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DIRECT OBSERVATION OF THE ROTATION IN A CONSTANT MAGNETIC FIELD OF HIGHLY ORGANIZED LAMELLAR STRUCTURES

J. D. CLEMENT-METRAL

Laboratoire de Photosynthèse, C.N.R.S., 91190 Gif-sur-Yvette, France

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1. Introduction

An increasing number of investigations is being devoted to the study of the orientation of the photosynthetic pigments in the functional membrane hoping for a better understanding of the structural conditions of the electric potential generation [1]. The vectorial properties of the electron transport is well substantiated at present. The desired information on the orientation of the chlorophyll in the membrane can be obtained from studies in membranes which are oriented with respect to the laboratory system. Most recent attempts were based on spreading or on drying technics or on the orienting effect of high magnetic field strength [2–5]. Chalazonitis et al. [6] have reported the direct observation of the rotation and alignment of a suspension of rod outer segments from frog retinas in a constant and homogeneous magnetic field. The phenomenon was explained by Hong et al. [7] in terms of magnetic anisotropy of the oriented constituent molecules of the rod disc membranes particularly rhodopsin. Owing to the extraordinarily regular array of disc membranes in the rod, the oriented anisotropy will be summed up.

The work reported here was aimed at the direct observation of the orientation of intact active structures in physiological conditions by magnetic field strength in correlation with its effect on linear dichroïsm or chlorophyll fluorescence. The emission anisotropy can be explained if it is assumed that the porphyrin head lies in or close to the plane of the lamellae oriented perpendicular to the magnetic field. The comparison of the effects observed and the structural organisation in such different organisms was an attempt to generalize the existence of a magnetic anisotropy in photosensitive pigmented membranes.

2. Experimental

The choice of the organisms was based on the following criterions:

(1) a rod, ellipsoid or discoid shape allowing the rotation to be observable, and (2) a regular stacking of lamellae in a determined direction. Lettuce chloroplasts and two types of photosynthetic bacteria: *Rhodopseudomonas palustris* and *Rhodopseudomonas viridis* which present the desired characteristics [8] were used. They were compared to *Porphyridium cruentum* algal cells which are spherical and contain a lamellar plastid of somewhat spherical symetry, therefore presumably isotropic.

Intact chloroplasts were isolated from lettuce obtained at a local market. They were isolated as described by Arntzen et al. [9]. *Rps palustris* and *Rps viridis* were grown anaerobically under illumination in Hutner's modified medium, harvested in the late log phase and suspended in the same medium. *Porphyridium cruentum* was grown as previously described [10] and suspended in 0.6 M NaCl. Series of consecutive photographs of the rotation of the cells and chloroplasts suspensions were performed at the Laboratoire de Neurophysiologie cellulaire, CNRS, 13-Marseille in Chalazonitis et al.'s previously described apparatus [11,12]. The fluorescence measurements were determined, at the Laboratoire de Magnétisme, CNRS, 92190 Meudon, Bellevue, with a device derived from that of Geacintov et al.

