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Evidence of monomeric photosystem I complexes and phosphorylation of chlorophyll *a/c*-binding polypeptides in *Chroomonas* sp. strain LT (Cryptophyceae)

Julia Janssen, Erhard Rhiel*

Geomicrobiology, ICBM, Carl-von-Ossietzky-University of Oldenburg, Oldenburg, Germany

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Summary. Thylakoid membranes of the cryptophyte *Chroomonas* sp. strain LT were solubilized with dodecyl-β-maltoside and subjected to sucrose density gradient centrifugation. The four pigment protein complexes obtained were subsequently characterized by absorption and fluorescence spectroscopy, SDS-PAGE, and Western immunoblotting using antisera against the chlorophyll *a/c*-binding proteins of the marine cryptophyte *Cryptomonas maculata* and the reaction-center protein D2 of photosystem II of maize. Band 1 consisted mainly of free pigments, phycobiliproteins, and chlorophyll *a/c*-binding proteins. Band 2 represented a major chlorophyll-*a/c*-binding protein fraction. A mixture of photosystem II and photosystem I proteins comprised band 3, whereas band 4 was enriched in proteins of photosystem I. Western immunoblotting demonstrated the presence of chlorophyll-*a/c*-binding proteins and their association with photosystem I in band 4. Phosphorylation experiments showed that chlorophyll-*a/c*-binding proteins became phosphorylated. Negative staining electron microscopy of band B4 revealed photosystem I particles with dimensions of 22 nm. Our work showed that PSI-LHCI complexes of cryptophytes are similar to those of *Chlamydomonas rheinhardtii,* the diatom *Phaeodactylum tricornutum*, and higher plants. [**Int Microbiol** 2008; 11(3):XXX-XXX]

Key words: *Chroomonas* · cryptophytes · chlorophyll-*a/c*-binding proteins · light-harvesting complex · photosystems I and II

Introduction

Oxygenic photosynthesis is mediated by two photosystems, photosystem I (PSI) and photosystem II (PSII). The core complexes of both are rather conserved and harbor the reaction-center proteins, the components of the electron transport chain, and the inner antennae. The structure of the outer antennae of PSI and PSII, the light-harvesting complexes (LHCs), varies significantly between different phylogenetic groups [8,9]. Thus, the LHCs of green plants consist of chlorophyll (chl)-*a*/*b*-binding proteins (Lhc proteins). Chromophytic algae, i.e., brown algae and diatoms, harbor fucoxanthin chl-*a/c*-binding polypeptides (Fcps), while chl-*a/c*-binding proteins (Lhcc) are found in cryptophytes [1,2,12,13].

The Lhc proteins of higher plants and green algae have distinct functions and locations within PSII and PSI. The LHC of PSII (LHCII) consists of the monomeric minor Lhc proteins CP29, CP26, and CP24 and the more distal major LHCII formed by trimeric Lhc proteins (LHCb1, LHCb2, and LHCb3). The Lhc proteins of the antenna of PSI (LHCI), LHCa1, LHCa2, LHCa3, and LHCa4, are organized as dimers [3,7,13,14]. Ultrastructural studies on PSI-LHCI complexes of higher plants and the green alga *Chlamydomo-*

***Corresponding author:** E. Rhiel
Geomikrobiologie, ICBM
Carl-von-Ossietzky-Universität Oldenburg
P.O.B. 2503
D-26129 Oldenburg, Germany
Tel. +49-4417983389. Fax +4904417983384
E-mail: erhard.rhiel@uni-oldenburg.de

nas rheinhardtii revealed particles measuring 20 × 16 nm [4] or 22 × 18 nm [16] in top-view projections. Recently, PSI-LHC complexes of 21 nm were described for the diatom *Phaeodactylum tricornutum* [30]. All these complexes were shown to represent monomers, in contrast to the trimeric PSI complexes of cyanobacteria. Currently, no ultrastructural data exist for PSI-LHC complexes of cryptophytes, although such complexes have been isolated using dodecyl-β-maltoside (DM) as the solubilizing detergent [1]. Typical Lhc proteins may also function in photo-protection. The main mechanisms in these cases are the xanthophyll cycle and photo-phosphorylation of LHC subunits [18,22]. Phosphorylation experiments on chl-*c*-bearing algae are restricted to studies on the brown alga *Laminaria saccharina* and the diatom *Cyclotella cryptica* [5,6], both of which demonstrated phosphorylation of Fcps. No phosphorylation experiments have been carried out on cryptophytes yet; thus, whether Lhcc subunits become phosphorylated remains unclear.

