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ORIENTATION OF PIGMENTS IN THE THYLAKOID MEMBRANE AND IN THE ISOLATED CHLOROPHYLL-PROTEIN COMPLEXES OF HIGHER PLANTS

IV. THE 100 K LINEAR DICHROISM SPECTRA OF THYLAKOIDS FROM WILD-TYPE AND CHLOROPHYLL *b*-LESS BARLEY THYLAKOIDS

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Using a polyacrylamide gel squeezing technique, linear dichroism spectra of thylakoids from wild-type and chlorophyll-*b* less barley have been obtained at 100 K. The calculated difference linear dichroism spectra, based on normalization at 690–695 nm, are identical to those of the light-harvesting complex (LHC) isolated by Triton solubilization. This observation is in agreement with previous conclusions (Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta 682, 339–344) regarding: (i) scattering artifacts are absent in linear dichroism spectra determined using polyacrylamide gels, (ii) the in vivo orientation of LHC pigments is maintained in the isolated complex and (iii) the largest dimension(s) of the isolated LHC is (are), in vivo, parallel to the plane of the photosynthetic membrane.

The technique of linear dichroism (LD), which measures the differential absorption of plane-polarized light, is used to obtain information on the specific orientation of a given chromophore in an anisotropic sample [1]. In the photosynthetic membrane of green plants these chromophores, mainly chlorophylls, are noncovalently attached to proteins in specialized pigment-protein complexes [2]. Previous papers in this series [3–5] have analyzed the orientation of these pigments both in isolated complexes and in the native membrane. We have shown that the LD spectrum of pea thylakoids can be resolved into the spectra of its three major complexes [4,5] and that specific chlorophyll orientations are maintained in these complexes [5].

The existence of a *Chlorina* mutant of barley which does not contain Chl *b* [6,7] has prompted us to analyze some spectral features we previously ascribed to Chl *b* in the LD spectra of pea membranes [4,5]. This mutant, because of the lack of Chl *b*, does not contain functional light-harvesting complex (LHC), which in normal membranes contains most of the Chl *b* [6,7]. In the present paper we demonstrate that the absence of this major pigment-protein complex is also revealed by analysis of the low-temperature LD spectra.

Stroma-free thylakoids were isolated from both the wild-type and mutant barley in 10 mM Tricine-NaOH (pH 7.8), 0.1 M sorbitol and 5 mM $MgCl_2$ as described by Burke et al. [7]. The thylakoids were then oriented for LD measure-

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Abbreviations: LHC, light-harvesting complex; Chl, chlorophyll; Tricine, *N*-tris(hydroxymethyl)methylglycine.

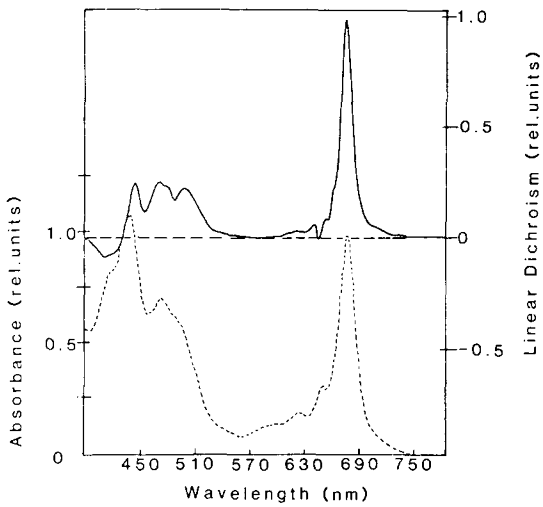

Fig. 1. The 100 K absorption (------) and LD (———) spectra of wild-type barley thylakoids. Both spectra are normalized to arbitrary values of 1.0. Data determined as described in Ref. 4. Dichroism ratio at the red maximum is 1.2–1.4.

ment using the polyacrylamide gel squeezing technique [3.8]. LD and absorption spectra of the samples were taken at 100 K as described by Haworth et al. [4]. The determination of chlorophyll concentrations and Chl *a*/*b* ratios was by the method of Arnon [9].

Fig. 1 shows the 100 K absorption (- - -) and LD spectra (———) of wild-type barley thylakoids. The spectra have been normalized at the red maximum to an arbitrary value of 1.0 to facilitate comparison of the different samples. Both the absorption and LD spectra of Fig. 1 appear to be in very close agreement with the data presented for pea thylakoids (Fig. 3 of Ref. 4); the Chl *a*/*b* ratio of these membranes is also 3 : 1. In Fig. 2 we present the corresponding data for thylakoids isolated from the Chl *b*-less mutant. There are several obvious spectral differences between the two membranes in both absorption and LD. In the absorption spectra the major differences are the absence of the peak at 650 nm in the mutant and some variations in the absorption in the Soret region. The nondichroic Chl *a* absorption at 673 ± 1 nm [4] can be observed in both wild-type and mutant thylakoids. The LD spectra of these two types of membranes show some distinct differences. Firstly, the long-wavelength maximum, associated with Chl *a*, is red shifted (by approx. 5 nm) in the thylakoids of the mutant; secondly, the group of LD signals we termed the 'fingerprint' for LHC [4] is absent in Fig. 2. At shorter wavelengths we can see there is no signal at 482 ± 1 nm in the Chl *b*-less mutant, again a signal previously assigned to Chl *b* [4]. Demeter et al. [10] reported differences in the room-temperature LD spectra of wild-type and Chl *b*-less thylakoids at 650 and 483 nm, which are consistent with the data reported here. However, both the presence of larger scattering artifacts in their spectra than in those presented here [3] and the lower resolution inherent to

