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Structural organization of chlorophyll *b* in the prochlorophyte *Prochlorothrix hollandica*

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Circular dichroism spectra of thylakoid membranes and isolated chlorophyll *a/b*-protein complexes from *Prochlorothrix hollandica* and from spinach were different. The negative deflection below 650 nm was absent in *Prochlorothrix*. Hence, the molecular organization of the chlorophyll *a/b*-protein complexes of the prochlorophyte appeared strikingly different from the one in chloroplasts of higher plants and green algae. These data are consistent with the relatively high chlorophyll *a/b* ratio in whole cells of prochlorophytes as well as in its isolated chlorophyll *a/b*-protein complexes.

The recently discovered free-living Chl *b* containing photoautotrophic prokaryote *Prochlorothrix hollandica* has been reported to morphologically resemble filamentous cyanobacteria [1]. Hitherto, the symbiont *Prochloron didemni* was the only example of an oxygenic prokaryote to bear Chl *b* [2]. Chl *b* is ubiquitous in chloroplasts of higher plants and green algae, it is incorporated in the light-harvesting complexes (LHC) [3]. Chl *b* in prochlorophytes is organized in a LHC-type complex with a distinctive molecular mass of 31–34 kDa [4,5]. These complexes are immunologically distinct from higher plant LHC II [4,5]. The LHC of higher plant chloroplasts exhibits characteristic bands in the red and the Soret region of CD spectra which have been attributed to excitonic interactions between Chl *b* molecules and which has been proposed to indicate a trimer structural organization of chlorophyll *b* within the LHC complex of chloroplasts [6–9]. The question is whether or not Chl *b* in *Prochlorothrix* is contained in a pigment-protein complex with a molecular structure similar to that of the LHC of chloroplasts. To answer this question we compared the CD spectra of chloroplasts and prochlorophyte thylakoid membranes, as well as spectra from the isolated chlorophyll protein complexes. A mono algal culture of *Prochlorothrix hollandica* [1] was grown in an airlift fermentor at 22°C in BG 11 medium [10] at a light intensity of 40 $\mu E \cdot m^{-2} \cdot s^{-1}$. The cultures were bubbled with air and harvested in the linear phase of growth by centrifugation at 4000 $\times g$ (4°C, 5 min). The pellets were resuspended and washed twice in a medium with 10 mM Tricine/NaOH (pH 7.8), 0.1 mM EDTA, 10 mM NaCl and 0.1 mM PMSF. The suspension was passed through a precooled French press cell at 70 MPa, sufficient to break over 95% of the cells. Unbroken cells and cell-wall fragments were discarded (5000 $\times g$, 10 min) and the thylakoid membranes were collected (40000 $\times g$, 1.5 h). The thylakoids were resuspended in the isolation medium plus 5% glycerol (v/v) to a Chl concentration of 60 μM. Spinach thylakoid membranes were prepared from intact chloroplasts [11]. Non-denaturing 'green' gels were run on single concentration 7.5%/0.2% acryl/bisacrylamide gels using conditions as in Ref. 5. Bands were excised, mounted to the side of a 1 cm pathlength glass cuvette and fixed in position with polyacrylamide with the same composition as used in the gels. CD spectra were recorded on an apparatus as previously described in Ref. 12 or on a modified Cary 61 instrument, absorbance spectra on an Aminco DW 2000 spectrophotometer. Chl *a*-to-*b* ratios were determined by HPLC.

The *a*-to-*b* ratio in the prochlorophyte thyhlakoids was 8.0 ± 0.3 in HPLC, whilst the ratio in the spinach thylakoid preparations was 3.1. Thus, if the Chl *b*

Abbreviations: CD, circular dichroism; Chl, chlorophyll; HPLC, high-performance liquid chromatography; LHC, light harvesting chlorophyll *a/b* complex; PMSF, phenylmethanesulfonylfluoride.

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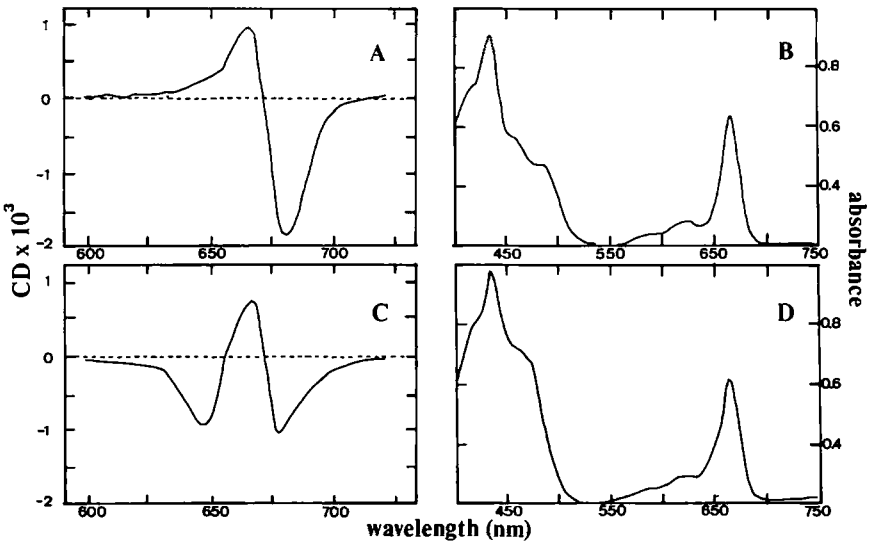