The current work focuses on the photosynthetic apparatus of a *Chroomonas* species, Using DM, we isolated pigment protein complexes from a limnic phycocyanin-bearing cryptophyte in order to determine whether Lhcc subunits become phosphorylated, and thus investigate if PSI-LHCI complexes of cryptophytes are similar to those of *Chlamydomonas rheinhardtii, Phaeodactylum tricornutum*, and higher plants.

Materials and methods

Culture and growth conditions. *Chroomonas* sp. strain LT (referred to in the following as *Chroomonas* LT) was isolated from the pond located in front of the main entrance of the building for Natural Sciences of the University of Oldenburg by Dipl. Biol. Gerke Kunz. The alga was grown in Moor-CHU medium [28] at 15°C in Erlenmeyer flasks of 100–800 ml culture volume without aeration. The photon flux density was adjusted to 5 μmol photons m^{-2} s^{-1}. The light:dark regime was 14 h:10 h.

Isolation of pigment protein complexes by sucrose density gradient centrifugation. All steps were done at 4ºC and in dimmed light. The cells (800–1000 ml) were harvested by centrifugation at 6500 ×*g* for 10 min in a Sorvall RC-5B refrigerating centrifuge equipped with a GSA rotor (DuPont, Newtown, CT, USA), washed once in 20 ml buffer A (20 mM Tris-HCl pH 7.5, 1 mM $MgCl_2$, 5 mM EDTA) and spun for 15 min at 1600 ×*g* with a Sorvall SS34 rotor. The resulting cell pellet was resuspended in 15 ml buffer A and broken at 6.9 MPa with a pre-cooled Aminco 8000 French pressure cell (SLM Aminco, Urbana, IL, USA). The membrane fraction was pelleted from the supernatant by centrifugation at 31,000 ×*g* for 30 min and resuspended in 1 ml buffer A. Aliquots were withdrawn to estimate the chl concentrations. The membrane fraction was adjusted with buffer A to a final chl concentration of 100 μg chl/ml and solubilized with 10% (w/v) DM at chl:DM ratios of 1:25, 1:50, or 1:100 (w/w) for 30 min on ice and in the dark. Aliquots equivalent to 100 μg chl were loaded onto 5–12% (w/v) sucrose gradients in buffer A containing 0.15% DM. The gradients were centrifuged for 16 h at 228,000 ×*g* (Beckman L8-55 ultracentrifuge equipped with a SW40 TI swinging-bucket rotor, Beckman, Palo Alto, CA, USA). The resulting colored bands were harvested using a syringe with an injection needle. The content of the bands was either used immediately for spectroscopy and electron microscopy or frozen for analysis by SDS-PAGE.

SDS-PAGE, Western blotting, and immunodecoration. SDS-PAGE was carried out as described previously [1,2,12]. Aliquots of the bands obtained by sucrose densityradient centrifugation were adjusted to equal chl concentrations, mixed with loading buffer [1.54% (w/v) dithiothreitol, 2% (w/v) SDS, 80 mM Tris-HCl, pH 6.8, 60% (v/v) glycerol, and 0.0012% (w/v) bromphenol blue] and heated for 3 min at 85ºC. Fifty μl (equivalent to 200 ng chl) of the samples were loaded onto 15 or 17.5% (w/v) polyacrylamide gels using a previously described buffer system [19]. The gels were run at a constant voltage of 20 V overnight and then silver-stained [31]. Western blotting [29] was done in a BIO-RAD Trans-blot SD semidry transfer cell. Antiserum against the PSII core complex polypeptide D2 (anti-D2) of maize was the generous gift of Prof. Dr. R. Barbato (University of Padua, Italy) via Dr. J. Marquardt. Antiserum against the major LHC of *Cryptomonas maculata* was described earlier [12,26]. Rabbit IgG fractions that detect phosphorylated serine or threonine residues within polypeptides were obtained from Zytomed (Berlin, Germany) and used according to the manufacturer's recommendations. Immunodecoration was carried out as described previously [1].

