Chlorophyll *a/b* proteins of Photosystem I

Eric Lam, William Ortiz* and Richard Malkin*+

*Department of Biophysics and *Division of Molecular Plant Biology, University of California, Berkeley, CA 94720, USA*

Received 27 December 1983

The chlorophyll *a/b*–protein complex (LHCPI) associated with Photosystem I (PS I) has been isolated from spinach thylakoids and further fractionated into two chlorophyll-containing complexes by sucrose gradient centrifugation. The lighter fraction contains two polypeptides with relative molecular masses of 23 and 22 kDa and has been designated as LHCPIa. The denser fraction is enriched in a 20 kDa polypeptide and has been named LHCPIb. Both fractions have a chlorophyll *a/b* ratio of 3.5 ± 0.5. The absorption spectra and 77 K fluorescence emission spectra of the fractions show distinct characteristics with LHCPIb having a fluorescence maximum at 730 nm at 77 K while LHCPIa shows a maximum at 680 nm. The optical activities of the chlorophyll *a/b* complexes and the antenna-depleted PS I have been examined by circular dichroism (CD) in the near-UV and visible regions of the spectrum. All the LHCPI complexes show strong CD signals at 648, 485 and 340 nm which are absent in the antenna-depleted PS I complex.

Chlorophyll b *Pigment protein* *Photosystem I* *Low-temperature fluorescence* *Circular dichroism*

1. INTRODUCTION

Chlorophyll *b* in higher plants has been generally thought to be associated exclusively with Photosystem II (PS II) [1]. Authors in [2] first reported the presence of chlorophyll *b* in CPIa, the aggregated PS I pigment–protein complex isolated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) under mildly dissociating conditions. Evidence for specific chlorophyll *b*-containing protein(s) associated with PS I has recently been obtained in *Chlamydomonas reinhardii* [3,4]. It was demonstrated in [5] that chlorophyll *b* transfers excitation energy efficiently to the PS I reaction center from spinach and that this form of chlorophyll is most likely associated with proteins other than those of the PS II antenna complex (i.e., LHCPII). Authors in [6] were the first to describe an isolation of a chlorophyll a/*b*–protein from a resolved PS I complex. The pigment–protein complex (LHCPI) had a chlorophyll a/*b* ratio of 3.5–4.0 and showed a low-temperature fluorescence maximum at 735 nm. SDS–PAGE showed LHCPI contained 3 predominant polypeptides in the molecular mass range 20–25 kDa in addition to several lower molecular mass polypeptides. No relationship between chlorophyll *b* and specific PS I peptides has yet been reported for this complex from PS I. Evidence is presented here that indicates at least two different chlorophyll-associated protein complexes are present in LHCPI and initial characterization of these complexes is described.

2. MATERIALS AND METHODS

The isolation procedure of LHCPI was essentially that in [6] with the following modifications: instead of centrifuging at 100000 × *g* for 15 h in the final sucrose gradient step, we found that a more complete separation between the LHCPI fraction and the P700-containing fraction could be achieved by centrifugation at 360000 × *g* for 3 h in a fixed-angle rotor. The polypeptide compositions

+ To whom correspondence should be addressed

Published by Elsevier Science Publishers B V

of the two chlorophyll-containing bands obtained from this step are shown in lanes 1 and 2 of fig.1. The LHCPI fraction (lane 2) is free of the P700 pigment–protein complex (~63 kDa in our gel system) while the P700-containing fraction (lane 1) is depleted of the LHCPI polypeptides.

The LHCPI preparation has been further fractionated by the following treatment: the preparation was dialyzed against 0.05% Triton plus 50 mM potassium phosphate buffer (pH 7.2) for 2 h at 10°C. The dialyzed solution was then centrifuged overnight on a sucrose gradient (0.1–1.0 M) containing phosphate buffer and Triton, as above, at 190000 × *g*. Two distinct green zones were present with the top zone designated LHCPIa and the lower zone LHCPIb. LHCPIa sometimes migrates as a more diffuse green zone. At present, we do not know the reason for this variability.

