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Novel type of red-shifted chlorophyll *a* antenna complex from *Chromera velia*. I. Physiological relevance and functional connection to photosystems

Eva Kotabová [a,b,*,1], Jana Jarešová [a,b,1], Radek Kaňa [a,b], Roman Sobotka [a,b], David Bína [b,c], Ondřej Prášil [a,b]

[a] Institute of Microbiology ASCR, Centrum Algatech, Laboratory of Photosynthesis, Opatovický mlýn, 379 81 Třeboň, Czech Republic
[b] Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic
[c] Institute of Plant Molecular Biology, Biology Centre ASCR, Branišovská 31, 370 05 České Budějovice, Czech Republic

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ABSTRACT

Chromera velia is an alveolate alga associated with scleractinian corals. Here we present detailed work on chromatic adaptation in *C. velia* cultured under either blue or red light. Growth of *C. velia* under red light induced the accumulation of a light harvesting antenna complex exhibiting unusual spectroscopic properties with red-shifted absorption and atypical 710 nm fluorescence emission at room temperature. Due to these characteristic features the complex was designated "Red-shifted *Chromera* light harvesting complex" (Red-CLH complex). Its detailed biochemical survey is described in the accompanying paper (Bina et al. 2013, this issue).
Here, we show that the accumulation of Red-CLH complex under red light represents a slow acclimation process (days) that is reversible with much faster kinetics (hours) under blue light. This chromatic adaptation allows *C. velia* to maintain all important parameters of photosynthesis constant under both light colors. We further demonstrated that the *C. velia* Red-CLH complex is assembled from a 17 kDa antenna protein and is functionally connected to photosystem II as it shows variability of chlorophyll fluorescence. Red-CLH also serves as an additional locus for non-photochemical quenching. Although overall rates of oxygen evolution and carbon fixation were similar for both blue and red light conditions, the presence of Red-CLH in *C. velia* cells increases the light harvesting potential of photosystem II, which manifested as a doubled oxygen evolution rate at illumination above 695 nm. This data demonstrates a remarkable long-term remodeling of *C. velia* light-harvesting system according to light quality and suggests physiological significance of 'red' antenna complexes.

1. Introduction

In an aquatic environment, photosynthetic organisms are distributed through the euphotic zone where the light conditions are variable and depend mainly on the absorption and scattering of water and various dissolved and particulate matter, including phytoplankton. In the oligotrophic areas, the light environment is driven by the optical properties of the water itself as the spectrum of light changes with depth. Red light and far-red light are strongly attenuated in the upper layer of the water column and blue light penetrates much deeper [1,2]. In optically more complex environments, such as shallow coastal waters, the spectrum of light is affected by the higher concentration of organic or inorganic particles and by the reflection from the sea floor [3,4]. Photosynthetic organisms that live in aquatic environments have developed strategies to sense and respond to variable ambient spectral conditions. In algae and protists, several sensory photoreceptors are used to detect the spectral quality of irradiance (for review, see [5]). The mechanisms responding to changes of the ambient spectral light quality were described as chromatic adaptation (CA) [6]. This phenomenon has been well documented for cyanobacteria (for recent review see [7]), but for eukaryotic organisms the current knowledge is rather inconsistent (see discussion in [8]). There are several studies describing CA in diatoms [8–13], dinoflagellates [14] and in colonies of coral reefs [1]. The CA in diatoms was identified as an inverse type [8], which is typically accompanied by compensatory changes within the photosystems at the thylakoid level. This is different in comparison to the complementary type of CA in cyanobacteria that involves preferential synthesis of major light-harvesting phycobilin pigments such as phycocyanin or phycoerythrin (reviewed in [7]).

Abbreviations: CA, chromatic adaptation; *C. velia-B*, *Chromera velia* grown on blue light; *C. velia-R*, *Chromera velia* grown on red light; Chl*a*, chlorophyll *a*; CLH(c), *Chromera* light harvesting (complex); ETR, electron transport rate; FCP, fucoxanthin chlorophyll *a*/*c* protein; LHC, light harvesting complex; $NPQ_{(\lambda)}$, non-photochemical fluorescence quenching (spectrally resolved); PAR, photosynthetically active radiation (400–700nm); PS I (II), photosystem I (II); Red-CLH(c), red-shifted *Chromera* light harvesting (complex); RT, room temperature; SRFI, Spectrally Resolved Fluorescence Induction

