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ENERGETIC ASPECTS OF PHOTOPHOSPHORYLATION CAPACITY AND REACTION CENTER CONTENT OF *RHODOPSEUDOMONAS CAPSULATA*, GROWN IN A TURBIDOSTAT UNDER DIFFERENT IRRADIANCES

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Cells of *Rhodopseudomonas capsulata* were grown in a turbido-stat and adapted to high (1400 W/m^2) or low (40 W/m^2) light intensities. In high-light-grown cells the specific BChl content was about 10-times lower, the number of intracytoplasmatic membrane vesicles smaller by a factor of about 20, the photosynthetic unit smaller by a factor of 1.9 and the reaction center content about 5-times lower than in low-light-grown cells. However, the photophosphorylation rate per reaction center under saturating light was higher in high-light-grown cells by a factor of 7.7, apparently compensating the lower amount of reaction centers. Adaptation of the cells to different irradiances not only seems to comprise a variation of the size and composition of the antennae, but also a change in the affinity of the photosynthetic system to light, as concluded from saturation curves obtained from the two adaptation stages of cells.

Introduction

Cells of *Rhodopseudomonas capsulata* growing photoheterotrophically under high light intensities contain lower amounts of pigments (bacteriochlorophyll and carotenoids) in their light-harvesting system compared with cells grown in low light [1]. This low level of pigments in high-light-grown cells is accompanied by a low amount of intracytoplasmic membranes [2] and a small photosynthetic unit (total BChl per reaction center). The antennae of *Rps. capsulata* are composed of the light-harvesting complexes B870 and B800–850. It is assumed that the first one has a fixed ratio to the reaction centers, but the latter forms a pool with variable size depending on the light offered to the cells [3,5].

At very low irradiances, light as energy source becomes growth limiting. Cells adapted to saturating irradiances are growing with a maximal growth rate [5] and their specific BChl content becomes independent of the growth rate [4]. Originally it was postulated that the regulation of the pigment level maintains the number of light quanta absorbed [3]. Later it was shown that the quantum yield of growth is not constant, but reaches an optimum when the irradiance is just saturating and decreases to higher irradiances [4]

Chromatophores isolated from various species of nonsulfur purple bacteria were active in photophosphorylation [5,6]. The term 'chromatophores' was introduced for pigment-bearing membranes isolated from low-light grown cells, where intracytoplasmic membranes are predominant. We prefer the terms 'high-light membranes' and 'low-light membranes' for the membrane fractions isolated from cells grown at the respective illuminations.

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Abbreviation: BChl, bacteriochlorophyll.

High-light membranes exhibited a 3–15-fold higher photophosphorylation rate on the basis of membrane bacteriochlorophyll and a 1–4-fold higher photophosphorylation rate on the basis of protein than low-light membranes [5,7]. The rates vary, because they depend on growth conditions, isolation procedures, cytochrome c_2 content of the membranes and the phosphate potential used [8,9].

In this paper we try to associate pigment levels of growing cells with data obtained from isolated membranes to elucidate the background of cell adaptation. In order to obtain homogeneous cell material, a turbidostat was constructed, allowing to grow cells under a constant mean irradiance. Irradiance conditions were selected to give a maximal differentiation of the photosynthetic apparatus but allowing growth without energy limitation.

Materials and Methods

Organism and culture conditions

Rps capsulata 37b4 (german collection of microorganisms, Göttingen strain DSM 938) was grown anaerobically at 30°C under a high irradiance of about 1000 W/m^2 (2 × 300 W Osram concentra) in 100 ml screw-cap flasks containing malate medium [10]. The turbidostat filled with the same medium was warmed to 30°C, gassed with nitrogen (99.99%) and inoculated (Fig. 1). The culture was kept 0.5 h in dark to obtain anaerobiosis. Phototrophic growth was started with one central bulb (200 W Osram, 250 W/m^2). Irradiance values were measured with a bolometer (Laboratory Data Control, Model 68, Riviera Beach, FL, U.S.A.) calibrated against a thermopile. Measuring points were chosen behind the layer of the culture (3 cm thickness) at the final bacterial density of 0.25–0.30 mg dry mass per ml. After the culture had reached its final density, fresh malate medium, gassed with nitrogen, was pumped from a reservoir to the fermenter to maintain a constant bacterial density. Pumping intervals were recorded to determine the cell doubling time. After a few hours, when a constant pigment level was established, the irradiance was changed either to low- (40 W/m^2) or to high-light (1400 W/m^2) conditions. At high-light conditions the total irradiance was estimated, measuring the irradiance passed through the layer of bacteria, first on the outside, then on the inside of the vessel (Fig. 1). Finally, both values were added up. After 4 h of adaptation, the cell suspension leaving the fermenter was cooled immediately to about 4°C and collected in dark vessels standing on ice. The time of this storage was at most 12 h.