SDS–PAGE was done with a 10–15% gradient gel as in [7]. The samples were solubilized at room temperature for 1 h in the presence of 4 M urea before application to the gel.

Chlorophyll was determined as in [8]. Absorption spectra were recorded with a Cary 219 spectrophotometer with automatic baseline correction. 77 K fluorescence emission spectra were obtained with a Perkin-Elmer spectrofluorometer (courtesy of C. Krasnow, AGS, Berkeley). CD spectra were recorded at room temperature with a home-built spectropolarimeter (courtesy of K. Sauer, UC, Berkeley).

3. RESULTS

The polypeptide compositions of LHCPIa and LHCPIb are compared in fig.1 (lanes 3 and 4, respectively). It is evident that LHCPIa is depleted in a 20 kDa polypeptide, while the LHCPIb fraction contains primarily this polypeptide. For comparison, the LHCPI preparation is also shown. It has been demonstrated that a 20 kDa polypeptide from PS I is associated with chlorophyll a and *b* [9]. Although similar amounts of chlorophyll were loaded onto each lane of the gel in fig.1, the absence of a significant amount of this 20 kDa polypeptide in LHCPIa strongly suggests that the chlorophyll in LHCPIa does not originate from a contamination by LHCPIb, but that this chlorophyll is associated with the major peptides

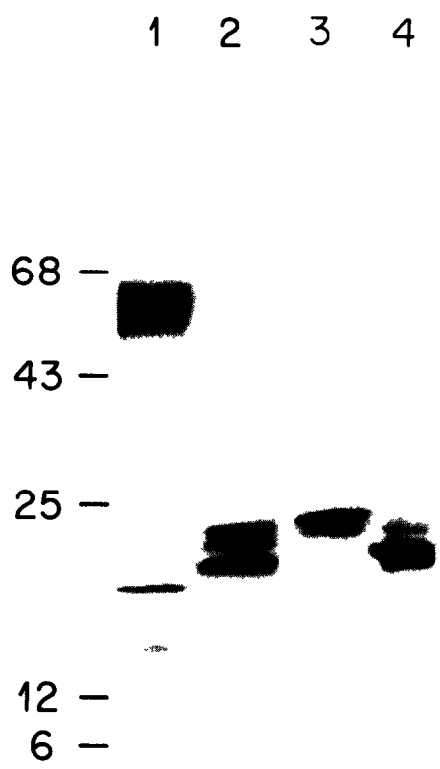

Fig.1. SDS–PAGE of PS I chlorophyll–protein complexes. Lane 1, antenna-depleted PS I complex; lane 2, LHCPI; lane 3, LHCPIa; lane 4, LHCPIb. The total amount of chlorophyll loaded onto each lane is 9, 9, 9 and 12 μg, respectively.

of molecular mass 22 and 23 kDa in this fraction.

The absorption spectra of the two PS I chlorophyll protein complexes are shown in fig.2. LHCPIb shows a broad absorption maximum at about 676 nm and a pronounced shoulder at 650 nm. A sharp maximum at 467 nm is also observed. LHCPIa, however, shows very different characteristics. A sharp absorption maximum at 670 nm is observed while only a shoulder at 465 nm is evident. Both fractions have a chlorophyll a/*b* ratio between 3.0 and 4.0 with LHCPIb being slightly lower than that of LHCPIa. The LHCPI preparation shows a spectrum qualitatively intermediate between the two separate complexes. The two chlorophyll complexes contain about equal amounts of chlorophyll based on the starting material (i.e., half of the original chlorophyll in LHCPI is recovered in each band from the gradient). However, since these

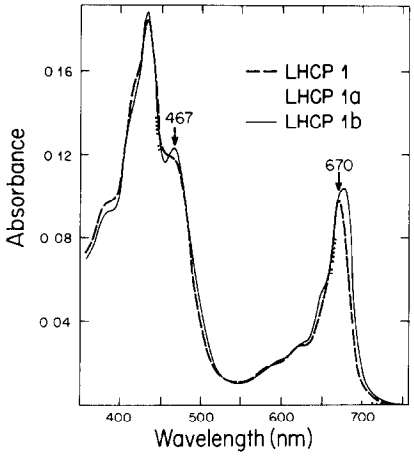

Fig.2. Absorption spectra of chlorophyll a/*b*–protein complexes of PS I. The chlorophyll–protein fractions are diluted with water and the spectra taken at room temperature.

