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Biochimica et Biophysica Acta 1707 (2005) 170–178

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Evidence for dissociation of chlorophyll *b* from the main light-harvesting complex in the oligomerization state isolated from marine alga, *Bryopsis corticulans*

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Received 22 June 2004; received in revised form 27 November 2004; accepted 1 December 2004
Available online 12 January 2005

Abstract

We investigated the composition and organization of chlorophylls in monomers, trimers and oligomers (small aggregates) of the main light-harvesting complex (LHC II) isolated from marine alga, *Bryopsis corticulans*, using a combination of measurements with reversed-phase high performance liquid chromatography (RP-HPLC) and steady-state spectroscopy of absorption, circular dichroism (CD) and low temperature fluorescence. The composition and organization of the chlorophylls in monomeric and trimeric LHC II were essentially identical to those of LHC II from higher plants. For LHC II oligomers, a large decrease of chlorophyll (Chl) *b* absorption and of CD signals corresponding to Chl *b* was consistent with the quantitative analysis of Chl *b* by RP-HPLC, indicating that oligomerization of the LHC II proteins significantly influenced spectroscopic properties and led to the dissociation of Chl *b* molecules from LHC II. Our data strongly suggested that protein oligomerization constitutes a structural basis for the decrease of Chl *b* molecules in LHC II of *B. corticulans*. The LHC II of *B. corticulans* might play a photoprotective role with the reduction of the ability of light absorption via alteration of its own structural conformation.

Keywords: Light-harvesting complex; Chlorophyll; Oligomerization; Spectroscopy; *Bryopsis corticulan*

1. Introduction

The light energy for the photosynthesis of plants and green algae is collected by an array of chlorophyll-binding proteins encoded by a light-harvesting complex multigene family [1]. The most abundant of the proteins is the main Photosystem II associated light-harvesting complex (LHC II) harboring about 50% of the total chlorophylls in thylakoid membranes. The structure of LHC II from spinach has recently been resolved at 2.72 Å using X-ray crystallography [2]. There are three transmembrane α-helices connected by hydrophilic loops and a short amphiphylic helix exposed to the inner surface of the thylakoid membrane. Eight chlorophylls (Chls) *a*, six Chls *b*, two luteins and one neoxanthin have been identified and accurately located within one monomeric LHC II. The significant differences between the binding sites of Chl *a* and Chl *b* have also been proposed from this structural model of LHC II [2]. Except for one form of Chl *b*, almost all Chls *b* in the complex are selectively hydrogen-bonded to the polypeptides or to the coordinated water through their C7-formyls. For the selective binding of Chl *a*, the environment surrounding the C7-methyl groups of Chl *a* molecules is likely nonpolar. In the center of the complex,

Abbreviations: LHC, light-harvesting complex; Chl, chlorophyll; CD, circular dichroism; DM, *n*-Dodycel-β-D-Maltoside; OG, *n*-Octyl-β-D-glucopyranoside; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RP-HPLC, reversed-phase high performance liquid chromatography

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0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2004.12.001

Chl *b* is in close contact with Chl *a* for rapid energy transfer [2]. Energy transfer from Chl *b* to the closely related Chl *a* (both compounds differ only in a formyl group instead of a methyl group at the 7-position in the chlorine ring of Chl *a*) [3] can take place because of the different energy contents and decay times of their excited states [4,5]. Energy absorbed by Chl *b* is transferred to Chl *a* within less than 1 ps, while the Chl *a* fluorescence decay time remains 1–3 ns, as shown in the purified, detergent-solubilized LHC II [6]. Thus, the ratio of Chl *a*/Chl *b* of LHC II reflects its light absorption ability.

LHC II is not only very important for the overall light-harvesting process, but also plays an important role in various regulatory mechanisms of photosynthesis [7]. The dynamic properties of LHC II are observed when trimers form higher aggregates upon exposure to a variety of conditions. The aggregation causes non-photochemical quenching: almost all excitation energy is rapidly dissipated as heat [8,9]. The physiological significance of this rests upon the evidence that LHC II exists in an oligomerization state in vivo [10–12] and that the regulation of LHC II in vivo is detected as a decline in the yield of chlorophyll fluorescence [13]. Therefore it has been suggested that this feature of LHC II is the basis for the control of the light-harvesting function of photosynthetic membranes [13–16].

