Complementary chromatic adaptation alters photosynthetic strategies in the cyanobacterium *Calothrix*

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The cyanobacterium *Calothrix* sp. strain PCC 7601 drastically changes phycobiliprotein composition and colour in response to light quality, through complementary chromatic adaptation (CCA). Red light promotes phycocyanin-II and inhibits phycoerythrin synthesis, while green light has the opposite effect, through changes in transcription regulated by a putative green/red photoreceptor(s). The effects of CCA on photosynthesis were characterized by measuring oxygen evolution and chlorophyll fluorescence parameters. Cells fully acclimated to either red or green light achieve a similar photosynthetic quantum yield of oxygen evolution (light-use efficiency). Shifting acclimated cells from green to red or from red to green light caused similar 40% drops in photosynthetic quantum yield. Therefore, full CCA significantly increases light use efficiency, which is of great importance under light-limited growth. Cells growing under red light are in state I, with very low PS II to PS I energy transfer, since red light is absorbed both by phycocyanin in the phycobilisome/PS II supracomplex and by PS I chlorophyll. Cells growing under green light are in state II, with high transfer of excitation energy from the phycobilisome/PS II supracomplex to PS I. This transfer allows green light captured by phycoerythrin to ultimately drive both PS I and PS II photochemistry.

Keywords: chlorophyll fluorescence, light-harvesting, phycobiliproteins, state transition

INTRODUCTION

Various cyanobacteria strongly modulate the composition of the rods of their phycobilisomes in response to the relative levels of red and green light. This acclimation process is termed complementary chromatic adaptation (CCA). The molecular mechanism for the red/green light regulation of CCA receives considerable attention (reviewed by Grossman, 1990; Grossman *et al.*, 1994; Tandeau de Marsac *et al.*, 1988; Tandeau de Marsac & Houmard, 1993), particularly in the strain *Calothrix* sp.

PCC 7601 (*Calothrix* 7601, syn. *Fremyella diplosiphon*) (Bennett & Bogorad, 1973; Capuano *et al.*, 1988; Gendel *et al.*, 1979; Grossman *et al.*, 1988; Oelmüller *et al.*, 1988a, b) and the closely related *Tolypothrix tenuis* (Fujita & Hattori, 1960; Hattori & Fujita, 1959a, b). Tandeau de Marsac (1983) distinguished three functional groups of cyanobacteria with respect to CCA. *Calothrix* is a representative of group 3, with the synthesis of both phycocyanin and phycoerythrin under chromatic control. A putative photoreversible pigment responds to red and green light, and regulates the expression of the phycoerythrin and phycocyanin-II operons through a system involving reversible phosphorylation of DNA-binding proteins, some of which have been characterized (Schmidt-Goff & Federspiel, 1993; Schyns *et al.*, 1994; Sobczyk *et al.*, 1993, 1994). Under pure green light, phycoerythrin gene expression is induced and expression of phycocyanin II genes is nearly completely repressed (Mazel *et al.*, 1986). Under red light the opposite occurs, with repression of phycoerythrin synthesis and induction

Abbreviations: CCA, complementary chromatic adaptation; Chl *a*, chlorophyll *a*; F_0 , minimal fluorescence; $F_M(\text{dark})$, maximal fluorescence in the dark-adapted state; F_s , steady-state fluorescence under illumination; F_0' , minimum fluorescence in a light-adapted state; F_M' , maximal fluorescence in a light-adapted state; F_M , true maximal fluorescence; PS I, photosystem I; PS II, photosystem II; q_N , non-photochemical quenching of chlorophyll fluorescence; q_P , photochemical quenching of chlorophyll fluorescence.

of phycocyanin-II. Pigment content under white light falls between these extremes and depends on the balance of green and red in the light source (Tandeau de Marsac, 1983).

In *Calothrix*, CCA interacts with nitrogen metabolism and hormogonium differentiation in a complex regulatory network. On top of the light quality regulation, the environmental source of nitrogen modulates pigment content to favour phycoerythrin when nitrate is supplied, or phycocyanin if ammonium is supplied (Erokhina, 1992; Liotenberg *et al.*, 1996). Furthermore, the pigment composition of the cells helps determine their susceptibility to induction of hormogonium differentiation (Damerval *et al.*, 1991). This link apparently arises because differentiation can be triggered by imbalances in electron transport, which depend upon the light qualities captured by the pigment systems (Campbell *et al.*, 1993). Furthermore, during differentiation all phycobiliprotein gene expression is transiently repressed (Damerval *et al.*, 1991).

