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The membrane-intrinsic light-harvesting complex of the red alga *Galdieria sulphuraria* (formerly *Cyanidium caldarium*): biochemical and immunochemical characterization [1]

Jürgen Marquardt [a,*], Erhard Rhiel [b]

[a] *Fachbereich Biologie / Botanik, Philipps-Universität Marburg, Karl-von-Frisch-Strasse, 35032 Marburg, Germany*
[b] *Geomikrobiologie, ICBM, Carl von Ossietzky Universität Oldenburg, P.O. Box 2503, 26111 Oldenburg, Germany*

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Abstract

The membrane-intrinsic light-harvesting complex of the red alga *Galdieria sulphuraria* (formerly *Cyanidium caldarium*) could be isolated by gel-electrophoresis as a green band with an apparent molecular mass of about 20 kDa. The band had a long-wavelength absorption maximum at 672 nm and a fluorescence maximum (77 K) at 680 nm and reacted with an antibody against light-harvesting proteins of higher plant Photosystem I. Screening of thylakoid membranes with antisera directed against various chlorophyll *a / b* and chlorophyll *a / c* light-harvesting proteins indicated the existence of at least 4 distinct light-harvesting polypeptides with apparent molecular masses between 17 and 20 kDa. Isolation of Photosystem I and of a fraction enriched in Photosystem II showed that these polypeptides are exclusively bound to Photosystem I, thus forming a holocomplex which binds at least 205 molecules of chlorophyll *a*, and 33 and 37 molecules of zeaxanthin and β-carotene, respectively. Additionally, there is some evidence for the existence of a second Photosystem I pool without light-harvesting complexes. In-vitro translation experiments showed that at least two of the five polypeptides which constitute the membrane-intrinsic light-harvesting complex of *Galdieria sulphuraria* are translated from the poly(A)-enriched RNA fraction. They could be immunoprecipitated as preproteins being 3 to 4 kDa larger in size than the mature polypeptides.

Keywords: Light-harvesting complex; Photosystem I; Red alga; In-vitro translation; *Galdieria sulphuraria*; *Cyanidium caldarium*

Abbreviations: Chl, chlorophyll; DM, dodecyl-β-D-maltoside; HPLC, high-pressure liquid chromatography; IEF, isoelectrofocussing; LHC, light-harvesting complex; LHC I and LHC II, light-harvesting complex of Photosystem I and Photosystem II; OG, octyl-β-D-glucopyranoside; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonylfluoride; PS I and PS II, Photosystem I and Photosystem II; SDS, sodium dodecylsulphate; TMBZ, 3,3′,5,5′-tetramethylbenzidine; Tris, tris(hydroxymethyl)aminomethane

* Corresponding author. Fax: +49 6421 282057; E-mail: moersche@mailer.uni-marburg.de

[1] Dedicated to Professor W.E. Krumbein on the occasion of his 60th birthday.

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1. Introduction

All oxygen-evolving photosynthetic organisms harbour two photosystems, Photosystem I and Photosystem II (PS I and PS II), which mediate the transduction of light energy into chemical energy. While the reaction centres and the core-antennae are apparently highly conserved throughout the cyanobacteria and the photosynthetic eucaryotes, the additional peripheral antenna complexes exhibit a greater variability.

Green plants and chromophytes contain membrane-intrinsic chlorophyll (Chl) *a* / *b* or Chl *a* / *c* light-harvesting complexes (LHCs) (for reviews, see [1,2]), respectively, while cyanobacteria and red algae possess phycobilisomes, which are attached to the outer surface of the thylakoid membranes (for a review, see [3]). The existence of an additional, membrane-intrinsic LHC was demonstrated by Wolfe et al. [4,5] for the rhodophyte *Porphyridium cruentum*. This complex which is functionally associated with PS I could not be found in cyanobacteria. It harbours Chl *a* as the only Chl type and the carotenoids zeaxanthin and β-carotene. The authors showed that the polypeptides composing the LHC I of *P. cruentum* were immunologically related to the polypeptides of Chl *a* / *b* and Chl *a* / *c* LHCs. Furthermore, this antenna complex seems to be involved in acclimation processes towards different light intensities [6]. Only a partial amino acid sequence of one of the subunits has been published [7]. Models proposing the secondary and tertiary structure are missing for this red algal LHC I due to the lack of molecular data. On the contrary, the structure of higher plant LHC II has been resolved up to 0.28 nm resolution [8]. It is not known whether the red algal polypeptides are nuclear- or plastid-encoded. Red algae deviate in this respect from higher plants and green algae, as the genes for several polypeptides which are nuclear-encoded in chlorophytes are still located on the red algal chloroplast genome [9,10]. LHCs, however, were not encoded by the red algal chloroplast genom of *Porphyra purpurea* [9], suggesting that LHCs in red algae are nuclear-encoded.

Up to now, the existence of a red algal LHC I has been exclusively reported for *P. cruentum* and *Aglaothamnion neglectum* [4], while it has not been demonstrated for other red algae such as *Galdieria sulphuraria* (formerly *Cyanidium caldarium*) [11]. *Galdieria sulphuraria* deviates from most other rhodophytes in several aspects; it reproduces via autospores and has a cell wall composed of protein. As a thermoacidophilic microorganism, the alga lives in an extreme habitate with temperatures of up to 56°C and a pH value of 1.5 [12]. Its systematic position has been a matter of debate. Some authors proposed *Galdieria* and its closest relatives, compiled in the taxon 'Cyanidiaceae', to represent glaucophytes or even an acidophilic colourless type of *Chlorella* that engulfed a thermophilic cyanobacterium (for a review, see [12]). Other authors (e.g., [12,13]) deviate from both assumptions and place the Cyanidiaceae as a primitive line of the red algae within the taxon Prerhodophyceae. From this point of view one might expect that *G. sulphuraria* contains a very ancient type of LHC, if any.

