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Separation of Photosystems I and II from the oxychlorobacterium (prochlorophyte) *Prochlorothrix hollandica* and association of chlorophyll *b* binding antennae with Photosystem II

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Photosystem I and Photosystem II plus associated chlorophyll *a*/*b*-protein complexes were isolated from washed thylakoid membranes of the oxychlorobacterium (prochlorophyte) *Prochlorothrix hollandica*. Separation was achieved by solubilization with Zwittergent 14 and sucrose gradient centrifugation. Characterization involved non-denaturing and SDS- gel electrophoresis, immunodecoration, pigment content estimation by HPLC and analysis by absorbance and fluorescence spectroscopy. The data obtained indicate a functional positioning of the chlorophyll *a*/*b* antennae in the vicinity of Photosystem II. Fractions enriched in these antennae contain at least four polypeptides, of 28 to 30, 32, 34 and 36 kDa. The chlorophyll *a* to *b* ratio of the separated antennae was estimated to be about 4. As opposed to higher plant and green algal chloroplasts, Photosystem I of *Prochlorothrix* does not appear to have its own separate chlorophyll *b* containing antenna.

Introduction

Oxychlorobacteria (prochlorophytes), e.g., *Prochloron* and *Prochlorothrix*, are oxygenic phototrophic prokaryotes containing chlorophylls *a* and *b* [1,2,3]. Because of this evolutionary interesting set of properties prochlorophytes have been related to both higher plant and green algal chloroplasts as well as to cyanobacteria [1,2]. This classification has raised questions about the architecture of PS I and PS II and the adjoining chlorophyll-protein complexes. In chloroplasts, the lateral heterogeneity of PS I and PS II has been explained to be the result of the hydrophobic interaction of the LHC II complexes [4]. Interestingly, such a lateral heterogeneity has also been documented for *Prochloron* and *Prochlorothrix* [5,6,7]. In contrast, PS I and PS II in cyanobacteria are fully mixed in a regular pattern [8].

In chloroplasts, most of the Chl *b* is incorporated in the light-harvesting complex II (LHC II), which has a Chl *a* to *b* ratio of about unity and comprises up to 50% of the total Chl. Three minor PS II associated Chl *a*/*b*-binding complexes (CP 29, CP 26 and CP 24) have been described as well [9–11]. Approx.15% (as calculated from [12]) of the Chl *b* is associated with PS I. LHC I has a Chl *a*/*b* ratio of about 3.5 and is comprised of apoproteins with molecular masses ranging from 20 to 24 kDa [9,11]. In cyanobacteria such a separate PS I antenna is not present [13,14].

Prochlorophytes have been portrayed as possible ancestors of higher plant chloroplasts [15,16]. Comparison of the antennae with various antibodies indicated a distinct diversity in that the complexes of *Prochloron* and *Prochlorothrix* were shown to be related [17], but to be both different from chloroplast LHC II. Even if antenna polypeptides are different, functional alikeness would require a major role for Chl *b* as antenna pigment of PS II. However, experimental data obtained by different groups rather surprisingly pointed to an appreciable association of Chl *b* with PS I [18,19].

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Abbreviations: CHAPS, (3[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate; Chl, chlorophyll; cyt, cytochrome; LHC, light-harvesting complex; LiDS lithium dodecyl sulphate; PMSF, phenylmethylsulfonyl fluoride; PQ, plastoquinone; PS, Photosystem; SB 14, Zwittergent 14 (*N*-tetradecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulphonate).

Maltoside solubilisation of thylakoids from *Prochlorothrix hollandica* yielded five chlorophyll containing complexes on non-denaturing ('green') gels [19]. The excitation spectrum of the 717 nm fluorescence emission of the excised PS I area revealed a peak at 468 nm. This was interpreted as a functional coupling of Chl *b* to PS I.

LHC II phosphorylation is a way in which light energy distribution towards PS I and PS II can be regulated in chloroplasts [20–22]. In a study on the in vitro phosphorylation of antenna polypeptides in *Prochloron* the activity of the kinase appeared to be independent of the light conditions [23]. This phenomenon was interpreted as an adaptation to the light conditions in the habitat of *Prochloron* [23], it remains arrested in state 2, with part of the antenna located in the unstacked regions associated with PS I [23]. Antenna phosphorylation in *Prochlorothrix* has also been shown [24–26]. The model proposed for *Prochloron* [23] would suggest a constant association of the phosphorylated antenna with PS I. In case of *Prochlorothrix* an in vivo reversibly phosphorylated 35 kDa polypeptide was found to copurify with PS I [26]. The dephosphorylated form of this protein was found to be predominantly associated with PS I [25]. The phosphorylation of this polypeptide coincided with state transitions [26]. This would imply that it is the phosphorylated antenna that moves away from PS I, opposite to the state 2 conditions suggested to be prevalent in *Prochloron* [23].

With reference to these observations, a study on the actual presence of Chl *a*/*b* antennae near PS I or PS II was addressed in this work. We present evidence that the major Chl *a*/*b* binding antenna is associated with PS II. No antenna was found to be associated with PS I.