In spite of the long-term efforts to define the molecular elements involved in cyanobacterial CCA (Grossman *et al.*, 1994; Kehoe & Grossman, 1996; Houmard *et al.*, 1996), functional characterization of the significance of CCA to photosynthesis has been lacking. Indeed, some ecologically oriented workers regard the phenomenon as a manifestation of low-light acclimation, with limited relevance to natural populations (Dring, 1981, 1990; Dring *et al.*, 1996; reviewed by Wyman & Fay, 1987). The dialogue is complicated by differing applications of the term CCA. In the original ecological sense, CCA referred to depth- and illumination-dependent segregation of algal groups according to pigmentation differences. This concept, although widely cited, has not withstood critical examination (Dring, 1981). In contrast, in many cyanobacteria, predominantly colonial or benthic strains from fresh water (Rippka & Herdman, 1992), chromatic regulation of gene expression qualitatively alters phycobilisome composition (Dring, 1981; Tandeau de Marsac, 1983). This CCA process is quite distinct from the overall regulation of phycobiliprotein content, which involves the allophycocyanin and phycocyanin-I operons as well as phycoerythrin and phycocyanin-II. CCA in cyanobacteria is of interest not only as an example of chromatic regulation of gene expression, but also for photosynthetic studies because it allows drastic and reproducible manipulation of pigment content and overall absorbance within a single strain.

In this study I used three complementary approaches to characterize photosynthesis of *Calothrix* 7601 cells acclimated to red or green light, measured under their growth light quality but also immediately after a shift to the alternative light quality. Oxygen evolution was used to monitor the rate and light-response of electron transport. Room-temperature fluorescence quenching analysis and 77 K fluorescence emission spectra provided information on the balance of electron transport and excitation between PS II and PS I. These results provide a functional characterization of CCA, and demonstrate alternate light-harvesting strategies and changes in electron transport upon changes in light quality.

METHODS

Cell growth. Cultures of *Calothrix* 7601 were grown in flat Roux bottles in 300 ml BG 11 medium (Rippka *et al.*, 1979) buffered to pH 7.5 with 10 mM MOPS, and bubbled with 1% CO₂ in air at about 1 ml s⁻¹. The Roux bottles were laid on their side in a Plexiglas shaking water bath regulated at 27 °C, with fluorescent and incandescent light sources shining up from below. The lower sides of the bottles were wrapped in red or green coloured filters (for spectra see Fig. 1), with the rest of the bottles wrapped in aluminium foil to exclude extraneous light. For both red- and green-light cultures the light intensity was about 15 µmol m⁻² s⁻¹. Cultures were inoculated with 10 ml from axenic precultures and then grown for 6–7 d, at which point samples were taken for pigment, fluorescence and oxygen evolution analysis. No clumping occurred and in all cases the cultures contained only long, cylindrical filaments with no hormogonia or pro-heterocysts.

Sampling and pigments. Fifteen-millilitre samples were taken from the culture flasks. One millilitre was mixed with 9 ml methanol to form a 90% methanolic extract for chlorophyll assays, while 13 ml were loaded directly into the measurement cuvette, along with 100 µl 1 M NaHCO₃, added to ensure the culture did not become CO₂ limited during the measurement. Each point presented on the graphs is the mean of three measurements on the same culture. The entire oxygen and room temperature fluorescence experiment was repeated three times on independently grown cultures, with similar results in all three replicates. Chlorophyll *a* (Chl *a*) content was measured as the absorbance at 665 nm of methanolic extracts of cells, using the equation 13.9 µg Chl *a* ≡ 1 A₆₆₅ unit (Tandeau de Marsac & Houmard, 1988). The cultures contained 3–4.7 µg Chl *a* ml⁻¹. Whole-cell spectra of typical red- and green-light grown cells were measured using a Shimadzu MPS 2000 dual-beam scanning spectrophotometer.

Oxygen measurements. The measurement cuvette used (Hansatech) allows precise control of light and temperature for simultaneous measurement of oxygen consumption or evolution, with a Clark-type electrode (Hansatech), and of fluorescence, with a PAM fluorometer (Walz) (Campbell *et al.*, 1996; Clarke *et al.*, 1993, 1995). Samples were first incubated in the dark to measure dark respiration. Then a Bjorkmann-type lamp (Hansatech) was used with filter combinations to provide increasing levels of green or red light to the culture. Measurement of the rate of oxygen evolution at seven to nine light intensities took about 40 min overall.