* Corresponding author at: Institute of Microbiology ASCR, Centrum Algatech, Laboratory of Photosynthesis, Opatovický mlýn, 379 81 Třeboň, Czech Republic. Tel.: +420 384 340 435; fax: +420 384 340 415.
E-mail addresses: kotabova@alga.cz (E. Kotabová), jaresova@alga.cz (J. Jarešová), kana@alga.cz (R. Kaňa), sobotka@alga.cz (R. Sobotka), bina@umbr.cas.cz (D. Bína), prasil@alga.cz (O. Prášil).
[1] E. Kotabová and J. Jarešová contributed equally to this study.

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Diatoms possess several photoreceptors sensing both blue as well as red lights (see [2] and citations therein), however only the perception of blue light was found to be essential for acclimation to high light [13]. On the other hand, red light was reported to induce specific expression of Lhcf15, the fucoxanthin chlorophyll *a*/*c* protein, [13] and the appearance of the 710 nm PS II fluorescence emission at room temperature [9]. Although the mechanism(s) responsible for this red emission remain to be elucidated, available data suggests that photoprotection in diatoms is regulated not only by the light intensity but also by its quality and includes a large reorganization of antenna systems.

Focusing on the photosynthesis of coral reefs, the majority of photosynthetically active radiation (PAR) is absorbed by symbiotic algae of corals (zooxanthellae). The light environment inside the coral tissue and under the coral-reef invertebrates (e.g. ascidians) is strongly depleted in PAR, but enriched in far-red wavelengths [15–17]. Thus phototrophs associated with corals and didemnid ascidians had to extend their absorption capacity to the far-red region. This can offer a strategic advantage in niches where the PAR is limited [18]. For example, the ascidian associated cyanobacterium *Acaryochloris marina* uses chlorophyll *d* instead of chlorophyll *a* (Chl*a*) as a major pigment [19,20] that allows it to utilize available far-red light (700–750 nm). Recently, an even more far-red shifted chlorophyll *f* has been discovered in stromatolite samples [21]. The endolithic alga *Ostreobium* sp. is another oxygenic phototroph that can persist below a dense layer of other phototrophs due to extremely large number of red-shifted forms of Chl*a* [22–24]. The capacity to absorb light with wavelengths longer than 700 nm and the ability to utilize it for photosynthesis in *Acaryochloris* [25], *Acaryochloris*-like cyanobacteria [16] and in *Ostreobium* sp. [24] represents an important ecological advantage in coral-reef environment.

The role of red light absorbing pigments in the photochemistry of photosynthesis is still not clear. So far, the red forms of Chl*a*, discovered by Butler in the 1960s [26,27], were considered to be related mostly with the activity of photosystem I (PS I). Although relatively small in number (they form about 3–10% of the total chlorophylls), they have a pronounced effect on energy transfer and trapping in the PS I [28]. Nevertheless, they seem to be involved as well in PS II activity, as oxygen evolution induced by irradiance above 700 nm was also observed in Chl*a* containing phototrophs from regular habitats, such as green alga *Chlorella vulgaris* [29,30] and even for higher plants including sunflower, bean and spinach [31–33]. Despite clear evidence of far-red photochemistry [24,29–33], the role of red Chl*a* in PS II photochemistry is not generally accepted and there is only poor evidence for its presence in the PS II antennae. The only known exceptions are represented by the atypical association of PS I antenna Lhca1 with PS II in the endolithic alga *Ostreobium sp.* [23] and red-shifted antenna protein LHCb9 in the moss *Physcomitrella patens*, which possesses a typical motif for PS I antennae but associates with PS II [34,35]. However, the existence of such antennae in diatoms and brown algae was presumed for a long time [9,36–39].

In the present work, chromatic adaptation of *Chromera velia* and its physiological importance has been explored in detail. This recently discovered alga [40,41], together with *Vitrella brassicaformis* [42], represent a new phylum of algae called *Chromeridae* that are closely related to non-photosynthetic apicomplexan parasites [40,43–45]. *C. velia* is a coral associated alga [46,47] with highly efficient photosynthesis [48]. Its pigment composition is very simple, consisting of Chl*a*, isofucoxanthin-like carotenoid, violaxanthin and β,β-carotene [40]. Phylogenetically, the majority of light-harvesting complexes (LHCs) of *C. velia* form a separate cluster closest to dinoflagellate LHCs, and to the fucoxanthin chlorophyll *a*/*c* binding proteins (FCPs) of diatoms [49]. These complexes were designated recently as "*Chromera* light harvesting" (CLH) complexes because of their unique properties [50]. In addition, *C. velia* contains red alga-related PS I bound LHCs (PSI-LHCr) [49,50] and also LI818-like proteins [49] known to be induced during various stress conditions.