Fig. 1. CD spectra of isolated thylakoid membranes of the Prochlorothrix hollandica (A) and spinach (C). Frames B and D demonstrate the absorbance spectra of A and C, respectively. For CD spectra, the Chl concentration was 60 μM (A) and 42 μM (B), 0.25% (w/v) SDS was added to the samples about 10 min before the start of the measurement. Absorbance spectra are of extracts of isolated thylakoid membranes in 80% acetone and have been normalized at 663 nm.

containing complex(es) in the prochlorophyte would be structurally similar to the LHC type complexes of higher plants and if the low Chl *b* content would be accounted for by a low concentration of the LHC-like complex(es), we expected to see the characteristic negative deflection of the CD bands at around 650 nm area of the spectrum of the prochlorophyte albeit at relatively low amplitudes. Fig. 1 shows CD spectra of *Prochlorothrix* (A) and spinach thylakoid (C) membranes in the red area, along with the absorption spectra (B, D). The negative band below 650 nm dominates the CD spectrum of the spinach thylakoid suspension, but is totally absent in the CD spectrum of the prochlorophyte thylakoids. To exclude possible compensation of the negative peak by a positive peak of similar amplitude which could be present in the preparation of whole thylakoids of *Pro-*

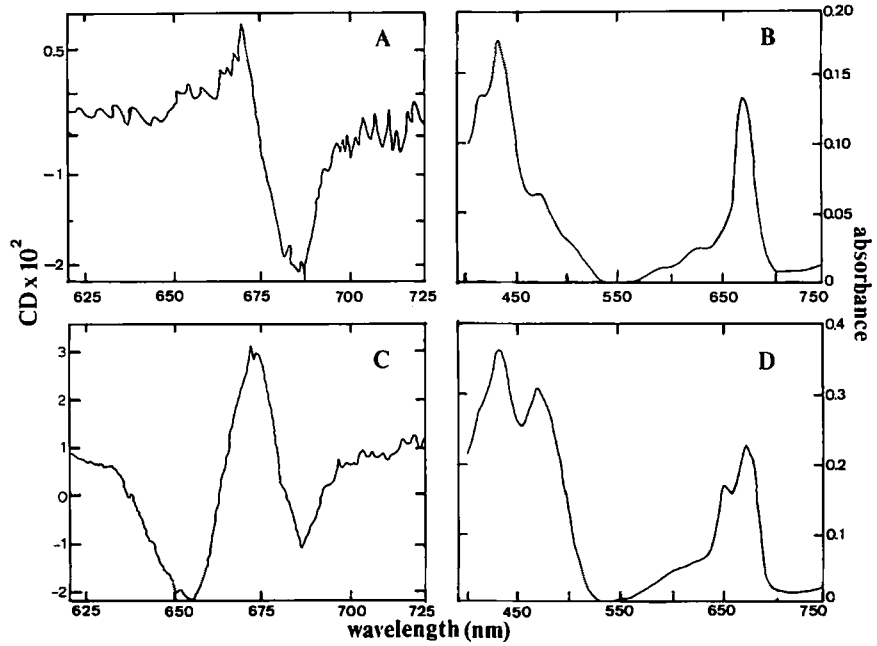

Fig. 2. CD and absorbance spectra of chlorophyll protein complexes from *Prochlorothrix* and spinach. Both CD and absorbance spectra were obtained from gel slices (cf.text). *Prochlorothrix*: A, CP3 (CD); B, CP3 (absorbance). Spinach: C, LHC II (CD); D, LHC II (absorbance). For reasons of identification, all green bands from the non-denaturing gel were excised and analysed on SDS-PAGE gels essentially as in Bullerjahn et al. [5], (not shown). Chl and protein were eluted overnight in upper reservoir gel electrophoresis buffer in the dark at 4° C and Chl was extracted with diethylether, followed by HPLC analysis on an ISCO model 2350 instrument using methanol 90%/ethylacetate 10% as eluant on a Chrompack C 18/5 μm column.

chlorothrix, we also studied the CD spectra of the isolated chlorophyll-protein complexes (Fig. 2). The negative deflection has been clearly demonstrated to be part of the LHC II band from spinach thylakoids, but such a peak could not be identified in any of the green bands resolved from *Prochlorothrix* membranes. Fig. 2D demonstrates the enrichment of chlorophyll *b* in the isolated LHC II complex from spinach (Chl *a/b* ratio 1.2). The Chl *a/b* ratio of the analogous band from *Prochlorothrix* has been determined not to decrease below 3.7 (Fig. 2B, also Ref. 5).

The experimental results presented here demonstrate that the relatively high Chl *a/b* ratio in *Prochlorothrix hollandica* is reflected in the molecular organization of the Chl *b* binding LHC-type complex(es) and thus was not solely due to a limited presence of these complexes in this prochlorophyte. The lack of those bands which have been related to a trimeric structural organization of chlorophyll *b* in the LHC of chloroplasts [6–9] in the CD spectra of *Prochlorothrix* membranes and LHC may exert effects on the molecular properties of the chlorophyll *a/b* complexes and their function in photosynthesis regulation such as thylakoid membrane stacking and light-state transitions.

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