The aims of the present study are (i) to look if the outstanding rhodophyte *G. sulphuraria* harbours a light-harvesting antenna similar to the LHC I described for *P. cruentum*, and, if present, (ii) to examine its composition and (iii) to investigate from which type of mRNA its polypeptides are translated.

2. Materials and methods

2.1. Growth conditions

The experiments were performed with a *G. sulphuraria* (formerly *C. caldarium*, cf. [14]) strain from the culture collection of Prof. W. Wehrmeyer (Marburg, Germany). Its former origin could not be elucidaded with certainty. It is probably a descendant of strain SAG 107.79 from the Göttingen algae collection ('Sammlung von Algenkulturen', Göttingen, Germany, cf. [15]). The cells were grown at 35°C in glass tubes containing 200 ml culture medium [16] and bubbled with air enriched with 2% (v/v) CO_2. The alga was cultured under white light at a light intensity of 25 $W \cdot m^{-2}$.

2.2. Isolation of thylakoid membranes and photosystem preparation

Thylakoid membranes were isolated as described before [17], with the exception that cells were broken

at 4°C by vigorous shaking with glass beads (1 mm diameter) in a Vibrogen cell homogenizer (Bühler, Tübingen, Germany) for 15 min. For isolating PS I and a fraction enriched in PS II, thylakoid membranes (600 μg Chl *a*) were incubated on ice with 6 ml 0.2% (w/v) dodecyl-β-D-maltoside (DM) in 0.1 M Tris-HCl, pH 7.8, at a detergent:Chl *a* ratio of 20:1 for 15 min. Subsequent centrifugation at 45,000 *g* for 30 min yielded a brownish pellet, while most of the Chl *a* was in the supernatant. The supernatant was loaded in aliquots of approximately 1 ml (equivalent to 100 μg Chl *a*) onto 35 ml-gradients of 5–20% (w/v) sucrose in 0.1 M Tris-HCl, pH 7.8, 0.03% DM, 0.1 mM phenylmethylsulfonylfluoride (PMSF) and centrifuged at 100,000 *g* and 4°C in a Kontron TST 28/38 swinging-bucket rotor for 20 h. The gradients yielded 3 coloured bands. The bands were harvested with a syringe, diluted with 0.1 M Tris-HCl, pH 7.8, 0.1 mM PMSF and sedimented by centrifugation at 200,000 *g* over night. By this centrifugation all Chl was recovered in the pellets which were resuspended in 200–300 μl of the supernatants. To determine the percentage of pigments bound to the single bands gradients were harvested with the aid of a peristaltic pump. Fractions of 0.5 ml were collected and the absorbance at 500 nm and 675 nm of each sample was measured with a Hitachi U-3200 spectrophotometer.

2.3. Polyacrylamide gel electrophoresis and immunochemical procedures

Partly denaturing polyacrylamid gel electrophoresis (PAGE) was performed according to [18]. The samples were solubilized on ice with 0.25% (w/v) sodium dodecylsulphate (SDS) and 1% (w/v) octyl-β-D-glucopyranoside (OG) at a SDS:OG:Chl *a* ratio (w/w/w) of 10:40:1. Per well 10 μg of Chl *a* were loaded. The gels were 45 mm in width, 80 mm in length and 1 mm thick and contained a 7–20% (w/v) acrylamid gradient. The total migration distance was approximately 25 mm. Fully denaturing PAGE was performed as described previously [19]. The gels were stained with 3,3′,5,5′-tetramethylbenzidine (TMBZ) according to [20] to detect heme-containing proteins or Coomassie-stained with Serva Blue R (Serva, Heidelberg, Germany).

For immunoassays the polypeptides were transferred onto Schleicher & Schuell (Dassel, Germany) BA 85 nitrocellulose membranes in a semi-dry system using a buffer given in [21]. The blots were immunodecorated as described before [22]. Antibodies against PS II core-complex polypeptides from higher plants were provided by Dr. R. Barbato (Dipartimento di Biologia, University of Padua, Italy). Antibodies against Chl *a*/*b* proteins of PS II from higher plants and LHC I-680 were from Prof. R. Bassi (Biotecnologie Vegetali, University of Verona, Italy). A detailed specification of their origin is given in [22]. The antibody against LHC I-730 of *Chenopodium rubrum* was a kind gift of Drs. V. Schmid and C. Schäfer (Lehrstuhl für Pflanzenphysiologie, University of Bayreuth, Germany). The antibodies directed against the main LHCs of the cryptophyte *Cryptomonas maculata*, the prasinophyte *Mantoniella squamata* and of the diatom *Cyclotella cryptica* are described in [23–25].

2.4. Isoelectrofocussing

Non-denaturing flat-bed isoelectrofocussing (IEF) was carried out as described by Dainese et al. [26] using Servalyte carrier ampholytes (Serva, Heidelberg, Germany) in the pH range of 4–9. The gel (95 mm in length, 45 mm in width, 3 mm in thickness) was prefocussed for 1 h at 1 W. Bands harvested from gradients were concentrated approximately 20-fold with Filtron Microsep centrifugal concentrators (Filtron, Northborough, MA, USA) with an exclusion size of 50 kDa to a final volume of 500 μl and supplemented with 1.6% ampholytes just before application. Focussing was carried out for 2 h at 1 W. Coloured material was removed from the gel, mixed with sample buffer [19], heated to 90°C for 2 min and analysed by fully denaturing SDS-PAGE. Alternatively, for the measurement of PS I activity, proteins were eluted from the gel matrix with 0.1 M Tris-HCl, pH 7.8, containing 0.03% (w/v) DM.

