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THE LIGHT-HARVESTING CHLOROPHYLL A/B PROTEIN ACTS AS A TORQUE ALIGNING CHLOROPLASTS IN A MAGNETIC FIELD

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Abstract. Displacement of particles from the purified light-harvesting chlorophyll a/b protein aggregate (LHC) was studied in magnetic fields of various strengths (0 to 1.6 T) by polarized fluorescence measurements. Macromolecular aggregates of LHC have a considerable magnetic susceptibility which enables the particles to rotate and align with their nematic axes parallel with $\vec{H}$. As LHC is embedded in a transmembrane direction thylakoids should align perpendicular to $\vec{H}$, the mode of alignment experimentally observed in thylakoids. The value of the magnetic susceptibility could be estimated by relating it to the integral susceptibility of the chlorophyll molecules in LHC. The fitting of this value with the field strength dependency of the fluorescence polarization ratio (FP) revealed a relationship between the LHC content of various photosynthetic membranes and their capacity for alignment, which suggested that LHC might be the torque ordering chloroplasts in a magnetic field.

INTRODUCTION

LHC, the pigment-protein complex which controls the distribution of quanta between the two photosystems might have a dynamic structure enabling the complex to change its connectivity with the individual photosystems depending on the physiological demands of the plant [11]. Such mobility is expected in a system where polypeptides with the attached pigments can, under certain conditions, transmute to liquid crystal organizations. Such a structure was suggested by a giant CD signal of LHC [5], a characteristic of pigments embedded in liquid crystals [14]. A further attribute of

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Abbreviations: LHC: light-harvesting chlorophyll a/b protein; FP: fluorescence polarization ratio, I_z/I_y;

liquid crystals is their diamagnetic susceptibility and their orientation by electric fields [15]. The former can be easily demonstrated by the rotation of LHC macromolecules resulting in an alignment where the nematic axis in the particles is parallel to the direction of the magnetic field. Supposing that thylakoids are aligned in the magnetic field by LHC as a torque one would expect that the success and speed of alignment depends not only on environmental conditions (temperature and viscosity of the medium) but on the amount of LHC contained in the membranes.

In this paper experiments are reported which investigate the magnetic susceptibility of LHC in order to determine whether LHC in vivo can bring about the alignment of thylakoids in a magnetic field.

MATERIALS AND METHODS

Purified LHC was prepared [4] from chloroplasts isolated from spinach. The isolation medium contained 0.35 M sucrose, 0.015 M phosphate, 0.01 M KCl, pH 7.2 [1]. Mesophyll and bundle sheath chloroplasts of maize were prepared as in [3], chloroplasts grown under intermittent light were obtained by the method described in [2].

Magnetic alignment of the purified LHC and of various types of chloroplasts was carried out in an electromagnet (Type Phylatex 1316, DDR) equipped with a sample holder with a cuvette which could be illuminated by the 488 nm line of an Argon laser ILA 120 Zeiss Jena. The magnetic field

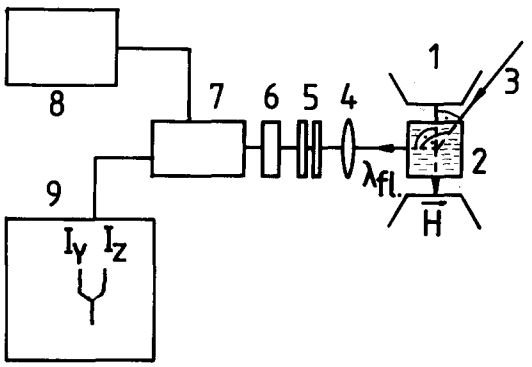

FIGURE 1. Block-scheme of the apparatus for measuring the alignment of thylakoids in a magnetic field. 1: magnet, 2: samples with membranes aligned with their plane perpendicular to the field, 3: light exciting fluorescence, 4: lens, 5: polaroid filters alternating the direction of observation between the two fluorescence components emitted parallel and perpendicular to the plane of the aligned membranes (I_z and I_y, respectively), 6: interference filter transmission at 680 ± 10 nm, 7: photomultiplier, 8: power supply, 9: recorder.

strength was regulated from 0 to 1.6 T and measured with a probe inserted into the cuvette by a Gaussmeter (RFL Industries Inc. Boston New Jersey USA).

Fluorescence was excited and observed perpendicular to the magnetic field direction. Polaroid filters separated the fluorescence components emitted parallel and perpendicular to the exciting beam [6]. The system is represented diagrammatically in Fig. 1. Measurements were made at room temperature.

LHC particles of different sizes were obtained from large aggregates of purified LHC by incubation with 2% v/v Triton X-100.

RESULTS

In these studies we postulated that in chloroplasts of various LHC content, as in isolated LHC particles and their fragments, fluorescence at 680 nm originates from Q_Y dipoles of chlorophyll a making the same angle of orientation with respect to the normal of the membrane plane. If so, then observing the alignment of various thylakoids in a steady

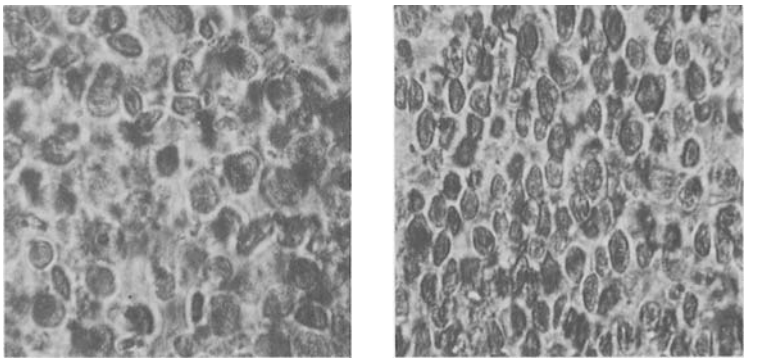

FIGURE 2. The geometry of thylakoids and chloroplasts suspensions prior to (a) and during (b) magnetic alignment. (Chloroplasts suspensions in the light microscope - x 1500 - at 0 (a) and 1.2 T (b) magnetic field strength.)

