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Brief communication

Uphill energy transfer from long-wavelength absorbing chlorophylls to PS II in *Ostreobium* sp. is functional in carbon assimilation

Christian Wilhelm* & Torsten Jakob
Biology I / Plant Physiology, University of Leipzig, Johannisallee 21–23, 04103 Leipzig, Germany;
**Author for correspondence (e-mail: cwilhelm@rz.uni-leipzig.de; fax: +49-341-9736899)*

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Abstract

From the algal genus *Ostrobium* two species are known which express a chlorophyll antenna absorbing between 710 and 725 nm to a different extent. In a comparative study with these two species it is shown that quanta absorbed by this long wavelength antenna can be transferred to PS II leading to significant PS II-related electron transfer. It is documented that under monochromatic far red light illumination growth continues with rather high efficiency. The data show that the uphill-energy transfer to PS II reduces the quantum yield under white light significantly. It is discussed that this strategy of energy conversion might play a role in special environments where far red light is the predominant energy source.

Abbreviations: Chl – chlorophyll; ETR – relative electron transport rate; F – fluorescence yield; F_m' – maximal fluorescence yield of illuminated sample; $\Phi_{PS\ II}$ – Photosytem II quantum yield; I_K – PAR-value characteristic for light saturation; LHC – light harvesting complex; PAM – pulse amplitude modulation; PAR – photosynthetically active radiation; P_{max} – maximal rate of photosynthesis; PS – Photosystem

Introduction

Engelmann (1884) was the first who measured photosynthetic action spectra by observing the migration of oxygen-requiring bacteria in the microscope under the influence of monochromatic light. He observed that the action spectrum followed in general the absorption spectrum of the photosynthetic pigments. Later on, this observation was modified. It was noticed by Haxo and Blinks (1950) that in red algae in the blue part of the spectrum photosynthesis is less efficient as expected from absorption. In the red the photosynthetic performance dropped down at shorter wavelengths as the absorption spectum. This 'red drop' in the photosynthetic action spectum has been explained later by Emerson (1958) who showed the cooperative action of two photosystems. Later on, after the discovery of photochemically inactive light harvesting antenna proteins, the photosynthetic unit was understood as a system compromising peripheral antenna complexes which transfer their energy to the reaction center down-hill like in a funnel. In such a funnel energy migrates in any case from short-wavelengths absorbing chlorophylls to long-wavelength tuned pigments. Long wavelengths absorbing chlorophylls in chloroplasts have been discovered by Butler (1961), starting a long discussion on their function until now. It was hypothesized that the far red absorbing pigments can either increase the absorption cross section of PS I (Trissl 1993) or act as quencher with a photoprotective function (Stahl et al. 1989).

A decade ago, the light-harvesting subpopulations of both photosystems had been described

on the biochemical and molecular level, giving evidence that PS I-core complex interacts with four peripheric antenna-proteins encoded in the nucleus as *lhca*1-4 (Durnford et al. 1999). Recent progress in structural biology has revealed the atomic structure of PS I-LHC I supercomplexes (Fromme et al. 2001; Ben-Shem et al. 2003). These data show that the LHC I subunits are attached to the reaction center core by evenly spaced lhca monomers. Mutation analysis of the lhca4 subunit has revealed clear evidence that the low energy absorption band of the most red shifted chlorophylls is due to a specific interaction between the chlorophylls A5 and B5 (Morosinotto et al. 2005).

Based on the excitation equilibrium model (Schatz et al. 1988) energy can migrate from 'red chlorophylls' back to the reaction center via a slow thermally activated transfer (Jennings et al. 2003). This uphill energy transfer is supported both structurally by the existence of the so-called gap chlorophylls which bridge the far red chlorophylls with the bulk chlorophylls of the inner core complex and biophysically by a slow equilibration phase in the excitation dynamic within the PS I-LHC I network (Ihalainen et al. 2002). From the atomic structure it has been concluded that in most cases the excitons were transferred through the pools of far red chlorophylls before being trapped (Ben-Shem et al. 2003). Therefore, up-hill energy transfer seems to be the normal case in higher plant PS I. A real up-hill energy transfer has been described in the case of cyanobacteria (Murata 1977; Shubin et al. 1995), purple bacteria (Trissl et al. 1999) and recently, in the Chl *d*-containing photosynthetic prokaryote *Akaryochloris marina* (Mimuro et al. 2000). In the case of *Akaryochloris* the exciton have to migrate from Chl *d* to Chl *a* acting in both reaction centers. To our knowledge it was not yet shown that long-wavelength absorbing Chl *a* molecules can transfer their energy 40 nm uphill (from 720 nm in the antenna to 680 nm in the RCII) to drive linear photosynthetic electron transport with the primary function to produce biomass.