In vitro phosphorylation experiments. These experiments followed a previously outlined protocol [5] with some modifications. Cell suspensions (800–1000 ml) of *Chroomonas* LT were harvested by centrifugation. The cells were washed once with 10 ml buffer B (50 mM Hepes-KOH, pH 7.4, 20 mM NaCl, 10 mM $MgCl_2$) containing protease inhibitor cocktail (Sigma, Munich, Germany) and resuspended into 10 ml buffer B containing protease inhibitor cocktail. The cells were disrupted in a French pressure cell. Unbroken cells and cell debris were removed by centrifugation (2 min at 2000 ×*g*, 4ºC). The membrane fraction was harvested by centrifugation (15 min at 10,000 ×*g*, 4ºC), washed once with buffer B containing protease inhibitor cocktail and finally resuspended in 500 μl buffer C (50 mM Hepes-NaOH, pH 7.6, 100 mM sorbitol, 5 mM NaCl, 5 mM $MgCl_2$) containing protease inhibitor cocktail. Aliquots were withdrawn to estimate the chl concentration. The membrane fraction was adjusted to a chl concentration of 5 μg chl/ml with buffer C. Aliquots of 100 μl, equivalent to 500 ng chl, were incubated in the dark for 25 min at 4ºC. Ten μCi [γ-^{32}P]ATP (specific activity 9.25 MBq, Hartmann Analytic, Braunschweig, Germany) and 40 nmol ATP were added and the membrane fractions were illuminated with white light (photon flux density of 150 μmol photons m^{-2} s^{-1}) for 25 min at room temperature. Two μl NaF (1 M stock) were added. The membrane fractions were pelleted by centrifugation (3 min, 13,000 ×*g*, table-top centrifuge), washed twice with 200 μl buffer C containing 20 mM NaF and protease inhibitor cocktail, and finally resuspended in 50 μl loading buffer. Samples were incubated for 5 min at 90ºC and then subjected to SDS-PAGE. Following electrophoresis, the gels were stained with Coomassie Blue R 250 [0.25% (w/v) in H_2O:methanol:acetic acid 5:5:1 (v/v)], dried, and subjected to autoradiography. X-ray films (Lumi Film, Boehringer Mannheim, Germany) were used and exposed for 1–14 days.

In vivo phosphorylation experiments. These experiments followed a previously outlined protocol [27] with some modifications. Since *Chroomonas* LT cells lysed when resuspended in buffer C containing protease inhibitor cocktail, cell suspensions (800 ml) were harvested by centrifugation, resuspended in 15 ml Moor-CHU containing 20 mM NaF, transferred into Petri dishes, and illuminated with white light (photon flux density of 150 μmol photons m^{-2} s^{-1}) for 20–30 min at 20ºC. Protease inhibitor cocktail was then added to the cell suspensions and the cells were disrupted in a French pressure cell. Unbroken cells and cell debris were removed by centrifugation. The membrane fraction was harvested by centrifugation and resuspended in buffer C containing 20 mM NaF. Aliquots were withdrawn to estimate the chl concentration. The membrane fraction was diluted with loading buffer to final chl concentrations of 1, 2, or 4 μg per 50 μl loading

buffer, heated for 3 min at 90ºC, and loaded onto polyacrylamide gels. SDS-PAGE and Western blotting using the anti-phosphoserine and anti-phosphothreonine IgG fractions followed the protocols outlined above.

Spectroscopy. Chl concentrations were determined using the equations described in a previous publication [15]. Absorbance spectra of the pigment protein complexes obtained by sucrose density gradient centrifugation were recorded from 340 to 740 nm at 20ºC with a Hitachi U-3000 spectrophotometer. Fluorescence excitation and emission spectroscopy were carried out at –196ºC with a Hitachi F-4500 spectrofluorometer. Excitation and fluorescence light were guided by fiber optics. The excitation spectra were recorded from 400 to 600 nm at the emission maximum of each sample and were corrected using a rhodamine B spectrum as reference. The emission spectra were recorded from 600 to 800 nm. The excitation wavelengths for the emission spectra were 440, 460, and 480 nm. P700 concentrations were determined as described earlier [1] by measuring the absorbance differences at 700 nm of chemically oxidized and reduced samples [24]. Calculations were made using an extinction coefficient of 64 $mmol^{-1}\,cm^{-1}$ [10].

Electron microscopy. Fifty-µl droplets of band B4, obtained by sucrose density gradient centrifugation, were used for negative staining. Particles were allowed to adsorb for 60 s onto freshly prepared carbon-coated grids, stained for 60 s with 2% (w/v) uranyl acetate, and washed in two drops of distilled water. Images were taken as digitized photographs with a Zeiss EM 902A electron microscope operated at 80 kV using a digital camera (Proscan High Speed SSCCD camera, 1kx1k, Proscan elektronische Systeme, Lagerlechfeld, Germany). The digital camera was operated by the iTEM Five software (Olympus Soft Imaging System, Münster, Germany). Particle dimensions were measured using the iTEM Five software.

Results

Sucrose density gradient centrifugation. When thylakoids of *Chroomonas* LT were solubilized with DM and fractionated in sucrose density gradients, they yielded four colored bands at a chl:DM ratio of 1:50 (Fig. 1A). A chl:DM ratio of 1:25 lead to poor solubilization of thylakoid membranes, whereas a ratio of 1:100 caused loss of pigments bound to the complexes (not shown). The uppermost band 1 (B1) was bluish-green, band 2 (B2) was yellowish-green in color. Band 3 (B3) appeared approximately in the middle of the sucrose gradients and was green. Band 4 (B4) was yellowish-green in color and migrated in the lower parts of the gradients.