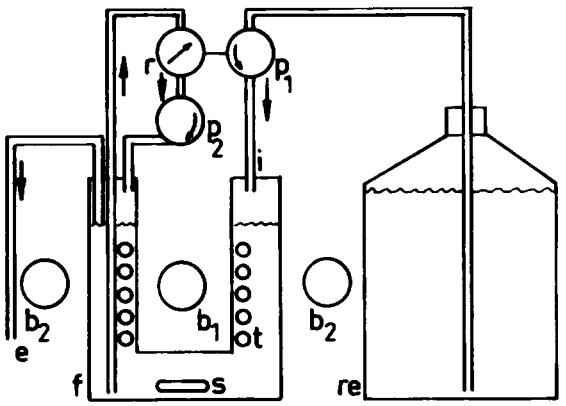

Fig.1. Turbidostat for phototroph cultures. The turbidostat consists of a culture vessel, f, with cooling tubes, t, and a stirring bar, s, a reservoir, re, illumination system, b, pumps, p and efflux, e. To maintain a constant cell density, the culture was diluted with fresh medium by pump p_1. The density of the cell suspension was recorded in a simple photometer, r, which also controlled pump p_1. Illumination or low-light culture was provided by one central bulb, b_1 (40 W). For high-light conditions one central bulb, b_1 (200 W), was fixed in the center and six reflector bulbs, b_2 (6×300 W Osram concentra), were directed to the center of the vessel.

Membrane isolation

Harvested cells were washed twice in a Tris-buffer (0.05 M, pH 7.6). The cells were resuspended in 50 mM glycylglycine buffer (pH 7.5)/5 mM $MgCl_2$ to an apparent absorbance of 40 (1 cm, 660 nm). Desoxyribonuclease (1 mg/ml) was added and the cells were broken in a French pressure cell (110 MPa, 4°C). All further isolation steps were carried out at 4°C. The crude extract was centrifuged at 30000 × g for 20 min. The supernatant was sedimented at 200000 × g for 90 min. The sediment was resuspended in 100 mM glycylglycine buffer (pH 7.5)/10 mM $MgCl_2$ and mixed with glycerol (55% v/v final concentration). This stock solution of membranes with a protein concentration of 3–10 mg per ml was stored at

−20°C without loss of phosphorylation activity [11].

Photophosphorylation

The photophosphorylation rate was determined with luciferin-luciferase [12]. The stock solution of membranes was diluted to 4 μM BChl with 100 mM glycylglycine buffer (pH 7.5)/10 mM $MgCl_2$/100 μM phenylmethylsulfonylfluoride/100 μM dithioerythritol/1 mM *p*-aminobenzamidine (solution A). *p*-Aminobenzamidine was necessary to avoid the loss of coupling factor in the diluted membrane suspension [13]. Solution B contained 100 mM glycylglycine (pH 7.75), 10 mM $MgCl_2$, 2 mM K_2HPO_4, 0.2 mM sodium-succinate, 0.1% bovine serum albumin, 25 μM ADP, 250 μM AMP, 100 μM luciferin and 10 μg/ml luciferase (last four reagents from Boehringer, Mannheim). To 1.9 ml solution B (25°C, air saturated) was added 0.1 ml of solution A in an optical cuvette (1 cm). The bioluminescence of this assay was measured with a photomultiplier (EMI 9658) through band-pass filters (NAL 550, SKF 11, Schott, Mainz) and a short pass filter (550 nm, Balzers, Liechtenstein) under continuous stirring. During irradiation of the cuvette ($\lambda \geq 780$ nm, 3 × RG 780, Schott, Mainz), the bioluminescence was recorded. Immediately after illumination the sensitivity of the luciferase system was calibrated by addition of 220 pmol ATP (20 μl solution). Care was taken to use saturating concentrations of luciferin resulting in signals constant in time. Furthermore, we made sure that the phosphate potential was not altered significantly by raising the ATP level. For this, illumination times were chosen as short as possible (10–100 s depending on the irradiance) and concentrations of membranes, corresponding to 100 nM BChl, were kept low. With this precaution the registered ATP level never exceeded 500 nM. Dark production of ATP, probably due to adenylate kinase activity was suppressed by the presence of AMP.

