefore, it may be concluded that the reduced rate of NADH dehydrogenase-dependent electron flow in anaerobically/dark-grown membranes is due to the diminished capacity of the cytochrome *c* oxidase-containing pathway. This assertion is strongly supported by the fact that the antimycin A-sensitive NADH-cytochrome *c* reductase activity in DMSO chromatophores is only 28% of that measured in photosynthetic membranes. Furthermore, the presence of a normal cytochrome *c* oxidase, i.e., ascorbate-TMPD oxidation, in anaerobic/dark membrane vesicles seems to localize the rate-limiting step of NADH respiration to the Q-low potential-cytochrome *b*-cytochrome *c* region of the chain. This conclusion is in agreement with a lack of stimulation induced by antimycin A in light-driven oxygen uptake, as shown in Table I. Under aerobic lighted assay conditions, two electron-transport pathways operate, one involving cyclic, the other noncyclic electron flow [13]. They branch at the Q-cytochrome *b* pool level, and in photosynthetic membranes antimycin A (5 μM) enhances light-driven oxygen reduction by interrupting the cyclic electron flow. Furthermore, oxygen reduction driven by light in anaerobically/dark-grown membranes suggests that in this type of chromatophore the photosynthetic apparatus, i.e., light-harvesting systems and reaction center bacteriochlorophyll, operates normally and that respiratory chain and photosynthetic components are connected as already demonstrated for photosynthetically and semiaerobically grown membranes of *Rps. capsulata* [13].

Light-driven energy transduction

Membranes from anaerobically/dark-grown cells of *Rps. capsulata* are able to maintain a high-energy state. We have used the quenching of 9-aminoacridine fluorescence [26,27] as a measure of proton gradient formation induced either by light or ATP hydrolysis in photosynthetically and anaerobically/dark-grown chromatophores.

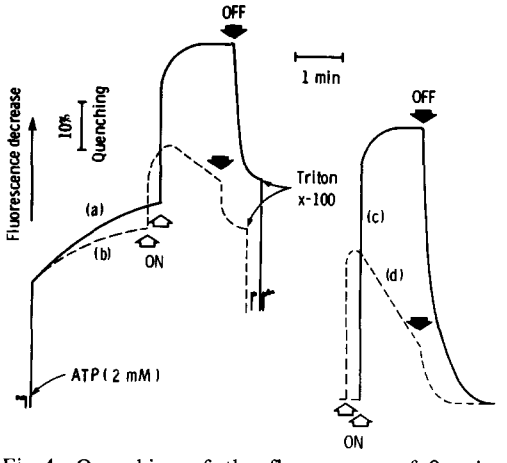

Fig. 4. Quenching of the fluorescence of 9-aminoacridine induced either by light or ATP hydrolysis in membranes from MT1131. The assay contained in a final volume of 2.5 ml: 60 mM glycylglycine (pH 8.0); 7 mM P_i; 10 mM $MgCl_2$; 50 mM KCl; 1.6 μg/ml valinomycin; 4 μM 9-aminoacridine. Both photosynthetic and DMSO chromatophores correspond to 14 μg of bacteriochlorophyll per ml. The following additions were made: ATP (2 mM); Triton X-100 (0.04%). (a and c) photosynthetic membranes, (b and d) anaerobic/dark membranes.

Fig. 4 shows that the addition of 2 mM ATP causes a decrease of the fluorescence due to a non-enzymatic interaction between the amine and ATP itself, followed by a slow decrease in fluorescence which reaches a steady-state value after 2–3 min. In both types of chromatophores actinic light causes a new level of energization greater than that which ATP alone could support. However, it is evident that this higher value cannot be maintained at a steady level by anaerobically/dark-grown chromatophores, unlike photosynthetically grown particles. This phenomenon is more striking when actinic light alone is used to create the high-energy state (curves c and d). In this case, fluorescence quenching induced by ATP hydrolysis does not mask the inability of light to maintain the proton gradient at the initial high level. This effect might be due either to a light-

