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The adaptation of the electron transfer chain of *Rhodopseudomonas capsulata* to different light intensities

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(1) Cells of *Rhodopseudomonas capsulata* (wild-type) were grown photoheterotrophically in a turbidostat under very high and very low light intensity. Membranes were isolated from cells adapted to the respective light conditions and fractionated by sucrose density centrifugation. The molar ratios of ubiquinone and cytochromes c_2, c_1, *b*-561 and *b*-566 per reaction center were 3-fold to 5-fold higher in high-light than in low-light membranes. (2) Most of the Cyt($c_1 + c_2$) and Cyt *b*-561 detected in dark redox titrations undergoes light-induced redox changes, both in high- and in low-light membranes. (3) The fractions of the total photooxidizable reaction center and Cyt($c_1 + c_2$) oxidized under continuous light in the absence of antimycin are higher in membranes from low-light- than from high-light-grown cells. (4) From these data and results of kinetic studies it is proposed that cyclic electron flow under saturating light intensities is faster in high-light-grown cells.

Introduction

It has been known for a long time that the bacteriochlorophyll content of facultative photosynthetic bacteria varies greatly in response to environmental factors, of which the most important are light intensity and oxygen tension [1]. BChl and carotenoids are non-covalently bound in stoichiometric ratios to pigment-binding polypeptides forming antenna and reaction center complexes of the photosynthetic apparatus in the intracytoplasmic membrane [2]. A decrease in light intensity or oxygen tension causes not only an increase in the cellular pigment content, but also an enlargement of the system of intracytoplasmic membranes (chromatophores) [3,4], and an increase in the average size of the photosynthetic unit, i.e., the molar ratio of BChl per reaction center [5]. Photophosphorylation is a major activity of the bacterial photosynthetic apparatus [6]. This activity has been reported to be higher in chromatophores from cells grown at high light intensity than in chromatophores from cells grown at low light intensity, when calculated on a BChl

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Abbreviations: BChl, bacteriochlorophyll; Cyt, cytochrome, DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine, Mops, 4-morpholineethanesulfonic acid; RC, photosynthetic reaction center; UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole.

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basis [7]. This difference was not so clear when calculated on protein basis [7].

In order to study more thoroughly the adaptation of the energy metabolism to variations of light intensity, we set up turbidostat cultures of *Rhodopseudomonas capsulata.* Exponentially growing cells adapted to high (about 1400 $W \cdot m^{-2}$) and low (40 $W \cdot m^{-2}$) light levels, respectively, both having a doubling time of 150 min, were used to isolate the membrane fraction [8]. In high-light cells the BChl content per cell was about 10-times lower, the number of intracytoplasmic membrane vesicles smaller by a factor of about 20, the photosynthetic unit smaller by a factor of 2, and the RC content (on a cell protein basis) about 5-times lower than in low-light cells [8,9]. Interestingly, the K_m for light (irradiance required for half-saturation of photophosphorylation, $W \cdot m^{-2}$) was in high-light membranes about 7-times higher and the V_{max} (mol ATP · (mmol RC)$^{-1} \cdot min^{-1}$) about 6-times higher than in low-light membranes [8,9]. The higher K_m for light was also observed in high-light membranes from mutant cells which lack the B800–850 light-harvesting complex and have the same size of the photosynthetic unit, irrespective of the irradiation conditions during growth [9]. Thus, high-light membranes have higher maximal rates of photophosphorylation under saturating light intensities, but lower efficiency of light energy transduction than low-light membranes. Considering the smaller number of photosynthetic units per cell it was calculated that high-light cells produce ATP, in saturating light, at the same rate per cell as low-light cells [8].

The observation that in high-light membranes the ratio of the ATPase activity per RC was about 7-fold that of low-light membranes [9], together with preliminary results on the re-reduction of RC [10], suggest the idea that in high-light membranes the rate of cyclic electron transport was higher than in low-light membranes [11]. In this study we show that, in fact, high-light membranes have a higher content of ubiquinol-cytochrome c_2 oxidoreductase, of cytochrome c_2 and of ubiquinone, when normalized to RC, as compared with low-light membranes. We also demonstrate that in high-light membranes the uncoupled electron transfer chain operates at a higher rate than in low-light membranes.

Materials and Methods

Rps. capsulata 37b4 (wild-type) (German collection of microorganisms, Göttingen strain DSM 938) was grown in a turbidostat at high (6×300 W and 1×100 W lamps) and low (1×50 W lamp) light intensities as previously described [8]. Cells were harvested and washed, and chromatophores were prepared by French press as in Refs. 8,9. The low-speed centrifugation supernatant (Sorvall SS34 rotor, 15000 rpm, 15 min) was submitted to a 17 h ultracentrifugation at 30000 rpm in a 60 Ti Beckman rotor through a discontinuous sucrose gradient in 50 mM Tris buffer (pH 7.6) consisting of the following layers: 3 ml of 1.75 M, 4 ml of 1.2 M, 5 ml of 1.0 M, 6 ml of 0.6 M and 5 ml of supernatant. Only a single band of chromatophores was seen (at about 1.2 M sucrose) in either low- or high-light preparations and was recovered with a peristaltic pump. Glycerol to 55% (v/v) was added and the fractions were stored at −20°C.