The marine alga *Ostreobium* sp. has been shown to possess an extremely large number of long wavelengths absorbing chlorophylls (Haldall 1968) bound to the Lhca1-type protein (Koehne et al. 1999). From this genus two different species are known *O*. sp. and *O*. *queketii*, where this Lhca1-type protein is expressed to different extent. Spectroscopic studies showed that in both species the antenna size is significantly larger than in higher plants; additionally, it was found that the ratio of F_v/F_m of dark adapted cells was only half of that found in higher plants indicating that the expression of long-wavelength absorbing chlorophylls is related to a decrease in quantum yield.

In this study we show that in *Ostreobium* the uphill energy transfer is a physiological mechanism to maintain growth under unfavorable light conditions and deliver arguments why this strategy is not of general use in oxygenic photosynthesis.

Materials and methods

Algal material and growth

O. *queketii* (strain B 14.86) was obtained from the Collection of Algal Cultures in Göttingen (Germany). *O*. sp. was isolated by Prof. Schnetter (University of Gießen, Germany) from corals in the Chilenean Pacific Ocean. Both strains were cultivated in a medium according to Müller (1961) at 20 °C in a 16:8 light dark cycle illuminated with 5 μmol photons m^{-2} s^{-1}. For cultivation the thallus was cut in several pieces and kept under the conditions mentioned for 6 weeks when the size of the thallus has reached a diameter of about 1–2 cm.

Absorption measurement

The thalli were settled on glass fiber filters which were placed into a 0.5 cm cuvette and measured with a SPECORD 500 (Zeiss, Germany). As reference blank filters have been used to correct for scattering. Spectrally resolved measurements of the light used for illumination together with the absorption spectra allowed the calculation of the quanta absorbed by a given sample (Gilbert et al. 2000).

Oxygen measurement

The thalli were settled on the surface of a sieve which was fixed perpendicular to the plane of illumination to prevent self-shading as much as possible. Oxygen measurement and illumination

was performed with an ILLUMINOVA light pipette (Illuminova Instruments, Sweden) with a temperature control to maintain 20 °C during measurement. The light intensity was adjusted to 40 μmol photons m^{-2} s^{-1} for *Ostreobium* and 50 μmol photons m^{-2} s^{-1} for *Chlorella*. Action spectra have been calculated on the basis of the quanta absorbed.

Fluorescence measurements

Chl *a* fluorescence was measured with a XENON PAM (Heinz Walz GmbH, Effeltrich, Germany) equipped with Optical Unit ED101-US featuring a standard 10×10 mm quartz cuvette (for a more detailed description of the instrument see Kolbowski and Schreiber (1995) and Schreiber (1998)). To measure up-hill energy transfer the monochromatic light produced by different filters has been used as actinic light source. We used band pass filters with a band width of 20 nm with the maximal transmission at 680, 700, 710, 720 and 730 nm. The quality of the filter was checked by measuring the transmission spectra showing that outside the spectral range defined the transparency was lower than 1%. Normally, the actinic light does not influence the fluorescence emission, because the measuring light is pulse-modulated whereas the actinic light is not. Using the coccoid green algae *Chlorella* as a reference organism having no significant contribution of long wavelength absorbing chlorophylls, this setup did not show any variable fluorescence in the case of *Chlorella* at excitation wavelengths higher than 710 nm. The absolute quantum yield was calculated by plotting the electron transport rates according to Genty et al. (1989) against the light quanta absorbed. The slope of the resulting linear relationship was designated as electron transport yield of PS II.