Polypeptide patterns and immunochemical tests of the bands. In the polyacrylamide gels (Fig. 1B), band B1 is dominated by polypeptides with relative molecular masses (M_r) of approximately 18–22 and 12 kDa. Polypeptides of higher M_r could also be detected. Band B2 consisted of two prominent protein bands with M_r of 22 and 18 kDa. Faint bands appeared in the low and high molecular mass range as well. The polypeptide pattern of band B3 comprised a multitude of polypeptides in the M_r range from below 10 kDa up to 60 kDa. Proteins with M_r of 12, 14, 18, 21, 30, and 54 kDa could be detected as well. Band B4 contained two major protein bands, one with M_r of 54 kDa and the other at 18–24 kDa.

Relative molecular masses of 18–22 kDa, as found for the dominant polypeptides of bands B1 and B2, are typical for Lhcc proteins. Polypeptides of similar size were also present in B3 and B4. To confirm the identity of these polypeptides, Western blots were treated with an antiserum against a total LHC fraction of *Cryptomonas maculata* (anti-Cmac) (Fig. 1C, "Cmac"). The antiserum recognized polypeptides with M_r of approximately 22 and 18 kDa in bands B1 and B2, with the

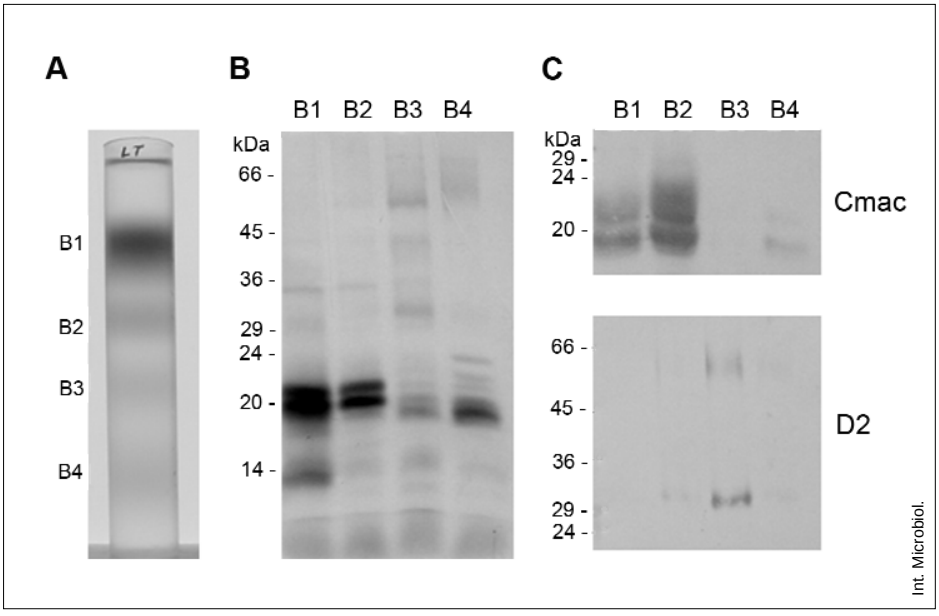

Fig. 1. (**A**) 5–12% sucrose gradients with fractionations of thylakoids solubilized with dodecyl-β-maltoside (DM) from *Chroomonas* LT. (**B**) SDS-PAGE of gradient bands B1, B2, B3, and B4 of *Chroomonas* LT. (**C**) Relevant details of Western blots of gels as in (B); immunodecoration with antisera against the polypeptides is indicated on the right. Relative molecular masses of the marker proteins (kDa) are indicated.