For further resolution of subpopulations of chromatophores differing in sedimentation coefficient, 2 ml of high-light and low-light membrane preparation (containing about 1 mg BChl) were subjected to a short ultracentrifugation at 4°C (40000 rpm in an SW 41 Beckmann rotor for 15 min) through a sucrose gradient in 50 mM Mops (pH 7.0) prepared with 0.5 ml of 1.75 M, 1.5 ml of 1.2 M, 2.5 of each 0.8 M and 0.4 M and 2 ml of 0.2 M. The prepared gradients were stored overnight before the run.

Quinones were extracted from chromatophores and measured by reversed-phase HPLC as described in Ref. [12]. The identify of the ubiquinone peak was controlled with UQ-10 standard through the retention time of the oxidized and $NaBH_4$-reduced species. BChl was measured as in Ref. 13.

Equilibrium redox titrations of cytochromes in chromatophores were performed in the dark in 50 mM Mops/100 mM KCl (pH 7.0) under a nitrogen atmosphere in the presence of the following redox mediators prepared daily: 20 μM of each 1,2-naphthoquinone, 1,4-naphthoquinone, *p*-benzoquinone, phenazine methosulfate, phenazine ethosulfate, 40 μM duroquinone, 100 μM DAD and 5 μM pyocyanine. Valinomycin and nigericin (10 μM each) were also present. The chromato-

phore concentration was equivalent to 70 μM BChl and 40 μM BChl for low- and high-light preparations, respectively. Ferricyanide was used as oxidant and ascorbate or dithionite as reductant. The redox titrations were performed by recording full spectra in the range 500–600 nm with a Jasco UVIDEC-610 double-beam spectrophotometer with a 3 nm bandwidth. Total content of Cyt($c_1 + c_2$) was measured at 551 nm, Cyt *b*-561 and Cyt *b*-566 at 561 nm and 565 nm, respectively.

A careful spectral analysis reveals that the differential redox spectra of cytochromes overlap not only with a small contribution due to pyocyanine in the *b*-type spectral region, but also with a component which titrates with a multiple phase pattern and exhibits a valley with a minimum at 588 nm on a reductive titration. The presence of this spectral signal, which is particularly prominent in low-light preparations and is probably due to changes of bacteriochlorophyll absorbance, causes aspecific baseline shifts. Redox titrations of this effect, measured at 588–570 nm, a wavelength pair not affected by cytochrome redox changes, indicate that this artifact occurs between 450 and 0 mV at pH 7, thus affecting both the titration of Cyt($c_1 + c_2$) and of Cyt *b*-561. The spectral contributions of cytochromes cannot therefore be simply evaluated on the basis of a single reference wavelength. Specifically, the trough produces an absorbance decrease at 551 and 542 nm, which, being larger at 551 than at 542 nm, causes a systematic underestimation of Cyt($c_1 + c_2$), when a single reference wavelength is used; the same trough also leads to an aspecific absorbance decrease at 561 and 569 nm (the wavelength pair normally utilized for the titration of Cyt *b*-561), which is larger at 569 than at 561 nm, thus producing an overestimation of Cyt *b*-561. For these reasons full spectra of cytochromes were recorded at controlled redox potentials and baselines were drawn on these spectra through 542 and 558 nm for Cyt($c_1 + c_2$) titration. The choice of this wavelength pair is supported by the spectrum of the light-induced oxidation of Cyt($c_1 + c_2$) obtained in the blue-green strain of *Rps. capsulata* A1apho$^+$ [14]. An analogous procedure was adopted for Cyt *b*-561 and *b*-566 choosing as isosbestic points respectively 547 and 569 nm for Cyt *b*-561 and 550 and 575 nm for Cyt *b*-566. These wavelengths were indicated by the spectra of photoinduced signals for the reduction of Cyt *b*-561 in *Rps. capsulata* A1apho$^+$ [14] and of Cyt *b*-566 for *Rps. sphaeroides* (as suggested in Ref. 16). Each titration was fully reversible and consisted of 30–50 experimental points. The individual extents and corresponding E_m values of each cytochrome species were determined utilizing computer routines based on non-linear least-square minimization. The global titration of Cyt c_2 and Cyt c_1 was performed in the E_h span from 440 to 160 mV and was analyzed for two components ($n = 1$). The midpoint potentials obtained with this analysis were identical in both types of membrane (high- and low-light) and in good agreement with values previously reported for *Rps. sphaeroides* [17]. Cyt *b*-561 titration in the E_h range from 200 to −100 mV was also analyzed for two components ($n = 1$), in order to account for Cyt *cc'* [18,19]. No evidence for significant spectral contributions due to Cyt *b*-150 was obtained [15,20]. Cyt *b*-566 titration was analyzed only from −50 mV down to −300 mV, as to avoid spectral interference with the titration of Cyt *b*-561. The same extinction coefficient (19.5 $mM^{-1} \cdot cm^{-1}$) was used for all cytochromes although the actual values in membrane are not known [21].