Chlorophyll a fluorescence transients were measured at room temperature using the PEA fluorometer (Hansatech, Norfolk, UK). The saturating light pulse was applied at 3500 μmol photons m^{-2} s^{-1} (1 s duration). Measurements were performed in a custom-made device for algal suspensions. Actinic illumination at 680 and 720 nm (4 min duration) was provided by a Schott lamp (KL1500, Schott, Mainz, Germany) in combination with band pass filters with an intensity of 120 μmol photons m^{-2} s^{-1}.

Growth measurement

The fresh weight of a set of thalli was determined under sterile conditions and then exposed to different light climates for 2 weeks. Far red light was produced by using white lamps (OSRAM TL 40) as the primary source filtered through a RG9 filter (Schott, Mainz, Germany) which cuts off all wavelengths shorter than 695 nm. White light was produced by a glass filter SP695 which has a 80% transmission from 430 to 680 nm cutting of all wavelengths higher than 700 nm. After 2 weeks growth in a 16:8 h under 10 μE m^{-2} s^{-1} the thalli were harvested, washed with fresh medium and the fresh weight determined.

Results

Figure 1a shows the normalized absorption spectra of both *Ostreobium* species in comparison to a typical green algae *Chlorella vulgaris*. In the far red

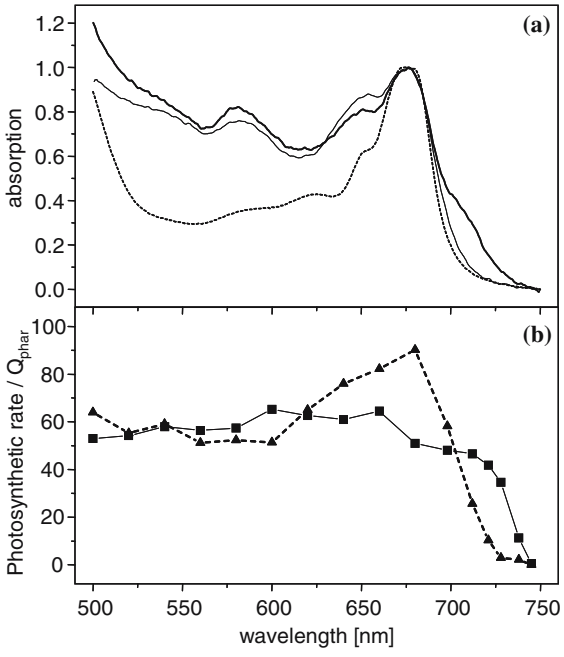

Figure 1. (a) *In vivo* absorption spectra of thalli of *O*. sp. (bold line), *O. queketii* (thin line) and of cell suspensions of *C. vulgaris* (dashed line). Absorption was normalized to the maximum at 680 nm. (b) Photosynthetic action spectra of *O*. sp. (filled squares, solid line) and *C. vulgaris* (triangles, dashed line). Actinic illumination was applied with an intensity of 40 (*O*. sp.) and 50 μmol photons m^{-2} s^{-1} (*C. vulgaris*) at different wavelengths using monochromatic filters.

region of the Chl *a* absorption *O. queketii* and *C. vulgaris* exhibited the typical red drop having only 7–10% absorption at 700 nm compared to the Chl maximum. In *O.* sp., however, the ratio of the absorption at 700/680 nm accounts to approximately 0.4. At 720 nm, when in typical green plants the absorption is negligible, in *O.* sp. the absorption still accounts to 30% of the maximum. In the green part of the spectrum both *Ostreobium* species behave similar and clearly different from *C. vulgaris*, because *Ostreobium* contains high amounts of siphonoxanthin and siphonein and due to its filamentous organization the light scattering is much higher leading to a much stronger apparent absorption in the short wavelength region.

In Figure 1b the action spectra (oxygen evolution per absorbed photon) is compared between *C. vulgaris* and *Ostreobium* sp.. At 680 nm the photosynthetic quantum yield of *Chlorella vulgaris* is found to be nearly twice as high as in *Ostreobium* sp., whereas the latter one does not show the typical red drop: even at 720 nm the oxygen production is nearly as efficient as in the red maximum. Interestingly, the highest photosynthetic quantum yield is found in the green region where the reaction center itself has only minor absorption and the energy is delivered from the LHC II antenna. However, oxygen evolution rates are influenced by the dark respiration and the true PS II quantum yield should be measured directly via fluorescence quenching analysis. For this purpose we excited the cells with monochromatic light higher than 680 nm and measured the fluorescence quantum yield of PS II. In the case of *C. vulgaris* we could not measure a significant closure of PS II reaction centers, if the cells have been excited with light above 680 nm (Figure 2). However, in *O.* sp. again, we observed a rather low quantum yield at maximal chlorophyll absorption but only a slight decrease in the yield between 680 and 710 nm. Even at 720 nm the quantum yield for PS II was found around 25%. This evidently shows uphill energy transfer from the long-wavelength absorbing chlorophylls in the PS I antenna up to the RC II.