2.5. Spectroscopy, measurement of PS I activity and pigment analysis

Fluorescence spectra at 77 K were recorded with the equipment described in [27]. The excitation wavelength was at 440 nm and spectra were recorded from

600–800 nm. Fluorescence data were collected with a computer using a home-made program. Absorbance spectra were recorded with a U-3200 spectrophotometer (Hitachi, Tokyo, Japan).

The concentration of PS I reaction centres was determined by measuring the light-induced absorbance changes at 700 nm with the Hitachi photometer as described previously [19]. Excitation light was obtained with a Prado Universal lamp (Leitz, Wetzlar, Germany) equipped with a DAL 446 interference filter (Schott, Mainz, Germany). The photomultiplier was protected with a Schott IL 701 interference filter. The sample contained 5–7 μM Chl *a*, 0.25 mM methylviologen and 5 mM sodium ascorbate in 0.1 M Tris-HCl buffer (pH 7.8).

Pigment concentrations were determined spectroscopically and by high-pressure liquid chromatography (HPLC) as described before [19].

2.6. RNA isolation, in-vitro translation and immunoprecipitation

Total RNA, and poly(A)$^-$ and poly(A)$^+$ RNA of *G. sulphuraria* were isolated and purified using the Qiagen RNeasy total RNA Isolation and Oligotex Poly(A)$^+$ mRNA Isolation Kits. For this, 1200 ml of a culture in the mid log phase were used. Cells were broken in a mortar cooled with liquid nitrogen. In-vitro translation was carried out according to [28] for 1 h at 30°C in 50 μl reaction volume containing 20 μl rabbit reticulocyte lysate, 20 μl of the salt mix (Amersham RPN 3151, Amersham, Braunschweig, Germany) and 30 μCi [^{35}S]methionine of >1000 Ci/mmol (redivue L-[^{35}S]methionine, Amersham). After complete translation 5 μl aliquots were withdrawn and spotted onto GF 92 glass fiber filters (Schleicher and Schuell). The rate of incorporation was determined by liquid-scintillation counting according to the suppliers protocol (Amersham, Braunschweig, Germany). The remaining samples were used for SDS-PAGE and immunoprecipitation. Indirect immunoprecipitation was performed according to Westhoff and Zetsche [29], modified by Batschauer et al. [30], using protein A-Sepharose (Pharmacia, Freiburg, Germany). The immunoprecipitates were resuspended in Laemmli sample buffer [31], heated for 2 min at 100°C and electrophoresed on 15% or 17.5% (w/v) polyacrylamide gels in comparison with the total in-vitro translation products and molecular mass marker proteins. Gels were stained, destained, impregnated with Amplify (Amersham) for 30 min, dried and fluorographed at −80°C for 24 h to 28 days.

3. Results

3.1. Partly denaturing gel electrophoresis

When solubilized by mild detergent treatment and subjected to partly denaturing gel electrophoresis, thylakoid membranes of *G. sulphuraria* yielded several green bands (Fig. 1a, lane 1). Most prominent were bands of apparent molecular masses larger than 100 kDa and a doublet at about 40 kDa. The apparent molecular masses, however, have to be taken with care as the complexes are not fully unfolded with SDS. Two-dimensional reelectrophoresis revealed that the upper bands consisted mainly of PS I complexes, while the 40 kDa bands contained the core-antennae of PS II, CP47 and CP43 (data not shown). Additionally, there was a faint green band migrating with an apparent molecular mass of approximately 20 kDa just above the bluish band of about 17 kDa containing remainder phycobiliproteins. The faint green band was excised from the gel and either characterized by its spectral properties or subjected to denaturing electrophoresis followed by Western-immunoblotting. The absorbance and low-temperature fluorescence emission spectra of the complex are shown in Fig. 2. The complex exhibited absorption maxima at 417, 439, 491 and 672 nm with the 417 nm absorption maximum being most probably caused by a co-migrating cytochrome as indicated by TMBZ-staining (Fig. 1a, lane 2) showing a heme-binding protein in the 20 kDa region of the gel. The low-temperature fluorescence maximum of the green band was at 680 nm. These spectroscopic values deviated significantly from the data obtained for free pigments which showed a long-wavelength absorbance maximum at 670 nm and a fluorescence emission maximum at 676 nm (not shown).

Reelectrophoresis of the band gave rise to at least three polypeptides with apparent molecular masses of 18–21 kDa (Fig. 1b). Beside these bands most probably aggregates thereof could be stained in the upper

part of the gel. The polypeptides were blotted onto nitrocellulose and immunodecorated with an antiserum raised against LHC I-730 of *C. rubrum*. One