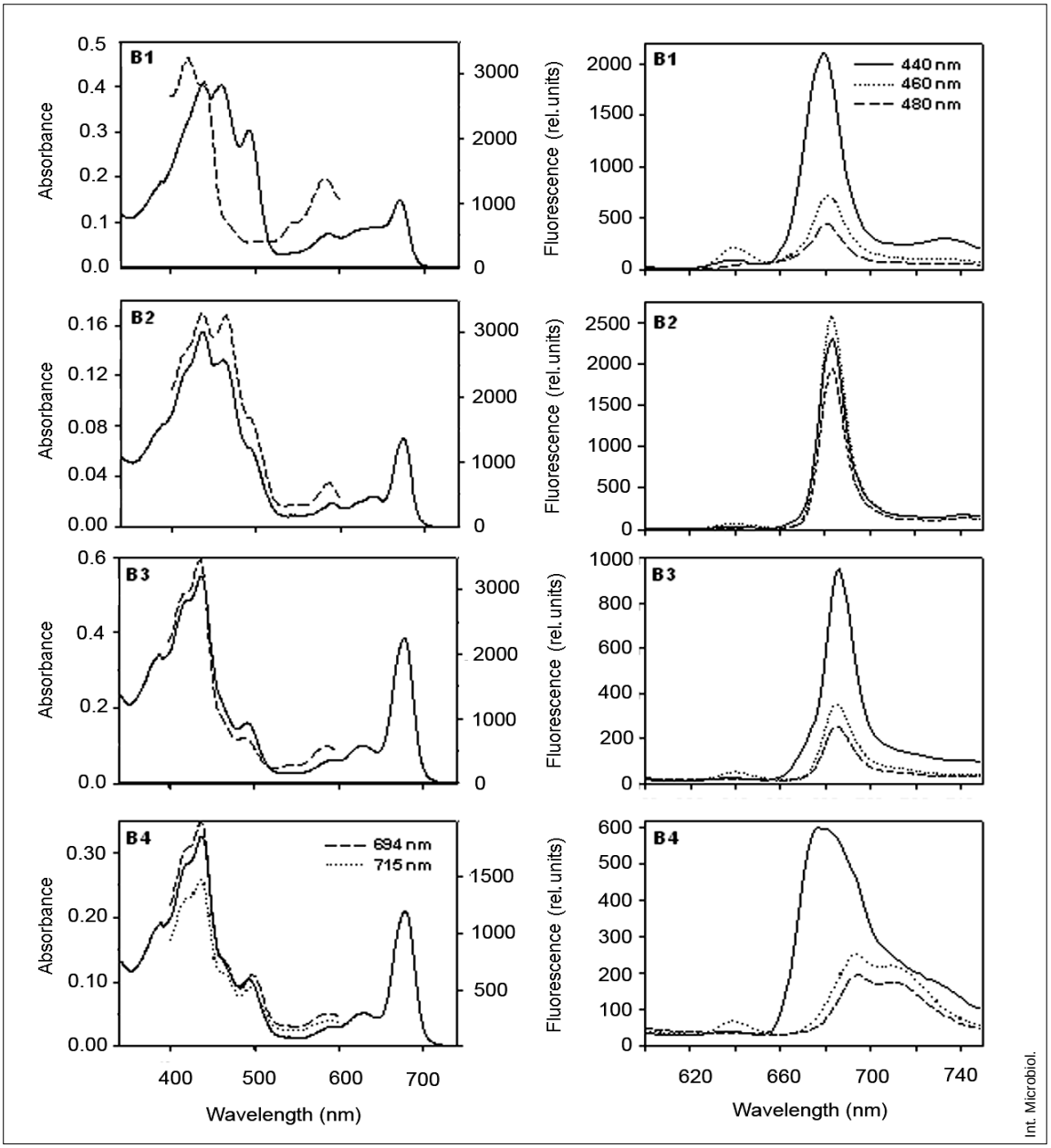

Fig. 2. Left column: Absorption (solid line) and fluorescence excitation spectra (dashed and dotted lines) of the gradient bands B1, B2, B3, and B4 of *Chroomonas* LT. **Right column:** Emission spectra of the gradient bands B1, B2, B3, and B4 of *Chroomonas* LT. The excitation spectra were recorded at the emission maxima for bands B1, B2, and B3. The emission spectra of band B4 were recorded at 694 and 715 nm (indicated in the plot of band B4, lowest left spectrum). The excitation wavelengths for the emission spectra are indicated in the plot of band B1 (upper right spectrum).

strongest signal intensities found for band B2. In addition, a diffuse polypeptide of M_r somewhat higher than 22 kDa was recognized in band B2. Polypeptides of 18–20 kDa were also recognized in band B4, but the signal intensities were rather weak in these cases. As the polypeptide pattern of band B3, especially the polypeptides in the 30-kDa range, pointed to PSII, Western blots were decorated with an antiserum against the D2 protein of the PSII core complex of maize. A 30-kDa polypeptide and a diffuse band of approximately 56 kDa in band B3 were recognized by the antiserum, indicating that band B3 harbored the major bulk of PSII complexes (Fig. 1C, "D2"). The banding pattern of band B4 pinpointed PSI complexes, with polypeptides with M_r larger than 50 kDa representing the core complex proteins PsaA and PsaB and sever-

al polypeptides of lower molecular masses. This assumption was confirmed by spectroscopy (see below) and by data obtained from other cryptophytes (see Discussion).