Redox controlled kinetic spectrophotometry of light-induced electron flow was performed as in Ref. 12. The redox components were monitored at the following wavelengths: RC at 542 nm ($E =$ 19.5 $mM^{-1} \cdot cm^{-1}$) and Cyt *b*-561 at 561–569 ($E = 19.5\ mM^{-1} \cdot cm^{-1}$) [12,14]. The specificity of the wavelengths utilized for Cyt($c_1 + c_2$) measurement was controlled at $E_h = 450$ mV; at this E_h a spectrum of RC photooxidation was obtained, exhibiting equal absorbance changes at 551 and 542 nm. The specificity of the wavelength pair utilized for Cyt *b*-561 was controlled in experiments performed in the presence of myxothiazol and antimycin A, which demonstrated the absence of any photoinduced signal due to RC and Cyt($c_1 + c_2$) at 561–569 nm. In all experiments, the concentration of chromatophores was adjusted so that flash actinic light was 90% saturating for all types of membrane. Flash saturation was determined by exciting chromatophores, poised at $E_h = 450$ mV, with a train of eight flashes, 20 ms

apart. At this potential, since Cyt c_2 is oxidized before excitation, the oxidation of RC on each flash is readily resolved and saturation can be determined from the extent of oxidation induced by the first flash, as compared to the total extent of RC oxidized by the subsequent flashes in the train. Continuous actinic light was provided by a 55 W halogen lamp shuttered with a Uniblitz electronic shutter mod.26L (1 ms closing time) and triggered by a shutter driver Uniblitz mod.SD1000 (Vincent Ass., U.S.A.). The RC concentration was measured at 542 nm by giving a series of eight 90%-saturating actinic flashes, 20 to 40 ms apart, in the presence of antimycin and UHDBT. The amount of oxidized RC reached a maximum pseudo-steady-state level for the last two or three flashes under these conditions. Essentially the same amounts of photoxidized RC were measured under the same conditions, when chromatophores were activated by continuous actinic light.

Results

Stoichiometry of the redox carriers

Isolated chromatophores from high- and low-light-grown cells of *Rps. capsulata,* wild-type 37b4, were compared with respect to their content in *b*- and *c*-type cytochromes and ubiquinone 10.

By running full spectra at selected redox potentials the following maxima were detected for both high and low-light membranes: 550.0 nm for Cyt c_2, 552.5 nm for Cyt c_1, 559.5 nm for Cyt *b*-561 and a double peak at 558.0 nm and 565.0 nm for Cyt *b*-566 [15–17]. Computer best fits of dark equilibrium potentiometric titrations allow the calculation of the relative amounts of *b*- and *c*-type cytochromes. The values shown in Table I were calculated using the extinction coefficients reported before for the *Rps. sphaeroides* Ga chromatophores [22,23], i.e., 19.5 $mM^{-1} \cdot cm^{-1}$ for *b*- and *c*-type cytochromes. The extinction coefficients in situ of cytochromes are, however, uncertain: e.g., in isolated bc_1 complex preparation values equal to 25 and 17.5 $mM^{-1} \cdot cm^{-1}$ were estimated for cytochromes *b* and c_1, respectively [24]. Assuming that the extinction coefficients of cytochromes and RC do not change under different growth conditions, the data presented in Table I can be taken as comparative values for the same organism grown under different conditions. In Fig. 1 dark equilibrium titrations of Cyt c_1 and c_2 in high and low-light chromatophores are compared. Titrations of similar quality were obtained for Cyt *b*-561 and Cyt *b*-566. As pointed out under Materials and Methods ,the titrations of Cyt *b*-561 were routinely analyzed for two components in order to resolve an eventual spectral contribution due to Cyt *cc'*. Only in high-light membranes did the best-fit procedure reveal, besides the Cyt *b*-561 component ($E_m = 50$ mV), a species titrating at zero potential, which showed an extent amounting to 25% of the total absorbance change. This component was interpreted as due to Cyt *cc'* (see below). The E_m values obtained for cytochromes are in good agreement with those previously reported for *Rps. sphaeroides* and are in general not changed by the growth conditions (see Table I). For Cyt *b*-566, an E_m value approx. 20 mV lower in low- as compared to high-light preparations was found. The E_m values for Cyt *b*-566 appear to be significantly lower than the E_m reported in *Rps. sphaeroides* [15,16]. The redox titration of this cytochrome was not changed when 40 μM juglone (5-hydroxy-1,4-naphthoquinone) was added to the mediator pool

TABLE I

THE SIZE OF THE PHOTOSYNTHETIC UNIT AND THE STOICHIOMETRIES OF THE REDOX COMPONENTS OF THE PHOTOSYNTHETIC ELECTRON TRANSFER CHAIN (MOLAR RATIOS) IN LOW-LIGHT AND HIGH-LIGHT MEMBRANES

For each redox species the E_m (mV) resulting from the computer analysis of the relative titration curve is given in brackets. UQ, ubiquinone.

