Biochimica et Biophysica Acta, 501 (1978) 545—551

BBA Report

BBA 41303

ABSORPTION AND CIRCULAR DICHROISM SPECTRA OF CHLOROPLAST MEMBRANE FRAGMENTS FROM SPINACH, BARLEY AND A BARLEY MUTANT AT ROOM TEMPERATURE AND LIQUID NITROGEN TEMPERATURE

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(Received September 23rd, 1977)

Summary

The absorption and CD spectra of chloroplast fragments from spinach, barley and a barley mutant (chlorophyll *b*-minus) were studied at temperatures of 23° C and −196° C. The CD spectrum of wild type barley and spinach at −196° C showed troughs at 640, 653, 676 and 695 nm and a maximum at 667 nm. The CD spectrum of the barley mutant at −196° C consisted of a large trough at 684 nm, a small trough at 695 nm and a positive peak at 670 nm. A new feature observed at −196° C but not at 23° C is the trough at 640 nm. This 640 nm CD signal is missing in the CD spectrum of the barley mutant. It is attributable to the light-harvesting chlorophyll *a/b* protein which appears to be missing in the mutant. Another new feature, the trough at 695 nm, was observed in the CD spectra of spinach, barley and the barley mutant at −196° C. The 695 nm trough appears to be sensitive to detergents and it may be due to a labile chlorophyll *a*·protein complex. Possible interpretations of these data are discussed.

Polyacrylamide gel electrophoresis of washed, SDBS-solubilized chloroplast lamellae reveals the presence of three principal chlorophyll-containing bands [1,2]. These are the *P*-700·chlorophyll *a* protein (40 chlorophyll *a*: 1 *P*-700; no pheophytin *a*, no chlorophyll *b*; molecular weight 110 000), the light-harvesting chlorophyll *a/b* protein (chlorophyll *a*: chlorophyll *b*, 1 : 1; molecular weight 35 000) and a free chlorophyll·detergent complex [3]. A mutant of barley lacking chlorophyll *b* was described by Highkin [4]. The apparent complete absence of chlorophyll *b* [5] is accompanied by a loss of

Abbreviations: SDBS, sodium dodecyl benzenesulfonate; CD, circular dichroism.

the light-harvesting chlorophyll *a/b* protein from the membranes of the mutant [6—8]. The molecular organization of the membranes of the mutant appears to be different from that of wild type barley [9]. It is photosynthetically active, yet, owing to a reduced concentration of chlorophyll, the intensity required to saturate its photochemical reactions is higher than that needed for the wild type [5,10]. In an effort to elucidate further the molecular organization of chlorophyll molecules in the membrane we investigated the optical properties of photosynthetically active chloroplast membranes of spinach, barley and of the mutant barley. The absorption and CD spectra described here corroborate previous observations made at 23° C [11,12] and extend them to liquid nitrogen temperature. At low temperature the spectra are generally better resolved due to the sharpening of the absorption bands.

Spinach (*Spinacea oleracea*), barley (*Hordeum vulgaris*) and barley mutant lacking chlorophyll *b* (*H. vulgaris*, strain Chlorina) were grown outdoors in vermiculite. Chloroplast fragments obtained by sonication were isolated in 0.02 M Tris buffer (pH 8.0) by the procedure of Park and Pon [13]. Because the samples were almost clear, scattering contributions to the CD were negligible [14]. Absorption and CD spectra were recorded with a Cary Model 14 and a Jasco J-20 spectrophotometer, respectively. The CD spectra are given in terms of $(A_L - A_R)$, the difference in the absorbance for left and right circularly polarized light, respectively.