Spectroscopic characterization. The absorbance, fluorescence excitation, and emission spectra of the bands are shown in Fig. 2. Band B1 had absorbance maxima at 437, 452, 492, 587, and 672 nm and a broad shoulder at 600–650 nm. A fluorescence emission maximum at 681 nm was registered upon excitation at 440 nm. Excitation at 460 and 480 nm caused an overall decrease in fluorescence yield, with excitation at 460 nm yielding an additional small emission maximum at 640 nm caused by chl *c* fluorescence. Fluorescence excitation measurements revealed that the emission at 681 nm was mainly caused by chl *a* and phycocyanin. No P700 activity was found in B1. Absorbance maxima of B2 were registered at 436, 461, 590, 640, and 674 nm as well as an absorbance shoulder at 490 nm. The fluorescence emission maximum was at 683 nm. Excitation at 460 nm caused an increase in emission at 683 nm, with an additional small emission at 640 nm. Fluorescence excitation measurements revealed that the emission at 683 nm was caused by chl *a*, chl *c*, and carotenoids (maxima at 437 and 461, shoulder at 492 nm). As for B1, no P700 activity was detected in B2. Band B3 had absorbance maxima at 437, 490, 628, and 677 nm. The low absorbance at 460 nm demonstrated that chl *c* was present in rather low amounts; its fluorescence emission maximum (excitation 440 nm) was at 685 nm. Excitation at 460 and 480 nm caused a decrease in the emission at 685 nm, with an additional small emission at 640 nm upon excitation at 460 nm. Fluorescence excitation measurements revealed that mainly chl *a* contributed to the emission maximum. For B3, P700 activity was observed, corresponding to a P700:chl *a* ratio of 1:131 (±13) (n = 5). Absorbance maxima and shoulders of B4 were registered at 437, 493, 629, and 678 nm, thus demonstrating the most red-shifted absorbance peak of the four bands, typical for PS I. Fluorescence measurements upon excitation at 440 nm showed an emission maximum at 685 nm and a shoulder at 715 nm. Excitation with light of either 460 or 480 nm wavelength resulted in an overall decrease and a shift of the 685-nm emission towards 694 nm, whereas the emission at 715 nm became more pronounced. Fluorescence excitation measurements revealed that the emission maxima at 694 and 715 nm were mainly caused by chl *a* and, to a lesser extent, by chl *c* and carotenoids. Band B4 showed P700 activity; a P700:chl *a* ratio of 1:160 (±32) (n = 5) was calculated.

Phosphorylation assays. In the *in vivo* labeling experiments Western blots were treated with antibodies against phosphorylated amino acids. The relevant details of immunodecorated blots are summarized in Fig. 3. The antibody against phosphoserine gave rise to rather weak signals, immunodecorating polypeptides of 18 and 29 kDa. An additional signal was registered for a polypeptide of 32 kDa. The antibody against phosphothreonine strongly immunodecorated polypeptides of 35 and 30–32 kDa. Additional but minor signals were found for polypeptides of 18, 22, 23, and 27 kDa. The relevant detail of an autoradiograph of an in vitro phosphorylation assay is also shown in Fig. 3 (marked ^{32}P). Protein bands of 30, 27, 22, 18 and approximately 14 kDa were labeled.

Electron microscopy. Results of the negative staining of band B4 are compiled in Fig. 4. The great bulk of particles had dimensions of 22.2 nm (± 3.1 nm) in top-view projection (n = 184). Particles in side view measured 22 nm (±1.4 nm) in length (n = 17) and 7.5 nm (±0.9 nm) in width (n = 11). Particles of either larger or significant smaller dimensions were also found and were probably due to superposition of particles or aggregation/disaggregation processes, which might have occurred starting from the point of band sampling and continuing up to the negative-staining stage of the experiment.