Membrane type	BChl/RC	UQ/RC	c_2/RC	c_1/RC	*b*-561/RC	*b*-566/RC
Low-light	121	19	0.69 (343)	0.40 (286)	0.62 (56)	0.33 (−143)
High-light	42	64	2.51 (343)	1.91 (286)	2.28 (50)	1.28 (−212)

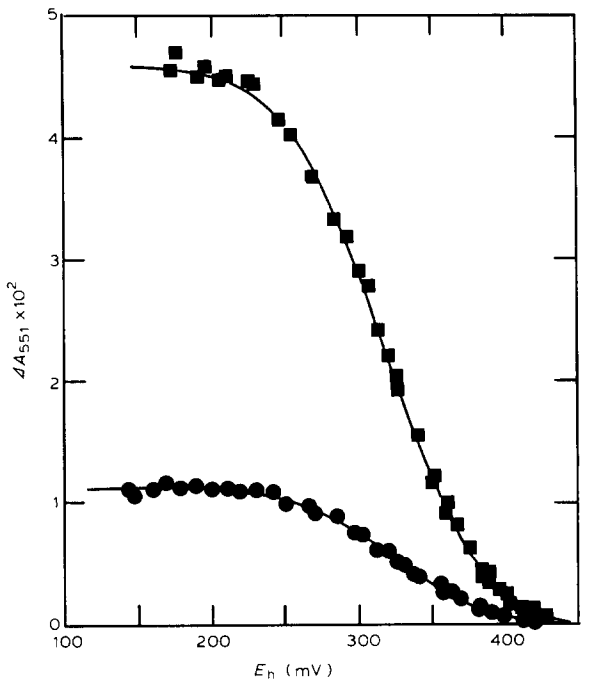


Fig. 1. Dark equilibrium redox titrations of Cyt c_1 and Cyt c_2 in high- (■) and low-light (●) chromatophores obtained from full spectra. The RC concentration was 0.57 μM in both types of preparation. For each titration, the continuous line represents the best fit to the data of the sum of two Nernst curves with $n = 1$. The midpoint potentials calculated are 286 mV and 343 mV for Cyt c_1 and Cyt c_2 preparations, respectively.

in order to improve redox equilibrium. As a whole, therefore, dark redox titrations do not give any evidence that cytochromes with different properties are present in high- vs. low-light membranes.

As shown in Table I, the content per RC of Cyt c_1, Cyt *b*-561 and Cyt *b*-566 are higher by a factor of approx. 4 in high-light chromatophores when compared to low-light chromatophores. Assuming that all the measured Cyt *b*-561, Cyt *b*-566 and Cyt c_1 are bound in a bc_1 complex and that their stoichiometries are independent of the light regime during growth, it becomes evident that about 4-times more bc_1 complexes per RC are present under high-light conditions.

It has been reported that the ubiquinone pool in *Rps. sphaeroides* amounts to 25–30 ubiquinone/RC [25,12]; for *Rps. capsulata*, grown photosynthetically, a similar value was recently published [26]. This ubiquinone content is usually determined in membrane preparations obtained from cultrues harvested in the late logarithmic phase of growth under moderate incident light intensities. As shown in Table I, this value is intermediate between the one determined for chromatophores from cells grown at very high and very low light intensities. The ubiquinone/RC ratio in high-light membranes is 3.4-fold larger than in low-light membranes. This ratio is continuously modulated between these extreme values by the light intensity during growth (not shown).

The Cyt c_2 content was also enhanced in high-light membranes by a factor of approx. 4. However, the amounts of Cyt c_2 measured in chromatophores are an underestimation of the actual content in the cells, since this cytochrome is lost upon cell breakage by French press. It is expected that the loss of Cyt c_2 is higher in high-light cells due to the higher ratio of cytoplasmic to intracytoplasmic membrane area [9]. Upon cell breakage the loss from the periplasmic space covered by the cytoplasmic membrane is higher than that from the inside of the intracytoplasmic vesicles, since it has been demonstrated that intracytoplasmic membrane consists of pre-formed vesicles, while the cytoplasmic membrane forms open membrane pieces besides vesicles [27]. In order to have a quantitative estimation of this loss, we have measured the total content of Cyt($c_1 + c_2$) present in low-light cells having a high proportion of intracytoplasmic membrane and in dark aerobically grown cells, where intracytoplasmic membrane is absent, a limiting case as compared to high-light cells. We found that 30% of Cyt c_2 is lost from low-light cells, but at least 80% is lost from dark-grown cells upon breakage. Although direct experiments were not performed, it can be estimated, therefore, that the 'in vivo' content of Cyt c_2 per RC is higher by a factor of 6–7 in high- vs. low-light cells.

The analysis of the soluble fraction of the cell-free extract of low-light cells and dark aerobically grown cells, after the preparative isopicnic centrifugation, shows the presence not only of Cyt c_2 ($E_m = 340$ mV), but also of a cytochrome which exhibits the redox and spectral properties of Cyt *cc'* (broad band in the 550–560 nm range, peak at 426 nm with a shoulder at 435 nm for the reduced species; peaks at 390 nm and 480 nm for the oxidized form [18,19]. This component in both

types of soluble fraction titrated at 5 ± 5 mV with $n = 1$ when analyzed at 561–569 nm. This figure is consistent with the E_m of the spectral component detected in Cyt *b*-561 titrations and attributed to Cyt *cc'*.