Materials and Methods

Prochlorothrix hollandica (PCC 9006) [3] was grown in aerated BG 11 mineral medium [27] at 20°C under continuous illumination at 30 μE m^{-2} s^{-1}. Cells were spun at 3000 × *g* and washed twice in buffer A (20 mM Hepes-NaOH, 200 mM sorbitol, 10 mM NaCl, 2 mM EDTA, 0.1 mM PMSF). All preparative procedures were done at 0 to 4°C. Cells were broken in the French press at 70 MPa, unbroken cells were pelleted at 5000 × *g*. The supernatant was layered on a cushion of 1.5 M sucrose in buffer A. After centrifugation at 40000 × *g* for 1 h the green material above the sucrose cushion was collected and pelleted at 200000 × *g*. The pellet was resuspended in buffer A to a chlorophyll concentration of 1 mg/ml. To remove extrinsic polypeptides, thylakoids were washed in 10 mM CHAPS (Sigma) [28]. Thylakoids were resuspended in buffer A containing 5 mM $MgCl_2$ instead of EDTA at a chlorophyll concentration of 2 mg/ml, quickly frozen and stored at −70°C.

For the isolation of photosystems, frozen membranes were quickly thawed, pelleted and dissolved in buffer B (buffer A without sorbitol). After addition of detergent (Zwittergent 14 was selected in this study, cf. Results), membranes were kept on ice for 30 min. The dissolved complexes were separated on a 0.1 to 0.8 M sucrose gradient containing 0.05% Triton X-100 (Bio-Rad) and buffer B at 270000 × *g* for 18 h. The collected green bands were bound to Fractogel TSK DEAE-650 (S) (Merck) which was preequilibrated with buffer B containing 0.02% dodecyl maltoside (Calbiochem). The bound material was washed with buffer B containing 50 mM NaCl, 0.02% dodecyl maltoside, which did not release any green material, and eluted by a final NaCl concentration of 250 mM. Dodecyl maltoside (0.2%) was added to the green fractions and these were fractionated on a second sucrose gradient containing buffer B and 0.02% dodecyl maltoside.

Room temperature absorption spectra were recorded on an Amilco DW 2000 spectrophotometer. 77 K absorption spectra were recorded on a Cary 219 spectrophotometer. 77 K fluorescence spectra were measured on a laboratory built apparatus, as described in Ref. 29. For measurements at 77 K, samples were adjusted to 50% glycerol in buffer B containing 0.02% dodecyl maltoside. For fluorescence measurements, samples with a maximal absorbance of 0.3 between 435 and 440 nm were used.

Sucrose density was measured on an Abbe refractometer. P700 content was estimated from reduced minus oxidized spectra according to [30], a mmolar extinction coefficient of 64 was used for calculations. Cytochromes were estimated according to [31].

The polypeptide composition of the isolated bands was analysed by SDS-polyacrylamide slab gel electrophoresis on 15% acrylamide, 0.2% bisacrylamide gels containing 0.75 M Tris-HCl (pH 8.8) [32] and 10% glycerol. The gels were washed twice with 5% acetic acid, fixed for 1 h with 12% trichloroacetic acid, stained with Coomassie Brilliant Blue G 250 in 2% phosphoric acid, 8% ammonium sulphate and 20% methanol [33] and destained with 5% acetic acid. Gels were shrunk in poly(ethyleneglycol) 2000 before photographing [34]. Chlorophyll-protein complexes were analysed by non-denaturing electrophoresis [19] on pre-chilled 2 mm thick 7.5% acrylamide, 0.1% bisacrylamide slab gels using a Mini Protean II apparatus (Bio-Rad). During electrophoresis the buffer tank was placed on ice. Green bands were excised from the gel, and the chlorophyll-protein complexes were eluted overnight in buffer B containing 0.02% dodecyl maltoside. After concentration in Centricon 30 microconcentrators (Amicon), pigments were extracted with 90% actone for pigment analysis. Immunological analysis was es-

sentially done according to [35]. Blots were stained with Ponceau Red in 5% acetic acid to indicate molecular mass markers and transfer efficiency. The antibodies against the reaction centres of PS I and PS II from maize were a kind gift by Dr. Roberto Bassi. Antibodies against the apoproteins of the oxygen-evolving complex were made available by Dr. H. Pakrasi. Chl concentrations were determined in 80% acetone [36].

Pigments were analysed by HPLC on a ODS-hypersil column (250 × 4.6 mm) with 5 μm particles. Samples were extracted with 90% acetone and spun at 12000 × *g* for 2 min. To aliquots of the supernatant 0.3 vol. of an ion-pairing mixture (tetrabutylammonium acetate (1.5%, w/v) and ammonium acetate (7.7%, w/v)) was added 3 min before injection into the HPLC. Gradient elution was achieved by linear mixing of phase A comprising 90% methanol (Merck, gradient grade) and 10% water with phase B containing 80% methanol and 20% acetone reaching 100% phase B in 15 min, and subsequent elution with phase B during 16 min. A flow rate of 1.5 ml/min was used throughout. Phase A contained a 10-fold dilution of ionpairing reagents with respect to the sample. Peak areas were used to calculate pigment contents.

Zwittergent (chainlength $n = 8$ to 16), dodecyl maltoside and octyl glucoside were purchased from Calbiochem. Deriphat 160 was obtained from Serva. Digitonine was obtained from Merck and was recrystallized twice from ethanol. All other chemicals were analytical grade.