induced instability of the photosynthetic apparatus present in DMSO chromatophores or to the relatively slow electron flow rate between cytochrome *b* and cytochrome *c* shown by this type of membrane (Table II). After many turnovers of light-induced electron flow under steady-state conditions of illumination, an inadequate flow rate in the cytochrome *b*-cytochrome *c* region could result in a redox state of the chain inhibitory to cyclic electron flow coupled to the proton pump. Alternatively, the possibility that membranes from anaerobically/dark-grown cells are leaky for protons could be another explanation for the lack of high-energy steady level in this type of chromatophore. We have sought to determine which one of these hypotheses may be accepted by measuring the photophosphorylation activities under different experimental conditions: (1) with an artificial dye present, (ii) in aerobiosis and (iii) in anaerobiosis.

Table III shows that the rate of light-induced ATP synthesis in chromatophores from anaerobically/dark-grown cells, which normally is about 15–16% of the rate obtained with photosynthetic membranes, can reach about 45% of that of photosynthetic chromatophores when PMS (0.5 mM) is added. In this connection, it has been recently demonstrated that a high concentration of PMS is able to induce an appreciable restoration of cyclic photophosphorylation in chromatophores treated with antimycin A (5 μM) [15]. In our experiments, the PMS stimulation of the light-induced ATP synthesis in anaerobically/dark-grown chromatophores may involve the formation of an artificial electron flow bypassing the rate-limiting step of the chain at the cytochrome *b*-cytochrome *c* level. The high value of photophosphorylation obtained with PMS suggests that dark-grown chromatophores are able to maintain a proton gradient. From Table III it is also evident that after a period of aerobic preillumination (5 min) the rate of ATP synthesis in both types of membrane is repressed to the same extent. Since this inhibitory effect induced by light seems to be related to a conformational change in the ATPase structure under lighted conditions in photosynthetically grown membranes [28], it is likely that anaerobically/dark-grown membranes show sensitivity to aerobic preillumination for the same reason.

TABLE III

PHOTOPHOSPHORYLATION ACTIVITIES IN MEMBRANE VESICLES FROM PHOTOSYNTHETIC AND ANAEROBICALLY/DARK-GROWN CELLS OF *RPS. CAPSULATA* MT1131

ATP formed expressed in μmol/h per mg BChl. To obtain anaerobiosis the buffer was flushed with pure nitrogen for 1 h, then transferred to the cuvette and flushed continuously during the experiment. Very similar results were obtained replacing the nitrogen with 5 mM KCN, which inhibits both terminal oxidases.

Experimental conditions	ATP formed	
	Photosynthetically grown membranes	Anaerobically/dark-grown membranes
Aerobiosis	281	49
Aerobiosis + 0.5 mM PMS	429	192
Preillumination aerobiosis	142	23
Preillumination anaerobiosis	147	3

In constrast to the behavior of photosynthetically grown membranes, however, anaerobically/dark-grown membranes show a marked decrease in photophosphorylation activity when the preillumination is conducted anaerobically (Table III). This finding suggests that in anaerobically/dark-grown chromatophores, the presence of oxygen is essential for maintaining an ambient redox potential able to support photophosphorylation activity.

The function of the cytochrome b_{260}-containing pathway during light-induced ATP synthesis in anaerobically/dark-grown chromatophores

As previously reported, a redox chain in which Q_{10} and the low mid-point potential *b* type cytochromes are completely oxidized while cytochrome c_{340} is reduced represents the best redox state for the cyclic, light-induced electron flow [29]. During steady-state illumination in anaerobiosis, the Q_{10}-cytochrome *b* region could become excessively reduced in anaerobically/dark-grown chromatophores because of the partial block between cytochrome *b* and cytochrome *c*. It seemed reasonable to expect the alternative pathway of oxygen consumption that has been recently shown to participate in light-

