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Magnetic field effect on the chlorophyll fluorescence in Chlorella

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SUMMARY

The chlorophyll *a* fluorescence in *Chlorella pyrenoidosa* can be enhanced by 4–9% if the excitation light beam is parallel to an external magnetic field or decreased by 4–9% if the light beam is oriented perpendicular to a magnetic field of about 16 kgauss or more. These effects cannot be explained in terms of the small changes in light absorption which are also observed. It is suggested that these observations are due to a reorientation of pigment molecules in the magnetic field.

The weak fluorescence of chlorophyll *a* in the green algae *Chlorella pyrenoidosa* has been found to be sensitive to an external magnetic field. The red fluorescence of an *in vivo* suspension of Chlorella cells may be either increased or decreased depending on the direction of the exciting light beam with respect to the magnetic field.

The experiments were performed at the Francis Bitter National Magnet Laboratory at the Massachusetts Institute of Technology in one of the Bitter coil solenoid magnets★. The Chlorella was grown in Pirson and Ruppel's[1] medium. The experiments were performed in the same medium. The age of the culture at the time of the experiments varied from 3 to 6 days. The experimental arrangement is shown in Fig.1. The Chlorella suspensions were placed in a 1 cm × 1 cm × 5 cm square bottomed cell (E) to which were attached two prisms C and C′ (at right angles to each other) and two 3-cm-long, 7-mm-diameter light guides A and B. A was cemented to the prism C and B was cemented to the bottom of the cell. The entire assembly was situated in the 2-inch bore of the magnet J. The magnetic field was homogeneous and variable from 0 to 145 kgauss. The light source was a 100-W mercury lamp; the light was filtered with a Corning CS 4-72 filter (band pass 340–620 nm). A 9-ft-long 7-mm-diameter bent quartz rod G was used as a light guide. By

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea.

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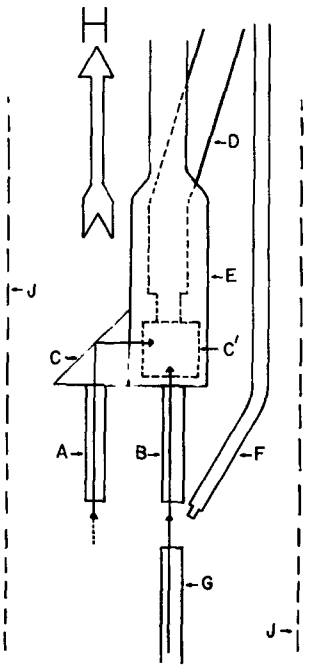

Fig.1. Schematic representation of experimental arrangement. A and B, 3-cm light guides; G, tip of 9-ft light guide; E, cell; C and C', 90°–45°–45° prisms; D, fluorescence monitoring fiber optic; F, incident light intensity monitoring fiber optic; J, walls of magnet bore; *H*, magnetic field direction.

moving the cell assembly, the short light guide A or B could be positioned over the light guide G. In the position shown in Fig.1, the direction of the exciting light beam is parallel to the magnetic field *H* and will be referred to as the parallel orientation ($h\nu//H$). When A is positioned over G, the light beam is reflected by prism C into the cell and the light beam direction is perpendicular to *H* (perpendicular orientation, $h\nu \perp H$). The exciting light was unpolarized in all experiments. The fluorescence was viewed by the second prism C' and a 20-ft-long 0.25-inch fiber optic D fitted with a red cut-off (640 nm) Corning CS 2-64 filter. A second 20-ft fiber optic F was used as a light intensity monitor. The unusual lengths of the light guide and fiber optics were required to eliminate effects of stray magnetic fields on the lamp and photomultipliers (negligible below 60 kgauss). The fluorescence was viewed with a cooled RCA 7102 and the light intensity with a 1P28 photomultiplier. The signals were fed into two Keithley 417 picoammeters (with zero-suppress and expanded range capability) which in turn were connected to strip chart or X–Y recorders.