Results

Several detergents, including dodecyl maltoside, octyl glucoside, lithium dodecyl sulphate, Deriphat 160 and digitonin were used in a first attempt to achieve solubilization and fractionation of the photosystems of *Prochlorothrix hollandica* on a sucrosegradient. None of these detergents yielded satisfactory results. With dodecyl maltoside or octyl glucoside the complexes were not very well separated. Prolonged exposure to LiDS, as occurs during centrifugation, induced formation of pheophytin. With Deriphat 160 or digitonin solubilization was very poor (not shown). Out of a series of zwittergent-type detergents with different alkyl chain length ($n = 8$ to $n = 16$) solubilization and separation of isolated thylakoid membranes from *Prochlorothrix* was successful with Zwittergent 14. It was used in different detergent to chlorophyll ratios to determine the optimal solubilisation of thylakoids without loss of complex integrity. Two discrete green bands with different polypeptide composition were obtained on a sucrose gradient (Fig. 1). The upper band at 13% sucrose was enriched in PS II and Chl *b*; the lower band at 14.5% sucrose was enriched in PS I (chlorophyll to P700 ratio of 135 as compared to the ratio of

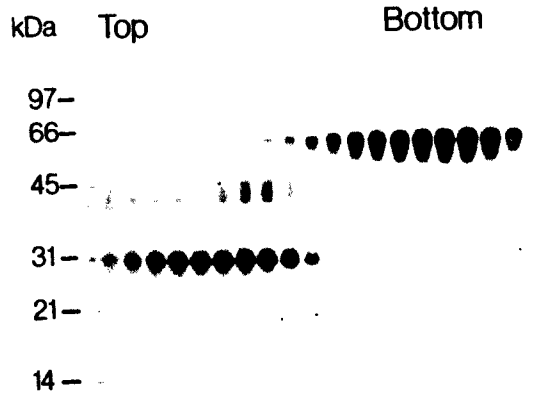

Fig. 1. Polypeptide composition of membranes solubilized with SB 14 at a detergent to chlorophyll ratio of 4 to 1 and separated on the (first) sucrose gradient containing 0.05% Triton X-100. Fractions of 300 μl were collected. Only green fractions (fraction 11 to 30) were analysed.

210 in whole thylakoids) and depleted in Chl *b* (not shown). At detergent to chlorophyll ratios above 8 an additional band appeared on top of the gradient at 10.3% sucrose. This band was enriched in carotenoids and contained some Chl. A further increase of the SB 14 to chlorophyll ratio correlated with an increase in the chlorophyll content of this band at the expense of the PS II enriched band. Apparently, higher SB 14 concentrations affected the complex integrity of the PS II enriched fraction. For an optimal solubilization a detergent to chlorophyll ratio of 6 was negotiated.

Since Triton X-100 was known to affect the spectroscopic characteristics of isolated chlorophyll-protein complexes [37,38], we replaced Triton X-100 by maltoside during the further purification steps. In order to concentrate the samples, to remove sucrose and to exchange the detergent, the selected fractions were bound to Fractogel and eluted with 250 mM NaCl. The eluted material was further purified on a second sucrosegradient containing dodecyl maltoside. The PS I fraction produced one green band with a chlorophyll to P700 ratio of 90 at 14.5% sucrose (PS I-2) and a thin, yellow green band at 10.3% sucrose (PS I-1). The PS II fraction yielded a yellow green band at 10.3% sucrose (PS II-1) and a green band at 12.6% sucrose (PS II-2). In some cases a faint green band at 13.2% sucrose could be detected (PS II-3). No cytochromes could be detected in the PS I enriched fraction (PS I-2). In the PS II-antenna fraction (PS II-2) cytochrome *b*-559 (low potential) could be demonstrated at a chlorophyll to cytochrome *b*-559 ratio of 165. No other cytochromes were detectable in this fraction.

The polypeptide pattern of the major green bands is displayed in Fig. 2. The yellow green bands on top of

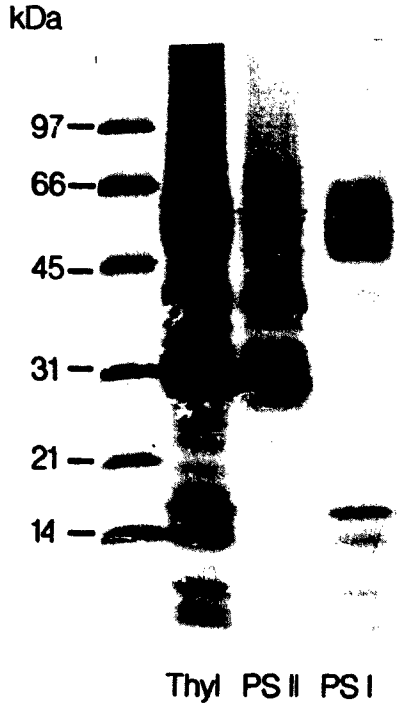

Fig. 2. Polypeptide composition of PS I and PS II enriched fractions. Lanes were loaded with 8 μg chlorophyll of the photosystem fractions and 20 μg chlorophyll of the thylakoids. (1) Molecular mass markers; (2) thylakoids (Thyl); (3) PS II enriched fraction PS II-2 (PS II); (4) PS I enriched fraction PS I-2 (PS I).