The absorption and CD spectra of normal spinach at room temperature and at −196° C are shown in Fig. 1. The absorption maximum at room temperature is located at 678 nm. At −196° C, it is blue shifted to 673 nm. The absorption peak at low temperature is narrower, and the shoulder owing to chlorophyll *b* at 650 nm is more pronounced. The CD spectrum of normal spinach chloroplast fragments at 23° C (Fig. 1b) closely resembles that of normal barley [12], and is characterized by minima at 654 and 686 nm and a maximum at 669 nm, which is in good agreement with previously reported spectra [14,15]. In the CD spectrum at −196° C, the signal is resolved into additional components, and the band width narrows appreciably. Troughs at 640, 653, 676 and 695 nm and a maximum at 667 nm are seen. The corresponding features are shifted slightly to a shorter wavelength at the low temperature.

In the barley mutant, the absorption maximum at 678 nm at 23° C (Fig. 2a) is blue shifted to 672 nm at −196° C (Fig. 2c). There is no chlorophyll *b* shoulder at 650 nm in the barley mutant spectrum. Its CD consists of a large trough at 686 nm and a smaller positive peak at 670 nm. These results are in good agreement with the room temperature CD spectrum reported by Dratz et al. [12]. The CD pattern of the barley mutant at −196° C is similar to that at 23° C except for a small minimum (shoulder) at 695 nm (Fig. 2). The general character of the CD spectrum of the mutant is similar to that of the isolated *P*-700·chlorophyll *a* protein [15,16], with the exception that in the isolated protein, the negative (690 nm) and positive (677 nm) CD components are of almost equal magnitude [16] while in the mutant (Fig. 2) the negative component is about 2.5 times larger than the positive one. A small trough at 653 nm is seen in the isolated protein [16] but not in the mutant.