Saturation curves were obtained by using different irradiances, Φ. The data were replotted Φ/PP versus Φ (PP, photophosphorylation rate). The data points in this plot could not be fitted in one straight line indicating a non-hyperbolic saturation curve. For that reason, a fit using two straight lines with different slopes was calculated. The half-saturating irradiance was derived from the low-irradiance straight line and the maximal photophosphorylation was obtained from the high-irradiance straight line.

Reaction center bleaching

The reaction center content was estimated by reversible bleaching using the extinction coefficient 113 $mM^{-1} \cdot cm^{-1}$ [14]. Light-harvesting BChl in membranes shows an electrochromic band shift responding to a membrane potential which may superimpose the bleaching signal [15]. To prevent this we added valinomycin to collapse the membrane potential [16]. Re-reduction of the photooxidized reaction center was inhibited by blocking the cyclic electron flow and poising the ambient redox potential at about 400 mV [17]. Membranes were diluted to 1–5 μM BChl with 50 mM Tris-HCl buffer (pH 7.6) containing 100 mM KCl, 5 μg/ml valinomycin and 5 μg/ml antimycin A. The ambient redox potential was poised with ferricyanide and ferrocyanide (final concentrations approx. 100 μM). Bleaching of the reaction centers (ΔA), recorded at 870 nm in a spectrophotometer, was achieved by cross-illumination with monochromatic light ($\lambda = 590$ nm, NAL 590 Schott, Mainz). Bleaching was recorded at irradiances between 0.1 and 100 W/m^2. Saturation curves were converted by plotting $\Phi/\Delta A$ against Φ. The resultant plot gave a straight line with exception of the low-irradiance region. These data points were omitted for the straight line fit. The half-maximal bleaching irradiance and the maximal bleaching value were obtained from the fitted line. Reaction center concentrations were corrected for chemical preoxidized reaction centers.

Morphometric methods

Electron micrographs of bacteria from different culture conditions were examined with a semiautomatic image analysis system ASM (Leitz, Wetzlar, F.R.G.) interfaced with a computer [2]. For calculation of number and surface area of intracytoplasmic membrane vesicles per cell the same assumptions were used as in Ref. 2 with exception of the vesicle shape in high-light-grown cells, where ellipsoidal forms were used.

Protein and bacteriochlorophyll determination

Protein was measured using bovin serum albumin as standard by the method of Lowry et al. [18]. BChl was extracted with aceton/methanol (7 : 2, v/v). Pigmented precipitate was extracted a second time. The extinction was measured at 770 nm and BChl was calculated using $\epsilon = 76\ mM^{-1} \cdot cm^{-1}$ [19].

Results and Discussion

Turbidostat culture

BChl concentrations, specific BChl content and cell doubling time were recorded in two different experiments to follow the adaptation to low light and to high light conditions (Fig. 2A and B).

After switch over to low irradiance, two phases of adaptation were observed. The first 4 h were characterized by a fast increase of cellular BChl content and an initial increase of doubling time for cell mass (Fig. 2A). During the second phase the BChl content increased slowly until the end of the experiment. The cell doubling time between 4 and 16 h was about 150 min.