Room temperature fluorescence. When a pigment absorbs the energy of a photon and enters an excited state, there are essentially four routes for a return to the ground state: (1) photochemical reactions; (2) heat dissipation; (3) transfer of the energy to another pigment; and (4) emission of a fluorescence photon. These four processes are in competition, such that the most rapid de-excitation pathway predominates. For biological systems the chlorophyll fluorescence yield is usually very low (less than 3% of absorbed photons re-emitted as fluorescence), and, *in vivo*, fluorescence from PS II predominates (Krause & Weis, 1991), although in cyanobacteria non-variable fluorescence from phycobiliproteins also strongly contributes (Campbell *et al.*, 1996). Fluorescence, although a minor de-excitation pathway, is in competition with the quantitatively more important routes of photochemistry, transfer and heat dissipation. Therefore changes in the rates of photochemistry or in the two non-photochemical dissipation routes (transfer or heat) can alter (quench) the fluorescence yield from PS II (Bolhr-Nordenkamp & quist, 1993; Krause & Weis, 1991). Minimum fluorescence, F₀, was determined by illuminating the

dark-adapted cells with a low-intensity light modulated at 1.6 kHz (average intensity of $0.14 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) from a light-emitting diode (peak emission 650 nm). A 1 s flash of saturating white light ($8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was then given to determine maximal fluorescence in the dark-adapted state, $F_M(\text{dark})$. After a further 30 s, the actinic light was activated. Steady-state fluorescence, F_s , was achieved within 2 min, and minimum fluorescence in the light-adapted state, F_0' , was measured by briefly interrupting the actinic beam. After F_s was re-established, a saturating light pulse was given to determine maximal fluorescence in the light-adapted state, F_M' . The actinic light intensity was then increased and the process was repeated in steps. Finally, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) ($0.5 \mu\text{M}$ final) was injected into the cuvette to stop electron transport, for determination of the true maximal fluorescence, F_M . The parameters F_0 , $F_M(\text{dark})$, F_s , F_0' , F_M' and F_M were used for calculation of photochemical (q_p) and non-photochemical (q_n) quenching coefficients (van Kooten & Snel, 1990).

q_p is approximately equal to the proportion of PS II reaction centres which are open and ready to perform photochemistry. It reflects the balance between capture of light energy, which tends to close the reaction centres, and dissipation of energy (by any route), which re-opens reaction centres. The non-photochemical quenching coefficient, q_n , reflects any process other than photochemistry which lowers the fluorescence yield, and therefore is a measure of the transfer or heat dissipation de-excitation routes.

77 K fluorescence emission spectra. Fifty-millilitre samples of the same cultures as used for oxygen and fluorescence-quenching measurements were harvested and concentrated to $12.1\text{--}12.5 \mu\text{g Chl } a \text{ ml}^{-1}$ by centrifugation. Thirteen millilitres were loaded into the cuvette used for oxygen and fluorescence-quenching measurements. The sample was dark-adapted for 10 min, and then, under very dim ambient light, a clear acrylic rod sample holder for 77 K fluorescence was dipped briefly into the cuvette to collect about 100 μl ; this sub-sample was plunged directly into liquid nitrogen. The sample holder was attached to a fibre-optic-based fluorometer and 77 K fluorescence emission spectra were collected as described by Ögren & Öquist (1984), with excitation at 430 and 574 nm. The sample in the cuvette was then incubated under green or red light light equivalent to the growth light intensity of $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and sub-sampling for 77 K fluorescence was repeated. Through this procedure, the 77 K spectra are from cells under precisely the same conditions as those used for room-temperature fluorescence quenching and oxygen measurements.

RESULTS

Different pigmentation of cells grown under red and green light

The transmission spectra of the red or green filters used for growth and photosynthetic measurements are presented in Fig. 1. After 6 d growth under $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of either red or green light, the cultures reached similar cell densities of about $4 \mu\text{g Chl } a \text{ ml}^{-1}$, although the whole-cell absorbance spectra presented in Fig. 2 show that the phycobiliprotein composition of the red- and green-light cultures was very different. The cultures grown under red light contained large amounts of phycocyanin, with a peak absorbance at 622 nm, but only a trace of phycoerythrin. In contrast, the green-light cultures contained much less phycocyanin but had high

Fig. 1. Transmission spectra of the red (—) or green (---) coloured filters used for growth and photosynthetic measurements of the *Calothrix* PCC 7601 cultures.