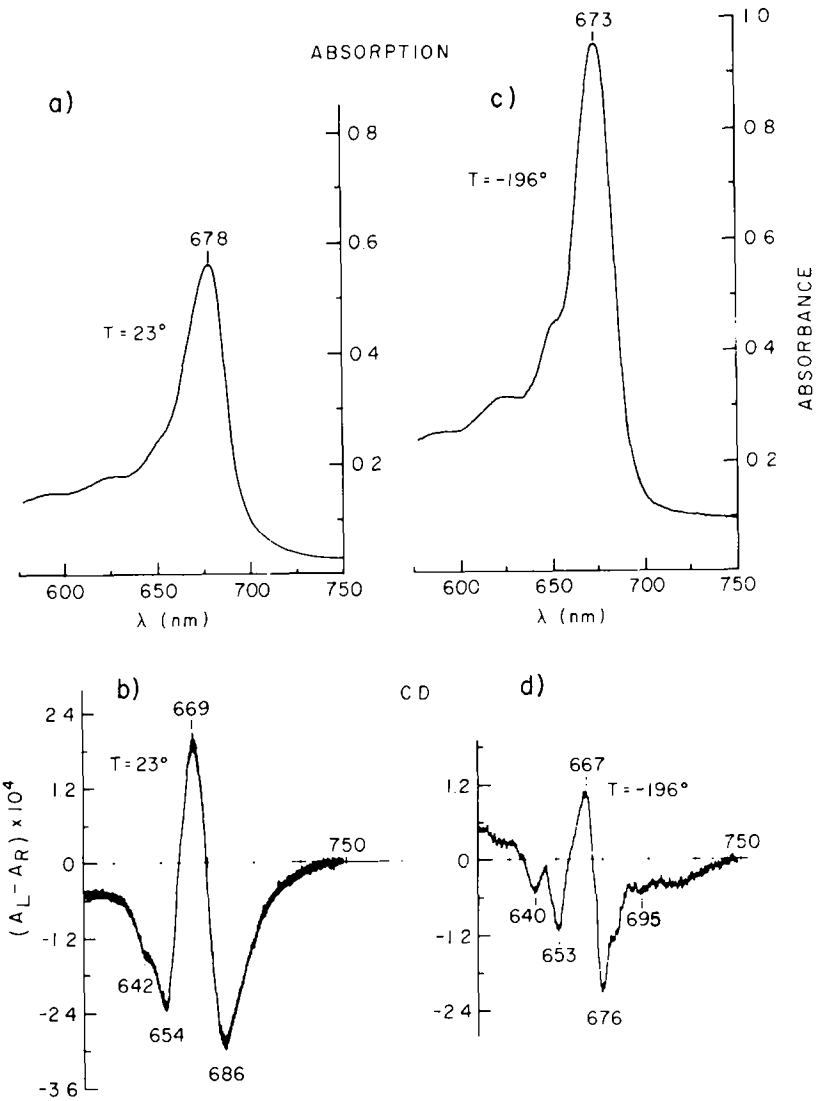

Fig. 1. Absorption and CD spectra of chloroplast fragments of spinach at 23°C (optical path length, 1.00 cm) and a suspension in 60% glycerol at −196°C (optical path length, 0.20 cm). (a) Absorption at 23°C. (b) CD at 23°C. (c) Absorption at −196°C. (d) CD at −196°C. Chloroplast fragments were prepared essentially by the procedure of Park and Pon [13]. The buffer used in this study was 0.02 M Tris (pH 8.0), containing 0.5 M sucrose and 0.01 M NaCl. Sonication of the chloroplast suspension was followed by isolation of a fraction sedimenting between 14 000 × *g* (30 min), resuspension in 0.02 M Tris buffer (pH 8.0), and clarification at 14 000 × *g* (10 min).

The CD spectrum of normal barley at −196°C is illustrated in Fig. 3. The peak positions for normal barley are identical to those for spinach (Fig. 1d) except for the trough at 676 nm (spinach) that appears at 680 nm (barley).

Based on these data it appears that there are three main differences between the CD spectra of normal spinach and barley compared to the mutant.

(1) Normal spinach and barley possess a somewhat enhanced 667 nm peak relative to the 676—680 nm trough compared with the mutant.

(2) A trough at 653 nm is present at both 23° C and —196° C temperature (Figs. 1b, d, 3) in the CD spectrum of the normal species but not in the mutant.

(3) A new feature observed at liquid nitrogen temperature for normal barley and spinach is a negative component at 640 nm (Figs. 1d, 3). This component is seen as a shoulder in the room temperature CD spectra of normal spinach (Fig. 1b) and it can be seen as a shoulder in the barley CD spectra