After switch over to high-light conditions, the BChl concentration in the culture decreased immediately to reach a lower level after about 4 h (Fig. 2B). For about 1 h growth apparently stopped, although a change in pigment content might have influenced cell density recording during this time. Cell doubling time was about 170 min between 4 and 16 h.

Reaction-center bleaching

In order to study energy transfer in the photosynthetic apparatus membranes were isolated from high-light- and low-light-grown cells. Reaction-center bleaching was recorded as a function of irradiance (Fig. 3A). The values of maximal bleaching, derived from Fig. 3B and C, were used to calculate the size of the photosynthetic unit using the specific BChl content of the membranes (Table I). High-light membranes had a photosynthetic unit of 40 compared with 74 of low-light membranes. Moreover, the plots in Fig. 3B and C revealed a remarkable difference in the affinity of

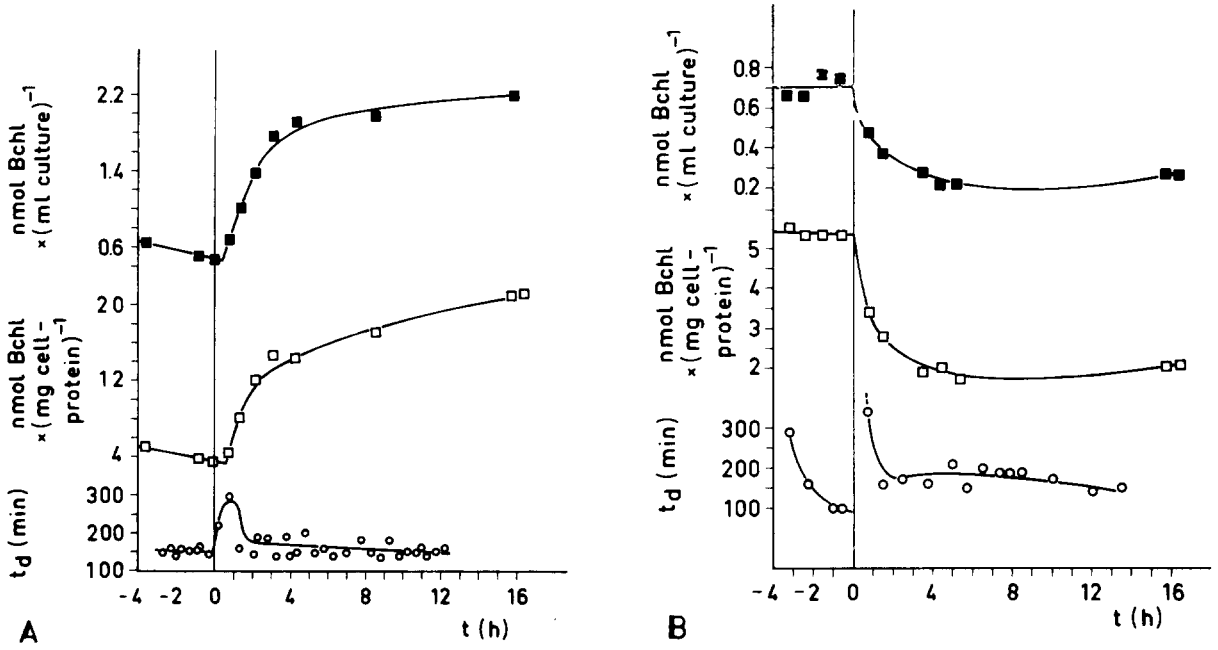

Fig. 2. BChl concentration in the culture (■), specific BChl of the cells (□) and cell doubling time (○) of high-light and low-light adapting cultures. Cells collected between 4 and 17 h were used for membrane isolation. (A) Switch over from 250 to 40 W/m² (low-light conditions). Mean values for adapted cells between 4 and 16 h: 2.0 (±7%) nmol BChl/ml, 17.5 (±19%) nmol BChl/mg cell protein, 154 (±11%) min cell doubling time; (B) Change from 250 to 1400 W/m² (high-light conditions). Mean values for adapted cells between 4 and 16 h: 0.24 (±11%) nmol BChl/ml, 1.9 (±7%) nmol BChl/mg cell protein, 173 (±13%) min cell doubling time.

