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Novel type of red-shifted chlorophyll *a* antenna complex from *Chromera velia*: II. Biochemistry and spectroscopy

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ABSTRACT

A novel chlorophyll *a* containing pigment–protein complex expressed by cells of *Chromera velia* adapted to growth under red/far-red illumination [1]. Purification of the complex was achieved by means of anion-exchange chromatography and gel-filtration. The antenna is shown to be an aggregate of ~20 kDa proteins of the light–harvesting complex (LHC) family, unstable in the isolated form. The complex possesses an absorption maximum at 705 nm at room temperature in addition to the main chlorophyll *a* maximum at 677 nm producing the major emission band at 714 nm at room temperature. The far-red absorption is shown to be the property of the isolated aggregate in the intact form and lost upon dissociation. The purified complex was further characterized by circular dichroism spectroscopy and fluorescence spectroscopy. This work thus identified the third different class of antenna complex in *C. velia* after the recently described FCP-like and LHCr-like antennas. Possible candidates for red antennas are identified in other taxonomic groups, such as eustigmatophytes and the relevance of the present results to other known examples of red-shifted antenna from other organisms is discussed. This work appears to be the first successful isolation of a chlorophyll *a*-based far-red antenna complex absorbing above 700 nm unrelated to LHCI.

1. Introduction

The pigment–protein complexes involved in the photosynthetic energy conversion fall into two functional classes: the reaction centers (RCs) that carry out the photoactivated charge separation reaction and the light-harvesting complexes (antennas) that capture radiation and deliver the energy to the reaction centers in an efficient and regulated manner. Large numbers of chlorophyll (Chl) molecules are bound within the light-harvesting complexes relative to the reaction centers in order to increase the absorption cross-section, moreover, other pigment classes, carotenoids (tetraterpenoids) and phycobilins (open-ring tetrapyrols) accompany chlorophylls (cyclic tetrapyrols) in different groups of light-harvesting complexes in order to expand the useful range of wavelengths beyond the blue and red regions absorbed by chlorophylls.

Generally, the energy transfer in a photosynthetic system proceeds downhill along an energy gradient towards a pool of pigment isoenergetic with the excited state of the reaction center [2]. This energy corresponds to wavelengths of about 700 nm and 680 nm for photosystem I (PSI) and photosystem II (PSII), respectively. However, it has been known that light-harvesting systems connected to both photosystems contain chlorophyll molecules absorbing at longer wavelengths than the respective primary donors of the RCs. Hence, the transfer of excitation energy from these low-energy forms to the reaction center is a thermally activated process. The nature of these red-shifted forms of chlorophylls has been more thoroughly investigated in the case of PSI of plants and cyanobacteria, where both the peripheral antenna (LHCI in plants) and the light-harvesting domains of the core complex have been shown to contain the low-energy pigments [3].

As for the red-shifted chlorophylls associated with the PSII antenna systems, the situation appears less clear. Antenna systems providing excitation to PSII while absorbing above 690 nm were found in green alga *Ostreobium* (Bryopsidales). In the case, the far-red chlorophylls were located in a variant of Lhca1 proteins, normally a component of the PSI light-harvesting machinery [4,5]. Recently, red-shifted Chl *a*, was described in association with a form of the LHCII subunit in the moss *Physcomitrella patens* [6]. Among cyanobacteria, *Acaryochloris marina* can be thought of as an organism relying solely on red-shifted chlorophylls, although in this case the spectral shift is achieved by replacing Chl *a* with chlorophyll *d* as a major light-harvesting pigment [7]. In diatoms and brown algae, the production of a far-red (>700 nm) absorbing

Abbreviations: CD, circular dichroism; CLH, *Chromera* light-harvesting [complex]; FCP, fucoxanthin-chlorophyll protein; NPQ, non-photochemical quenching of fluorescence; Red-CLHc, red-shifted *Chromera* light-harvesting [complex]; XLH, *Xanthonema* light-harvesting [complex]; PS, photosystem

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and emitting antenna in adaptation of changing light quality has been studied since at least 1960s [8–15]. However, to our knowledge, no study reporting purification of the heterokont red-shifted antenna and its characterization has been published. The relatively best understood appears to be the antenna complex from the pennate diatom *Phaeodactylum tricornutum*. In this case a partial isolation was presented [16]. In this organism, the chromatic adaptation was shown to be accompanied by a formation of a protein complex exhibiting fluorescence emission around 710 nm at room temperature. The complex was unstable and dissociated rapidly after cell breakage, however, the authors managed to perform sucrose gradient centrifugation showing the complex fractionating towards the lower part of the band containing the light-harvesting complexes indicating that it is a larger complex. Moreover, these authors suggested that the 710 nm emitter might co-purify with PSII.

In the accompanying paper [1] it is shown that upon transition from blue to red cultivation light, *Chromera (C.) velia* undergoes a chromatic adaptation that shares many similarities with diatoms. The chromatic adaptation manifests itself by a pronounced change in the emission spectrum of the cells, with a maximum of fluorescence emission shifting from 686 nm to 714 nm with a concomitant appearance of an absorption band around 705 nm. The far-red emitter appeared to be an aggregate of ~17 kDa protein, most probably without direct association with either photosystems, although it was shown that *in vivo* the far-red excitation was capable of driving photosystem II photochemistry in the red-light adapted cultures.

