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Composition and activity of the photosynthetic system of *Rhodopseudomonas capsulata*. The physiological role of the B800–850 light-harvesting complex

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Cells of *Rhodopseudomonas capsulata* 37b4 (wild-type) and NK3 (mutant lacking the B800–850 light-harvesting complex) were grown phototrophically in a turbidostat under high- and low-light conditions. Low-light cells of both strains showed higher specific reaction center content (related to cell protein) than high-light cells. Specific H^+-ATPase activities were the same in all cells tested. We conclude that the ratio of H^+-ATPase to reaction centers is altered following changes in the light conditions. The irradiance needed for half-maximal photophosphorylation (apparent K_m for light) was estimated. Membranes derived from low-light grown NK3 cells showed a significantly smaller K_m than membranes derived from high-light grown NK3 cells although the size of their photosynthetic units (mol BChl / mol reaction center) under both culture conditions were identical. Under low irradiances the K_m for light of wild-type cells was 3–4 times smaller than the one of NK3 cells. Apparently, the presence of the B800–850 complex decreased the K_m for light in wild-type cells thus allowing growth under lower irradiances.

Introduction

The adaptation of phototrophically growing *Rhodopseudomonas capsulata* to different light conditions comprises a differentiation of the intracytoplasmic membrane system [1], variations in the size of the photosynthetic unit (mol BChl/mol reaction center; Ref. 2) and a change in specific reaction center content [3,4].

It is now well established that the two light-harvesting complexes, B870 and B800–850, found in *Rps. capsulata* are coded by different regions of the genome thus being regulated independently [5].

The total membrane area (intracytoplasmic plus cytoplasmic membrane area) was nearly doubled when high-light adapted *Rps. capsulata* cells were transferred to low-light conditions [3,4].

Why do the cells take the metabolic expense of synthesizing such large amounts of membranes, BChl, and proteins [6]? What is the advantage of two different light-harvesting complexes when in some purple bacteria apparently one complex is sufficient (i.e., *Rhodospirillum rubrum*)?

To approach these questions, we used a mutant of *Rps. capsulata* lacking the B800–850 light-harvesting complex which was able to grow under high- and low-light conditions. By means of this mutant we investigated if there exists a mechanism altering electron-transport rates under different growth conditions which could explain the changes in light requirement of photophosphorylation observed.

Abbreviations: P_i, inorganic phosphate; BChl, bacteriochlorophyll; Cyt, cytochrome.

It was reported that the mutant *Rps. capsulata* A1a$^+$ (B800–850$^-$, carotenoid$^-$) showed faster kinetics in the reduction of Cyt c_2 compared to *Rps. sphaeroides* Ga which was attributed to a change in the electron-transport rate in the absence of the B800–850 light-harvesting complex [7].

Materials and Methods

Cells of *Rhodopseudomonas capsulata* 37b4 (German collection of microorganisms, Göttingen, DSM 938) and NK3 [5] were grown in a turbidostat as described earlier [4]. Transition from high-light conditions (1 × 200 W central lamp, Osram, plus 6 × 300 W peripheral lamps, Osram concentra) to low-light conditions (central lamp only – 40 W for 37b4, 75 W for NK3 culture) was performed after 12 h continuous culture at constant cell density.

Membranes were isolated by disrupting the cells by one passage through a french press. Care was taken to remove all cell-wall fragments in the following centrifugation (20 min, 30000 × g). The supernatant was concentrated by one ultracentrifugation step (90 min, 200000 × g). Further washing of the membranes was avoided in order not to loose photophosphorylation activity. No effort was made to separate cytoplasmic and intracytoplasmic membranes.

The other analytical and preparative methods used were the same as described earlier [4].

Photophosphorylation was measured in a medium of 100 mM glycylglycine buffer (pH 7.75)/10 mM magnesium acetate/0.7 mM KH_2PO_4/0.1% bovine serum albumin/0.05 mM ADP/0.25 mM AMP/1 unit per ml of myokinase (Sigma)/50 mM KCN/15 μM phenylmethylsulfonyl fluoride/0.4 mM dithioerithritol/150 μM luciferin/20 μg per ml luciferase (last three reagents from Boehringer, Mannheim). Membranes in general were added corresponding to 0.5 μM BChl; 37b4 low-light membranes were used with 2 μM BChl to obtain activities comparable to the other samples. The cuvette was irradiated with continuous monochromatic light ($\lambda = 846$ nm, NAL 846, or $\lambda = 872$ nm, IL 872 Schott, Mainz) for 5–30 s. A repeated estimation of photophosphorylation was performed using different irradiances with dark times of 2 min (or more) in between. It was observed that photophosphorylation activity decreased with time in some samples. Photophosphorylation rates were corrected for this loss (about 20% in 30 min) which was estimated by repeated measurements at one fixed irradiance.