the gradients barely contained polypeptides (not shown), confirming the assumption that these bands are comprised of free pigment. The PS II (PS II-2) fraction was dominated by polypeptides of 58, 38, 34 and 32 kDa and a broad band at 28–30 kDa. Notable is the absence of polypeptides in the 20 to 28 kDa area. In chloroplasts this area is dominated by the antenna apoproteins [9–11], in contrast the antenna apoproteins from *Prochlorothrix* all have molecular masses above 28 kDa [19, this study]. By this coincidence, the antenna polypeptides, the reaction centre polypeptides D1 and D2 and the 33 kDa apoprotein of the oxygen-evolving complex all appear in the same molecular mass range. Furthermore, immunoblot analysis with antibodies directed against the 33, 23 and 17 kDa apoproteins from the oxygen-evolving complex in chloroplasts indicated that the 23 and 17 kDa polypeptides are absent in *Prochlorothrix* (not shown). These two polypeptides are part of the oxygen-evolving complex in chloroplasts, but appeared absent in cyanobacteria [39]. To exclude accidental loss of these extrinsic polypeptides during the isolation steps, this analysis was also done with extracts from whole cells.

The PS I fraction was dominated by a broad band centering at around 60 kDa, the psaA and psaB gene products which are not well separated, and minor polypeptides at 16.5, 14.5, 11 and 9 kDa. This polypeptide composition is similar to the one found in cyanobacteria [13,14] and *Prochloron* [40]. The polypeptides of 55 and 56 kDa are either the α and β subunits of the ATPase or the apoproteins of the carotenoid-binding complex [41], which were not completely removed during the isolation and washing of the thylakoids. In thylakoids which were not washed in EDTA or CHAPS these two polypeptides are among the most abundant bands, and during non-denaturing electrophoresis they comigrate with PS I derived bands (not shown). Presumably these two polypeptides were erroneously identified as the psaA and psaB gene products in a first attempt to analyse the chlorophyll-protein complexes of *Prochlorothrix* [19].

To establish the purity of the preparations, the different fractions were challenged with antibodies directed against core complexes of PS I and PS II from maize (Fig. 3). With an antibody preparation directed against a PS II core complex, *Prochlorothrix* thylakoids showed strong crossreaction at 34 and 32 kDa (D2 and D1). The band at 58 kDa is probably the D1-D2 dimer [42]. The PS II enriched fraction showed the presence of D1, D2 and a major band at 14 kDa, probably cytochrome *b*-559. The PS I enriched fraction did not show crossreaction with the PS II antibody. On a blot decorated with antibodies against the PS I core in the PS I fraction the polypeptides at 60 kDa showed crossreaction (Fig. 3B, lanes 1 and 3), but the PS II fraction lacked this crossreaction (lane 2).

The pigment contents of the bands from the second sucrose gradient were determined by HPLC. The PS I and the PS II fractions both contain β-carotene (Table

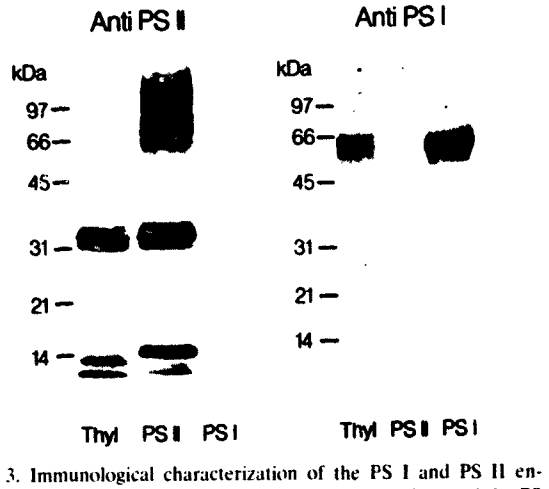

Fig. 3. Immunological characterization of the PS I and PS II enriched fractions. Thylakoid membranes of *Prochlorothrix* and the PS I and PS II enriched fractions were challenged with antibodies against reaction centres of PS I and PS II from maize. Thyl: thylakoids.

TABLE I

Pigment composition of the bands from the second sucrose gradient

Pigments were determined by HPLC. PSI-1 and PSII-1: upper yellow-green bands from the PS I and PS II fractions respectively, dominantly comprised of free pigment. PSI-2 and PSII-2: major green bands from the PS I and the PS II fractions, these bands were used for further analyses. PSII-3: faint green band from the PS II fraction, which was not subjected to further analysis. All data are expressed per 100 molecules Chl *a*. n.d., not determined. The detection limit for the chlorophyll to P700 ratio and the chlorophyll to cyt *b*-559 ratio were estimated to be 2000 and 1000, respectively.

	PS I-1	PS I-2	PS II-1	PS II-2	PS II-3
Chl *a*	100.0	100.0	100.0	100.0	100.0
Chl *b*	5.7	1.9	6.9	16.5	18.4
Zeaxanthin	94.7	1.4	78.7	10.3	17.8
β-Carotene	36.0	16.7	13.9	8.2	14.2
Chl\P700	n.d.	90	n.d.	> 2000	n.d.
Chl\cyt *b*-599	n.d.	> 1000	n.d.	165	n.d.

1), the results point to a somewhat larger distribution in the PS I fraction. In the PS I fraction some Chl *b* and zeaxanthin were present. The free pigment zones were enriched in carotenoids. The PS II bands were enriched in chlorophyll *b* (Chl *a* to *b* ratios of 5 to 6).