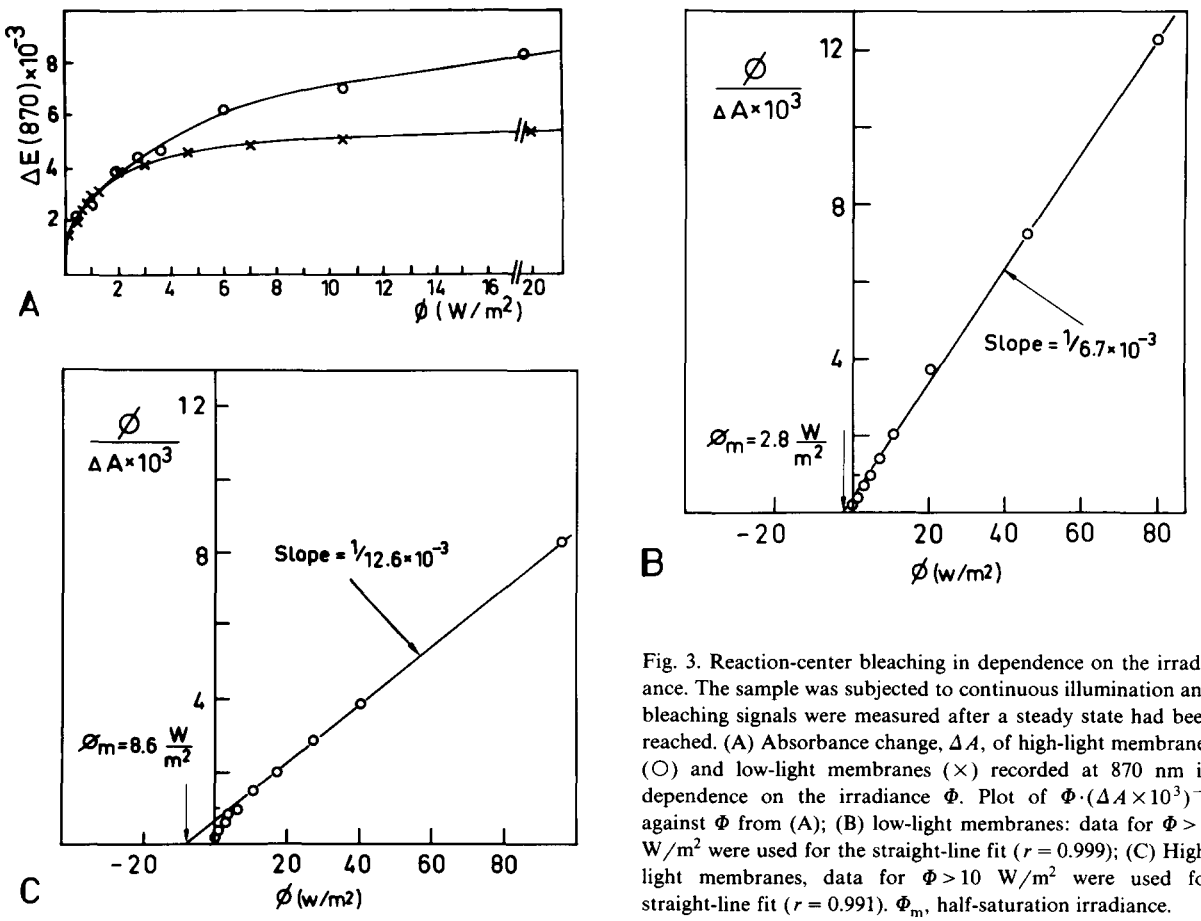

Fig. 3. Reaction-center bleaching in dependence on the irradiance. The sample was subjected to continuous illumination and bleaching signals were measured after a steady state had been reached. (A) Absorbance change, ΔA, of high-light membranes (○) and low-light membranes (×) recorded at 870 nm in dependence on the irradiance Φ. Plot of $\Phi \cdot (\Delta A \times 10^3)^{-1}$ against Φ from (A); (B) low-light membranes: data for $\Phi > 3$ W/m^2 were used for the straight-line fit ($r = 0.999$); (C) High-light membranes, data for $\Phi > 10$ W/m^2 were used for straight-line fit ($r = 0.991$). Φ_m, half-saturation irradiance.

both samples to light. High-light membranes needed an irradiance about 3-times higher than did low light membranes to bleach half of their reaction centers.