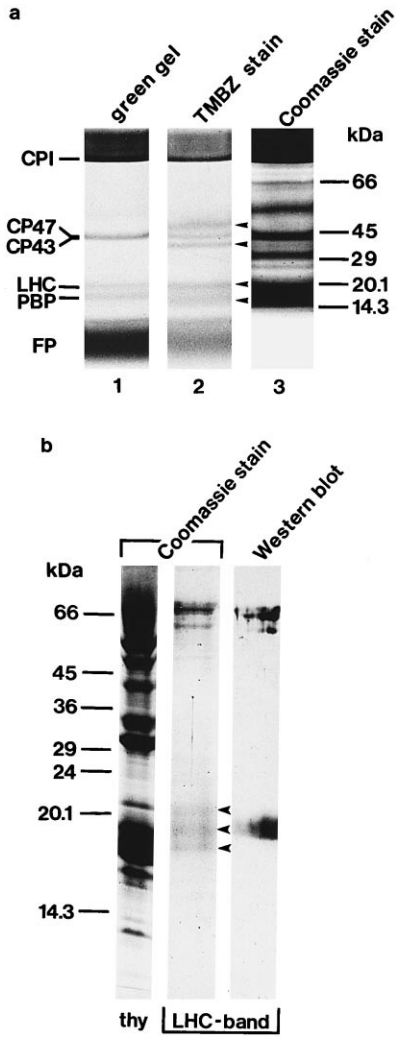

Fig. 1. Isolation and identification of the LHC. a: partly denaturing gel electrophoresis of *Galdieria sulphuraria* thylakoids. The figure shows an unstained 'green gel' (lane 1), the same gel after heme-staining with TMBZ (lane 2) and after Coomassie-staining (lane 3). Heme-containing bands which appeared bright blue after TMBZ-staining are marked with arrowheads. The identity of the coloured bands in the green gel is given on the left (PBP, phycobiliproteins; FP, free pigments), the positions of the molecular mass marker proteins are indicated on the right. b: reelectrophoresis of the LHC band under denaturing conditions. The figure shows the Coomassie-stained gel and a Western blot immunodecorated with an antiserum raised against LHC I-730 of *Chenopodium rubrum*. For comparison an SDS-PAGE of thylakoid membranes (thy) run under the same conditions is given on the left.

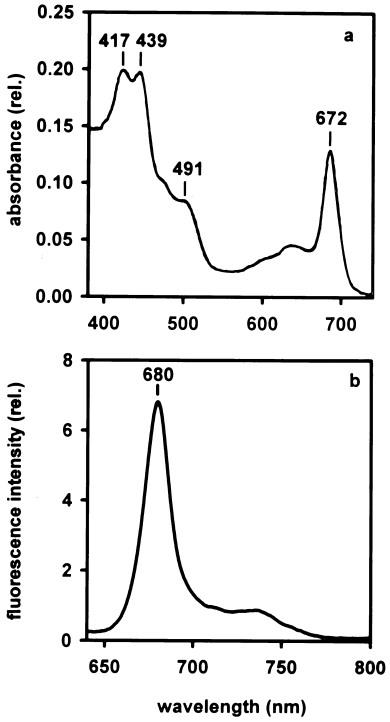

Fig. 2. Spectroscopic characterization of the LHC band from the green gel. a: absorption spectrum at room temperature; b: fluorescence emission spectrum at 77 K. The excitation wavelength was at 440 nm.

of the three bands showed a strong cross-reactivity with the antiserum. From this we conclude that the green band represented the LHC of *G. sulphuraria*.

3.2. Immunochemical screening

In order to see whether additional polypeptides were recognized by antisera directed against other Chl *a*/*c* or Chl *a*/*b* LHCs, thylakoid membranes of *G. sulphuraria* were subjected to SDS-PAGE and Western-immunoblotted. The results are compiled in Fig. 3. At least four different polypeptides could be immunodecorated with the various antisera. The strongest reactions were obtained with antisera directed against the Chl *a*/*c* LHCs of the diatom *C. cryptica* and the cryptophyte *C. maculata* and those raised against the minor Chl-protein CP24 and LHC I-730 of higher plants. Weaker immunoreactions were found in experiments with antisera against LHC I-680, LHC II and CP26. No immunoreactivity could be demonstrated with an antiserum directed against CP29

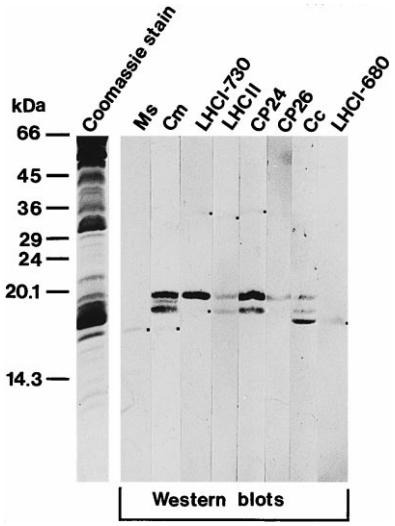

Fig. 3. Immunochemical screening of thylakoid membranes. Thylakoid membranes (10 μg Chl *a* per well) were fractionated by SDS-PAGE. A Coomassie-stain of a gel is shown on the left. The gels were blotted and the Western blots immunodecorated with antisera against the Chl *a* / *b* and Chl *a* / *c* complexes indicated above (Ms, LHC of *Mantoniella squamata*; Cm, major LHC of *Cryptomonas maculata*; Cc, major LHC of *Cyclotella cryptica*). Faint bands which are hardly visible on the photograph are marked with small dots.

while that against the LHC of the prasinophyte *M. squamata* gave rise to a very faint signal only.

The antibodies against LHC II and CP24 recognized two polypeptides with apparent molecular masses of about 20 and 19 kDa while the antiserum against CP26 immunodecorated exclusively the uppermost of these bands. The antibody against LHC I-730 recognized predominantly the 20 kDa band, while the reaction with the 19 kDa polypeptide was rather weak. The antibody against the LHC of the diatom *C. cryptica* additionally reacted with a polypeptide of 18.3 kDa which was also recognized by the antibody against LHC I-680. The antiserum raised against the LHC of the cryptophyte *C. maculata* bound to an additional band with an apparent molecular mass of 19.5 kDa. Furthermore, a faint signal was detected in the 17 kDa region. This protein was also recognized by the antibody against the LHC of the prasinophyte *M. squamata*. With some antisera (especially with the antibodies against LHC II, CP29 and LHC I-730) a weak reaction with a band of about 35 kDa was found. It might be caused by aggregated LHC proteins.

3.3. Sucrose density gradient centrifugation

In order to investigate whether the LHC was functionally attached to either PS I or PS II or to both, we separated the photosystems by sucrose density centrifugation. A schematic presentation of the density gradient obtained and elution profiles recorded either at 675 nm for Chl *a* or at 500 nm for carotenoids are given in Fig. 4. The gradient yielded three coloured bands. The pigment distribution in the gradient was determined in four independent experiments. Band 1 in the upper part of the gradient always contained 12–15% of the Chl and approximately 40% of the carotenoids loaded; the rest of the pigments was found in band 2 and band 3. Both bands contained almost equal amounts of Chl, but band 3 seemed to be enriched in carotenoids.