The composition of the PS I and PS II/antenna fractions from the second sucrose gradients was analysed by non-denaturing electrophoresis (Fig. 4A). The PS II fraction was resolved into 3 bands. PS I yielded two bands. Even by illumination with ultraviolet light, a sensitive method to detect PS II or antennae complexes, no fluorescence could be seen on the gel in the PS I fraction (not shown). The same conditions induced brigh fluorescence of all bands in the PS II

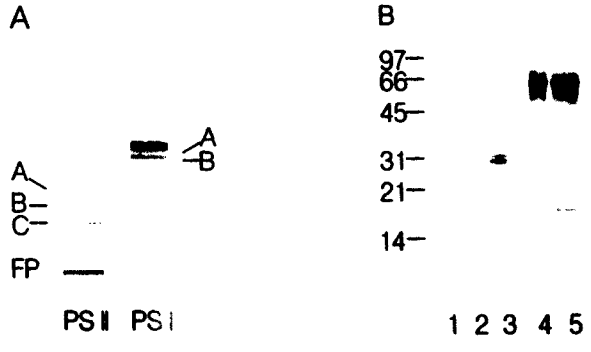

Fig. 4. Analysis of the chlorophyll-protein complex composition of the PS I and PS II enriched fractions. (A) Separation of chlorophyll-protein complexes from the PS I and PS II enriched fractions from the second sucrosegradient containing dodecyl maltoside on a non-denaturing gel. LiDS was added to the sample at a final concentration of 0.1% before electrophoresis. FP, free pigment. (B) Polypeptide composition of the separated chlorophyll protein complexes. Green bands were excised and subjected to denaturing electrophoresis. Lane 1 to 3: PS II-A to PS II-C. Lane 4: PS I-A. Lane 5: PS I-B.

TABLE II

Pigment composition of the chlorophyll-protein complexes isolated by non-denaturing electrophoresis (Fig. 4)

Green bands were cut from the gel, eluted overnight in buffer A plus 0.02% maltoside, concentrated and extracted with 90% acetone for pigment analysis (see Materials and Methods). The thin free pigment zone from PS I was not analysed.

	Chl *a*	Chl *b*	β-Carotene	Zeaxanthin
PS I-A	100.0	1.5	6.2	1.4
PS I-B	100.0	1.7	13.8	1.1
PS II-A	100.0	24.8	10.7	4.2
PS II-B	100.0	21.0	9.8	5.6
PS II-C	100.0	4.8	11.1	2.4
PS II-FP	100.0	46.5	82.6	133.3

fraction. After excision, the polypeptide composition of these green bands was analysed on non-denaturing gels (Fig. 4B). The PS I derived bands showed the dimer at 60 kDa and the small apoproteins connected with PS I. In one of the green bands, PS I-A, the 16.5 kDa component is missing. This polypeptide, presumably the psaD gene product, is apparently loosely attached to the PS I reaction centre. It has been demonstrated for spinach [43] and for *Synechococcus* [44] that the psaD gene product is an extrinsic polypeptide, that can be removed from the PS I complex.

In the PS II derived bands at least three polypeptides can be attributed to the Chl *a*/*b* binding antenna, a major band between 28 and 30 kDa and two weaker bands at 32 and 34 kDa. A fourth band at 36 kDa, visible in PS II-C, is presumably an antenna apoprotein too. The band PS II-C in addition contained polypeptides at 44 and 48 kDa, presumably CP 43 and CP 47.

The pigment composition of the green bands from the non-denaturing gel (Fig. 4A) was analysed by HPLC (Table II). The PS I derived bands both contained β-carotene. Both green bands contained trace amounts of Chl *b* and zeaxanthin, similar to the Chl *b* content of the PS I enriched fraction isolated from the sucrosegradient. This indicates that no specific Chl *b* or zeaxanthin containing component has been removed from the PS I complex during electrophoresis. The PS II derived bands all contained Chl *b*. The antenna band PS II-A contained Chl *b* in a Chl *a* to *b* ratio of approx. 4, the Chl *a* to *b* ratio of PS II-B is slightly higher. The higher Chl *a* to *b* ratio of band PS II-C confirms the presence of Chl *a*-binding complexes in addition to the antenna, as was judged from the polypeptide composition of this band. All green bands contained β-carotene as well. During electrophoresis relative more Chl *b*, β-carotene and zeaxanthin were removed from the complexes (PS II-FP). As a result of conversion of chlorophyll to pheophytin during elec-

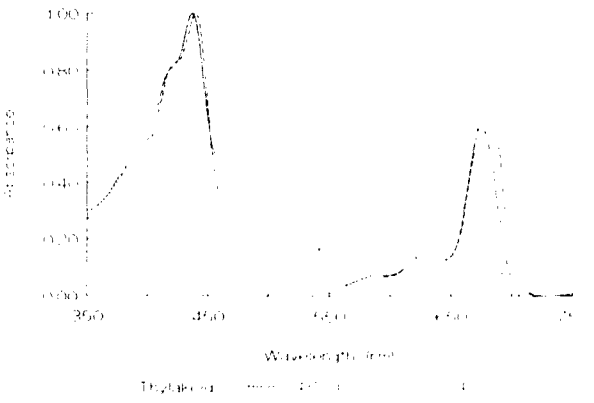

Fig. 5. Room temperature absorbance spectra of thylakoid membranes and Photosystem I and Photosystem II enriched fractions. ———, thylakoid membranes; ·····, the PS II enriched fraction; - - - - -, the PS I enriched fraction.