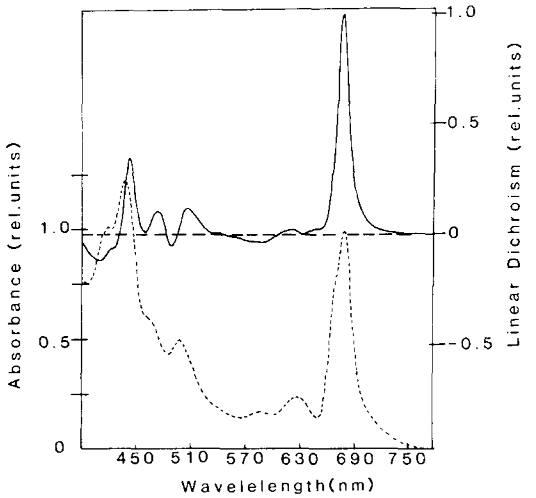

Fig. 2. The 100 K absorption (------) and LD (———) spectra of thylakoids from the Chl *b*-less mutant of barley. Dichroism ratio at the red maximum is 1.1–1.2.

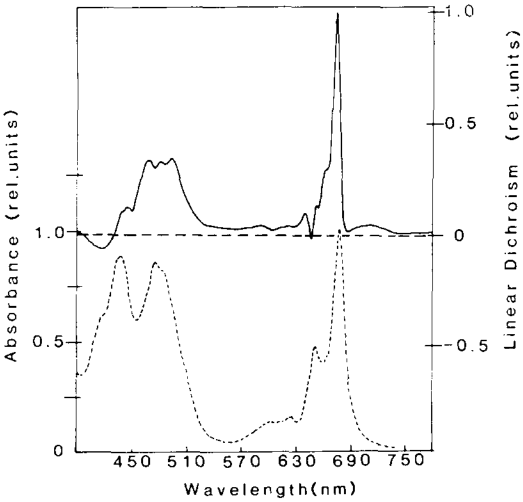

Fig. 3. Computer-determined difference spectra obtained by subtracting Fig. 2 from Fig. 1. The spectra were normalized in the 690–700 nm region. Absorption (------), LD (———).

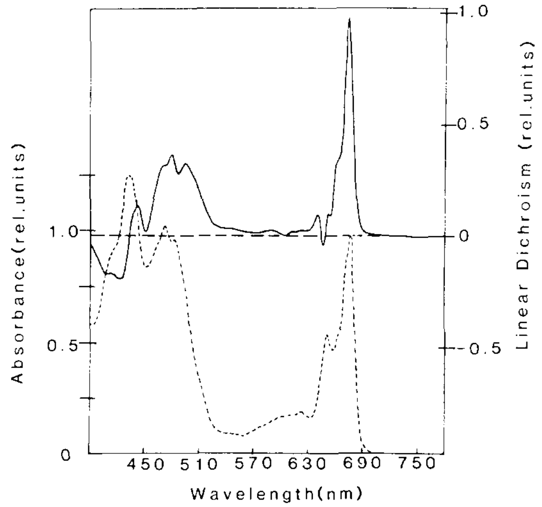

Fig. 4. The 100 K absorption (------) and LD (———) spectra of the LHC isolated from wild-type barley thylakoids. Dichroism ratio at the red maximum is 1.3–1.6.

room-temperature measurements precluded more specific conclusions in their case.

By using computer-fitting procedures described by Tapie et al. [5] we have been able to determine the difference between the spectra presented in Figs. 1 and 2, normalized at 690–700 nm, i.e., in a region where the contribution of the Photosystem I signal to the total LD was maximum [4,5]. These calculated difference spectra are presented in Fig. 3. For comparison, the 100 K LD and absorption spectra of LHC isolated from wild-type barley are presented in Fig. 4.

The calculated difference for the absorption spectra of the two types of thylakoids (dashed line, Fig. 3) in the 570–700 nm range is very similar to that of barley LHC (Fig. 4) or pea LHC (Fig. 3 of Ref. 4). This spectrum exhibits all the minor spectral features previously reported in this region [4]. However, in the Soret region of Chl *a* and in the carotenoids bands there is some discrepancy; the possible source of these errors has been discussed by Tapie et al. [5]. As with the difference absorption spectrum, the difference LD spectrum (solid line, Fig. 3) is quite similar to that of pea and barley LHC in the 570–700 nm region and demonstrates a clear LHC fingerprint [4]. Again at lower wavelength there is less agreement but recognizable components can be observed [4].

The close similarity between the low-temperature LD difference spectrum of thylakoids from normal and Chl *b*-less barley and the LD of isolated LHC, reported in the present study, allow us to confirm several conclusions presented in previous articles of this series [3–5]:

(1) There is little or no contribution from scattering artefacts to the LD spectra of intact thylakoids as determined under our conditions.

(2) The in vivo orientation of the LHC pigments is maintained in the isolated complexes.

(3) The largest dimension(s) of the isolated LHC is (are), in vivo, parallel to the plane of the photosynthetic membrane.

In addition, the results presented in this report demonstrate that the LD spectra of Photosystem I and II complexes of thylakoids from normal and Chl *b*-less barley are quite similar. This observation indicates a small magnitude for the contribution of Chl *b* to the LD spectra of these complexes.

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