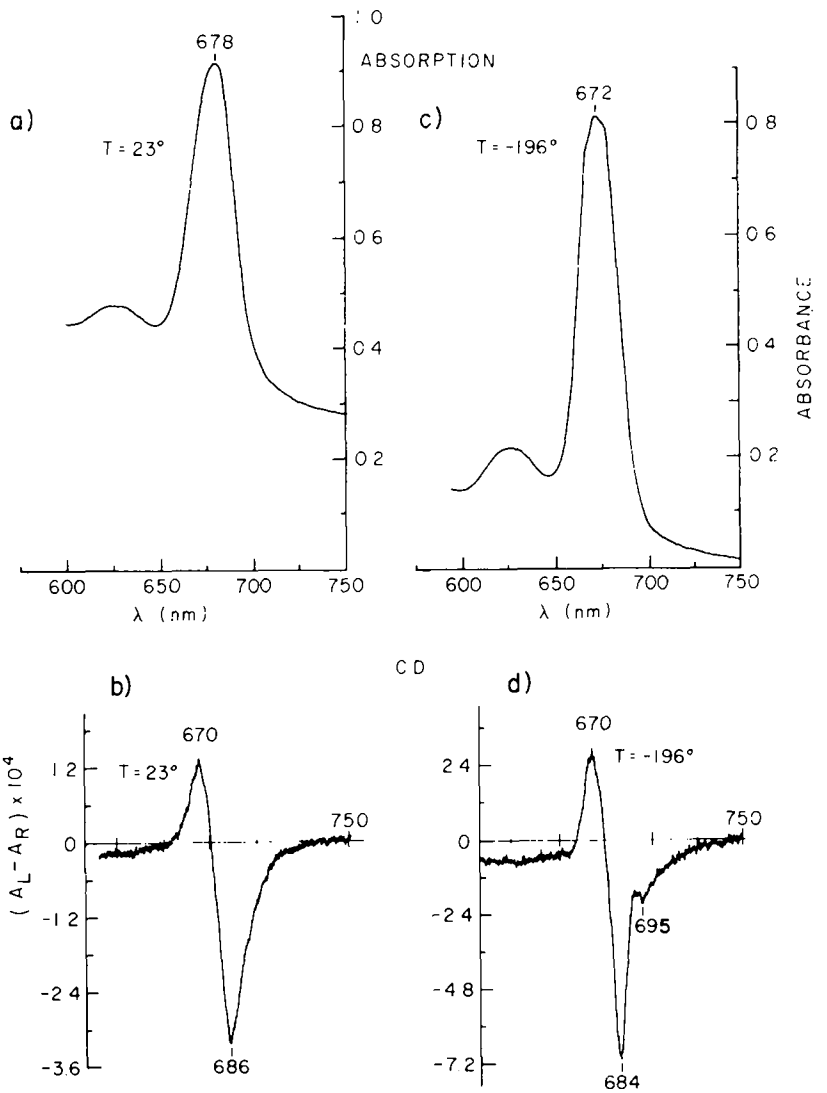

Fig. 2. Absorption and CD spectra of chloroplast fragments of a barley mutant which lacks chlorophyll *b* at 23° C (optical path length, 1.00 cm) and a suspension in 60% glycerol at —196° C (optical path length 0.20 cm). (a) Absorption at 23° C. (b) CD at 23° C. (c) Absorption at —196° C. (d) CD at —196° C. Sample preparation as described in Fig. 1.

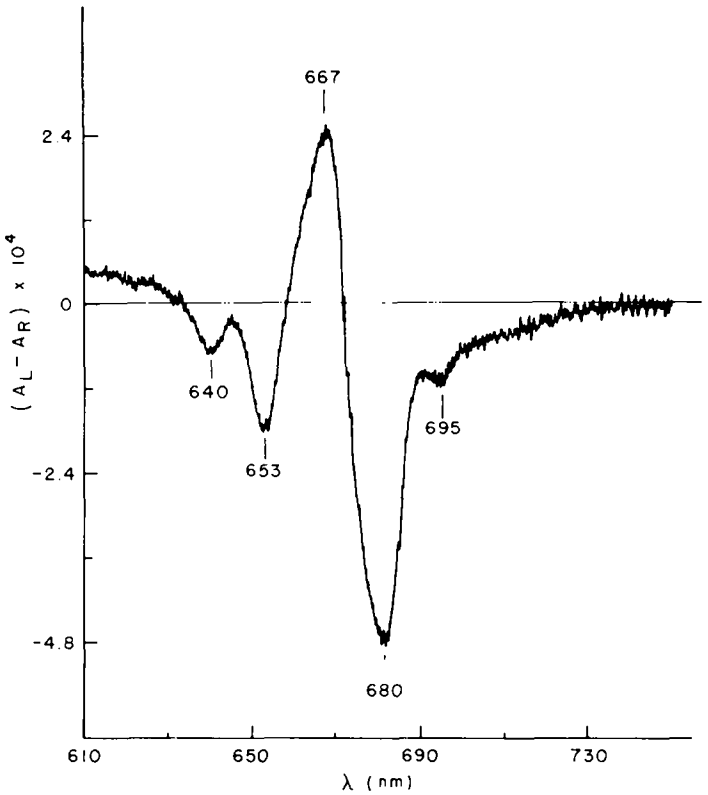

Fig. 3. The CD spectrum of chloroplast fragments of wild type barley at −196°C in 60% glycerol. Optical path length, 0.20 cm.

reported by others [12,15]. However, this 640 nm trough is absent from the CD spectra of barley mutant both at 23°C and at −196°C temperature (Figs. 2b, d).