Membrane fractions

The chromatophores used in the present study have been purified by sucrose isopicnic centrifugation as a final step, obtaining only one band in both types of cell. However, these preparations could be further resolved into different fractions by a short sucrose gradient centrifugation (see Materials and Methods). Three different bands were generally found: fractions I, II and III at 0.15 M, 0.22 M and 1.20 M sucrose, respectively. The fraction distribution of the membrane fragments was different in high- vs. low-light cells. In the high-light preparation, bands I, II and III represented, on a BChl basis, 27%, 38% and 35% of the total sample, whereas in low-light membranes the distribution was 0%, 80% and 20%, respectively. The three fractions have different amounts of antenna BChl as judged from the absorption spectra in the near infrared (not shown). A lower B800–850/B870 ratio was observed in fraction III. This fraction is very turbid, indicating possibly the presence of cell wall material adhering to the membrane, suggesting that it is derived from the cytoplasmic membrane. Therefore, the intermediate running fraction (fraction II), in which a large amount of B800–850 exists, would have a different origin, possibly intracytoplasmic membrane. Fraction I is similar to fraction III and could be also attributed to the cytoplasmic membrane.

In membranes from low-light cells essentially the same situation holds. The higher proportion of band II observed in this case is in agreement with the enhanced biosynthesis of intracytoplasmic membrane in low-light cells.

It is interesting the hypothesis that the membrane system of the cell can be heterogeneous in the distribution not only of the different pigment complexes, but also of the redox carriers.

Light-induced electron transfer

The results of the dark redox titrations show that in high-light membranes the stoichiometric ratio bc_1 complex/RC is much higher when compared with low-light membranes. This situation is confirmed by the extent of the photoinduced redox changes observed when electron flow is blocked by antimycin (Table II). The maximum amount of Cyt($c_1 + c_2$) per reaction center photooxidized in continuous light is about 4-times larger in high-light membranes than in low-light membranes. Similarly, the amount of photoreduced Cyt *b*-561 per reaction center is doubled in high light membranes *. In both types of preparation the extent of photoinduced redox changes parallels the amount of cytochromes revealed by the dark redox titrations and amounts to 70–80% and 70–90% for Cyt($c_1 + c_2$) and Cyt *b*-561, respectively. These results demonstrate that the functional stoichiometry of bc_1 complexes and reaction centers can be different from the proposed 1 : 2 ratio [23,20,28], according to the growth conditions. Moreover, it appears that a change in the stoichiometry does not preclude delocalized redox interactions between the two types of complex.

TABLE II

MAXIMUM AMOUNTS OF PHOTOXIDIZED CYT($c_1 + c_2$)/RC AND PHOTOREDUCED CYT *b*-561/RC (MOLAR RATIOS) IN HIGH-LIGHT AND LOW-LIGHT MEMBRANES, FOLLOWING A 600 ms PERIOD OF ILLUMINATION IN THE PRESENCE OF ANTIMYCIN, AT DIFFERENT INITIAL REDOX POISES

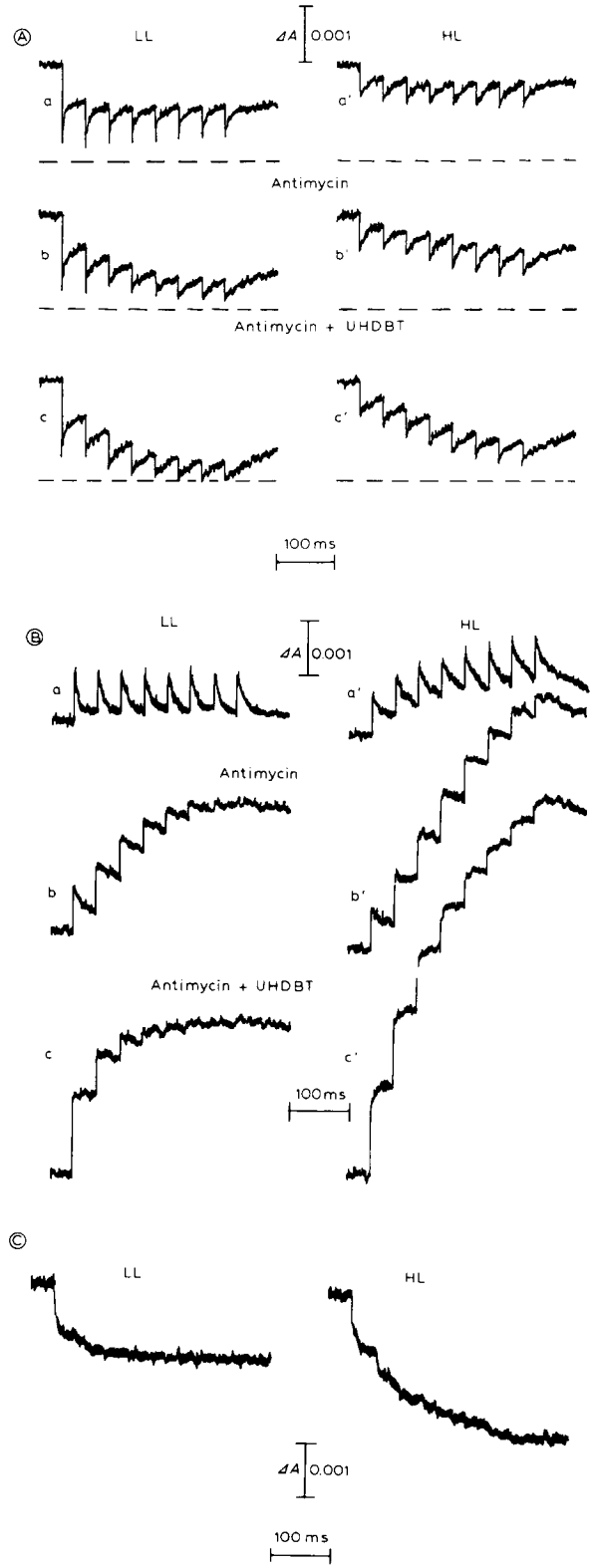
The assay conditions are described under Materials and Methods. Valinomycin and nigericin were present at 10 μM.