Furthermore, with the measurement of chlorophyll fluorescence induction kinetics (Figure 3) we found evidence that electrons released at PS II are transferred into the redox carrier system between PS II and PS I. Whereas, in *C. vulgaris* excitation with

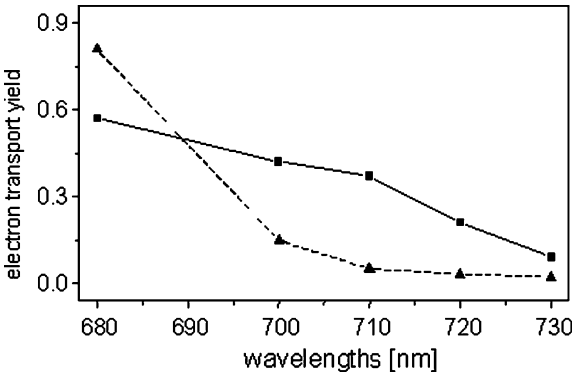

Figure 2. Quantum yield of electron transport in *O.* sp. (filled squares, solid line) and *C. vulgaris* (triangles, dashed line). The data are derived from the slope of electron transport rates during illumination with different light intensities and at different wavelength.

far-red light (720 nm) did not lead to a significant reduction of the PQ-pool (relative reduction state increased by 9% compared to excitation at 680 nm set as 100%) in *O.* sp. we found a strong increase in the reduction state of PQ (56%) at 720 nm compared to the reduction state at 680 nm (set as 100%).

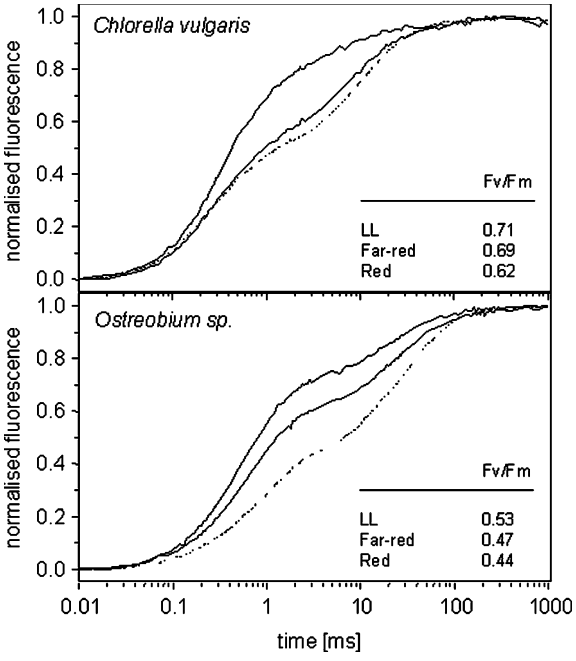

Figure 3. Chlorophyll fluorescence induction kinetics in *C. vulgaris* and *O.* sp. Data were normalized to the initial and the maximum fluorescence. Cells were measured after low light-adaptation (dashed line) and after actinic illumination with far-red (720 nm, thin solid line) and red light (680 nm, bold solid line).

Table 1. Relative growth rates in *O. queketii*, *O.* sp. and *C. vulgaris* during a 14 days-cultivation under white light (400–700 nm) and under far-red light (> 700 nm) illumination

Species	White light [relative growth rate 14 days]	Far-red [relative growth rate 14 days]
Ostreobium queketii	100	21
Ostreobium sp.	100	78
Chlorella vulgaris	100	−21

Finally, we were interested to know if the far red driven photosynthesis is able to promote growth. For this purpose we have grown both *Ostreobium* species in two different light climates at quantum corrected fluxes: one in white light and one in far red light with a cut-off filter at 700 nm. The growth rate in white light was taken as 100%. In *O. queketii*, having only a minor far-red absorption the relative growth rate was reduced to 21%, whereas in *O.* sp. growth was similar in both light climates (Table 1). In the green alga *C. vulgaris* no growth was observed at wavelengths above 700 nm.