Stray light effects were less than 3% of the fluorescence. The spectral distribution of the fluorescence was checked by removing the 2-64 cut-off filter and interposing a monochromator between fiber optic F and the 7102 photomultiplier. A peak at 685 nm and a broad shoulder at 740 nm were obtained which correspond to the well-known fluorescence spectrum of chlorophyll *a* in Chlorella.

A 4-min (or longer) illumination period in zero-field preceded the actual experiments to minimize induction effects. Experiments were started after the fluorescence

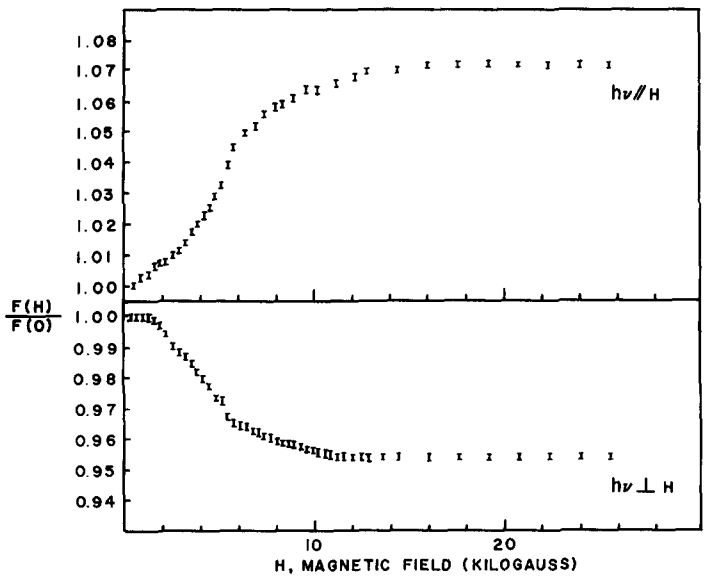

Fig.2. Magnetic field dependence of fluorescence (F) of Chlorella. $h\nu \,//\, H$ - - - light guide B (Fig.1) positioned over light guide G. $h\nu \perp H$ - - - light guide A positioned over G. Sweep rate 6 kgauss/min. The exciting light was unpolarized and its intensity was 15 000 ergs/cm^2 per sec.

had reached a steady level. A typical result is shown in Fig.2. In the parallel orientation the fluorescence F increases and saturates at a value 7.2% higher than in zero-field. In the perpendicular orientation the effect saturates at −4.6%. The maximum change varies from sample to sample, and ranges from about +4 to +9% and −4 to −9% for the parallel and perpendicular orientations, respectively. The magnetic field sweep rate used in Fig.2 was 6 kgauss/min. The positive effect saturates at 16 kgauss and the negative effect at about 11 kgauss. No further changes were observed when the field was swept up to 145 kgauss. If the sweep rate is increased to 10 kgauss/min or faster, $F(H)/F(0)$ reaches a limiting value at higher field strengths for both orientations. Magnetic field induced changes in the fluorescence cannot follow the field unless the sweep rate is 6 kgauss/min or less. When the magnetic field is rapidly swept back to zero, the fluorescence does not immediately settle back to its zero-field value; this is shown in Fig.3 for both orientations. This relaxation effect is slower in the perpendicular orientation.

Experiments were also performed with a 0.3-msec light flash. The experimental arrangement was as before, except that the fluorescence and intensity monitor signals were fed into two pulse stretching amplifiers which were connected to two separate inputs of an Intertechnique Didac 800 digital analyzer. Integration of the signals led automatically to two numbers which were proportional to the total number of fluorescence and flash intensity photons, respectively. The small variations (1–2%) in the intensity of the flashes could thus be accounted for. Each experiment consisted of a single flash with the magnetic field off or on, preceded by a 3-min dark period. The wavelength of the exciting light for both the flash and steady-state source (100-W mercury lamp) was 436 ± 7 nm for this particular set of experiments and was done between the poles of an iron core magnet. The intensity of the flash was 1200 ergs/cm^2 per flash, and that of the mercury lamp was