Assuming hyperbolic saturation curves, maximal photophosphorylation rates and the irradiance needed for half-maximal photophosphorylation were derived from a Woolf plot [8].

H^+-ATPase (EC 3.6.1.34) activity was measured under day light at 25°C in 50 mM glycylglycine buffer (pH 8.0) with 3 mM Mg_2SO_4/0.4 mM dithioerythritol, and membranes corresponding to 5–30 μM BChl. The reaction was started with 5 mM ATP and stopped with 2% trichloroacetic acid (final concentrations). The increase of inorganic phosphate was determined after Taussky and Shorr [9] photometrically at 820 nm (where bacteriopheophytin has a low absorbance) using sufficient sodium dodecyl sulfate (Serva, Heidelberg) to keep protein in solution.

Reaction center content, photophosphorylation and H^+-ATPase activities obtained from isolated membranes were used to calculate the corresponding parameters in cells by using the specific BChl content of cells and membranes [4].

Results

Isolated membranes from high- and low-light grown *Rps. capsulata* wild-type (37b4) and mutant (NK3) cells were compared with respect to photophosphorylation and H^+-ATPase activities, reaction center and BChl content (Table I). The size of the photosynthetic unit (mol BChl/mol reaction center) was the same in high- and low-light NK3 membranes. Wild-type membrane photosynthetic units differed in size depending on the culture conditions. In these membranes the photosynthetic units were larger than the one found in the mutant (Table I, line 2). Specific reaction center content in low-light membranes was 4-times higher than in high-light membranes of both strains (line 3). Under comparable culture conditions, NK3 membranes contained 2-times more reaction centers (related to membrane protein) than the 37b4 membranes (line 3).

As reported earlier [4], photophosphorylation

TABLE I

PIGMENT CONTENT, PHOTOPHOSPHORYLATION RATES, AND H^+-ATPASE ACTIVITIES OF ISOLATED MEMBRANES FROM HIGH-LIGHT AND LOW-LIGHT GROWN WILD-TYPE (37b4) AND MUTANT (NK3) CELLS

Specific reaction center content and BChl-content of other experiments varied up to ±30% with respect to the data presented here. Comparable data were obtained in these experiments. Photophosphorylation and H^+-ATPase activities are mean values of up to five estimations from one experiment. Variations are mainly due to loss in activity with time. Error limits are only given for primary results. We consider results as significantly different when they are distinct by a factor of at least 2.

	Strain:	37b4	37b4	NK3	NK3
	Irradiation of the culture:	low light	high light	low light	high light
	Pigments				
1	nmol BChl/mg membrane protein	25.9	3.4	10.3	2.7
2	mol BChl/mol reaction center	126	65	26	28
3	pmol reaction center/mg membrane protein	205	52	400	100
	Photophosphorylation:				
4	mol ATP/mol BChl per min	10 ±2	66 ±17	77 ±57	109 ±34
5	mol ATP/mmol reaction center per min	1.3	4.3	2.0	3.1
6	nmol ATP/mg membrane protein per min	260	220	790	290
7	K_m (W/m^2; 846 nm)	4.3±0.95	22 ±5.4	15 ±1.5	46 ±13
8	K_m (W/m^2; 872 nm)	5	36	18	56
	H^+-ATPase:				
9	mol P_i/mol BChl per min	1.1±0.62	17.0±3.7	3.7±1.3	24.0±8.0
10	mol P_i/mol reaction center per min	140	1100	100	670
11	nmol P_i/mg membrane protein per min	28	58	38	65

rates (related to reaction centers) of high-light membranes were higher than the one of low-light membranes (line 5). This was true not only for the wild-type strain, but also for the mutant NK3. Photophosphorylation rates (based on membrane protein) were about the same for all samples tested with the exception of NK3 low-light membranes which showed about a 3-fold higher activity (line 6).