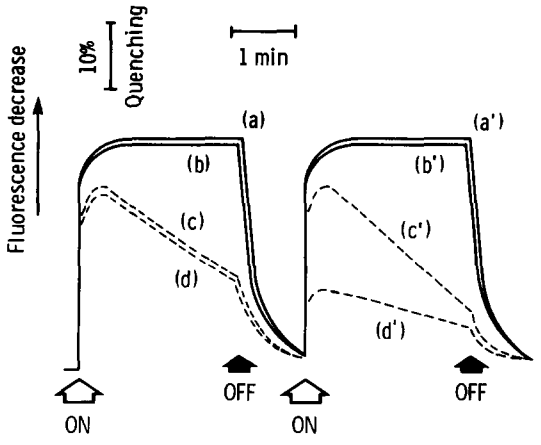

Fig. 5. Quenching of the fluorescence of 9-aminoacridine induced by two sequential periods of light in membrane chromatophores from photosynthetic and anaerobically/dark-grown cells of *Rps. capsulata* MT1131 and ZM6. Conditions as in Fig. 4 except for the amount of membranes added (about 24 μg/ml of BChl). Traces: continuous line corresponds to photosynthetic membranes from MT1131 (a) (a') and ZM6 (b) (b') strains; dashed line correspond to anaerobic/dark chromatophores from MT1131 (c) (c') and ZM6 (d) (d') cells. The samples are exposed to atmospheric oxygen throughout.

induced oxygen uptake [13] to keep the Q_{10}-cytochrome *b* pool in a partially oxidized state under aerobic/lighted conditions.

To test this possibility, we have examined the light-induced proton gradient in membrane fragments from *Rps. capsulata* ZM6, a strain lacking cytochrome b_{260}, the alternate oxidase.

The traces in Fig. 5 represent 5-aminoacridine quenching measured in photosynthetic and anaerobically dark-grown chromatophores from *Rps. capsulata* ZM6 and wild-type cells during two sequential illumination periods in aerobic suspensions. Membranes from both mutant and wild-type cells show the same behavior during the first illumination period. As shown above, membranes from anaerobically/dark-grown cells are unable to achieve the high and steady level of light-induced fluorescence quenching characteristic of photosynthetically grown membranes. A second period of illumination reveals a dramatic difference between the mutant and wild-type membranes. While anaerobically/dark-grown wild-type membranes are able to recover during the intervening aerobic/dark period, so that they again show a strong initial response to illumination, anaerobically/dark-grown membranes from ZM6 rapidly resume their downward trend upon re-illumination. These results are in excellent agreement with the data on photophosphorylation given in Table III showing that under anaerobic/lighted experimental conditions, anaerobically/dark-grown membranes from wild-type cells synthesize ATP at a very low rate. Therefore, the alternative oxidase pathway in membrane fragments from *Rps. capsulata* cells grown anaerobically in the dark seems to operate as a redox valve for the Q_{10}-cytochrome *b* pool. The necessity of a proper redox poise for optimal turnover of cyclic electron flow has been described by Bose and Gest [30] and later reported for photophosphorylation in chromatophores of *Rhodospirillum rubrum, Rps. sphaeroides* and *Rps. capsulata* [15,31,32].

To study whether the low rate of light-induced ATP synthesis of anaerobically/dark-grown chromatophores may depend on the ambient redox potential, we have assayed photophosphorylation under controlled redox conditions in the presence of artificial redox mediators. Fig. 6 shows the E_h dependence of photophosphorylation in chromatophores from anaerobic/dark membranes of *Rps.*

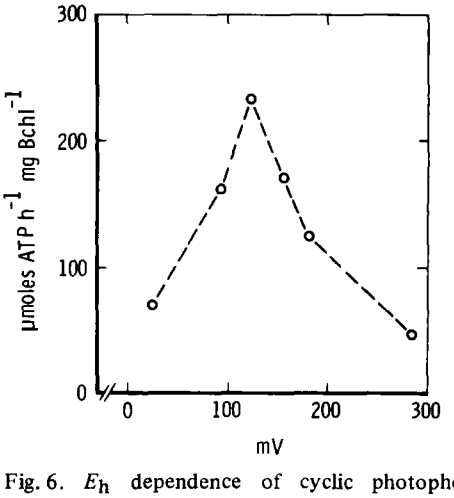

Fig. 6. E_h dependence of cyclic photophosphorylation in anaerobic/dark chromatophores from *R. capsulata* MT1131. The rate of light-induced ATP synthesis assayed without added redox mediators under anaerobic conditions, using the standard method, was about 10 μmol of ATP synthesized/h per mg BChl.