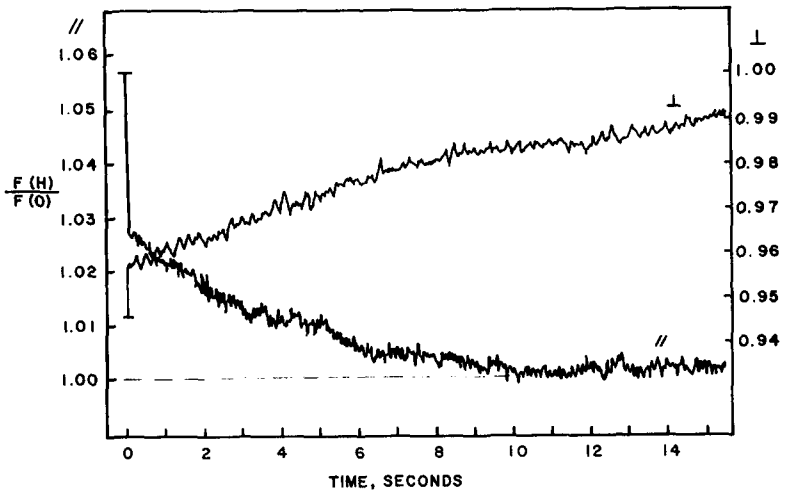

Fig.3. Relaxation of fluorescence (F) to zero magnetic field values. Fluorescence change during the 4 sec required to turn off the magnetic field indicated by vertical bars at time = 0 sec.

8000 ergs/cm^2 per sec. An average of six separate experiments with the field off and the field on (10.5 kgauss), yielded in the parallel orientation a fluorescence enhancement of +6.6 ± 0.4% with the flash and a +7.0% effect with steady-state illumination. In the perpendicular orientation a fluorescence decrease of −7.6 ± 0.4% was obtained with the flash and a −8.4% decrease with steady-state illumination. Qualitatively similar results were obtained at 40 kgauss in the Bitter solenoid magnet. These numbers (flash and steady-state magnetic effect) are sufficiently close to warrant the conclusion that the magnetic field is acting on an emission process which has an induction period of the order of 1 msec or less.

In another experiment, chlorophyll (Fischer Scientific) was dissolved in benzene solution. The fluorescence spectrum was determined and was found to coincide with that of chlorophyll *a* in benzene solution. No magnetic field effect on the fluorescence up to 145 kgauss was observed. (In this experiment, the voltage on the RCA 7102 photomultiplier was the same as in the Chlorella experiments.) It is concluded that the observed effects with Chlorella are therefore not due to a molecular property of chlorophyll.

The quantum efficiency of fluorescence φ of Chlorella is known to increase from about 0.03[2] to about 0.06[3] with increasing light intensity I. At low light levels an intensity dependent quenching process[4] with rate constant k limits φ to a value below the maximum of approx. 0.06. In the simplest model, the quantum efficiency may be written:

$$\varphi = \frac{F}{I} = \frac{k_F}{k_F + k_H + k[Q]} = \begin{cases} \rightarrow \sim 0.03, \\ \quad k[Q] \rightarrow \text{maximum} \\ \quad \text{at low } I \\ \\ \rightarrow \sim 0.06, \\ \quad k[Q] \rightarrow \text{minimum} \\ \quad \text{at high } I \end{cases} \qquad (1)$$

where $[Q]$ is the concentration of fluorescence quenching traps which decreases with increasing light intensity, $k[Q]$ is the term due to photosynthesis, k_F is the radiative rate constant of chlorophyll *a* in Chlorella and k_H is the sum of all other nonradiative rate constants.

We measured $F(H)/F(0)$ as a function of light intensity. In the perpendicular orientation the negative effect is nearly independent of light intensity. In a typical example, the effect is −6.3% when F/I is maximum (at high light intensity) and is −5.3 ± 0.5% at low light intensities where $\varphi = F/I \rightarrow 0.03$; at the low light intensity the signal is noisy and there is consequently a ± 10% uncertainty associated with the measured value of −5.3%. The magnitude of the effect in the perpendicular orientation does not change (within limits of ±0.7%) when the photosynthetic poison 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) is added. Since it is well known that DCMU eliminates the intensity dependence of φ (the quenching process $k[Q] \rightarrow 0$), the magnetic effect in the perpendicular orientation does not appear to operate on the term $k[Q]$.