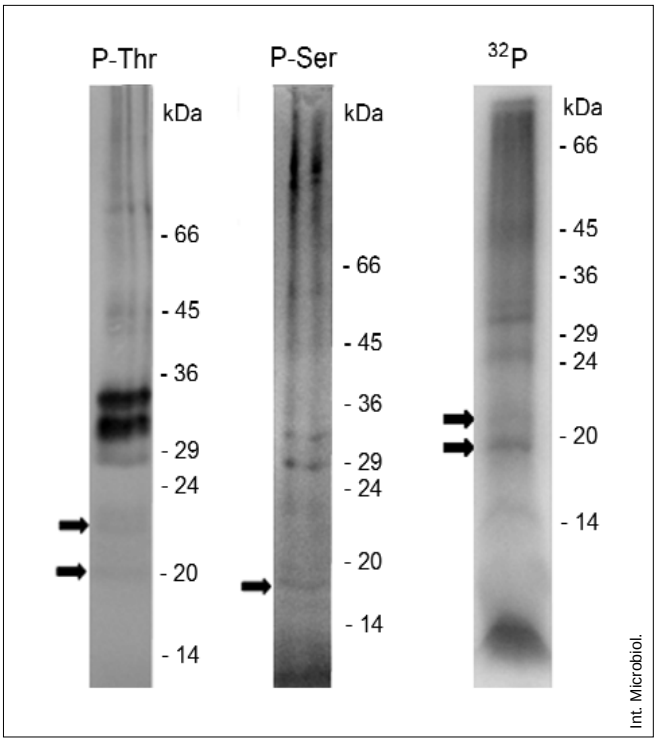

Fig. 3. Results of the phosphorylation experiments. In the in vivo phosphorylation assays, Western blots were immunodecorated with antibodies against phosphothreonine (P-Thr) and phosphoserine (P-Ser). The autoradiograph of an in vitro phosphorylation assay is shown on the right (^{32}P). Relative molecular masses of the marker proteins (kDa) are indicated. Arrows indicate Lhcc subunits.

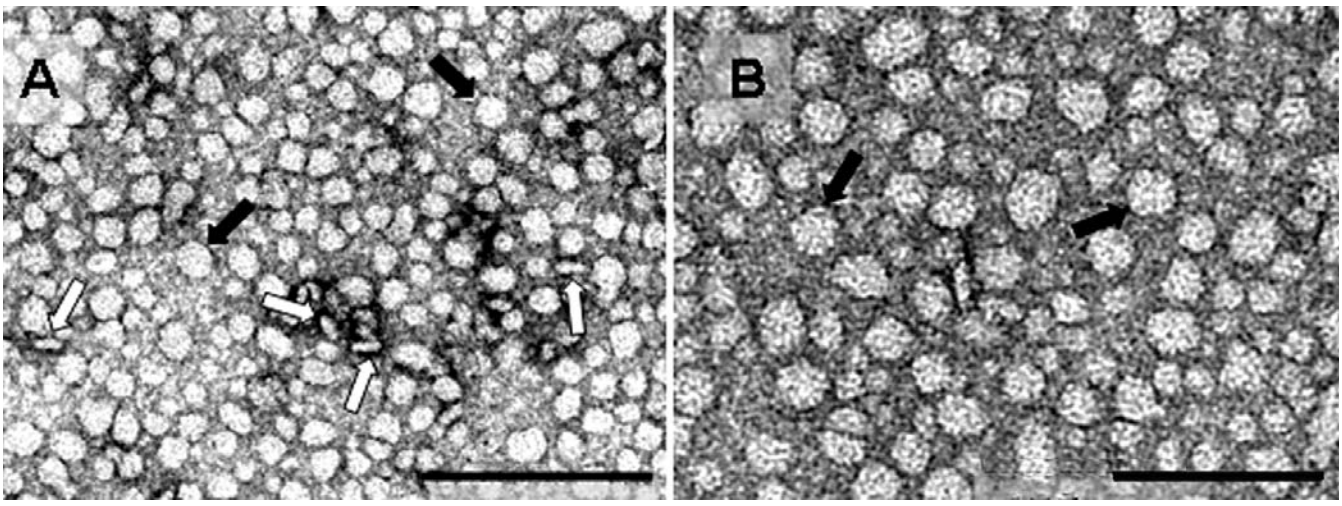

Fig. 4. Electron microscopy of isolated PSI-Lhcc complexes (sucrose gradient band B4) of *Chroomonas* LT (**A, B**) negatively stained with 2% (w/v) uranyl acetate. Particles in top and side view are marked with black and white arrows, respectively. Scale bars are 200 nm in (A) and 100 nm in (B).

Discussion

Cryptophytes are quite common in aquatic ecosystems. They are the sole organisms that contain both phycobiliproteins and Lhcc subunits for light-harvesting. In contrast to cyanobacteria and red algae, phycobiliproteins are located in the thylakoid lumen and organized as heterodimers [21,23]. Investigations on the photosystems and Lhcc subunits of cryptophytes have been carried out in two limnic and two marine species, and fractions enriched in PSI, PSII, or Lhcc subunits were isolated either by gel electrophoresis or by sucrose density centrifugation after solubilization with Triton X100, digitonin, or DM. The use of different detergents in these studies is reflected in the varying numbers of pigment protein complexes obtained. To date, DM had not been used for solubilizing thylakoid membranes of a limnic cryptophyte.

