*Biochimica et Biophysica Acta*, 591 (1980) 356—360


BBA 47862

# MACROMOLECULAR ORGANIZATION OF CHLOROPHYLL *a* IN AGGREGATED CHLOROPHYLL *a/b* PROTEIN COMPLEX AS SHOWN BY CIRCULAR DICHROISM AT ROOM AND CRYOGENIC TEMPERATURES

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(Received September 28th, 1979)

*Key words: Chlorophyll organization; Carotenoid; Circular dichroism; Light-harvesting chlorophyll-protein complex; (Chloroplast)*

## Summary

This report concerns the large circular dichroic (CD) signal of intact chloroplasts of higher plants. The CD spectra of chloroplasts are compared with the aggregated form of the light-harvesting chlorophyll *a/b* complex at 25°C and −250°C. The light-harvesting chlorophyll aggregate has a CD of magnitude equal to or greater than chloroplasts, but of opposite sign, and it is not related to the CD of the unaggregated form, and hence its arrangement is an artefact compared to the arrangement in the chloroplast. We suggest that this preparation, which has pseudo-lamellar structure, is a clear example of a large CD signal being generated by macromolecular association. The asymmetry of organization in the chloroplast has an opposite sense to that of the aggregate, but affects only chlorophyll *a*, not chlorophyll *b*.

## Introduction

Whole chloroplasts have been shown previously to have a very prominent CD signal at 688—676 nm, and it has been suggested that it is due to architectural features such as thylakoid stacking [1,2]. Disrupted chloroplasts do not show the big signal but do show a less intense and different CD spectrum in which contributions of known chlorophyll protein complexes can be seen [3]. CD is therefore established as a valuable non-destructive means of investigating the organization of chlorophyll in chloroplasts. Recently, low

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temperatures have been shown to assist in the resolution of chloroplast CD spectra [4,5].

In this paper we show that big CD signals can be created, of closely similar wavelength and magnitude, by forming an aggregate of the chlorophyll-protein known as chlorophyll *a/b* light harvesting complex [6]. This aggegate can be seen in the electron microscope to have an extensive organization somewhat resembling chloroplast lamellae.

The big signals from both chloroplasts and the aggregate indicate asymmetric organizations of chlorophyll *a*; we show that the chlorophyll *b* contribution can only be clearly observed when low temperatures are employed.

## Materials and Methods

Chloroplasts were isolated from New Zealand spinach (*Tetragonia tetragonioides* Pall.), from the mesophyll of 3 weeks-old seedlings of maize (*Zea mays* L. cv. KSC 360) and from the leaves of a chlorophyll *b*-less barley (*Hordeum vulgare* cv. Donaria, strain 3613, Gatersleben). The isolation medium contained 0.35 M sucrose, 0.05 M potassium phosphate and 0.01 M KCl, pH 7.2 [7]. Chloroplasts were collected at $1500 \times g$ for 10 min, and were suspended in the isolation medium.

Isolation and purification of the light-harvesting chlorophyll aggregate was carried out by the method of Burke et al. [6]. Spinach leaves were used and the pellets obtained were resuspended in sucrose phosphate buffer [7] or subjected to fixation, dehydration, staining and sectioning by the conventional methods of electron microscopy [8]. Chlorophyll determinations were carried out in ethyl ether solutions spectrophotometrically by the multiwavelength method [9].

The measurement of CD was carried out in a spectropolarimeter similar to that described by Breeze and Ke [10]. The 50 kHz modulated component of the photomultiplier signal was selected by an ORTEX-Brookdeal 9501 lock-in amplifier and plotted on an *X-Y* recorder, or logged in a Solartron-Teletype digitizing unit. The sample holder used in room temperature measurements allowed the chloroplast suspension to be placed 3 mm away from the end-window of the photomultiplier EMI 9558 A.

Low temperature measurements were performed in a Displex closed-cycle refrigeration system (Model DE-202) equipped with a sample holder of 1 mm thickness. The temperature was measured in the sample by means of a gold-chromel thermocouple. In order to obtain transparent samples, chloroplast suspensions were mixed with glycerol to a final concentration of 67%. The samples were precooled with liquid $N_2$.