Discussion

In higher plants Rivadossi et al. (1999) have demonstrated that in a shady environment up to 40% of the photons can be harvested by far red absorbing chlorophylls. The increase in absorption capacity of PS I may have a double function. First, it can induce the up-regulation of LHC II units leading to a typical shade type chloroplast. Second, an increased PS I absorption cross section can serve cyclic photophosphorylation. Alternatively, the red spectral forms of chlorophyll have been discussed to be photoprotective, because the energy being stored in this pigment matrix will become dissipated mainly as heat (Stahl et al. 1989). In higher plants and green algae the uphill-energy migration from the red forms to the reaction center is restricted to PS I. Kühl et al. (2005) have reported that the Chl *d*-containing cyanobacterium *Acaryochloris* can grow in biofilms beneath didemnid ascidians where only far red light is available. Therefore, Chl *d* located in the antenna drives the photochemistry of PS I and PS II in this species.

In the case of *Ostreobium* it was shown by Koehne et al. (1999) that the peptide responsible for the accumulation of red-shifted Chls is the Lhca1 subunit. Therefore, the species- and growth dependent far red absorption in *Ostreobium* strains clearly show that only stoichiometric differences of well known subunits are responsible for the differences in the far-red absorption. However, although the molecular basis appears similar in higher plants and *Ostreobium* the function seems to be basically different. First, the red Chl forms contribute to a much higher extent to total absorption. From the absorption spectrum one can roughly estimate that 30% of all Chl *a*-molecules are red-shifted leading to a situation that nearly all excitons become associated with the red-shifted forms. Second, the red forms are obviously not only associated with PS I but also with PS II serving as PS II antenna system. To our knowledge, this is the first report giving clear evidence that PS II can be closed by excitons harvested at wavelengths more than 40 nm down-hill. This is proved by three facts reported in the results: In *O.* sp. monochromatic far red light above 710 nm is able to close PS II with a concomitant reduction in the PQ-pool, to drive water splitting and to promote growth. In *O. queketii* theses features are present in much lower extent and clearly absent in the typical green alga *C. vulgaris*.

These observations lead to several challenging questions: How does the photosynthetic machinery realize the uphill energy transfer and prevents the overexcitation of PS I? Finally, why the nature does not use this strategy in general in all far red light enriched environments?

Uphill energy transfer in the PS I-LHC I network operates in a densely packed cluster of chlorophylls associated with a fast and efficient reaction center (Trissl and Wilhelm, 1993). The atomic structure of the PS I-LHC I network can explain how the excitation energy around PS I can be concentrated and the funnelled into two different pathways acting competitively between trapping and heat emission. The regulation between both pathways might be due to oligomerisation or by non-light harvesting carotenoids (Melkozernov and Blankenship, 2005). However, in higher plants and algae there is no up-hill transfer from Lhca to PS II. By contrast, it was suggested that the psaL-related cluster in PS I is an adaptation to enhance down-hill energy transfer

from LHC II to PS I. Therefore, up to now there is no polypeptide yet identified which can support the energy migration in the backward direction. Therefore, we postulate that the difference between *O*. sp. and higher plants is due to an atypical PS II-Lhc1a association which is spatially separated from PS I to prevent energy migration to the latter. In addition to these conditions one has to expect that the maximum quantum yield will be decreased. This is obviously the fact in *Ostreobium*: the maximal quantum yield of PS II expressed as F_v/F_m is only two thirds of *C. vulgaris* (see Figure 3). This observation delivers the explanation for the question why nature did not use this strategy more often. Although it is well known that in a canopy the lower parts face a far red light enriched environment, such a molecular organization would led to a strong reduction in the maximum quantum yield during light flecks. As long as the far red light is not permanent such a strategy may not be successful. In the case of *Ostreobium* which grows below other algal mats, this strategy seems to be the only way to survive, similar to the situation of *Acaryochloris marina*.

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