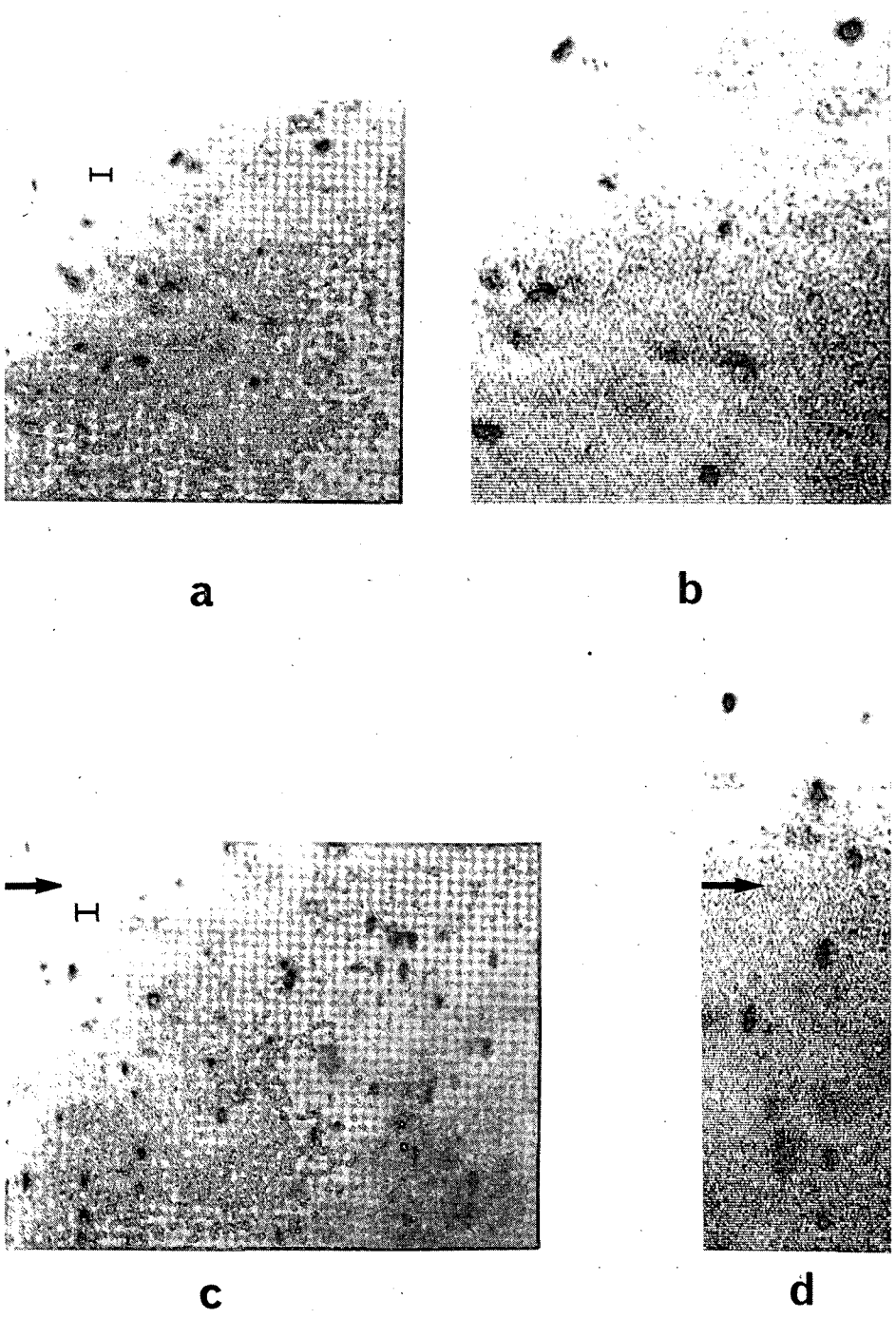

Fig. 1. Photographs on television screen of lettuce chloroplasts flotting in Arntzen et al. medium [9] containing 20% Ficoll. a: In the absence of magnetic field (b: enlargment of a, × 2); c: in a homogeneous magnetic field of 14 kg (d: enlargment of c × 1.6). Arrows indicate magnetic field direction. Scale —— = 10 μ.

[2]. The entire assembly was situated in the 5 cm air-gap of a TEK ELEC TE 350 electromagnet. This 4 t. magnet is equiped with a 30 kw current stabilized power supply feed-back by a HALL pack. The magnetic field is homogenous and variable from 0 to 20 kg. The stray magnetic fields are very low, so that it was not necessary to use quartz light guides (SCHOTT) longer than 1.5 m; they were fitted with amagnetic tips made out of NS 22 S steel (UGINE). The stray magnetic fields around the photomultiplier and light were checked and found less than 0.5 G. The light source was a 150 W stabilized Xenon lamp. The fluorescence was viewed with an EMI extended S-20 cathode 9659 B photomultiplier, with high infra red response.

The LD spectra were recorded at the Service de Biophysique, Dept de Biologie, CEA de Saclay, 91190 Gif-sur-Yvette, using the technique previously reported by Breton and Roux [4].