For further characterization the bands were harvested with a syringe. In order to minimize cross-contaminations between the bands 2 and 3 only the lower part of band 3 (corresponding to fractions 25–32 in Fig. 4) and the upper part of band 2 (corresponding to fractions 37–45) was used, while the part of the gradient corresponding to fractions 33–36 was discarded. For the characterization of band 1 the material corresponding to fractions 50–67 was used. The Chl yield per gradient was about 20 μg for bands 2 and 3 and 10 μg for band 1. A subsequent SDS-PAGE of the three bands is shown in Fig. 5, while pigment amounts, pigment ratios, P-700 estimations and ab-

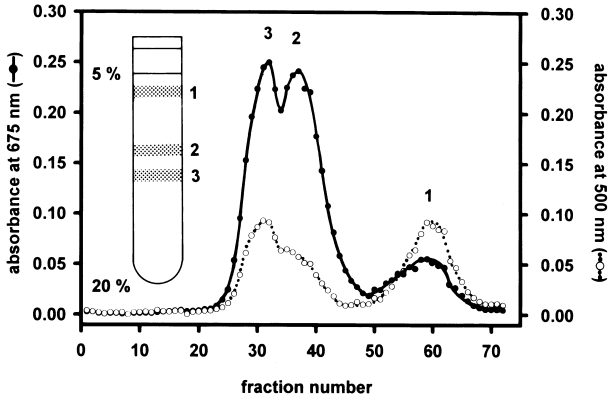

Fig. 4. Fractionation of thylakoid membranes by sucrose gradient centrifugation. The figure shows the elution profile of the gradient recorded either at 675 nm (solid line, filled circles) or at 500 nm (dotted line, open circles). A schematic presentation of the gradient is given as insert.

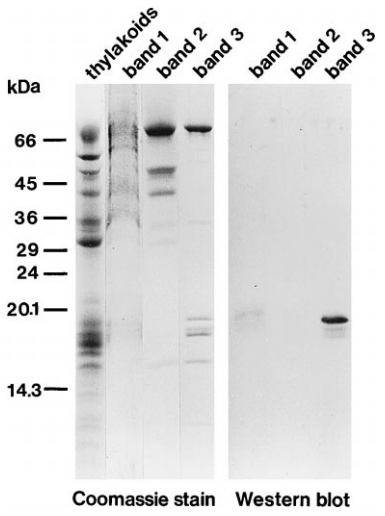

Fig. 5. Polypeptide composition of the bands isolated by sucrose gradient centrifugation. Coomassie-stain of an SDS-PAGE of thylakoid membranes and the three bands from the gradient and Western blot of the gradient bands. The blot was immunodecorated with a mixture of the antisera raised against LHC I-730 of *Chenopodium rubrum* and the main LHCs of *Cyclotella cryptica* and *Cryptomonas maculata*. For the Coomassie-stain 5 μg Chl *a* of each sample were loaded, for the Western blot 1.5 μg Chl *a* of band 1 and 4.25 μg Chl *a* of bands 2 and 3, respectively. The positions of the molecular mass markers are indicated on the left.

sorbance properties of the bands are summarized in Table 1. The fluorescence emission spectra of the three bands are given in Fig. 6.

Band 1 was almost devoid of proteins. Only traces of the LHC and polypeptides of the PS II core-complex could be detected and identified by Western-immunoblotting. The band is characterized by an absorption maximum at 670 nm and a fluorescence maximum at 682 nm (Fig. 6a). As deduced from the 2nd derivative the fluorescence maximum seems to consist of two components, one with a maximum at 672 nm, the other one emitting at 682 nm. The band was highly enriched in carotenoids, especially zeaxanthin (Table 1). The pigment stoichiometry, the low content of polypeptides and the spectroscopic data indicate that most of the pigments in this band were not protein-bound, although it is unusual that free pigments penetrate that far into a gradient. Therefore we tested the migration behaviour of unbound pigments by heating the sample before loading. This harsh treatment should release most of the pigments from the proteins. Nevertheless, after centrifugation there was no additional free-pigment band at the top of the gradient, but the overwhelming part of the pigments was now found in the position of band 1.

Band 2 was mainly composed of PS II polypeptides and PS I core-complexes. SDS-PAGE demonstrated bands with apparent molecular masses of 48, 42, 34 and 30 kDa, thought to represent PS II core-complex polypeptides. This could be confirmed by using antisera directed against CP47, CP43, D1 and D2 (data not shown). A band of approximately 60 kDa might represent CPI, the high molecular mass component of the PS I core-complex. This assump-

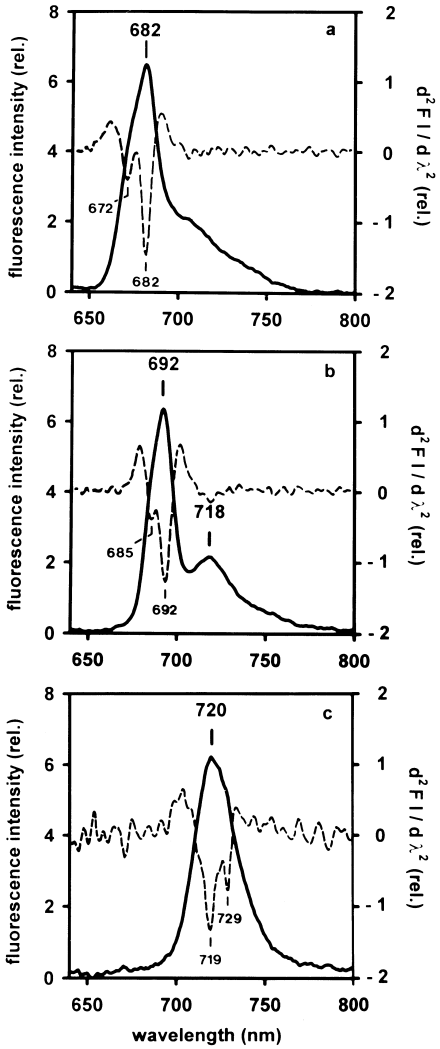

Fig. 6. Fluorescence spectra of the gradient bands. Fluorescence emission spectra at 77 K (solid lines) and the second derivatives thereof (broken lines) of band 1 (a), band 2 (b) and band 3 (c). The excitation wavelength was at 440 nm.