Most probably the antenna system, altered in size and composition, is responsible for the change in affinity (Tables I and II). But also differences in the re-reduction rate of reaction centers must be considered to explain the observed change.

Photophosphorylation

High-light and low-light membranes showed striking differences in their dependence of photophosphorylation on irradiance (Fig. 4A and B). High-light membranes yielded a saturation type curve, but low-light membranes showed a decrease of photophosphorylation rate at irradiances above 180 W/m^2 (Fig. 4A). Under saturating illumination, high-light membranes exhibited higher photophosphorylation rates per BChl, per membrane protein and per reaction center than did low-light membranes (Table I, Fig. 4C and D). The photophosphorylation rate per reaction center, a measure of the productivity of the photosynthetic system, is higher in high-light membranes by a factor of about 8.

Under the assumption that the turnover of reaction centers and the ATP synthesis are coupled at the same degree in both samples, different rates of the cyclic electron transport may explain the finding. Otherwise and altered H^+/e^- ratio or a variation in number and/or activity of the coupling factor could be not excluded.

The higher productivity of high-light reaction centers is also reflected by the about 4-times higher level of light intensity for half-saturation of photo-

TABLE I

BACTERIOCHLOROPHYLL AND REACTION-CENTER CONTENT OF MEMBRANES, PHOTOSYNTHETIC UNIT AND PHOTOPHOSPHORYLATION

	A Low-light membranes	*B* High-light membranes	*A/B* factor
Bacteriochlorophyll (nmol BChl/ mg membrane protein)	20.0	4.7	4.3
Photosynthetic unit (mol BChl/mol RC)	74 (±1.5%)	40 (±1.0%)	1.85 (±1.8%)
Reaction center content (pmol RC/ mg protein)	270	118	2.3
Irradiance required for half-saturation of bleaching (W/m^2)	2.8 (±19%)	8.6 (±6%)	1/3.1 (±20%)
Photophosphorylation mol ATP/mol BChl × h	150 (±7.4%)	2130 (±6.4%)	1/14.2 (±10%)
μmol ATP/ mg protein × h	3.0	10.0	1/3.3
nmol ATP/ pmol RC × h	11.1 (±7.5%)	85.2 (±6.5%)	1/7.7 (±10%)
Irradiance required for half-saturation of photophosphorylation (W/m^2)	25 (±7%)	93 (±8%)	1/3.7 (±11%)

RC, reaction center

TABLE II

MORPHOLOGY OF HIGH-LIGHT- AND LOW-LIGHT-GROWN CELLS

Cells were obtained from cultures adapted to high-light- and low-light-conditions for 18 h. Surface area of intracytoplasmic membranes from low-light-grown cells was calculated assuming spherical vesicles (diameter 51 nm). In high-light-grown cells, ellipsoid-shaped vesicles were found (half axes: $a = 32$ nm, $b = 28$ nm, $c = 17$ nm) and used for calculation of the surface area. The number of vesicles per cell was obtained after the method described in Ref. 2. The surface of the protoplast was calculated assuming a cylinder with two hemispheres on the ends.

	A Low-light--grown cells	*B* High-light--grown cells	*A/B* factor
Number of vesicles per cell	624 (±30%)	32 (±100%)	19.5 (±104%)
Intracytoplasmatic membrane area (μm^2) per cell	5.1	0.26	19.5
Protoplast surface area (μm^2) per cell	3.1	3.5	0.9
Total membrane surface area (μm^2) per cell	8.2	3.8	2.2
Specific bacteriochlorophyll content (nmol BChl/mg protein)	33	2.5	13.2