Fig. 2. Representative whole-cell absorbance spectra of *Calothrix* PCC 7601 cultures grown under red (—) or green (---) light.

levels of phycoerythrin, with peak absorbance at 568 nm. Comparison of the spectra of the growth lights and the whole-cell spectra shows that the overlapping absorbance peaks of both phycocyanin and chlorophyll fall within the wavelength range of the red light supply, which transmits wavelengths greater than 605 nm. In contrast, only phycoerythrin absorbance is appropriate for capture of the green light supply, peaking at 533 nm.

Similar oxygen evolution in cells grown under red or green light

The light response curves of oxygen evolution were measured for cultures grown under red (Fig. 3a) or green (Fig. 3b) light. Oxygen evolution was measured under both red and green light, so that each culture was measured under the light quality used for its growth, but also under the alternative light quality. When oxygen evolution is plotted versus the supplied light intensity, the initial slope of the curve is proportional to the quantum yield of photosynthetic oxygen evolution. As the light intensity exceeds the level to which the cultures are

Fig. 3. Light response curves for oxygen evolution measured with red (—■—) or green (---○---) light. The results are means of three measurements on the same culture, $\pm$ SE. (a) Culture grown under red light; (b) culture grown under green light. (c) Similar oxygen evolution at low light intensities for cultures measured under the light quality used for their growth, red (—■—) or green (---○---).

acclimated, the curve deviates below the initial slope, since factors other than light supply start to limit photosynthetic oxygen evolution. In these experiments the light intensities were not raised to levels high enough to saturate electron transport, in order to avoid any photoinhibition of the cells or changes in light quality at high intensity. The initial slopes, and hence the quantum yield of photosynthetic electron transport, were almost identical for red- and green- light-acclimated cultures when measured under their respective growth lights (Fig. 3c). Therefore, when a culture is properly acclimated the overall light-use efficiency of net photosynthetic electron transport is similar under green and red light.

Shifts of cells acclimated to red growth light to green measurement light, or vice versa, from green growth light to red measurement light, had similar effects on oxygen evolution. After either light shift the quantum yield of photosynthetic electron transport dropped to about 60 % of the value under the respective growth light. This drop drove up the light compensation point from 9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for cells under their growth light quality to 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (upon a shift of red light cells to green light) or to 12.5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (upon a shift of green light cells to red light) (Fig. 3a, b). Since the cells were acclimated to growth under only 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ these changes in the light compensation point and quantum yield have major influences on photosynthesis after a shift from one light quality to the other.

PS II reaction centres remain largely open under red or green light

For cultures grown under either red or green light, photochemical quenching of fluorescence, q_P , is somewhat lower when measured under green light than when measured under red light (Fig. 4a, b). This difference is particularly evident for the cultures grown under red light and shifted to green light (Fig. 4a). However, in common with other cyanobacteria, q_P remained high even at light intensities 10-fold higher than the growth light intensity of 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 4a, b). This indicates that most PS II reaction centres (> 85 %) remained open under all conditions, whereas in plants PS II centres close more readily in response to increasing light (Öquist *et al.*, 1995).

Energy transfer from the phycobilisome/PS II to PS I under green light

Fig. 5 presents light response curves for q_N , a measure of the non-photochemical quenching of fluorescence. In these *Calothrix* 7601 cultures, as in most cyanobacteria, q_N was relatively high in dark-acclimated cells, reflecting state II, a condition of low PS II fluorescence yield. Generally, upon illumination PS II fluorescence yield increases and q_N drops, falling to a minimum at around the growth light intensity, as the cells enter state I (Fujita *et al.*, 1994). This typical pattern of a state II to state I transition was very pronounced in the *Calothrix* 7601

Fig. 4. Light response curves of photochemical quenching measured under red (—■—) or green (---○---) light. The dashed vertical line marks the growth light intensity ($15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The results are means of three measurements on the same culture, $\pm \text{SE}$. (a) Culture grown under red light; (b) culture grown under green light.

cultures upon illumination with red light (Fig. 5a, b). In contrast, under green illumination q_N remained high, at or near the dark-acclimated level, for cultures grown under either light. There was little evidence of a state II–state I transition under green light and the cells remained in state II throughout the light-response curve (Fig. 5a, b). Nevertheless, cells grown under green light were fully competent to perform a large state transition when shifted to red light (Fig. 5b).