The irradiance needed to obtain half-maximal ATP synthesis (K_m for light, $\lambda = 846$ nm) was lowest in 37b4 low-light membranes and highest in NK3 high-light membranes. Remarkably, the K_m values of NK3 high-light and NK3 low-light membranes were different by a factor of about 3 (line 7). Similar values were obtained using light of 872 nm (line 8).

The K_m values of NK3 low-light membranes were 3–4 times higher than the one of 37b4 low-light membranes indicating the influence of the B800–850 complex (lines 7 and 8).

H^+-ATPase activities (related to reaction center) were higher in high-light membranes than in low-light membranes of both strains (6–8-fold; line 10). When related to membrane protein, H^+-ATPase activities were in the same order of magnitude in all samples tested (line 11).

Photophosphorylation, H^+-ATPase activity and reaction center content of membranes were used to derive the corresponding quantities in cells (Table II, lines 2, 3 and 5). The specific reaction center content of low-light cells of both strains was about 4-times higher than the one of the corresponding high-light cells. Under comparable culture conditions, NK3 cells showed about doubled specific reaction center content compared to wild-type cells (line 2).

Photophosphorylation rates extrapolated to infinite irradiance and related to cell protein were about the same for all strains and growth conditions, with the exception of low-light grown NK3 cells which showed a 2-fold higher rate (line 3).

Growth rates also were comparable in all cultures, with the exception of NK3 cells which showed a considerably increased cell doubling time under low-light conditions (line 4).

The morphometric data reveal that high-light grown cells of both strains contained less intracy-

TABLE II

PIGMENT CONTENT, PHOTOPHOSPHORYLATION CAPACITY, H^+-ATPase ACTIVITY, AND MORPHOLOGICAL DATA FROM LOW-LIGHT AND HIGH-LIGHT GROWN 37b4 AND NK3 CELLS

Data in lines 2, 3, 5 were calculated from values presented in Table I.

	Strain: / Irradiation of the culture:	37b4 low light	37b4 high light	NK3 low light	NK3 high light
1	nmol BChl/mg cell protein	18.3	2.14	5.4	1.53
2	pmol reaction center/mg cell protein	145	30	210	55
3	nmol ATP/mg cell protein per min	180	140	420	170
4	cell doubling time (min)	190	190	270	190
5	nmol P_i/mg cell protein per min	20	36	20	37
	Morphologic data				
6	intracytoplasmic membrane vesicles per cell	325	42	75	22
7	cytoplasmic membrane area (μm^2)	2.5	1.7	2.6	2.3
8	intracytoplasmic membrane area (μm^2)	2.5	0.4	0.2	0.1

toplasmic membrane vesicles than the corresponding low-light grown cells (line 6). Both high-light and low-light grown NK3 cells showed considerably smaller intracytoplasmic membrane areas (4% and 7% of the total membrane area, respectively) than 37b4 cells (19% and 50%; lines 7 and 8).

Discussion

Cells of the B800–850 less mutant NK3 are less adapted to grow under low-light conditions than wild-type cells. Their cell-doubling time is significantly prolonged compared to the wild-type demonstrating energy limitation. NK3 low-light cells try to overcome their reduced ability to absorb light by synthesis of large quantities of reaction centers which apparently do not require much space in the intracytoplasmic membranes (Table II, line 6–8). The high photophosphorylation rate together with the low growth rate demonstrates that the photosynthetic system of this mutant in vivo receives less light than applied in vitro for estimation of photophosphorylation. Thus the photosynthetic system of low-light growing NK3 cells works below its maximum capacity.

On the other hand, 37b4 cells grew with maximal rate even under lower irradiances (see Materials and Methods) because they could increase the size of their antennae. For this, the cells had to synthesize large quantities of intracytoplasmic membranes to house all the light-harvesting proteins (Table II, lines 6–8).

Having at hand no data which indicate how many of the coupled H^+-ATPases are recovered during the isolation, we will assume in the following that the content of H^+-ATPases in the membranes was representative for the cells. Similarly, we assume the amount of photosynthetic active membranes isolated also to be representative for the cells they originate from.

Photophosphorylation activities of all non energy-limited cells were roughly the same when related to cell protein which approximately corresponds to biomass. This shows again that the cells try to adapt the photosynthetic apparatus to keep their ATP synthesis rate proportional to their biomass [4].