capsulata MT1131. The optimum for activity with very low concentrations of redox mediators added (5 μM each) lies between 100 and 150 mV. It has been recently shown that redox dyes (PMS, TMPD) are able to overcome the inhibition by antimycin A of light-induced electron flow [15]. However, in the case of antimycin A-treated membranes, relatively high concentrations of redox dyes are necessary to provide an artificial electron flow bypassing the antimycin A block, while in anaerobically/dark-grown chromatophores the endogenous light-induced electron flow alone seems to support a high rate of ATP synthesis when the system is poised around the optimal E_h by much lower concentrations of redox mediators.

Energy conservation driven by respiration

As previously reported, membrane fragments from aerobically grown cells or *Rps. capsulata* exhibit respiratory electron transport coupled to ATP synthesis [33]. Although the P : O ratios obtained are invariably low (0.45 for NADH and 0.15 for succinate oxidation), we have been able to compare the efficiency of the energy conservation in photoheterotrophically and anaerobically/dark-grown chromatophores of *R. capsulata*, which perform low-level respiration (Table IV). Both types of membrane are able to couple respiratory electron flow to energy transduction; however, a most striking difference between the two types of chromatophores was observed in the effect of antimycin A on NADH-ubiquinone-1 reductase-dependent phosphorylation. While the reduction of Q_1 in both cases is partially inhibited by antimycin A, the ATP synthesis associated with this activity is much more depressed in membranes from anaerobically/dark-grown cells than photosynthetic ones. Since the phosphorylation coupled to Q_1 reduction in the presence of antimycin A can be assumed to measure ATP synthesis at the first site, it is evident from these data that in anaerobically/dark-grown chromatophores some mechanism bypassing the energy conservation in this site operates.

To examine the nature of the respiratory metabolism during anaerobic/dark growth on glucose and DMSO, two mutants of *Rps. capsulata* (strains R126 and MT113) were tested for their ability to grow in this mode. They both lack NADH-cytochrome *c* reductase, MT113 being deficient in cytochrome c_2, while R126 is blocked somewhere in the redox chain between Q_{10} and cytochrome c_2. These mutants have been shown to be capable of growing anaerobically in the dark, and therefore, neither the energy conservation nor the electron-transport flow associated with the Q_{10}-cytochrome *b*-cytochrome *c* oxidoreductase system seems to be directly

TABLE IV

NADH-DEPENDENT OXIDATIVE PHOSPHORYLATION BY PHOTOSYNTHETIC AND ANAEROBICALLY/DARK-GROWN MEMBRANES FROM *RPS. CAPSULATA* MT1131 CELLS

The measurements of NADH-ubiquinone-1 reductase activities and linked phosphorylations were performed as follows: the reaction mixture containing all the components, except NADH, carrier-free ^{32}P and Q_1, was made anaerobic by flushing with nitrogen as described for Table III. In order to avoid any incorporation of ^{32}P due to oxidation of NADH by traces of oxygen still present in the reaction, NADH was added first to the measuring cuvette followed by the addition of carrier-free ^{32}P. The reaction was started by adding Q_1 (0.1 mM) and allowed to proceed for 3–4 min. Particles were suspended at about 0.5–1 mg of proteins per ml. The measurements in the presence of oligomycin or in the absence of substrate were taken as background. ATP formed and substrate oxidized are expressed in μmol/h per mg protein. ant A, antimycin A.