Fig. 6 presents 77 K fluorescence emission spectra for cells after pre-incubation in the dark or after pre-incubation under $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of red or green light. Cells were pre-treated in the same cuvette and in the same manner as for the room-temperature fluorescence measurements used for calculation of q_P (Fig. 4) and q_N (Fig. 5). Excitation of fluorescence was with 569–579 nm light, absorbed by the phycobilisome. The major emission peaks were from PS I (726–729 nm); PS II (682–685 nm, shoulder at 690–693 nm); phycocyanin (655–659 nm) and phycoerythrin (642–647 nm). In the single-celled cyanobacterium *Synechococcus* sp. strain PCC 7942 the PS II fluorescence is resolved into two clear peaks (Salehian & Bruce, 1992), whereas in *Calothrix* 7601 the 690 nm fluorescence is only a shoulder. Whether this difference represents an important distinction in fluorescence emission from PS II, or if it arises simply from less favourable

Fig. 5. Light response curves of non-photochemical quenching measured under red (—■—) or green (---○---) light. The dashed vertical line marks the growth light intensity ($15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The results are means of three measurements on the same culture, $\pm \text{SE}$. (a) Culture grown under red light; (b) culture grown under green light.

optical properties in the filamentous *Calothrix* 7601, is unknown.

Cells pre-incubated in the dark are in state II, with relatively low PS II and phycobilisome fluorescence, supporting the room-temperature fluorescence measurements which showed high non-photochemical quenching of PS II fluorescence (Fig. 5). Upon illumination with green light, PS I fluorescence increased somewhat but PS II fluorescence increased only very slightly (Fig. 6), since as also shown by Fig. 5, the cells remained in state II under green light. Under red illumination, PS I fluorescence fell and PS II fluorescence increased very significantly (Fig. 6), reflecting the transition to state I. Phycobilisome fluorescence increased above the dark-acclimated level under both green and red light, by approximately the same extent.

Fig. 7 presents further evidence that the cells strongly modulate energy flow from the phycobilisome to PS I depending on the light environment. Fig. 7(a) plots PS I fluorescence at 77 K versus non-photochemical quenching of *in vivo* PS II fluorescence, q_N , measured under the

Fig. 6. 77 K fluorescence emission spectra for dark-incubated cells (.....), cells pre-incubated under green light (-----) and cells pre-incubated under red light (—). Excitation of fluorescence was in all cases with a 574 nm light beam. Dashed lines emphasize changes in relative peak heights. (a) Culture grown under red light; (b) culture grown under green light.

same treatment conditions. PS I fluorescence excited by phycobilisome absorbance of light at 574 nm increased with increasing q_N . This shows that the changes in q_N measured in living cells result from modulation of the excitation flow from the phycobilisome to PS I. In contrast, PS I fluorescence excited by chlorophyll absorbance of light at 430 nm declined slightly as q_N increased. Fig. 7(b) plots the ratio (PS I fluorescence excited by phycobilisome absorbance)/(PS I fluorescence excited by chlorophyll absorbance) versus q_N . As q_N increases, energy flow from the phycobilisome to PS I becomes more important relative to energy flow from chlorophyll to PS I.

DISCUSSION

CCA to red or green light results in cells with very different absorbance spectra, but the same initial slope of oxygen evolution versus light intensity, and, therefore, the same quantum yield of photosynthetic electron transport. Transfer to the alternative light lowers the quantum yield to about 60% of the acclimated level. This drop occurs because the cells have reduced ability to

Fig. 7. PS I fluorescence emission at 77 K correlates with non-photochemical quenching of *in vivo* PS II fluorescence. (a) PS I fluorescence excited by phycobilisome absorbance (○) (574 nm) or by chlorophyll absorbance (■) (430 nm), plotted versus non-photochemical quenching of *in vivo* PS II fluorescence. (b) Ratio of (PS I fluorescence excited by phycobilisome absorbance)/(PS I fluorescence excited by chlorophyll absorbance), plotted versus non-photochemical quenching of *in vivo* PS II fluorescence. ○, Green light cultures; ■, red light cultures.

capture photons in the new wavelength range and it represents the maximum effect of CCA on overall electron transport. Such a large change in quantum yield is of considerable importance for cells growing under low light. CCA in cyanobacteria is probably a special means to increase light capture in species which frequently encounter low, and chromatically imbalanced, light (Wyman & Fay, 1987), particularly in filamentous species such as *Calothrix* which naturally occur in dense layers with steep gradients of light intensity and quality (Villbrandt *et al.*, 1990). Examination of the spectra of the light sources and the whole-cell spectra shows that only phycoerythrin is able to capture the green light range. Therefore, a mechanism exists to allow green light captured by phycoerythrin alone to drive photochemistry in both photosystems (Fujita *et al.*, 1994; Zhao *et al.*, 1992).