Here, we show that the prolonged growth of *C. velia* under monochromatic red light leads to the reversible appearance of an additional "Red *Chromera* light-harvesting" complex (Red-CLHc). This complex is assembled from a 17 kDa antenna protein and exhibits a remarkable red-shift in Chl*a* absorbance and fluorescence emission at room temperature. The far-red absorption/fluorescence nature of Red-CLHc is caused by a 17 kDa protein aggregation as shown in the accompanying paper [51]. This novel antenna complex is functionally connected to PS II and contributes to the effective light harvesting of far-red wavelengths.

2. Materials and methods

2.1. Culture conditions

C. velia (strain RM 12) was grown at 28 °C in artificial seawater medium with f/2 nutrient addition. Cells were kept in aerated glass tubes in semi-continuous batch growth with 24 h continuous irradiation. Panels made from LED strips were used for illumination with monochromatic red ($\lambda = 635$ nm) and blue ($\lambda = 460$ nm) lights of incident intensity of 20 μmol photons m^{-2} s^{-1}. All physiological measurements were performed with culture densities of 1.8–2.6 × 10^6 cells ml^{-1}. For biochemical analysis cells were harvested in late exponential phase.

2.2. Fluorescence and absorbance spectroscopy

Absorption spectra were measured in a glass cuvette using a Unicam UV 550 spectrophotometer (Thermospectronic, UK) equipped with an integration sphere. Absorbance was recorded with a scan rate of 30 nm/min with a 4 nm detection bandwidth.

Room temperature fluorescence emission spectra were measured in a cuvette with a SM-9000 spectrophotometer (Photon Systems Instruments, Czech Republic) for blue light excitation ($\lambda = 464$ nm) with a dark acclimated sample in the F_M (maximum fluorescence) state induced by a saturating pulse according to Kaňa et al. [52,53]. For the proper F710/F685 ratio calculation, the experimental data was deconvoluted using Origin Pro 8.0 "Peak Analyzer" (OriginLab, USA), using Gaussian curves. During the fitting procedure, only positions of peak maxima were restricted to a 4 nm range around the predicted maxima; all other parameters were set to be free for the minimization of procedure, driven by Chi-square (with 10^{-6} precision). The whole-cell emission spectra at varying excitation wavelengths (Fig. 9) were measured with a Spex Fluorolog-2 spectrofluorometer (Jobin Yvon, Edison, NJ, USA) using a slit width of 1.6 nm. The sample OD was <0.1 in the Chl*a* Q_y band. In the case of the far-red excitation (720 nm) the emission spectrum was composed of separate measurements of the uphill (<720 nm) and downhill (>720 nm) parts to avoid damage of the instrument by excitation radiation passing directly into the detector when excitation and emission monochromators were set to the same wavelengths.

77 K fluorescence emission spectra were measured using an Aminco–Bowman Series 2 spectrofluorometer (Thermo Fisher Scientific, USA) using standard instrument geometry. Diluted cell suspensions (in order to avoid fluorescence reabsorption) were placed in a sample holder and immersed in an optical Dewar flask filled with liquid nitrogen. The excitation was at 435 nm with a 4 nm slit width. The emission spectra were scanned with a 4 nm slit width. The instrument function was corrected by dividing the raw emission spectra by the simultaneously recorded signal from the reference diode.

2.3. Preparations of cell membranes

C. velia cells (200 mL, optical density at 750 nm ~ 0.5) were washed and resuspended in buffer containing 1 mL of 25 mM MES/NaOH, pH 6.5, 5 mM $CaCl_2$, 10 mM $MgCl_2$, and 25% glycerol. The concentrated cell suspension was mixed with 0.5 mL of glass beads (0.1 mm diameter) in a 2 mL Eppendorf tube and broken in a Mini-BeadBeater (BioSpec,