bands migrate close to each other on the sucrose density gradient, it is difficult to recover them quantitatively without contamination from the other band. This is manifested by the presence of a small amount of the 20 kDa polypeptide in LHCPIa and the presence of some 22 and 23 kDa polypeptides in LHCPIb.

The 77 K fluorescence emission spectra of LHCPIa and LHCPIb are shown in fig.3. For comparison, the spectrum for LHCPI is also presented. In contrast to [6], our LHCPI preparation shows two emission maxima at 680 and 730 nm. Since the centrifugation step in our preparation of LHCPI is only 3 h, compared to 15 h in the original procedure, it is unlikely that increased perturbation of chlorophyll from prolonged exposure to detergents is a factor in the presence of the two emission bands in our LHCPI preparation. The only other major difference between our procedure and that in [6] is that spinach leaves are used in our preparation while pea leaves were the starting material in [6]. It is possible that with spinach some of the pigment–protein complexes might be more susceptible to alteration by detergent treatment. Alternatively, the 680 nm fluorescence peak might represent a difference in antenna pigment organization between the two plants. In any case, as fig.3 shows, LHCPIa is the species with a 77 K fluorescence emission at 680 nm while LHCPIb has a maximum at 730 nm.

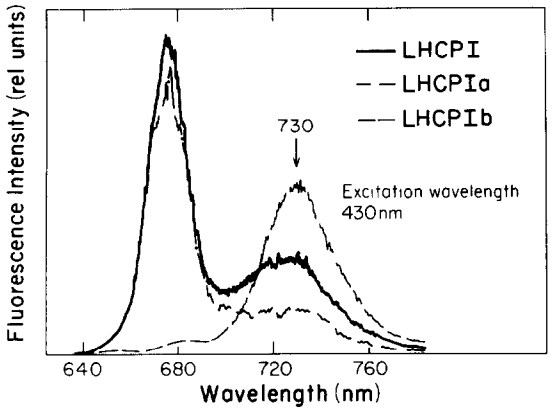

Fig.3. 77 K fluorescence spectra of chlorophyll *a*/*b*–protein complexes of PS I. The chlorophyll–proteins are diluted in 40 mM Tris–HCl (pH 7.5), and 60% glycerol to a final concentration of 10 μg Chl/ml each

The CD spectra in the visible spectral region of various PS I preparations have been reported [6]. One interesting feature of these spectra was a negative band at 647 nm observed in the native PS I complex that appeared to be indicative of the presence of chlorophyll *b*. LHCPI, however, although containing chlorophyll *b*, showed a CD spectrum almost identical to the antenna-depleted PS I complex prepared by Triton treatment (PSI-65 in the nomenclature of [6]). The only difference in the CD of these two samples was a small red shift to 685 nm of the negative peak at 680 nm in LHCPI. Because of the apparent absence of any chlorophyll *b* CD signal in LHCPI reported in [6], we have re-examined the CD properties of our PS I antenna and reaction center fractions. As shown in fig.4, the CD spectra of LHCPI and the antenna-depleted PS I are drastically different. The spectrum of the antenna-depleted PS I is similar to that for PSI-65 reported in [6], but the CD of LHCPI shows strong optical activity at 648 nm and 485 nm and both of these transitions are probably associated with chlorophyll *b* in the complex. CD spectra of LHCPIa and LHCPIb are shown in fig.5 for equal amounts of chlorophyll. These spectra are similar to that of LHCPI. It is obvious that LHCPIa has less optical activity than LHCPIb, suggesting the chlorophylls are less organized in LHCPIa, possibly due to decreased chlorophyll–chlorophyll or chlorophyll–protein interactions. An interesting feature of the CD spec-