## Results and Discussion

Figs. 1a—c show the absorption and CD spectra of normal granal chloroplasts of maize at room temperature. Preparations from spinach and normal barley were essentially similar and have been previously reported [1,11]. Fig. 1d shows the CD at —250°C of the same preparation in which there is the same big signal. There is a shoulder at about 710 nm, a positive peak probably with a

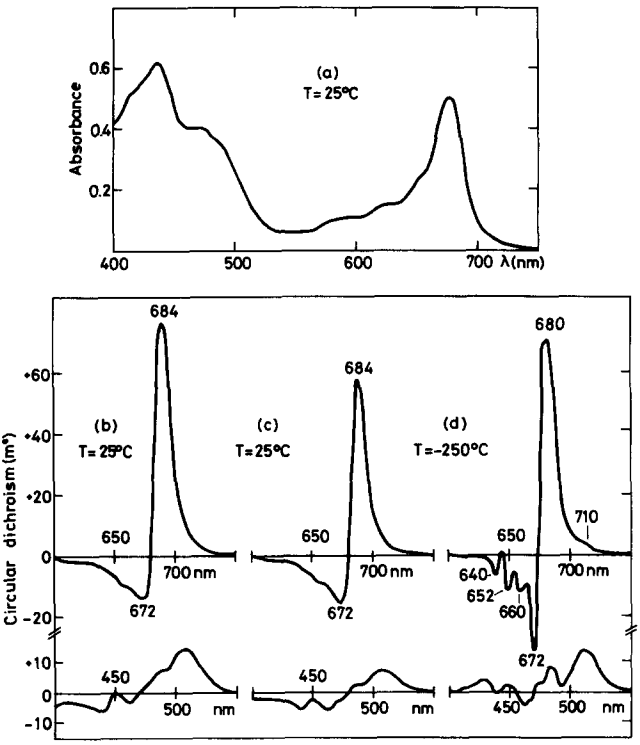


Fig. 1. Absorption and CD spectra of granal chloroplasts of maize: (a) absorption spectrum at 25°C in buffer, (b) CD at 25°C in buffer, (c) CD at 25°C in buffer containing 67% glycerol, (d) CD at −250°C in buffer containing 67% glycerol. Optical path lengths 1 mm, chlorophyll content 100 μg/ml; the suspending buffer contained 0.35 M sucrose, 0.05 M potassium phosphate, 0.01 M KCl (pH 7.2).

composite character at 680 nm, a negative peak at 672 nm and some fine structure between 662 and 640 nm. At 510 nm a positive band was observed and attributed to carotenoids. In the chlorophyll *b*-less mutant the same form of big signal was present but with a smaller magnitude [11]. The fine structure was however absent, and low temperatures did not resolve any further detail (Figs. 2a,b) which indicate that the fine structure is related to chlorophyll *b*. Our preparation of the aggregated light-harvesting chlorophyll *a/b* complex matched the previous description, including the electron micrographic appearance. The absorption and CD spectra at 25°C are shown in Figs. 3a,b. There is a big signal with peaks at 667 nm (+) and 684 nm (−) and a negative carotenoid band at 510 nm. At low temperature (Fig. 3d) on the 672 nm (+) band there is a shoulder at 662 nm and fine structure on the short wavelength side, some of which corresponds to that of normal chloroplasts. The band at 680 nm is very narrow, and is probably not of composite character.

When the aggregated light-harvesting chlorophyll *a/b* complex was dissociated by treating it with 0.25% sodium dodecyl sulphate the big signal diminished sharply (Fig. 3c, dotted line) and there appeared the positive and negative extrema at 670 and 650 nm, characteristic of the CP2 complex measured by Scott and Gregory [3]. The peak-to-peak magnitude of this CD spectrum agreed with these authors' data, within 20%. Thus the big signal of

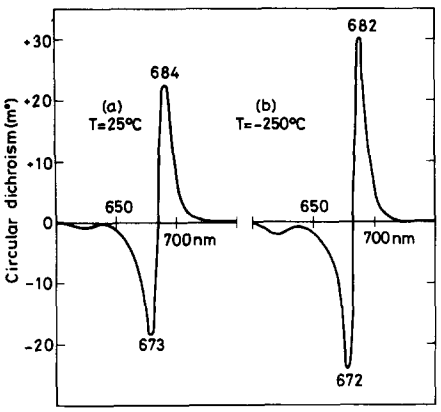


Fig. 2. CD spectra of chloroplasts of chlorophyll *b*-less barley measured at 25° C and —250° C respectively. (a) CD at 25° C, (b) CD at —250° C. Suspensions were prepared as Fig. 1c and d.

the light-harvesting chlorophyll aggregate can be considered separately from the CD of the monomeric form, and must arise from conditions specific to the aggregate.