In this work, we performed systematic comparative measurements on monomeric, trimeric and oligomeric LHC II isolated from marine green alga, *Bryopsis corticulans*. The Chl compositions in all three forms of LHC II were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC). Spectroscopic properties, absorbance, circular dichroism and low temperature fluorescence emission were also compared within LHC II subcomplexes. Our results showed that Chl *b* molecules were largely dissociated from the LHC II of *B. corticulans* upon the oligomerization of proteins.

2. Materials and methods

2.1. Isolation of LHC II

B. corticulans, a shiphonous green alga, was collected in tideland near Qingdao in China. All the following procedures were performed in dim light at 4 °C.

The whole plant of the green alga was homogenized and osmotically broken in TSN buffer (10 mM Tris–HCl, pH 8.0, 200 mM sucrose, 10 mM NaCl) followed by filtration through 8 layers of gauze and centrifugation at 10,000×*g* for 10 min. The pellets were suspended in 10 mM Tricine–NaOH, pH 8.0, and the broken membrane fragments were collected by centrifugation at 12,000×*g* for 10 min. For selective solubilization, the membrane fragments were treated with 3% *n*-Octyl-β-D-glucopyranoside (OG) in TMK buffer (20 mM Tricine–NaOH, pH 8.0, 40 mM $MgCl_2$, 80 mM KCl) for 15 min at a chlorophyll concentration of 1 mg/ml. After centrifugation at 180,000×*g* to remove unsolubilized membrane fragments, the supernatant was subjected to liquid chromatography.

The supernatant was subjected to anion exchange chromatography on a Q Sepharose Fast Flow column (from Amersham) equilibrated with TMK buffer containing 0.05% *n*-Dodycel-D-Maltoside (DM) (from Sigma). The column was eluted with the same buffer at a linear gradient of 0.1–1 M NaCl. The LHC II-enriched fraction was pooled together and concentrated with AMICON Centriprep-50 (50 kDa cut off). The concentrated samples were loaded on a Superose 12 column (from Amershem Biosciences) to implement gel-filtration chromatography. The column was isocratically eluted with TMK buffer containing 0.05% DM.

To obtain LHC II in monomeric, trimeric and oligomeric forms, 1 ml of the column-purified LHC II sample was loaded on top of a linear sucrose gradient prepared with 5%–25% sucrose in TMK buffer containing 0.05% DM at a chlorophyll concentration of 1 mg/ml and centrifuged at 270,000×*g* for 12 h with SW 40 rotor (from Beckman). The sucrose was removed from the collected band fractions using PD-10 column (from Amersham Biosciences).

2.2. Electrophoresis

Polypeptide composition was analyzed on 12% polyacrylamide gel containing 1% SDS and run in Laemmli system [17]. For partly denatured electrophoresis, homogeneous 12% acrylamide gel was prepared without SDS and run at 15 mA and 4 °C in the dark. Samples were loaded without SDS. The SDS concentration in the running buffer was 0.1%.

2.3. Amino acid sequencing

After SDS-PAGE, the protein plot was transferred to a polyvinylidene difluoride membrane (Millipore) for N-terminal sequence analysis by automated Edman degradation on a 477A Protein sequencer (from Applied Biosystems).

2.4. MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry)

The protein samples were prepared in 0.5% trifluoroacetic acid (TFA) (from Sigma) and desalted with $ZipTipC_4$ cartridges (from Millipore) prior to MS analysis. The protein solution was mixed with a matrix solution, the supernatant of a 50% acetonitrile solution saturated with sinapinic acid (from Sigma), and then air-dried on the flat surface of a stainless steel plate. Calibrations were carried out using standard bovine serum albumin. MALDI-TOF MS measurement was performed on an AXIMA-CFRTM plus MALDI-TOF mass spectrometer (from Shimadzu Co.).