In fluorescence studies at 77 K, the emission spectrum of the PS II band showed a peak at 687 nm (Fig. 7A). The usually encountered fluorescence emission wavelength in chloroplasts (77 K) are at 680 (LHC II), 685 (PS II core, CP 43) and 695 nm (CP 47, PS II core) respectively [46]. *Prochlorothrix* does not display a clear separation of these peaks, regardless of the wavelength of excitation (434, 470 or 505 nm) except for a small additional shoulder around 670 nm in the 434 nm excited sample due to some uncoupled pigment (Fig. 7A). Illumination of PS I with 434 nm light demonstrated the typical peak at 718 nm associated with PS I in cyanobacteria and chloroplasts (in which the LHC I antenna additionally emits at about 735 nm [46]) and a broad emission centering at 677 nm including a minor emission at 687 nm which preferentially appears after 470 and 505 nm emission (Fig. 7B). The former peak

trophoresis and elution of the complexes, the relative β-carotene content after electrophoresis appears to be increased (compare Tables I and II). For unknown reasons, the PS II fraction is more susceptible to pheophytin formation. As judged from control experiments, the estimated Chl *a* to Chl *b* ratio is not altered as a result of pheophytin formation (not shown). Band PS II-C, which is relatively depleted in antenna polypeptides (Fig. 4B) as compared to bands PS II- A and B, contained less zeaxanthin. Because the absence of lutein in *Prochlorothrix* has been reported [2], it might be that zeaxanthin is associated with the Chl *a*/*b* binding antenna, rather than lutein as is the case in LHC II from chloroplasts [45]. Also given the ubiquitous presence in *Prochlorothrix* [41], the apparent loss of zeaxanthin during the isolation hampers further conclusions on the function of zeaxanthin in the photosynthesis of *Prochlorothrix* to be drawn.

The room temperature absorbance spectra presented in Fig. 5 demonstrate the differences between the thylakoid membranes and the PS I and PS II fractions. PS II demonstrated Chl *b* and zeaxanthin plus β-carotene as a peak at 465 nm and a shoulder at 500 nm. The chlorophyll peak in the red centered at 671 nm. The spectrum of the PS I band was red shifted. The chlorophyll peak in the red centered at 680 nm. A peak at 497 nm indicated the presence of β-carotene in the PS I band. The differences were more prominent in the 77 K absorbance spectra (Fig. 6). In the PS II fraction, the presence of Chl *b* was indicated by a small shoulder around 650 nm. The absorption maximum in the red centered at 670 nm, the spectrum of the PS I fraction showed a red shift of 10 nm, the usually attributed positions of these components in the red spectral region.

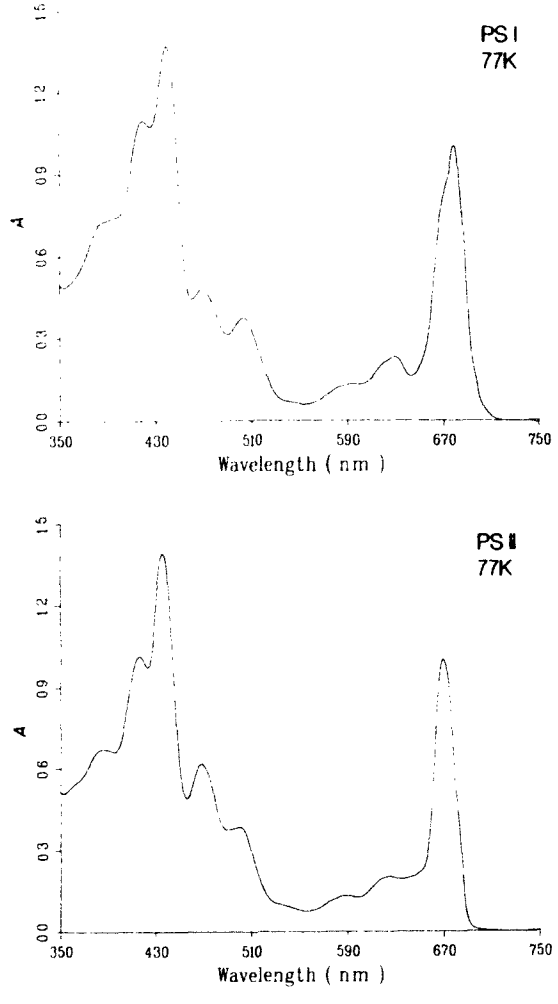

Fig. 6. 77 K absorbance spectra of the PS I and the PS II preparations.

reflects emission due to uncoupled Chl *a*, the latter peak corresponds with minor PS I emission at 685 nm as shown in a PS I core preparation of barley with 90 Chl *a* per P700 [47]. The 715 nm emission of PS I was excited by peaks at around 470 nm and 505 nm in addition to the main peak at 436 nm (Fig. 8A). The excitation spectrum of PS II (emission at 685 nm) showed peaks at 432 and 468 nm and a shoulder at 490 nm (Fig. 8B). A comparison between the excitation spectra shown in Fig. 8 clearly shows two differences.