Membrane type	($c_1 + c_2$)/RC		*b*-561/RC		
	120 mV	160 mV	120 mV	160 mV	350 mV
Low-light	0.74	0.77	0.48	0.46	0.55
High-light	–	3.40	1.08	1.10	1.56

Electron transfer in single turnover

The kinetics of electron transfer have been comparatively studied in single turnover resolu-

* The maximum extent of photoreducible Cyt *b*-561, which titrates in the dark with an $E_m = 50$ mV, appears to depend on the E_h value also for E_h values higher than 200 mV, increasing upon raising the E_h to 350 mV. This phenomenon is especially evident in high-light membranes, where approx. 30% of the maximum extent measured at 350 mV is not observed at 200 mV. At both E_h values, the reduction is blocked by myxothiazole.



tion. Both types of membrane have been activated by a train of eight flashes, spaced 40 ms apart. Following the first flash the amount of Cyt($c_1 + c_2$) oxidized in the presence of UHDBT and antimycin is slightly larger in high-light membranes as compared to low-light membranes (Fig. 2B) and is reflected in the amount of reaction center re-reduced after the first flash (Fig. 2A). The large excess of Cyt($c_1 + c_2$) present in high-light membranes becomes evident after the following flashes in the train: in fact, while, as previously described [29], in low-light membranes the photooxidation of Cyt($c_1 + c_2$) is saturated after the third flash, in high-light membranes saturation is not reached even following the eighth flash (Fig. 2B). Particularly in the first three to four turnovers, there is no evident difference in the rate of Cyt($c_1 + c_2$) photooxidation, suggesting that Cyt c_2 and the bc_1 complexes form kinetically homogeneous pools of electron donors for the reaction centers. This conclusion is supported by measurement of Cyt *b*-561 photoreduction in the presence of antimycin (Fig. 2C): while most of this cytochrome is reduced following the first flash in low-light membranes, an excess of photoreduced Cyt *b*-561 can be observed in high-light membranes following the subsequent flashes in the train.

The different stoichiometries of bc_1/RC, ubiquinone/RC and Cyt c_2/RC produce marked effects on the rate of cyclic electron flow in the

Fig. 2. Light-induced changes in the redox state of electron transport components in low- and high-light membranes (LL and HL), in the absence and in the presence of electron transfer inhibitors. The assay conditions are described under Materials and Methods. The ambient redox potential was poised at $E_h = 150 \pm 10$ mV. Valinomycin and nigericin were present at 10 μM. Traces are normalized to the same RC content (0.17 μM) Chromatophores were activated by a train of eight single turnover flashes, fired 40 ms apart. The traces are the average of four measurements, take with a sweep of 500 ms and an instrument time constant of 200 μs. Left columns: low-light membranes; right columns (hyphen letters): high-light membranes. (A) RC redox changes. The dashed lines give the maximum extent of photoxidizable RC, measured in continuous light and in the presence of antimycin A. (B) Redox changes of Cyt($c_1 + c_2$). (a), (a') without antimycin; (b), (b') plus 10 μM antimycin; (c), (c') plus 10 μM antimycin, plus 40 μM UHDBT. (C) Redox changes of Cyt *b*-561 in the presence of 10 μM antimycin.

TABLE III

PERCENTUAL REDOX STEADY STATES OF RC AND CYTOCHROMES, REACHED FOLLOWING A 600 ms PERIOD OF ILLUMINATION, AT DIFFERENT E_h VALUES, IN THE ABSENCE OF INHIBITORS, IN HIGH-LIGHT AND LOW-LIGHT MEMBRANES

The assay conditions are described under Materials and Methods. Valinomycin and nigericin were present at 10 μM. The 100% value for each component was taken from Table II.