We have no straightforward explanation for the differences in the ATP yield related to reaction centers (Table I, line 5, Ref. 4).

H^+-ATPase activities did not differ significantly when related to cell protein, in accordance with results found in *Rhodospirillum rubrum* [10].

It is known that H^+-ATPases do not show maximal activities under ATP-hydrolysis conditions due to the backpressure of the proton gradient in coupled membranes [11]. It was reported that lipophilic redox-mediators, light, low redox potentials, and optimal concentrations of uncouplers stimulate H^+-ATPase activities. The stimulation at one fixed redox potential did not exceed a factor of 1.5–2.0 even when a combination of the other stimulators was used [11].

High-light membranes might show a certain

degree of uncoupling as judged from fast reduction kinetics of P-870 and a fast decay of the carotenoid band-shift following flashes (Reidl, H. and Melandri, B.A., unpublished results). We consider the faster kinetics to reflect the adaptation of the photosynthetic system to high photon fluxes. Faster turnover kinetics were observed also in $A1a^+$ [7].

Membranes isolated from high-light-grown cells might have a higher content of cytoplasmic membranes (Table I). But the isolation conditions (see Materials and Methods) and the high photophosphorylation rate (Table I, line 5) rule out considerable contaminations with wrongly oriented vesicles or detached F_1-ATPase subunits.

We chose a redox potential of about 0 mV for the H^+-ATPase test, produced by the presence of dithioerythritol. This agent stimulates photophosphorylation as well as ATP-hydrolysis, probably by interaction with regulating thiolgroups in the H^+-ATPase [12].

It is conceivable that due to coupling not the maximal H^+-ATPase rates have been measured. But even when low-light membranes showed maximal and high-light membranes minimal coupling, the ratio of the activities would only deviate from the one of maximal rates by a factor of 1.5–2.0 which is smaller than the differences to be discussed below.

We assume that the H^+-ATPase activities measured are proportional within the errors discussed above to the number of H^+-ATP-synthetases responsible for photophosphorylation.

When related to reaction centers, H^+-ATPase activities differ by a factor of 8 in the wild type and by a factor of 6.7 in the mutant. We conclude that the smallest unit being able to convert light energy into ATP (light-harvesting complexes, reaction center, electron-transport chain and H^+-ATPase) is functioning with variable ratios of reaction centers to H^+-ATPases.

Depending on light conditions, the cells seem to adjust mainly the complexes responsible for primary photo reactions (light-harvesting complexes and reaction centers). H^+-ATPases being located at the end of reaction chain are not or only weakly changed in number or activity.

The irradiance needed to obtain half-maximal photophosphorylation in vitro (apparent K_m for light) is an appropriate parameter to compare the light requirement of differently grown cells [4]. We used light with two different wavelengths to excite separately both light-harvesting complexes (if present). Depending on extinction coefficients and energy-transfer rates, the K_m values obtained were only slightly different, indicating that not the primary light reactions were responsible for the differences found.

NK3 cells grown under high- and low-light conditions showed different K_m values. This is remarkable because the photosynthetic units of the different cell types were identical in size, and consequently the number of light quanta absorbed during estimation of photophosphorylation was the same (we used identical BChl concentrations in the test).

One possible explanation is that the cyclic electron transport is influenced by the activity of the H^+-ATPases via the backpressure of the proton-motive force (photosynthetic control; Ref. 13). More H^+-ATPases related to one electron-transport chain would decrease stronger an existing proton gradient, and would force the electron transport to work faster. Such a system which we believe is present in high-light-adapted membranes would require more light for half-maximal turnover. On the other hand, the low-light system has more reaction centers for each H^+-ATPase. This system would become saturated with lower irradiances.

The K_m can be used to quantify the adaptation capability of NK3 and 37b4 cells. NK3 cells subjected to transition from high-light to low-light decreased their K_m values about 3-fold, compared with a factor of 5 found in wild-type cells (Table I).

From this it follows that 37b4 cells are more flexible to adapt to different light conditions because they can vary both the size of their photosynthetic units and their reaction center content. Their small K_m might offer a decisive advantage over competitors in an ecological environment governed by dim light. This would explain the metabolic effort made by the wild-type cells to synthesize the extensive intracytoplasmic membrane system.

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