The main structural difference between normal and mutant barley is the absence of the light-harvesting chlorophyll *a/b* protein from the mutant [6—8]. The latter protein contains all the chlorophyll *b* in the chloroplast together with an equimolar quantity of chlorophyll *a*; it amounts to about 50% of the total chlorophyll in higher plants [17]. Therefore, we suggest that the three CD components at 640, 653 and 667 nm are due to chlorophyll interactions in the light-harvesting chlorophyll *a/b* protein. This protein was isolated and its CD spectrum at 23°C was reported [16]. It showed a positive peak at 670 nm, a large negative peak at 650 nm and a small negative peak at 683 nm. The broad trough at 650 nm observed in those studies [16] could easily contain a negative component at 640 nm, as we show here (Fig. 1); the troughs at 640 and 653 nm are resolved well only at low temperature.

A new feature in the CD spectra at liquid nitrogen temperature of normal spinach, barley and barley mutant chloroplast fragments is a trough at 695 nm (Figs. 1d, 2d, 3). This shoulder is not resolved in the CD spectra at room temperature. A similar component was previously observed in the room temperature CD spectrum of *Euglena* fragments [18]. A substantial part of

the 695 nm CD signal was removed upon the addition of SDBS to *Euglena* chloroplast fragments [18]. It is plausible that this 695 nm component is due to a labile chlorophyll *a* protein complex which dissociates during SDBS extraction of chloroplasts. The existence of such a labile chlorophyll *a*·protein complex has been suggested [3], and it was proposed to be a third chlorophyll·protein complex serving as a light-harvesting component of Photosystem I [3].

Based on our studies discussed above and the results reported by others [12,15,16,18], we suggest that the multi-component CD of normal spinach or barley can be treated as a superposition of three contributions:

(1) A double CD signal (negative at 686 nm, positive at 670 nm) at long wavelengths attributable to the chlorophyll *a* of the antenna of Photosystem I, as seen in the detergent preparations [16] or the *b*-minus mutant.

(2) A negative peak at 695 nm due perhaps to a labile chlorophyll *a* protein.

(3) A triple CD signal (positive at 667 nm, negative at 653 nm and negative at 640 nm) at shorter wavelengths due to chlorophyll *a* and chlorophyll *b* of the antenna of Photosystem II. The negative peak at 683 nm seen in the CD spectrum of isolated light-harvesting chlorophyll *a/b* protein may also be included in the above signal but was not resolved in our studies.

A similar suggestion was made by Scott and Gregory [16] who studied the CD spectrum of a SDBS extract of normal spinach chloroplast fragments and found it to fit closely to the combined CD of the SDBS isolated complexes of *P*-700·chlorophyll *a* protein, light-harvesting chlorophyll *a/b* protein and the free pigment [16]. However, the last CD spectrum did not fit well with the CD spectrum of chloroplast fragments untreated with SDBS [18]. This discrepancy can be explained by our observation that the CD spectra of normal and mutant barley chloroplast fragments probably contain contributions of labile chlorophyll protein which dissociate upon SDBS extraction.

The origin of the double CD signal of the barley mutant as well as that of the *P*-700·chlorophyll *a* protein were explained in terms of an exciton interaction in a dimer of chlorophyll *a* molecules [12,16]. The enhanced CD signals, band multiplicity and red shift in the absorption maxima observed here for normal spinach and barley chloroplast fragments suggest to us the existence of exciton interaction among chlorophyll molecules also in the antenna of Photosystem II. Another possibility to explain the origin of the CD signals of normal barley and spinach is chlorophyll-protein or chlorophyll-lipid interactions [12]. However, the current view from model system work is that chlorophyll-protein interactions per se do not produce spectral changes characteristic of in vivo chlorophyll; rather, the chlorophyll-chlorophyll interactions are the important ones [19]. The exact analysis of these CD spectra may turn out to be more complicated than previously thought, when one takes into account the environmental shifts of the transition energies which are effective in localizing the excitation on the donor or acceptor chlorophyll molecules resulting from exciton interaction [20].

This work was supported by the Division of Biomedical and Environmental Research of the U.S. Energy Research and Development Administration.

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