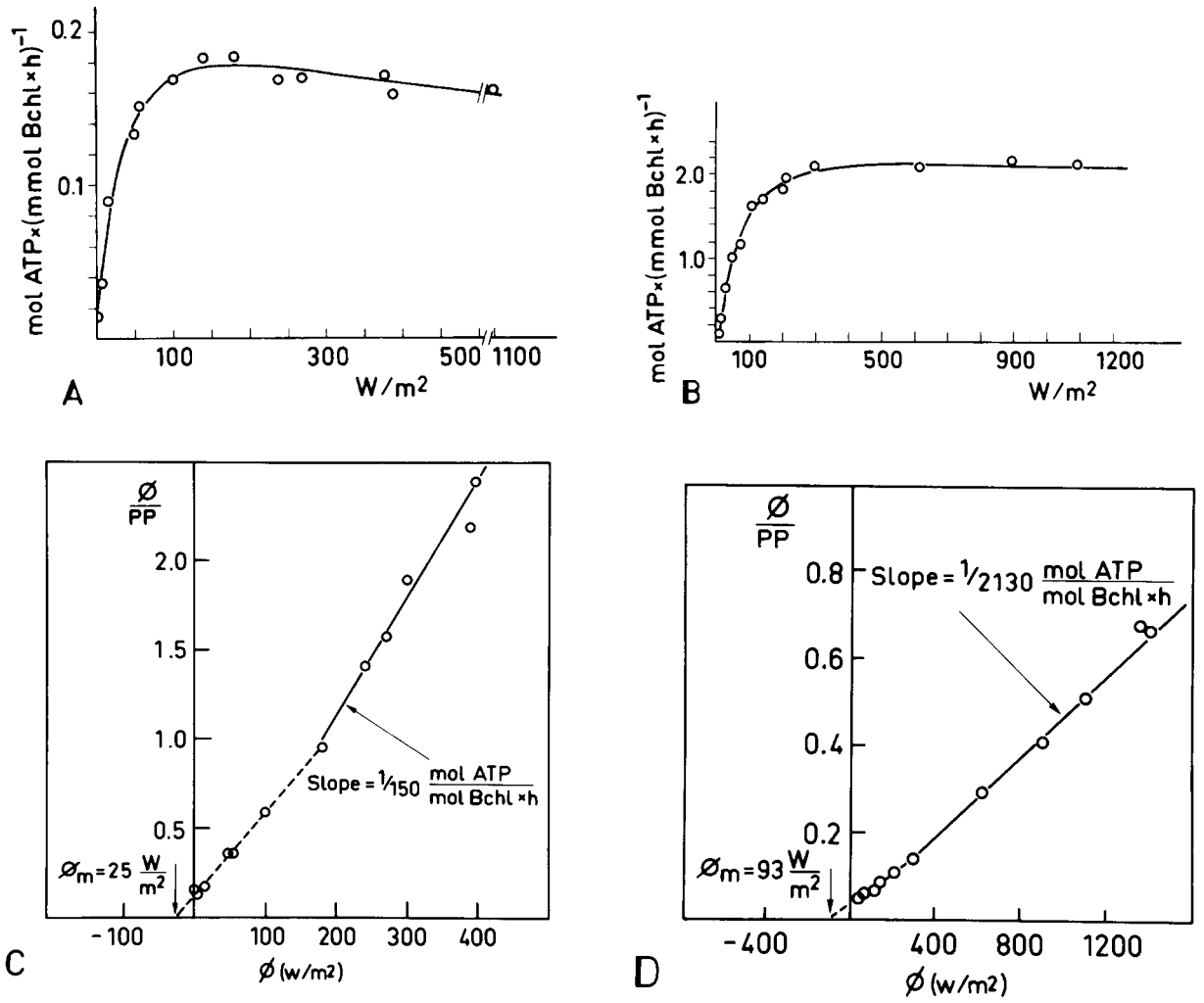

Fig. 4. Photophosphorylation in dependence on the irradiance. Membrane concentrations corresponding to 100 nM BChl. Photophosphorylation (PP) against irradiance (Φ): (A) low-light membranes; (B) high-light membranes. Plot of Φ/PP against Φ; (C) Low-light membranes: straight lines fitted to data at 0–180 W/m^2 ($r = 0.999$) and 180–390 W/m^2 ($r = 0.989$); (D) high-light membranes, straight lines fitted to data at 0–300 W/m^2 ($r = 0.995$) and 300–1500 W/m^2 ($r = 0.993$). Φ_m, half saturation irradiance.

phosphorylation calculated from Fig. 4C and D (Table I). This finding is in good agreement with the results of reaction-center bleaching, which also revealed a lower affinity to light in high-light membranes.