In the parallel orientation, there is a pronounced intensity dependence of $F(H)/F(0)$. For example, in a particular sample, the effect was +6.0% at high light intensities when F/I was maximum and decreased to 3.3 ± 0.5% at low light intensities. Addition of DCMU does not affect $F(H)/F(0)$ in the high intensity limit, but eliminates the intensity dependence which is observed in the absence of this poison. We first note that any intensity dependence of the magnetic effect is most likely due to a variation in one of the terms in the denominator of Eqn. 1. Furthermore, we assume that k_F is independent of the magnetic field, which is based on the lack of a magnetic field effect on the fluorescence of chlorophyll *a* in solution. If $k[Q]$ were the magnetic field sensitive term, $F(H)/F(0)$ would be expected to increase with decreasing light intensity since $[Q]$ increases. At high light intensities and in the presence of DCMU, $[Q] \rightarrow 0$, yet $F(H)/F(0)$ is largest under these conditions. We therefore concluded that at least part of the magnetic effect in the parallel orientation is operative on the term k_H in Eqn. 1.

The magnetic field may also be affecting the amount of light absorbed, which effectively would change I in Eqn. 1 and therefore F as well. Experiments currently in progress indicate that the absorption of polarized and unpolarized light is indeed magnetic field dependent; this effect depends on the wavelength of the exciting light and will be described in detail in a future publication. However, quantitative comparisons between magnetic field induced changes in both the absorption and the fluorescence, indicate that changes in I alone cannot account for the observed changes in the fluorescence (the parallel orientation effect in particular, which is intensity dependent, cannot be explained in this manner). For example, in the perpendicular orientation there is a decrease in the overall transmitted light, corresponding to an increase of 0.4–2.0% (depending on the sample) in the total amount of light absorbed by the Chlorella; yet the fluorescence always decreases when the magnetic field is turned on in the perpendicular orientation. In the parallel orientation there is a decrease of about 1–2% in the amount of light absorbed, yet the fluorescence increases by 4–9% in the presence of the magnetic field.

The changes in fluorescence (and light absorption) induced by magnetic fields may be due to a reorientation of the pigment molecules which may give rise to changes in energy transfer efficiencies. Such orientation phenomena can be explained in terms of cooperative effects between molecules with anisotropic magnetic susceptibilities, which are known to align small muscle fibers[5] and molecules in liquid crystals[6,7].

Conformational changes have been previously proposed to explain changes in the fluorescence intensity under varying experimental conditions. Papageorgiou and Govindjee[8] have invoked conformational changes in the lamellar system to explain the slow decline of fluorescence during the "second wave" in the fluorescence induction curve.

Murata[9] proposed the idea that the illumination of a photosynthetic organism with light preferentially absorbed by system I or system II changes the efficiency of excitation transfer between chlorophyll molecules. This author has also suggested that Mg^{2+} changes the mutual orientations of pigment molecules in chloroplasts, thus altering the rate of excitation transfer from chlorophyll molecules in pigment system II to those in pigment system I[10]. Bonaventura and Myers[3] have also proposed that conformational changes control the distribution of excitation energy between the two pigment systems. Duysens[11] explains the observed changes in the fluorescence following preillumination with photosystem I or II light in terms of a reorientation of pigment molecules in systems I and II. This gives rise to more efficient energy transfer from system II to I, and thus to a decrease in the fluorescence[11].

The magnetic field effects reported here, if they are indeed due to a reorientation of pigments as suggested above, may prove to be an important new tool in the study of conformational changes in *Chlorella pyrenoidosa*, and perhaps other organisms as well.

We finally note that Stacy *et al.*[12] examined the delayed fluorescence of Chlorella in a magnetic field of 18 kgauss and found no effect (within an error limit of ± 2%).

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