Membrane type	RC ox		$(c_1 + c_2)$ ox		*b*-561 red	
	120 mV	160 mV	120 mV	160 mV	120 mV	160 mV
Low-light	72	76	56	73	28	23
High-light	64	31	48	13	5	

absence of inhibitors and in the presence of uncouplers. In a train of flashes spaced 40 ms apart, not all reaction centers are re-reduced between flashes, but a pseudo-steady state-level of oxidized reaction centers is maintained. This level reflects a kinetic limitation in the delivery of electrons to the RC. The pseudo-steady-state level in high-light membranes is much lower than in low-light membranes (Fig. 2A), indicating a faster electron flow in the former case. The trace profiles also demonstrate clearly a faster re-reduction of RC, since in high-light membranes a much larger proportion of the re-reduction kinetics is not resolved at the time constant utilized in these measurements. This unresolved re-reduction phase is generally interpreted as due to electron transfer from reduced Cyt c_2 bound to the reaction-center complex [29,30,21]. The excess of reduced Cyt c_2 acting as electron donor to the RC is also clearly demonstrated by the observation that, when Cyt c_2 re-reduction is inhibited by antimycin, a large proportion of RC is re-reduced in a fast, non-resolved kinetics, even after the eighth flash in high-light membranes. On the contrary, in low-light membranes the maximum level of photooxidizable RC is reached already after the third flash in a train. This behavior is also evident, although less marked, in the presence of antimycin and UHDBT, and reflects the pattern of oxidation of Cyt$(c_1 + c_2)$ already described.

The difference in the stoichiometries of the redox carriers affects also the re-reduction kinetics of Cyt$(c_1 + c_2)$ in the absence of inhibitors (Fig. 2B). In low-light membranes, most of photooxidized Cyt$(c_1 + c_2)$ is re-reduced between flashes. In high-light membranes, oxidized Cyt c_2 gradually accumulates between flashes, reflecting presumably a stoichiometric limitation of bc_1 complexes over the Cyt c_2 pool. In spite of this accumulation of oxidized Cyt$(c_1 + c_2)$, an equal or larger amount of Cyt$(c_1 + c_2)$ is rapidly re-reduced in high-light membranes as compared to low-light membranes, as can be deduced by comparing the kinetic pattern following the first flash in the presence and in the absence of UHDBT.

Electron transfer in continuous light

The higher rate of uncoupled electron transfer in high-light membranes is also apparent from the steady-state redox level of RC and of Cyt$(c_1 + c_2)$ observed in continuous light in the absence of inhibitors (Table III). In fact, for both these electron carriers the percent reduction is higher in high-light membranes than in low-light membranes. The degree of reduction of Cyt *b*-561 appears to be slightly lower in high-light membranes than in low-light membranes. As already observed for the pseudo-steady-state reached under flashing light, the steady-state amount of oxidized Cyt$(c_1 + c_2)$ per RC is, however, larger in high-light membranes.

For both types of preparation, the initial ambient redox poise affects the RC and cytochromes redox state in continuous light in the absence of inhibitors. A lowering of the E_h from 160 mV to 120 mV causes a marked decrease in the steady-state level of photooxidation of RC and Cyt$(c_1 + c_2)$ and an increase in the level of Cyt *b*-561 reduction. Since an analogous decrease in the ambient redox potential does not affect the maximum extents of the light-induced redox changes in the presence of antimycin (see Table II), the E_h dependence of the steady states reached in continuous light in the absence of inhibitors seems to be a genuine effect, most likely related to the initial redox state of the ubiquinone pool, as a

parameter controlling the rate of electron transfer.

Consistent with the conclusions drawn from the analysis of redox steady states in continuous light, the decay kinetics of RC and Cyt($c_1 + c_2$) following a 600 ms period of illumination also indicate a faster electron flow in high-light membranes (not shown). In fact, the initial re-reduction rate of RC which at $E_h = 120$ mV is approx. 0.014 $e^- \cdot RC^{-1} \cdot ms^{-1}$ in low-light membranes is enhanced to 0.059 $e^- \cdot RC^{-1} \cdot ms^{-1}$ in high-light membranes. This re-reduction is completely inhibited by antimycin over the time-scale utilized for these measurements (sweep 30 ms, time constant 100 μs). It has to be noted that the recovery kinetics observed after continuous light are intrinsically different from those measured when chromatophores are activated by a train of single turnover flashes spaced 40 ms apart. The higher re-reduction rate observed after a period of continuous light in high-light membranes demonstrates a fortiori a faster electron flow, since the level of photooxidation reached in continuous light is higher in low-light as compared to high-light membranes.

The same conclusion can be reached from the re-reduction kinetics of Cyt($c_1 + c_2$) in high- and low-light membranes, which exhibits the same initial rates as RC do, when expressed as electrons entering Cyt($c_1 + c_2$) per RC per ms. Also these rates are sensitive to the redox poise as observed for redox steady states, being higher at $E_h = 120$ mV as compared to $E_h = 160$ mV.

Discussion

Electron transfer in high-light and low-light membranes

The results presented in this paper show that all constituents which participate in the light-driven cyclic electron flow and proton pumping increase relatively to the number of reaction centers in chromatophores from high-light cells. These components are ubiquinone 10, Cyt c_1, Cyt *b*-561, Cyt *b*-566 and Cyt c_2. Although the absolute values of cytochrome content are ill defined due to the uncertainty of the extinction coefficient in situ, the data of Table I are in broad agreement with the proposed stoichiometry for the components of the bc_1 complex [31,28,23]. It is noteworthy that the Cyt *b*-561/Cyt *b*-566/Cyt c_1 ratios remain approximately constant in high- and low-light membranes, and that a comparable fraction of the total dark titrating Cyt *b*-561 (about 80%) can be reduced in the light in the presence of antimycin.