state should result in saturation FP values of the same magnitude. The geometry of thylakoid alignment is shown in Fig. 2. According to data obtained for the field strength dependency of FP (Fig. 3) our presumption seemed to be correct: the orientability of the particles was determined by the magnetic susceptibility which induced the particles to reach the same level of FP. Calculations relating FP to the magnetic field strength and the magnetic susceptibility were performed using the theory developed in [10] and [13]. Regarding aligned particles as discs

$$p = \frac{I_z}{2I_z + I_y} = \frac{(1+\cos^2\gamma)}{4} + \frac{(1-3\cos^2\gamma)}{8}\left(\frac{e^{h^2}}{hD(h)} - \frac{1}{h^2}\right) \qquad (1)$$

$$\text{where } D(h) = \int_0^h e^{x^2}\,dx, \text{ and } h = \sqrt{\frac{\Delta\chi}{2kT}} \cdot |\vec{H}|$$

$\vec{H}$ is the field strength, $\Delta\chi$ is the magnetic anisotropy expressed as $\chi_y - \chi_z$, γ denotes the orientation angle of the Q_Y emission dipole with respect to the normal of the membrane plane.

FP used in our curve fitting procedure could be obtained as

$$FP = \frac{1}{\frac{1}{p} - 2}\ , \text{ where } p = \text{Eq (1)} \qquad (2)$$

The angle γ used in these calculations was throughout 58.6° (with respect to the normal of the membrane plane) which was found by us earlier to be characteristic for the fluorescence band localized at 680 nm [6].

As seen from Fig. 3 the calculated values of FP obtained from Eqs. (1) and (2) showed a reasonable fit with the experimental points of FP.

In order to know whether the diamagnetic moment is influenced by the size of the aligning particles allowing coupling between magnetic dipoles, we studied FP with LHC aggregates of different sizes. Size determinations were carried out on the basis of the speed of relaxation of alignment (after switching off the magnet, till FP has dropped to 1.0).

Presuming that fragmentation did not change the form of the particles.

$$r_1 = r_o \sqrt[3]{\frac{\tau_1}{\tau_o}}\ [9] \qquad (3)$$

r_1 = radius of the fragment
r_o = radius of the non-fragmented aggregate
τ_1 = relaxation time of the fragment
τ_o = relaxation time of the non-fragmented aggregate

The measurements show a straight line of direct relationship between the particle size and FP indicating that no

special coupling between the magnetic dipoles is represented by individual chlorophyll molecules.

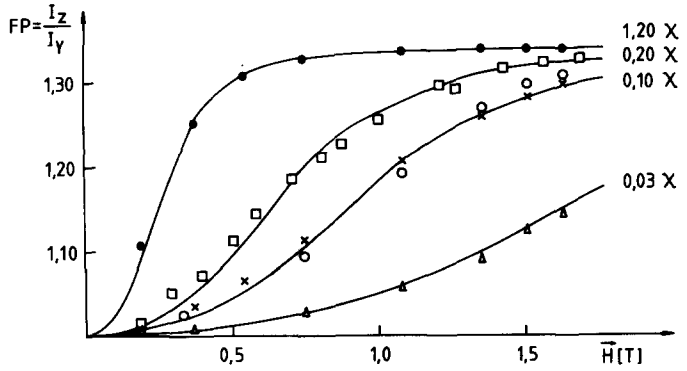

FIGURE 3. Field strength dependence of FP with thylakoids of different LHC contents (experimental points). Curves calculated according to Eqs. (1) and (2) with $\Psi = 58.6^{\circ}$ showing various susceptibilities fitted to the measured points. ●, mesophyll chloroplast of maize (Chl a/b = 2.8); □, macromolecular aggregate formed from purified light-harvesting chlorophyll a/b protein (Chl a/b = 1.16); x, bundle sheath chloroplast of maize (Chl a/b = 4.6); Δ, bean chloroplasts developed at a light region alternating periods of 98 min dark and 2 min light (Chl a/b=5.2); o, bean chloroplasts developed under weak continuous light for 24 h (Chl a/b=3.1). The suspension traversed by light was adjusted to OD: 0.15 at 680 nm which corresponds to an approx. chlorophyll concentration of $3 \cdot 10^{-6}$M. Temp. 25°C, $\chi = 3.4 \cdot 10^{-20}$ cm^3 [13] for chloroplasts.

DISCUSSION

The orientability of thylakoids has been recognized for many years [7] but the physical basis has not been elucidated. Here we could show that the magnetic susceptibility of LHC is commensurable with that of the chloroplasts. This suggests that LHC may act as a torque which aligns the chloroplasts in a magnetic field.

The value of the magnetic susceptibility estimated for chloroplasts [13] was $\chi = 3.4 \cdot 10^{-20}$ cm^3. For mesophyll chloroplasts the best fit (see Fig. 3) was found with the curve calculated at 1.2χ, but for the LHC only 0.2χ was applicable. This relatively low magnetic susceptibility of the purified LHC can be explained by the difference in structure

between the artificially aggregated macromolecule and that of the LHC in vivo [8]; the latter is suggested to be more asymmetric [12].

Functional importance of the liquid crystal like structure of LHC, mobile under the effect of magnetic and electric fields [15] can be the signalization between Photosystem II and LHC, hence high local electric fields around the reaction center [16] may induce LHC to separate from Photosystem II by moving away.

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