Changes in electron transport are relevant to the regulation of differentiation in *Calothrix* 7601. Shifting a culture from green to red light for several hours strongly induces hormogonium differentiation, which precedes the longer-term CCA process that changes pigment composition. The reciprocal shift of a culture from red to green light does not induce hormogonia (Damerval *et al.*, 1991; Herdman & Rippka, 1988), even though shifts in either direction cause a similar drop in overall electron transport. Therefore, the light effects on differentiation are not caused by changes in the rate of electron transport. Rather, the green to red light shift provokes specific imbalances in electron transport which trigger differentiation (Campbell *et al.*, 1993).

Photochemical quenching of PS II fluorescence measures the oxidation state of PS II. If excitation of PS II exceeds electron transport away from PS II, the reaction centres tend to close and q_P drops. In *Calothrix* 7601 q_P is somewhat lower under green light, particularly in cultures shifted from red to green light, reflecting the absorbance of green light exclusively by phycoerythrin associated largely with PS II. This preferential excitation tends to drive fractional closure of the PS II reaction centres. Nevertheless, even under strong green light, most PS II centres remain open, once again indicating that the cells are able to efficiently re-direct light energy captured by phycoerythrin, to maintain fairly balanced excitation of both photosystems (Zhao *et al.*, 1992).

In cyanobacteria, transfer of energy from the phycobilisome/PS II supracomplex to PS I is the predominant process contributing to non-photochemical quenching of PS II fluorescence (Campbell *et al.*, 1996). The behaviour of *Calothrix* 7601 grown under green light provides strong support to this interpretation. Green-light cultures grow with a high q_N , but electron transport is faster under green light than when measured under red light, where cells shift to a low q_N . If the high q_N under green light reflected energy lost as heat, the overall system would be less efficient under green than red light. Instead, under green light, energy captured by the phycobilisome/PS II is transferred to PS I, rather than being used to drive PS II photochemistry. This transfer appears as a high q_N and low PS II fluorescence, even though overall electron transport in these cells is more efficient under green light than under red light.

This energy flow can be detected directly using 77 K fluorescence emission from PS I. Light energy absorbed by the phycobilisome excites PS I fluorescence, and this emission increased in parallel with the q_N parameter, measured in the cells during the pre-treatment before freezing. In contrast, chlorophyll excitation of PS I fluorescence declined slightly with increasing q_N . Therefore, as q_N increases, as under green light, excitation of PS I activity via phycobilisome absorbance becomes increasingly important.

The green-light cultures operating in state II demonstrate a role for phycobilisome/PS II to PS I energy transfer under steady-state growth. Cyanobacteria are generally in state II with low PS II fluorescence and high q_N in the

dark and under very low light, shifting to state I with low q_N under the normal growth light. This typical pattern is found in *Calothrix* 7601 upon illumination with red light, which is captured by both phycocyanin and chlorophyll and can therefore directly excite both PS II and PS I. But under green illumination q_N remains high, and light captured by phycoerythrin drives both PS II and PS I photochemistry, for balanced electron transport. This strategy may also be reflected in the length of phycobilisome rods. In some strains under green light, each rod contains three hexameric discs of phycoerythrin, which under red light are replaced by only two discs of phycocyanin (Cohen-Bazire & Bryant, 1982; Tandeau de Marsac, 1983). The longer rod under green light may reflect the increased duty of the phycobilisome serving as antenna for both PS II and, indirectly, for PS I (Zhao *et al.*, 1992).

State transitions in cyanobacteria are regulated by the balance of electron transport between PS II and PS I (Mullineaux & Allen, 1986; Vernotte *et al.*, 1990), although the mechanistic details remain controversial (Fujita *et al.*, 1994; Sidler, 1994). In this case, green-light excitation of phycoerythrin drives overexcitation of PS II, which in turn causes establishment of state II with a high flow of excitation from the phycobilisome/PS II to PS I. *Calothrix* PCC 7601 thereby attains similar overall photosynthesis under either red or green light, but uses very different light-harvesting strategies to accommodate the chromatically imbalanced lights.

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