It is clear from the present data that the ratio between RC and bc_1 complexes can vary by a factor of about 4 under different light regimes. Mobile redox carriers, diffusable in the membrane (ubiquinone) or in the periplasmic space (Cyt c_2) can be increased even more than 3-fold in high-light membranes compared to low-light ones. The stoichiometric excess of bc_1 complex : RC over the 1 : 2 ratio proposed in previous papers [20,23,28], has, however, hardly any relevance for the redox events following one single turnover flash, since in both types of membrane comparable amounts of Cyt *b*-561/RC and of Cyt($c_1 + c_2$)/RC are reduced and oxidized in the presence of antimycin and antimycin plus UHDBT, respectively.

The prompt redox response of the cytochromes *b* and *c* present in excess in high-light membranes to subsequent flashes in a train supports the concept of a largely delocalized redox interaction between RC's and bc_1 complexes, mediated by the increased content of ubiquinone and Cyt c_2. It should be noted that the apparent higher ratio of Cyt c_2/Cyt c_1 in low compared with high-light membranes resulted from a preferential loss of Cyt c_2 during membrane isolation from high-light cells, as already discussed in Results.

Finally, it may be noticed that chromatophores prepared from high-light-grown cells provide a better system to study dark equilibrium and light-induced redox changes of cytochromes; in fact, the signal to background absorption ratio increases in high vs. low-light membranes due to a 3-fold decrease of the antenna pigments with a concomitant 4-fold enhancement of cytochrome content per RC.

The varying stoichiometry of the components of the redox chain as a mechanism of physiological adaptation to light intensity

The present study extends earlier observations showing that during adaptation of phototrophically growing cells of *Rps. capsulata* to low or high light intensities, a process of membrane differentiation takes place. Cells shifted to low light

become limited in energy supply, which results in a transient retardation of growth. The lowering of light intensity triggers a signal chain leading to an increased synthesis of additional photosynthetic units in the growing intracytoplasmic membrane, especially of antenna complexes [8,32]. This includes enhanced synthesis of phospholipids [33]. In contrast, cells which are shifted to higher light intensities become limited in processes beyond primary photochemistry in the reaction center. The physiological adaptation causes a strong repression of the RC synthesis and a relative increase in the ATPase activity per RC, i.e., presumably in the number of F_1-F_0 complexes per RC [9].

These variations must be considered in relation with a parallel modification in the membrane surface area per cell, which consists of a 5-fold decrease of intracytoplasmic membrane vesicles, when the growth conditions are shifted from a low-light to a high-light regime. Morphologic data [8,9] indicate that this adaptation corresponds to a decrease by approx. 60% of the total membrane surface per cell. This situation in vivo is reflected in the properties of the chromatophores obtained from high- and low-light cells. Although cytoplasmic and intracytoplasmic membranes form a continuous membrane system [3], membrane fractions having different sedimentation velocities can be obtained. The fast sedimenting fraction (fraction III) is enriched in RC/B870; while fraction II contains more B800–850 antenna complex. High-light cells have a larger portion of the fraction III, because the formation of intracytoplasmic vesicles is smaller than in low-light cells.

The rate of electron transport, when expressed per RC, is faster in high-light membranes than in low-light membranes, reflecting the stoichiometric excess of the other electron carriers over the RC's. The rate of RC re-reduction in the dark after a brief irradiation with continuous light indicates a 3-fold stimulation in high-light membranes: this figure should be related to a 5-fold decrease of RC per mg of cell protein, corresponding to about a 2-fold decrease per total membrane surface. Similar calculations yield an average increase of 150% in both bc_1 and ubiquinone per membrane surface area. A similar evaluation for Cyt c_2 is complicated by the unknown value for the volume of the periplasmic space and the massive losses of this carrier during the preparation of chromatophores from high-light cells.

These relatively small alterations in the composition of the photosynthetic chain are at the basis of the physiological adaptation to growth conditions in which the RC turns over at a faster rate and interacts rapidly with a larger number of bc_1 complexes. This mechanism of adaptation broadly resembles that observed upon transition from anaerobic-photoheterotrophic to semi-aerobic-chemotrophic growth conditions. Also in this case a repression of RC synthesis and a relative increase in the content of ubiquinone, Cyt c_2 and bc_1 complexes was detected [26].

The results of this paper therefore support the hypothesis that the high rate of photophosphorylation obtained in high-light membranes at saturating light intensities [8,9] is due mainly to an increased ratio of electron transport components per RC, resulting in a faster cyclic electron transport and proton pumping and in the utilization of the proton gradient by a higher number of H^+-ATPases. We have not studied whether an optimized antenna/RC organization and RC trapping contributed to the higher V_{max} of photophosphorylation in high- compared with low-light cells.

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