Summarizing the results from light saturation measurements we conclude that high-light and low-light membranes show a different organization of their photosynthetic apparatus. As known before, high-light membranes contain smaller antennae with a lower portion of B800–850 than low light membranes [3,7]. These variations seem to influence the affinity of the photosystem versus light, as seen in reaction center bleaching and photophosphorylation.

The highly increased photophosphorylation rates per reaction center in high-light membranes could be explained by an additional adaptation mechanism probably influencing the electron transport rate in steps following the primary photochemistry.

Morphometric measurements

The amount and form of intracytoplasmic

membrane vesicles were studied in cells grown at high and low light for 18 h. Low-light-grown cells contained spherical vesicles of 51 nm diameter; high-light-grown cells, however, formed ellipsoidal vesicles of about the same length. The latter were similar to the tubular intracytoplasmic membranes observed in aerobically-grown cells [20]. The number of intracytoplasmic membrane vesicles and their total surface area were both 19.5-times larger in low-light- than in high-light-grown cells (Table II). But in low-light-grown cells the total area of cytoplasmic plus intracytoplasmic membranes was only 2.2-times larger. This can be compared with the specific BChl content, which was 13-times higher in high-light- than in low-light-grown cells. The increase of BChl per cell protein correlates better with the increase of the total membrane area. These data support the hypothesis that the photosynthetic system is localized mainly in the intracytoplasmic membrane system [21].

Photophosphorylation capacities of cells

The photophosphorylation capacities of cells were calculated according to the formula given in the footnote of Table III, assuming that BChl found in the whole cells is totally membrane bound and also present in isolated membranes. The calculated rates of photophosphorylation on the basis of cell protein represent in vitro rates, but a comparison of the values is believed to reflect the relationship in vivo. The photophosphorylation rates on cellular basis have the same order of magnitude for the high-light- as for the low-light-grown cells (Table III). In high-light-grown cells the reaction-center content per cell protein was about 5-times lower than in low-light-grown cells. Nevertheless, a similar photophosphorylation rate per cell protein could be calculated in both low-light- and high-light-grown cells, because the latter are able to use higher irradiances producing ATP, probably with higher turnover rates of their reaction centers.

Assuming that, during the isolation of the membranes, the photophosphorylation activity is maintained or at least reduced at the same degree in both membrane types, we conclude that, in response to variations of the irradiance, the cells regulate pigment content, amount of intracytoplasmic membranes and size and composition of the photosynthetic system in order to maintain their photophosphorylation capacity.

TABLE III

PHOTOPHOSPHORYLATION

The rates are based on cell protein as: μmol ATP/mg cell protein $\times$ h = {μmol ATP/μmol BChl $\times$ h} $\times$ {μmol BChl/mg cell protein}. Results are based on measurements in vitro under saturating illumination. The isolation of membranes possibly includes loss of cytochrome c_2 and other factors needed for photophosphorylation, but comparison of the data is possible under the assumption that the loss of photophosphorylation activity during the isolation procedure is the same for high-light and low-light membranes. Specific reaction-center content is based on cell protein as: pmol RC/mg cell protein = (pmol BChl/mg cell protein)/(pmol BChl/pmol RC)

	A Low-light-grown cells	B High-light-grown cells	A/B Factor
Photophosphorylation (μmol ATP /mg cell protein $\times$ h	2.6 ($\pm$20%)	4.0 ($\pm$9.5%)	1/1.5 ($\pm$22%)
Specific reaction center content (pmol reaction center/mg cell protein	236 ($\pm$19%)	48 ($\pm$7%)	4.9 ($\pm$20%)

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