Table 1
Absorption characteristics and P-700 and pigment content of the bands obtained by sucrose gradient centrifugation

	Absorption maxima (minor peaks and shoulders in parenthesis)	Amount of Chl *a* (μg)	Chl *a*/P-700 (mol/mol)	Amount of P-700 (pmol)	Zeaxanthin/Chl *a* (mol/100 mol)	β-Carotene/Chl *a* (mol/100 mol)
Band 1	436, (458, 487), 670	14	n.d.	–	217 ± 23	43 ± 5
Band 2	436, (492), 676	43	163 ± 13	295	4 ± 1	19 ± 3
Band 3	436, (467, 497), 679	43	205 ± 5	235	16 ± 2	18 ± 3

Amounts of Chl *a* and P-700 were calculated from the Chl distribution in the gradient (cf. Fig. 4) and on the basis of a Chl load of 100 μg. The pigment ratios are means of at least six measurements ± S.D.; n.d., not detectable.

tion is strengthened by the presence of P-700 activity in the fraction (Table 1) and by the spectroscopic data. The long-wavelength absorption maximum of the band was at 676 nm, while the fluorescence spectrum (Fig. 6b) showed two maxima, a larger one at 692 nm (giving minima at 685 nm and 692 nm in the 2nd derivative), typical for the PS II core-complex, and a minor one at 718 nm, probably originating from PS I.

Band 3 obviously represented an almost pure PS I complex as shown by SDS-PAGE and spectroscopic data. It contained the 60 kDa band typical for CPI and additional low molecular mass polypeptides, especially a faint band at approximately 35 kDa and a number of polypeptides of 20 kDa and less. The long-wavelength absorption maximum was at 679 nm and the fluorescence spectrum had a maximum at 720 nm, typical for PS I (Fig. 6c). The molar ratio of Chl to P-700 was approximately 205 (Table 1). Taking into account the results obtained by HPLC analysis we calculated a carotenoid load of 33 molecules zeaxanthin and 37 molecules β-carotene per PS I complex.

The stoichiometry of Chl to PS I reaction centres was rather high, even higher than in band 2, which contained PS II polypeptides in addition to PS I. This indicates that in band 3 additional pigment-bearing polypeptides were present. This assumption was confirmed by Western-immunoblotting. The blots were immunodecorated with a mixture of the antisera against LHC I-730 and the LHCs of *C. cryptica* and *C. maculata*. As shown above (Fig. 3) this mixture should recognize all putative LHC polypeptides of *G. sulphuraria*. The antisera recognized at least 3 bands with apparent molecular masses between 18 and 20 kDa. With the polypeptides of band 2, however, an antibody signal was hardly visible (Fig. 5).

In order to test whether the LHC polypeptides were structurally bound to PS I of band 3 or just co-migrating with PS I core-complexes the band was further purified by isoelectrofocussing. The pigments focussed as a single band with an isoelectric point of 7.05 (Fig. 7a). The band showed PS I activity with a molar ratio of Chl *a* to P-700 of 208. When analysed by denaturing SDS-PAGE (Fig. 7b) it contained CPI as well as polypeptides of lower molecular masses. Western-immunoblotting confirmed that part of these bands represented the LHC polypeptides described above (Fig. 7b). Since it is very unlikely that two distinct protein complexes share the same sedimentation behaviour and the same isoelectric point, we assume that the LHC polypeptides must have been structurally bound to the PS I reaction centres. In addition to it, the fluorescence spectrum of band 3 had a single maximum at 720 nm, but did not show an emission maximum at 680 nm (Fig. 6c). This indicates that the LHC was not only structurally but also functionally bound to PS I.

Obviously the polypeptides reacting with the antibodies are exclusively bound to PS I of band 3, but neither to PS II nor to the PS I centres present in band 2. This raises the question whether the cells contain a PS I population devoid of an LHC or whether the loss of antenna proteins is a result of the isolation procedure. The presence of probably unbound LHC polypeptides in band 1 indicates that at least some PS I centres might have lost their antenna polypeptides during preparation.

In order to distinguish between these two possibilities we tried to correlate the amount of unbound LHC in band 1 with the amount of PS I centres lacking antenna polypeptides. In the gradient Chl is more or less equally distributed between band 2 and band 3. From the ratio of Chl per P-700 the amount of PS I