Solubilization of thylakoid membranes of *Chroomonas* LT with DM followed by sucrose density gradient centrifugation gave rise to four colored bands. Three pigment-bearing fractions were obtained from thylakoids of the marine species *Cryptomonas maculata* after solubilization with Triton X-100 [25]. Four bands were resolved when thylakoid membranes of the limnic species *Cryptomonas rufescens* were solubilized with digitonin [20]. For a limnic *Chroomonas* species, three fractions were obtained using digitonin [11]. The highest value, six pigment-bearing bands, was reported for a marine phycoerythrin-545-bearing *Rhodomonas* species following DM treatment [1].

Based on the results obtained by spectroscopy, SDS-PAGE, and Western immunoblotting, we conclude that band B1 consisted mainly of free pigments, phycobiliprotein, and Lhcc subunits. Band B2 was enriched in polypeptides with M_r of 18–22 kDa that cross-reacted with the anti-Cmac antiserum. Polypeptides of 18–24 kDa have been shown to be the basic units of the Lhccs of cryptophytes [1,11,25]. The absorption spectrum of band B2 resembled those of chl *a/c* LHCs isolated from the cryptophytes mentioned above, with marked absorption maxima at 640–642 nm, characteristic for high amounts of chl *c*. The energy transfer from chl *c* to chl *a*, as judged from the fluorescence spectrum, was much more efficient than in band B1, indicating that the LHCs in band B2 were structurally less changed. Based on the sedimentation behavior, it can be assumed that the Lhcc polypeptides in B2 are present in a higher aggregation state than those of band B1. Band B3 showed long-wavelength absorption and fluorescence maxima. SDS-PAGE, Western immunoblotting with the anti-D2 antiserum, and P700 measurements led to the conclusion that band B3 was enriched in PSII, but also contained PSI. The polypeptides in the 30-kDa range and the diffuse polypeptides of 50–60 kDa can be attributed to the reaction-center polypeptides D2 and the D1/D2 heterodimer usually found in PSII preparations. The chl *a*/P700 ratio was smaller than the one measured for band B4, suggesting that the PSI complexes in band B3 are less ordered or have lost some polypeptides, thus sedimenting at slower rates when subjected to sucrose density gradient centrifugation. This assumption is strengthened by the fact that band B3 did not show more long-wavelength emission, considering the measured chl *a*/P700 values. Band B4 exhibited the most red-shifted long-wavelength absorption of all bands, typical for PSI. Its fluorescence emission did not show a dominant emis-

sion at 715–718 nm, as described for PSI of *Rhodomonas* sp. and *Cryptomonas rufescens*, but it exhibited a chl *a*/P700 ratio similar to those reported for these two cryptophytes [1,20]. These values correspond to those of vascular plants [14] and indicate that antenna complexes, similar to the LHCI of chl *a/b*-containing organisms, must be associated with PSI of cryptophytes. This has been demonstrated for *Rhodomonas* sp. [1] and in the recent investigation for *Chroomonas* LT based on the fact that Lhcc polypeptides were identified by Western immunoblotting in band B4.

Almost all data on the phosphorylation of LHC subunits has derived from studies on green plants. Data on phosphorylation of LHCs of chromophytic algae are restricted to investigations on the brown alga *Laminaria saccharina* and the diatom *Cyclotella cryptica* [5,6]. In a previously reported in vitro study [6], phosphorylation was registered for a polypeptide of 21 kDa that cross-reacted with *Fucus serratus* LHC antibodies. For *Cyclotella cryptica*, phosphorylation of a fucoxanthin chlorophyll-*a/c*-binding polypeptide of 22 kDa was shown in vivo and in vitro [5]. In our in vivo and in vitro experiments, the reaction-center polypeptides D1 and D2 of PSII were strongly labeled, and the label was rather weak in both experimental setups for Lhcc subunits of 18–23 kDa. At least our data indicate that cryptophytes, like green plants, do phosphorylate Lhcc subunits. Currently it remains to be determined whether phosphorylation of Lhcc subunits plays a role in the mechanism of light protection.

A mean size of 22 nm was measured for the PSI particles of *Chroomonas* LT in band B4. Thus, these particles were significantly larger than the PSI particles of *Cryptomonas maculata*, which measured approximately 10 nm [25]. As these smaller particles showed a P700:chl *a* ratio of 1:100, they were assumed to represent PSI reaction-center complexes devoid of an antenna. The measured size of 22 nm is similar to the values reported for PSI-LHCI complexes of *Chlamydomonas rheinhardtii*, the recently described PSI-FCP complexes of the diatom *Phaeodactylum tricornutum*, and higher plants [4,16,17,30]. Thus, cryptophytes, like green plants and diatoms, seemed to harbor monomeric PSI-LHC (i.e., PSI-Lhcc) complexes.

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