Respiratory activities	Photosynthetically grown membranes			Anaerobically/dark-grown membranes		
	Substrate oxidized	ATP formed	P/2e	Substrate oxidized	ATP formed	P/2e
NADH-oxidase	4.1	1.3	0.31	1.8	0.28	0.15
NADH-Q_1 reductase	6.5	1.0	0.15	5.0	0.51	0.10
NADH-Q_1 (5 μM ant A) reductase	4.57	0.59	0.13	2.91	0.05	0.018

TABLE V

NADH-DEPENDENT OXIDATIVE PHOSPHORYLATION BY ANAEROBICALLY/DARK-GROWN MEMBRANES FROM *RPS. CAPSULATA* MT113 AND R126 CELLS

Conditions as described for Table III. ATP formed and substrate oxidized are expressed in μmol/h per mg protein. ant A, antimycin A.

Respiratory activities	MT113 memranes			R126 membranes		
	Substrate oxidized	ATP formed	P/2e	Substrate oxidized	ATP formed	P/2e
NADH-oxidase	2.4	0.3	0.12	2.3	0.22	0.09
NADH-ubiquinone-1 reductase	7.6	0.75	0.098	6.1	0.08	0.013
NADH-ubiquinone-1 (5 μM ant A) reductase	3.6	0.12	0.033	5.9	0.04	0.00

involved in the anaerobic/dark metabolism. In addition, chromatophores from both wild-type and mutant cells contain a non-energy-conserving NADH-dehydrogenase activity (Table V). This finding confirms the data reported above for wild-type membranes and indicates that while the nonenergy-conserving nature of ubiquinone-1 reductase activity excludes a priori a role of the first site of phosphorylation during anaerobic/dark metabolism, it does not eliminate the possibility that electron flow at the NADH-dehydrogenase level might be involved in this type of growth.

Discussion

The cytochrome content of membrane fragments from cells grown anaerobically in the dark with DMSO differs from that of photosynthetically grown membranes. The total amount of *b* type cytochromes in anaerobically/dark-grown membranes is 3-times higher while the cytochrome c_2 concentration in less than half of that in photosynthetically grown membranes. Since we do not have an accurate molar extinction coefficient for the *b* type cytochromes in the anaerobically/dark-grown membranes, and since the wavelength of maximum absorbance of some of these cytochromes is shifted several nanometers (data not shown) from the 561 nm used in our titrations, a precise description of the cytochrome *b* composition is not possible at this time.

Membrane vesicles from anaerobically/dark-grown cells contain a branched chain showing a rate-limiting step of respiration between the antimycin A-sensitive site and cytochrome c_2. This block, which could be related to the abnormal cytochrome *b* and *c* content, seems to affect light-dependent ATP synthesis. Anaerobically/dark-grown chromatophores show a low level of photophosphorylation, which is easily enhanced by adding an artificial electron carrier (PMS) bypassing the cytochrome *b*-cytochrome *c* region of the chain.

The inability of anaerobically/dark-grown chromatophores to support a high level of light-induced electron flow is most evident when photophosphorylation activites are measured under anaerobic conditions or in a mutant lacking the alternate oxidase. To explain this phenomenon, we propose that in anaerobically/dark-grown membranes, light-driven reduction of the Q-cytochrome *b* region of the electron-transport chain is not properly balanced by oxidation, and that this results in a decreased rate of proton pumping, even though a steep electrochemical gradient exists. We speculate that this might be the result of a shift in the chemistry of a 'Q-cycle', which requires oxidized Q for proton pumping in some formulations [34]. This inhibitory effect is less pronounced when oxygen is present because it acts as an acceptor of electrons from the Q-cytochrome *b* region via the cytochrome b_{260}-containing pathway. This conclusion is supported by the results obtained using chromatophores from *Rps. capsulata* ZM6, a strain lacking the alternative pathway of oxygen consumption, in which the presence of

oxygen does not relieve the light-induced inhibition of proton pumping. It has been shown previously that the redox states of the components of the photosynthetic system depend on the degree of aeration of cells [35] and more recently this hypothesis has been proposed to explain the lack of photoheterotrophic growth of a mutant of *R. rubrum* (F11) defective in photooxidative activity [36]. We have been able to show a high rate of light-induced ATP synthesis by anaerobically/dark-grown chromatophores by maintaining the system under controlled redox conditions. The E_h optimum of 120 mV reported corresponds to a redox chain in which Q_{10} and the low mid-point potential *b* type cytochromes are largely oxidized while cytochrome c_2 is reduced. These redox conditions represent the best state for the photophosphorylation and are in agreement with recently reported data for photosynthetically grown membranes of *Rps. capsulata* [15], however, our findings indicate that membranes from anaerobically/dark-grown cells cannot maintain that redox state as well as photosynthetically grown membranes can.