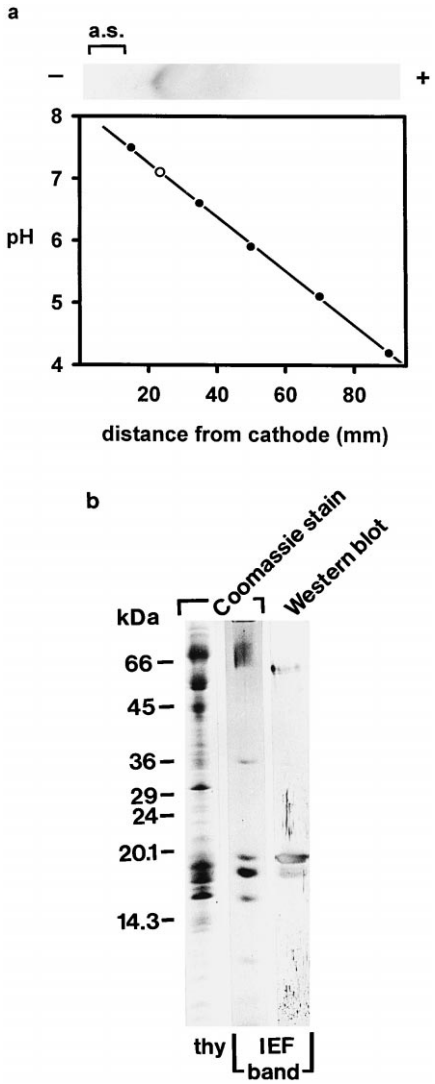

Fig. 7. Isoelectrofocussing of band 3. a: photograph of the gel (above) and the calibration curve (below) used to determine the isoelectric point of the green band. Closed circles represent sites of pH measurements, the open circle shows the position of the green band. The sample had been applied to the gel close to the cathode (a.s., application site). b: denaturing SDS-PAGE of the green band. The figure shows a Coomassie-stained gel and a Western blot immunodecorated with a mixture of the antisera raised against LHC I-730 of *Chenopodium rubrum* and the main LHCs of *Cyclotella cryptica* and *Cryptomonas maculata*. For comparison an SDS-PAGE of thylakoid membranes (thy) is given on the left.

centres in the bands can be calculated. It is higher in band 2 than in band 3 (approx. 295 pmol P-700 vs. 235 pmol P-700, cf. Table 1). If all these centres had lost their LHC during preparation, the amount of LHC in band 1 should be higher than in band 3. However, when the bands were loaded onto the gel according to their relative quantity in the gradient (1.5 μg Chl of band 1, 4.25 μg Chl of bands 2 and 3, respectively) and analysed by Western-blotting there was a strong immunodecoration of the polypeptides of band 3 and only a weak reaction with the LHC proteins of band 1 (Fig. 5). Thus the number of LHC polypeptides in band 1 is too small to explain for the amount of PS I centres depleted in antenna proteins. This indicates that the cells must contain a distinct PS I population devoid of an LHC I.

3.4. In-vitro translation / immunoprecipitation experiments

In-vitro translation/immunoprecipitation experiments were performed in order to look whether the LHC I polypeptides of *G. sulphuraria* are translated from poly(A)$^+$ or poly(A)$^-$ RNA. Approximately 1800 μg total RNA were isolated from an exponentially grown culture of *G. sulphuraria*. The RNA had an absorbance 260 nm:280 nm ratio of 1.76 and was further fractionated into a poly(A)$^-$ and a poly(A)$^+$-enriched fraction with total yields of 1500 μg and 37 μg, respectively. Total RNA, poly(A)$^-$ and poly(A)$^+$ RNA were translated in vitro with rabbit reticulocyte lysate. The synthesis rate found for the poly(A)$^-$ RNA was rather poor (approx. 7000 cpm incorporated per μg RNA), whereas the poly(A)$^+$ fraction and the total RNA fraction resulted in incorporation rates of approx. 210,000 cpm and 25,000 cpm per μg RNA, respectively. The newly synthesized polypeptides were electrophoresed and visualized by fluorography. The lanes 1 and 3 of Fig. 8 show the translation products of poly(A)$^-$ and poly(A)$^+$ RNA.

Indirect immunoprecipitation with protein A-Sepharose using the antiserum directed against LHC I-730 resulted in the precipitation of a distinct polypeptide with an apparent molecular mass of 23 kDa and an additional faint band of about 21 kDa. This highly specific precipitation reaction was found for the poly(A)$^+$ RNA (Fig. 8, lane 4) and not in in-vitro translation/immunoprecipitation experiments with poly(A)$^-$ RNA as template (Fig. 8, lane 2). The results indicate that the subunits of the LHC I of *G. sulphuraria* are translated from poly(A)$^+$ RNA and

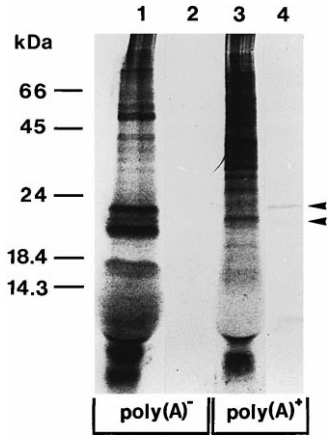

Fig. 8. In vitro-translation/immunoprecipitation products. Autoradiograph showing in vitro-protein synthesis from poly(A)$^-$ RNA (lane 1) and poly(A)$^+$ RNA (lane 3) and polypeptides immunoprecipitated thereof with antiserum against LHC I-730 (lanes 2 and 4). Ten μg of poly(A)$^-$ and 1 μg of poly(A)$^+$ RNA were used in the translation experiments. Equal amounts of translation products (equivalent to 100,000 cpm incorporated) were loaded in lanes 1 and 3. In lanes 2 and 4 the entire immunoprecipitated fractions were loaded. The putative precursor proteins are marked with arrowheads. The positions of molecular mass markers are indicated on the left.

immunoprecipitated as precursors 3–6 kDa larger in molecular mass than the mature polypeptides.

4. Discussion

The first evidences for a membrane-intrinsic LHC in red algae was found by Wolfe et al. [4] for *P. cruentum*. It consisted of polypeptides of 18–23.5 kDa and was associated with PS I. The antenna exhibited a low-temperature fluorescence maximum at 680 nm. Here we report the isolation of the LHC I of *G. sulphuraria* by partly denaturing gel electrophoresis. As demonstrated for the LHC I of *P. cruentum*, the isolated complex emits light at 680 nm. When it is associated with PS I, however, this emission band is not longer visible, indicating an efficient energy transfer. A similar result was obtained for the PS I holocomplex of *P. purpureum* (= *P. cruentum*) by Marquardt and Rehm [19].