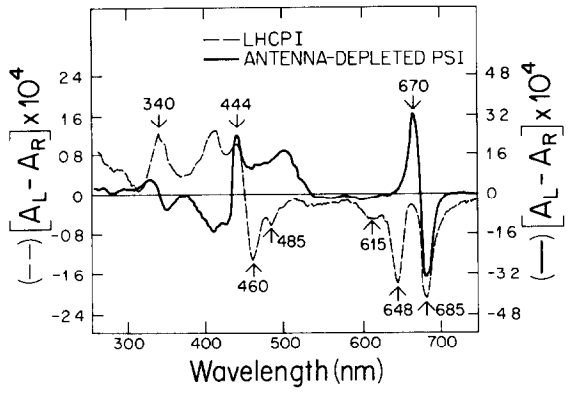

Fig.4. CD spectra of chlorophyll *a*/*b*–protein complexes of PS I. The samples are diluted in 20 mM Tricine–KOH (pH 7.6). Antenna-depleted PS I complex (13 μg Chl/ml) and LHCPI (15 μg Chl/ml).

tra common to all LHCP complexes, including LHCPII (not shown), is a strong positive peak at 340 nm. Since this peak is not observed in complexes lacking chlorophyll *b*, but containing chlorophyll *a*, it apparently arises from the former. It should also be noted that there are quantitative differences between the CD spectra of the two PS I LHCP complexes. The ratio of the 648 nm band to the 685 nm band, and the ratio of the 460 nm band to the 444 nm peak are greater in LHCPIb as compared to LHCPI and LHCPIa. This suggests that the chlorophyll molecules in LHCPIa differ in environment from those in LHCPIb.

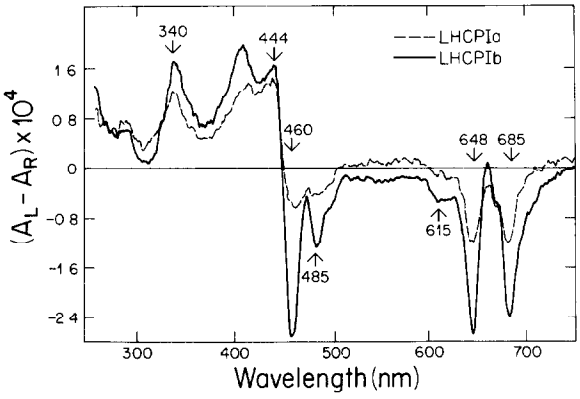

Fig 5 CD spectra of PS I chlorophyll *a*/*b*–protein complexes. Samples (each 15 μg Chl/ml) are diluted as in fig.4.

4. DISCUSSION

We have presented data here which suggest that there are at least two chlorophyll *a*/*b*-containing protein complexes in the LHCPI complex of PS I. As isolated, these complexes differ in terms of polypeptide composition, absorbance spectra, 77 K fluorescence emission spectra and CD characteristics. At present, we believe that the 680 nm fluorescence emission maximum of LHCPIa and the decrease in optical activities of its pigments are indications that the chlorophyll–protein in this complex is more sensitive to perturbations by detergent treatment as compared to that of LHCPIb. Consistent with this suggestion is the recent isolation of a chlorophyll–protein from a PS I preparation by mildly-denaturing SDS–PAGE [9]. This protein contained one polypeptide of about 20 kDa and thus represents LHCPIb of the present report. Under identical conditions, LHCPIa cannot be resolved as a separate chlorophyll-containing protein complex.

Our demonstration that the polypeptides of 20, 22 and 23 kDa are all involved in binding chlorophyll *b* as well as chlorophyll *a* correlates well with the observation in [10] that under conditions when chlorophyll *b* is not synthesized, all these polypeptides are drastically depleted. This is quite similar to the case of the polypeptides of LHCPII [11]. Thus, apparently the two LHCP complexes for PS I and PS II show a common control in that chlorophyll *b* synthesis is required for their accumulation.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the National Science Foundation. We would like to thank S. Worland and C. Krasnow for their help in obtaining CD and fluorescence spectra of samples in this work.

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