The necessity of a redox chain for anaerobic/dark growth on glucose and DMSO has been analyzed by means of two respiratory and photosynthetic mutants, R126 and MT113. Each is unable to grow photosynthetically because of the lack of cytochrome c_2 in the latter strain and a block of the electron flow between cytochrome *b* and cytochrome *c* in the former. These mutants grow under anaerobic/dark conditions with DMSO and therefore, the requirement of the electron transport chain at the cytochrome *b*-cytochrome *c* level does not seem to be essential for this mode of growth. However, the finding that anaerobically/dark-grown chromatophores from wild-type and mutant strains mentioned above have an active, nonenergy-conserving NADH dehydrogenase tends to suggest that although the complete respiratory electron-transport chain is not required for anaerobic/dark growth, membrane-bound NADH dehydrogenase activity may be involved in the reduction of DMSO. This activity may be catalyzed by the normal respiratory NADH dehydrogenase or by another enzyme catalyzing a similar reaction. An earlier study found that *Rps. capsulata* mutant M1, deficient in NADH dehydrogenase activity when grown photosynthetically, could grow anaerobically in the dark with DMSO [1]. Anaerobically/dark-grown cells of M1 show a parallel deficiency in NADH-ubiquinone-1 reductase activity (data not shown). Furthermore, the uncoupled NADH dehydrogenase activity observed in anaerobically/dark-grown wild-type membranes is inhibited by rotenone to the same extent as that in photosynthetically grown membranes (about 75% inhibition by 5 μM rotenone). Taken together these results suggest that the NADH dehydrogenase activity in anaerobically/dark-grown membranes is catalyzed by the normal respiratory enzyme, but further studies clearly are necessary.

Recently, electron transfer from NADH to trimethylamine-*N*-oxide has been demonstrated in cell-free extracts of *Rps. capsulata* grown anaerobically in the dark on fructose and trimethylamine-*N*-oxide. Trimethylamine-*N*-oxide reduction is relatively insensitive to rotenone and 2-(*n*-heptyl)-6-hydroxyquinoline-*N*-oxide and is entirely catalyzed by soluble components of cell extracts [7,37]. It is possible that dimethylsulfoxide reductase involves the membrane-bound, uncoupled NADH dehydrogenase activity that we described above, in which case trimethylamine-*N*-oxide- and DMSO-dependent anaerobic/dark growth might be analogous to each other, but not catalyzed by the same enzymes. A report from Ruben and Calvin [38] showing that in *Rps. sphaeroides* in the presence of DMSO a *b* type cytochrome undergoes rapid oxidation suggests that this may be the case, however, all attempts to obtain an NADH-dependent dimethylsulfoxide reductase activity in vitro have been unsuccessful for *Rps. capsulata* chromatophores.

In summary, the current study has shown that the membrane-bound electron-transport system of *Rps. capsulata* grown anaerobically in darkness is quantitatively, and probably qualitatively, different from that of photosynthetically grown membranes. Flux in the cytochrome *b*-cytochrome *c* region is relatively restricted, resulting in an inability to sustain cyclic electron flow-driven proton pumping or ATP synthesis without alternative routes of oxidation for the Q-cytochrome *b* level of the system. This suggests a physiological role for the alternate oxidase found in these and other photosynthetic bacteria, namely the maintenance of a favorable redox poise for photophosphorylation. The current

studies also show that while energy-conserving electron-transport components of the membrane are not essential for anaerobic/dark growth, a strong, uncoupled, membrane-bound NADH dehydrogenase activity is demonstrable in these cells. Further studies will be necessary to determine whether that dehydrogenase or other membrane-bound components are part of the dimethylsulfoxide reductase system.

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