Two polypeptides could be immunodecorated with an antiserum directed against the LHC I-730 of *C. rubrum*. An extension of the immunochemical screening using antisera directed against various LHCs from higher plants, a diatom, a cryptophyte and a prasinophyte indicate that the LHC of *G. sulphuraria* consists of at least four distinct polypeptides, which are recognized to various extents. The LHC I of *P. cruentum* was shown to be made up of six polypeptides. At least five of them were simultaneously recognized by antisera directed against CPIa of barley or the major LHC of the diatom *Phaeodactylum tricornutum* [4].

Our data differ in this respect and show that the single polypeptides of the *Galdieria* LHC were recognized to different degrees by the antibodies used. Thus only three of the four polypeptides seem to have epitopes which are recognized by the antisera against higher plant LHCs. These antisera recognized not more than two subunits simultaneously. The antisera against the diatom and cryptophyte LHCs recognized three subunits each. The antiserum against the main LHC of the prasinophyte *M. squamata* faintly immunolabelled a polypeptide which might represent a fifth subunit of the LHC. *Mantoniella squamata* differs from higher plants and chromophytes sinse its LHC harbours Chl *a*, Chl *b* and the Chl *c*-like pigment Mg-divinylphaeoporphyrin simultaneously. It should be mentioned that antibodies against antenna components of higher plant PS II recognized one of the polypeptides to a stronger extent than the antisera against higher plant LHC I.

The LHC of *G. sulphuraria* was exclusively copurified with PS I by sucrose density gradient centrifugation. Further purification by isoelectrofocussing showed that it was structurally bound to this photosystem. As shown by the fluorescence emission spectrum it transfers energy very efficiently to the reaction centre. An antenna size of 205 Chl molecules per PS I reaction centre was calculated. This result is rather high and differs from the findings of Wolfe et al. [4] who calculated for *P. cruentum* approximately 132 Chl *a* molecules per PS I holocomplex. For higher plants, however, PS I antenna sizes of about 200 Chl molecules have been described (for reviews, see [1,32]). Recent models propose that here the PS I antenna consists of two copies of four different gene products [1]. The discrepancy between our results and those of Wolfe and co-workers might thus either be species-specific or caused by the light regime applied or by loss of Chl *a* during photosystem isolation

from *P. cruentum*. *Galdieria sulphuraria* as a thermophilic organism might harbour photosynthetic protein complexes which are more resistent towards detergents and heat treatment.

Beside the PS I holocomplexes a mixture of PS II and PS I complexes banded in the sucrose gradients (band 2). This fraction contained no LHC as shown by immunolabelling. The rather low amount of LHC I found in the uppermost band of the gradient cannot explain the high quantities of PS I core-complexes in band 2. Thus we assume that beside PS I holocomplexes harbouring LHC I a distinct pool of PS I devoid of antenna proteins does exist in *G. sulphuraria*. Heterogeneity in the antenna size was observed for PS I [33] as well as for PS II (for a review, see [34]) of higher plants. While α-centers which have a larger antenna size are enriched in the appressed membranes of the grana stacks, β-centers with a smaller antenna are predominatingly found in stroma thylakoids. Up to now, there is no report about a PS I heterogeneity in red algae, but a heterogeneity in PS II antenna size has been reported for a facultative heterotrophic *C. caldarium* (= *G. sulphuraria*) by Diner [35].

Thus far, all genes encoding subunits of Chl *a* / *b* or Chl *a* / *c* LHCs in chlorophytes and chromophytes are found to be localized on the nuclear genome. This seems to be true for the red algal Chl *a* LHC as well, since two of the polypeptides of the LHC of *G. sulphuraria* were immunoprecipitated with antibodies against LHC I-730 in in-vitro translation experiments with poly $(A)^+$ RNA as template. However, mRNA containing extended internal poly(A)-rich sequences is present in some plastids and some chloroplast-encoded polypeptides are synthesized as precursors (e.g., [36,37]). Furtheron, posttransscriptional addition of poly(A)-rich sequences to a chloroplast mRNA has been shown by Lisitsky et al. [38]. Therefore the synthesis of precursor polypeptides from poly$(A)^+$ RNA cannot be used as a sure evidence for their nuclear encoding. It is true that red algae in this context often deviate from higher plants. Reith and Munholland [9] detected more than 125 genes on the chloroplast genome of *P. purpurea*; fifty-eight of them are not known to be located on land plant chloroplast genomes. Genes encoding for LHCs, however, were not found in the chloroplast genome of *Porphyra*.

Taking into account that *G. sulphuraria* is an ancient and primitive red alga we can assume that it contains an LHC that is more similar to that of the hypothetical progenitor of photosynthetic eucaryotes than the LHCs of many other algae and higher plants. This seems to be supported by the immunochemical data. The *Galdieria* LHC I consists of several subunits. Some of them obviously share more epitopes with chlorophytic LHC polypeptides while others seem to have more epitopes in common with subunits of chromophytic LHCs. From this we can speculate that the antenna system of the progenitor already consisted of various subunits, some of which were preferred in the evolution of the chlorophytic LHC, others in the evolution of chromophytic antenna systems. Possibly the membrane-intrinsic antenna systems were originally associated exclusively with PS I. During evolution these LHC I polypeptides might have replaced the extrinsic antenna of PS II, the phycobilisome, thus evolving towards LHC II. Further experiments, especially the cloning and sequencing of genes encoding the red algal LHC I are needed to reveal its phylogenetic relationship to LHCs of higher plants and chromophytic algae.

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