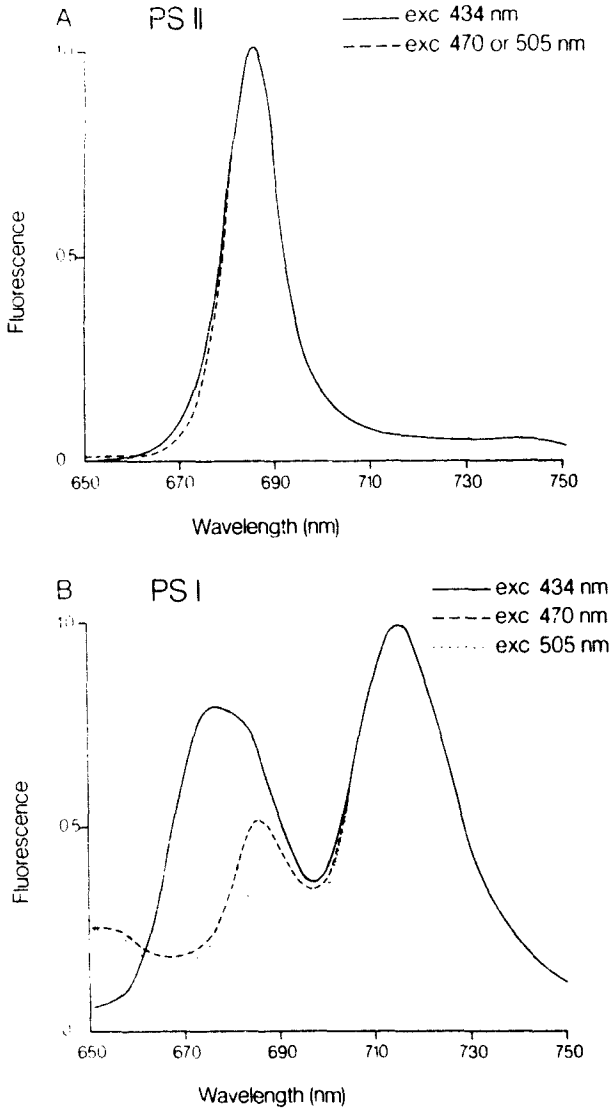

Fig. 7. Fluorescence emission spectra of the PS I and PS II enriched preparations. (A) 77 K spectrum of PS II. ——— (exc. at 434 nm), - - - - - (exc. at 470 or 505 nm). (B) 77 K spectra of PS I. ——— (exc. at 434 nm), - - - - - (exc. at 470 nm), ····· (exc. at 505 nm). All spectra are normalized. The maximal absorbance of the preparations was adjusted to 0.3.

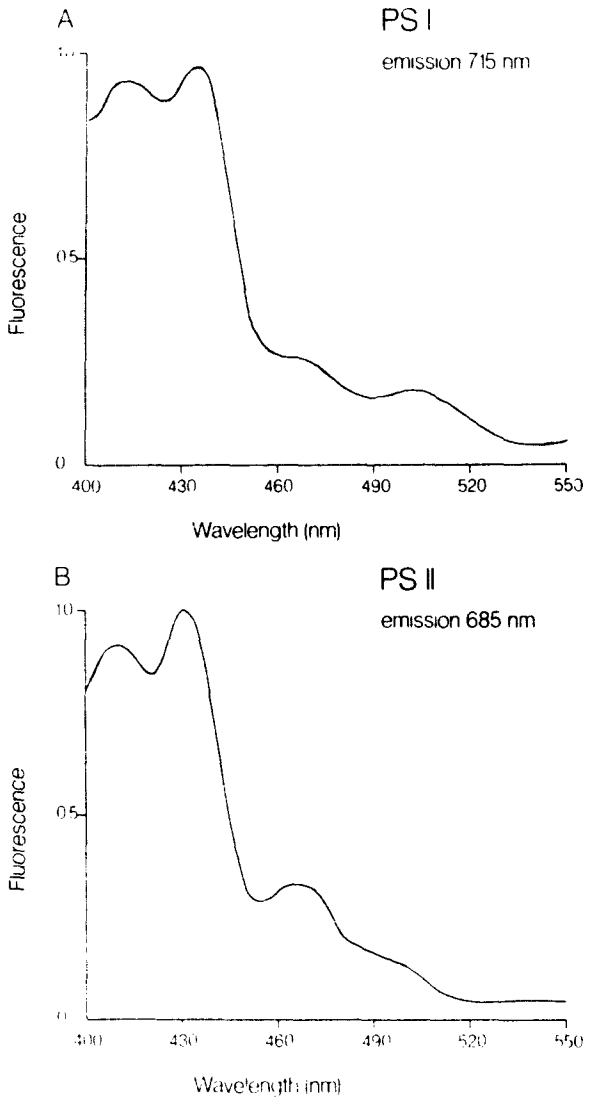

Fig. 8. Fluorescence excitation spectra of the PS II and PS I enriched fractions at 77 K. (A) PS I, emission at 715 nm; (B) PS II emission detection at 685 nm. The maximal absorbance of the preparations was adjusted to 0.3.

Firstly, in the PS I fraction the broad band centreed at 470 nm displays a shoulder at 460 nm, which is absent in the PS II fraction. Secondly, the difference between the spectra around 505 nm indicated coupling of β-carotene to PS I.