We note the following points. (1) The peak-to-peak magnitude of the CD of

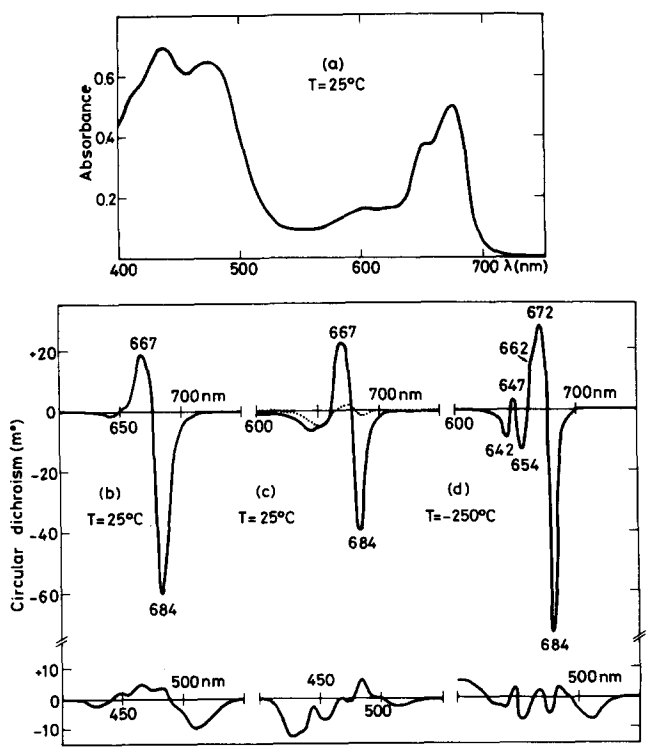


Fig. 3. Absorption and CD spectra of the aggregated light-harvesting chlorophyll protein complex: (a) absorption spectrum at 25° C in buffer, (b) CD at 25° C in buffer, (c) CD at 25° C in buffer containing 67% glycerol before (full line) and after disaggregation (dotted line), (d) CD at —250° C in buffer containing 67% glycerol. Other conditions as in Fig. 1.

the light-harvesting chlorophyll aggregate (200 to 250 millidegrees/absorption unit at the red peak) is about as much as that of chloroplasts. (2) These signals are of opposite signs, both in the red region of the chlorophyll and in the carotenoid band at 510 nm. Therefore the light-harvesting chlorophyll aggregate does not contribute to the CD of the intact chloroplast and is to be regarded as an analogue or artefact. (3) It is difficult to see the CD contribution of chlorophyll *b* at 25°C, either in the light-harvesting chlorophyll aggregate or in normal chloroplasts, while the dissociated light-harvesting chlorophyll *a/b* complex, and fragmented chloroplasts [1,2] have the clear CD signal of chlorophyll *b* at 650 nm.

We suggest that in both chloroplast grana and aggregated light-harvesting chlorophyll *a/b* complex there is large-scale ordering of chlorophyll *a*, of opposite helical senses, that causes the intense CD signals. The reason that the CD of the chlorophyll *b* is not enhanced in the same way as in chlorophyll *a* is possibly due to the arrangement of the two chlorophylls being different in degree and direction [12,13]. If the protein molecules were fixed in respect of one axis, corresponding to the average chlorophyll *a* absorption direction, we could expect to see a greater effect of the arrangement on chlorophyll *a* than on chlorophyll *b*. If the degree to which the protein molecules could rotate around that axis was significantly less at −250°C than at 25°C, there would be a greater ordering of chlorophyll *b*, and hence a greater CD signal, at the lower temperature. On this basis it appears from the data that some carotenoids share the organization of chlorophyll *a* rather than chlorophyll *b*. However a more quantitative analysis would require a crystallographic solution of the structure of the light-harvesting chlorophyll *a/b* complex.

## Acknowledgements

This work was performed under contract with the Hungarian Technical Development Committee, and in Manchester under Grant B/SR/8480.1 of the Science Research Council. RPFG is grateful to the University of Manchester for a travel grant. The authors wish to thank G. Eppeldauer from the Research Institute of Technical Physics, Budapest, for his valuable contribution in the construction of the spectropolarimeter.

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