3. Results

Series of consecutive micrographs of a suspension of lettuce chloroplasts in a small cuvette placed at the center of a constant magnetic field, attaining at maximum 20 kg intensity were made during the field action. At the onset of the experiment the chloroplasts sediment slowly and are seen as round or ovoid plates. As the field is on, they first rotate 90° round their big axis so that their stacking plane becomes vertical; they then rotate round their small axis until they are finally aligned perpendicular to the field direction. The plane of the stacked lamellae being perpendicular to that direction (fig.1). In order to prevent the Brownian motion and sedimentation 20% ficoll was added.

Due to too small dimensions it was not possible to take micrographs of the orientation of the bacterial suspensions, nor to saturate the orientation with the magnetic intensity used here. However with *Rps palustris* a 70–80% orientation was observed.

The LD spectra of the oriented chloroplasts and the photosynthetic bacteria are exactly as expected and similar to already published spectra [4,5,13]. On the contrary *Porphyridium cruentum* spectrum is completely flat. This result is in agreement with that of Geacintov et al. on *Phormidium luridum.* This blue green alga lacks also a regular stacking, the thylakoids being peripherally arranged.

The effect of the magnetic field strength on the fluorescence of *Rps palustris* is shown on fig.2. Although the magnetic field was not great enough to saturate the orientation, it is easy to see that the results are exactly similar to those obtained by Geacintov with Chlorella. Fluorescence emission was measured in steady state condition, eliminating the influence of fluorescence induction. When the direction of the exciting beam is parallel to the magnetic field H the fluorescence increase without saturating up to 5–9% at 16 kg. In the perpendicular orientation the negative effect varies from 9% up to 15%.

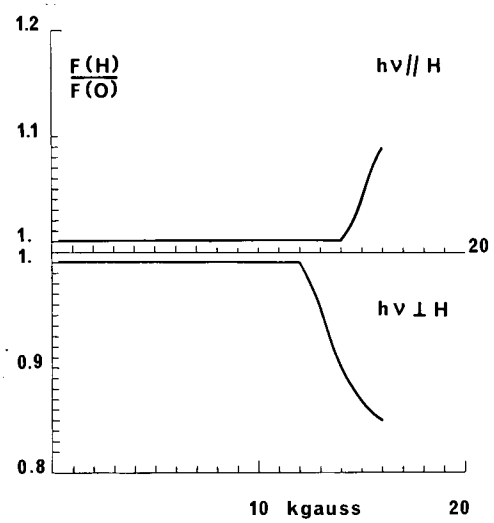

Fig. 2. Magnetic field dependence of bacteriochlorophyll fluorescence of *Rps palustris,* recorded in steady state conditions, eliminating the influence of induction phenomenon. F (o) = zero field initial steady state fluorescence level. In the upper part of the figure the direction of the exciting light beam was parallel to the magnetic field H ($h\nu$// H); in the lower part perpendicular. Sweep rate = 6 kgauss/min. Unpolarized exciting light between 350 nm–650 nm (max 585 nm) was obtained with a CORNING 4–76 filter plus an anticaloric Calflex RG 1 (350 nm–800 nm). Its intensity was 2000 ergs $cm^{-2} \cdot s^{-1}$. Fluorescence was isolated from exciting light with Kodak Wratten 87 c filter.

4. Discussion

This work confirms the hypothesis that the main bulk of the photosynthetic pigments has a considerable degree of orientation in vivo. Provided there exists an asymetry and a sufficient degree of regular array of the membranes in the cell a magnetic anisotropy will be noted in direct correlation with the spectroscopic effect of the magnetic field on the pigment LD or fluorescence. Intact viable cells are orientable as well as air-dried, spread, dead flattened cells. This is well illustrated by the fact that Breton [13] was not able to orient isolated chromatophores with 10.5 kg magnetic field as chromatophores are spherical vesicles formed by a closed membrane [8]. However air drying or spreading flattens the vesicles making double membrane discs, regularly stacked which are orientable.

The high degree of orientation of pigments with regard to the membrane plane seems to be a general feature of pigmented membranes, going from animal retinas to photosynthetic bacteria.

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