Discussion

Chl *b* has been found to be associated with the antennae derived bands on green gels of both *Prochloron* and *Prochlorothrix hollandica*; however, its

functional role in light harvesting has not yet been sufficiently revealed [18,19]. Typical differences in the polypeptide composition and structural assembly of the Chl *a/b* protein complexes of the prochlorophytes on the one hand and of chloroplasts from higher plants and green algae on the other hand have been established. For example, the major polypeptides of the Chl *a/b*-protein complexes demonstrate apparent molecular masses between 28 and 37 kDa (Refs. 18,19; this work), as opposed to the usual range of 25 to 29 kDa in chloroplasts [9–11]. The isolated complexes contain Chl *a* and *b* in a ratio estimated at about 2.4 for *Prochloron* [18] and about 4 for *Prochlorothrix* [19, this work] in contrast to the ratio of about unity encountered in LHC II in chloroplasts.

Thus far no functional role for Chl *b* in *Prochlorothrix* has been established. Bullerjahn et al. [19] demonstrated a substantial content of Chl *b* in the upper bands on green gels of maltoside solubilized thylakoid membranes from *Prochlorothrix*. These bands were demonstrated to contain PS I and some polypeptides with a molecular mass similar to the apoproteins of the antenna. The observed excitation peak in 77 K fluorescence spectra at around 470 nm present in these bands from green gels has been tentatively explained to indicate a functional role for Chl *b* in PS I activity, in contrast to this study as discussed below.

Detergents of the Zwittergent type have been applied in the isolation of PS II and PS I from *Synechococcus* [48,49]. In the present study Zwittergent 14 has been used as a suitable detergent for the isolation of the Chl-protein complexes from *Prochlorothrix* on a preparative scale. The results obtained were different from those arrived at by [19] in that the fraction containing PS I was isolated nearly devoid of the Chl *a/b*-protein complex, as judged by SDS gel electrophoresis and HPLC analysis. Thus, the peak at 470 nm in the PS I fluorescence excitation spectrum as seen in [19] and in Fig. 8A cannot be due to Chl *b*. The additional peak at 505 nm and the presence of β-carotene would rather point to involvement of β-carotene in PS I photosynthesis [45,50].

The comigration of PS II reaction centre and the Chl *a/b* -protein complex on two subsequent sucrose gradients indicates their possible association. PS II fluorescence excitation, as compared to PS I, shows a more pronounced peak at 470 nm, but only a small shoulder around 500 nm. This, on the one hand points to a minor contribution of β-carotene in PS II photosynthesis, and on the other hand demonstrates involvement of Chl *b* in PS II excitation. This contribution of Chl *b* matches with the absorbance spectrum of PS II. The Chl *a* to *b* ratio in the preparations of PS II plus adhering antennae was estimated at about 6, the Chl *a* to *b* ratio of the antennae is about 4. Assuming 50 molecules of Chl *a* per PS II core [11], the total Chl *a* plus *b* per P680 is calculated to be 175. This fits nicely with the estimation of one cyt *b*-559 low potential per each 165 molecules of chlorophyll (Table I), assuming one cytochrome *b*-559 per reaction centre [42].

A major role of Chl *b* in the excitation of PS I seems unlikely. We have found no indication for polypeptides in the molecular mass range usually corresponding to a PS I associated Chl *b* binding antenna. Interestingly, in *Prochloron* a considerable amount of Chl *b* was found to be associated with PS I after SDS-sucrose gradient centrifugation [18], despite the lack of any polypeptides other than a 70 kDa band, presumably composed of the psaA and psaB products. The low molecular mass subunits of PS I, which were demonstrated in *Prochloron* [40] and *Prochlorothrix* ([19] and this study) were not present in the study by Hiller and Larkum [18]. The harsh treatment with SDS used in [18] likely removed these components. From this, if Chl *b* were to be associated with PS I in *Prochloron* or *Prochlorothrix*, it would be bound to a 60 to 70 kDa band. Hitherto, such an association of Chl *b* with the reaction centre polypeptides of PS I has not been reported. The chlorophyll to P700 ratio of 90 in our PS I enriched fraction is in the range usually found for PS I core preparations with the psaA and psaB products as the sole chlorophyll binding polypeptides [13,14]. A coincidental overlap of the apparent molecular masses of the psaA and psaB products and a special PS I antenna polypeptide in *Prochlorothrix* cannot be excluded by our studies. However, an antenna with such high molecular weight would be unique given detailed studies on all polypeptides of various PS I preparations [14]. *Prochlorothrix* appeared to contain one or at most two Chl *b* per PS I (this study). Instead of unique binding to the PS I core, we tend to assume that the minimal contents of Chl *b* and zeaxanthin found, are entrapped in detergent micelles that copurify with PS I. In that case, the antenna organization in *Prochlorothrix* is similar to the one encountered in cyanobacteria, which lack a specific PS I antenna. Interestingly, *Prochlorothrix* adapts to different light intensities by varying the PS I to PS II ratio [51], like in cyanobacteria [52]. In conclusion, with regard to Chl *b* in *Prochlorothrix*, we propose a model in which it is associated with PS II. These observations on the photosynthetic apparatus of *Prochlorothrix* mark its interesting position in between two other groups of organisms with oxygenic photosynthesis, the Chl *b* containing eukaryotes and the cyanobacteria.

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