C. velia is a species associated closely with corals [17,18] that is a type of environment known to host other organisms shown to possess red-shifted antenna complexes connected to the PSII. Consequently, the presence of such antenna system in *C. velia* is not surprising. In addition a recent molecular phylogenetic study demonstrated that *C. velia* has a large number of genes coding for light-harvesting complexes [19] belonging to the light-harvesting (LHC) family, suggesting a possibility for large plasticity. Although majority of the genes appeared as a sister branch to a group comprising fucoxanthin-containing LHC (FCP, LHCf) from diatoms and LHC complexes from dinoflagellates, other complexes with less clear affiliation were also identified.

A complete biochemical and biophysical characterization of two major types of light-harvesting complexes isolated from cells grown in a natural illumination was presented recently [20]. One of these complexes was identified in a complex with PSI and shown to be homologous to PSI associated antenna of red algae (LHCr). The other light-harvesting complex did not form aggregates with either photosystem and its sequence exhibited homology to the FCP proteins. However, its structure as studied by electron microscopy and its spectroscopic properties indicated a strong similarity to antenna complexes of xanthophyte *Xanthonema debile* [21], hence it was labeled CLH (*Chromera* light-harvesting) complex. This complex, lacking Chl *c* contained iso-fucoxanthin-like pigment as the major carotenoid species.

In this study, a description of the complete purification procedure of a novel *C. velia* antenna complex is presented. This protein complex containing red-shifted chlorophyll *a* is expressed during transition of the culture from blue (or natural daylight) to red illumination and is responsible for the marked spectral changes that accompany the chromatic adaptation. The most pronounced of these being the shift of the emission maximum of the whole cells from 686 nm to ~714 nm. Furthermore, connection of the complex to PSII was demonstrated by the oxygen evolution and fluorescence induction kinetics driven by the far-red illumination. This to our knowledge represents a first successful complete isolation and characterization of a red-shifted antenna system other than LHCI. Hence we believe that the present pair of publications constitutes an important step in the understanding of processes of photo acclimation in photosynthetic members of the group Chromalveolata, which includes important species of marine and freshwater phytoplankton.

2. Material and methods

2.1. Culture and growth conditions

Cells of *C. velia* were grown in 5 L Erlenmeyer flasks at 28 °C in modified f/2 saline medium for diatoms [22] and bubbled with filtered air. The culture was irradiated by incandescent light provided by 60 W tungsten filament light bulb with an intensity of 15 μmol photons m^{-2} s^{-1}, the light regime was set to 15 hour light–9 hour dark cycle. Thylakoid membrane preparation, solubilization and separation of protein complexes on sucrose gradient were done as described in [20].

2.2. Chromatographic procedures

Purification procedures were based on those described earlier for the isolation of antenna complexes from diatoms, brown algae and *C. velia* [20,23–25]. All steps were performed in the following buffer solution (Buffer A): 50 mM Tris–HCl pH 7.5, 2 mM KCl, and 0.03% *n*-Dodecyl β-D-maltoside.

Gel-filtration was performed using Superdex 200 GL 10/300 gel-filtration column (GE Healthcare) at a flow rate of 1 mL/min using Econ 4020 FPLC system (Econ, Czech Republic) using Buffer A. The analysis was performed in the dark. The buffer was supplied from an ice-cooled storage flask.

Anion-exchange chromatography was performed on a 2 mL column packed with DEAE Sepharose CL-6B at 1 mL/min using Econo Pump EP-1 (Bio-Rad, Germany) using a locally made gradient mixer. Before applying the zone 2 from the sucrose density gradient to the anion-exchange chromatography column (DEAE Sepharose CL6, resin volume 2 mL) equilibrated with Buffer A, the samples were washed with the same buffer on an Amicon membrane filter (10 kDa cut-off) to remove sucrose. The whole procedure was performed in a cold room at 4 °C. The samples were eluted with a gradient of NaCl in Buffer A (for details see Fig. 2). Prior to the gradient start, the column was washed extensively with Buffer A without adding NaCl. The eluent at this stage did not contain any proteins. The chromatography was performed at 4 °C in the dark.

2.3. Spectroscopy

2.3.1. Absorption

Room temperature absorption spectra were recorded with a UV300 spectrophotometer (Spectronic Unicam, UK). Spectra of whole cells were recorded using Shimadzu UV-2600 spectrometer equipped with ISR-2600Plus integrating sphere (Shimadzu, Japan).

2.3.2. Fluorescence

Fluorescence emission and excitation spectra were recorded on the Spex Fluorolog-2 spectrofluorometer (Jobin Yvon, USA) with slit width of 2 nm. Room temperature spectra of whole cells were measured in the perpendicular geometry. Samples were stirred to prevent cell sedimentation. The concentration of the sample was adjusted to absorbance <0.1 to avoid distortion of spectra by reabsorption. Measurements of protein samples at 77 K were done in a reflection mode using locally made holders immersed in liquid nitrogen in a Dewar vessel.

2.3.3. Circular dichroism

Circular dichroism (CD) spectra were recorded with a Jasco J-715 spectropolarimeter using bandpass of 2 nm. Samples in quartz cuvettes were placed into a water-cooled sample holder and maintained at 4 °C for the duration of the measurement to prevent fast dissociation of aggregated state of the pigment–protein complexes.