CHLOROPHYLL *B* FLUORESCENCE AND AN EMISSION BAND AT 700 nm AT ROOM TEMPERATURE IN GREEN ALGAE

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1. Introduction

Chlorophyll (Chl) *b* is non-fluorescent *in vivo* as it transfers excitation energy with 100% efficiency to Chl *a* [1–3]. There is no record of Chl *b* fluorescence *in vivo*. We show here, for the first time, that Chl *b* is slightly fluorescent *in vivo* (at 660–665 nm) when the cells are exposed to bright light, or treated with DCMU (see footnote*); this emission was uncovered when we compared the ratios of "high" to "low" fluorescence yields as a function of the wavelength of light.

In red algae, a new band around 693 nm, observed upon excitation with high intensity system II light, or with DCMU treatment, was suggested to originate in the energy trap of system II [4, 5]. Earlier attempts in our laboratory by several investigators failed to reveal the presence of a similar band in green algae. We demonstrate here the existence of an emission band at about 700 nm in Chlorella and Chlamydomonas when we compare the ratios of "high" to "low" yield fluorescence as a function of the wavelength of light. We interpret the above findings as to be caused by a decrease in the efficiency of energy transfer from Chl *b* to Chl *a*, and from Chl *a* molecules near the trap to the trap of system II, when the latter are closed at high light and in the presence of DCMU.

2. Methods

Green algae *Chlorella pyrenoidosa* and *Chlamydomonas reinhardi*, used in this study, were cultivated as described earlier [6, 7]. Fluorescence measurements were made by the stop and flow method of Lavorel [8]. When a dark-adapted suspension of algae flows at a fast speed, the initial ("m") level is measured; this corresponds to the "low" yield of fluorescence. When the flow is stopped, fluorescence yield rises from the "low" value to a "high" value ("M"). If the low value is the true minimum, the cell is said to be in the "0" state; this fluorescence is also referred to as the "constant" fluorescence. If, however, the "high" value is the true maximum, the cell is said to be in the "P" state [7]. The difference Δm (M-m) is referred to as the "variable" fluorescence. Addition of DCMU (5×10^{-5} M) gives the true "P" state. The wavelength of excitation was 480 ± 4 nm, obtained from a large monochromator; the light source was a xenon lamp operated at 40 A. The intensity of excitation was 1.05×10^4 ergs cm^{-2} sec^{-1}. The concentration of Chl was about 20 μg per ml of suspension. Fluorescence was collected at right angles, passed through a Bausch and Lomb monochromator (half-band width 3.3 nm) and detected by an EMI 9558 A photomultiplier. Measurements were made point-by-point at every 2.5 nm intervals. No correction for the sensitivity of the photomultiplier was necessary as we report here the ratio spectra.

* DCMU: 3-(3,4-dichlorophenyl)-1, 1-dimethylurea.

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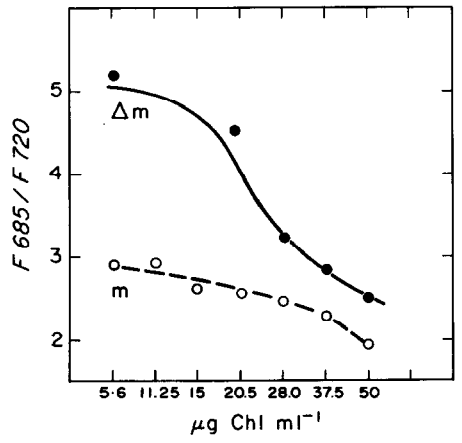

Fig. 1.

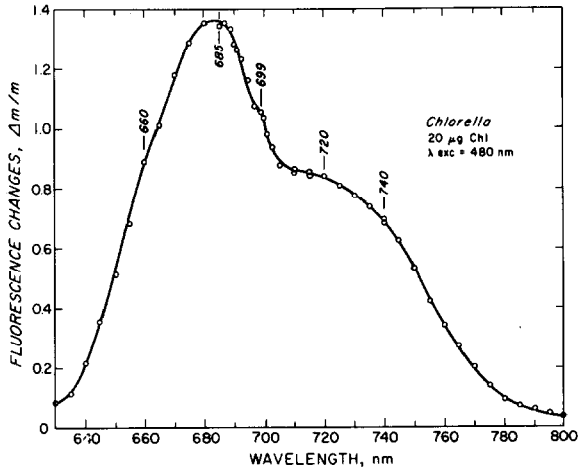

Fig. 2.

3. Results and discussion

Fig. 1. shows the ratio of fluorescence at 685 nm (F685) to that at 720 nm (F720) as a function of the Chl concentration of the sample. At 20 μg Chl ml^{-1} of suspension, the fluorescence signals are high enough, and the reabsorption of fluorescence is low. (Use of higher concentrations is not recommended as the reabsorption of fluorescence becomes significant.) Thus, we chose, for our study, a suspension having 20 μg Chl ml^{-1}.

Lavorel [9] was the first one to report Δm/M spectra for green algae; his main conclusion was the relative decrease of 720 nm fluorescence in the "P" state as compared to the "0" state.

Fig. 2 shows the Δm/M as a function of wavelength of light for a suspension of Chlorella. The main increase at 685 nm (due to bulk Chl *a*) is clearly observed. However, a closer inspection reveals a dip around 710 nm (Chl *a*), and minor shoulders around 660 nm (Chl *b*) and at 699 nm (Chl *a*). The latter, however, may not be convincing, but they must be trusted because they have been observed in all the seven experiments we made. As we noted under "Methods", the true "P" state is reached easily in the presence of DCMU. Therefore, we compared the ratio of ^{M}DCMU to ^{m}control to measure the ratio of the spectra at "high" to "low" yield of fluorescence (fig. 3). This ratio spectrum clearly shows the presence of shoulders at 665 nm (Chl *b*) and at 700 nm (Chl *a*). The dip at 710 nm is confirmed, and additional bands at 720 and 740 nm (perhaps, due to vibrational satellite bands of the main Chl *a* bands) are observed. These data have also been confirmed in seven experiments.

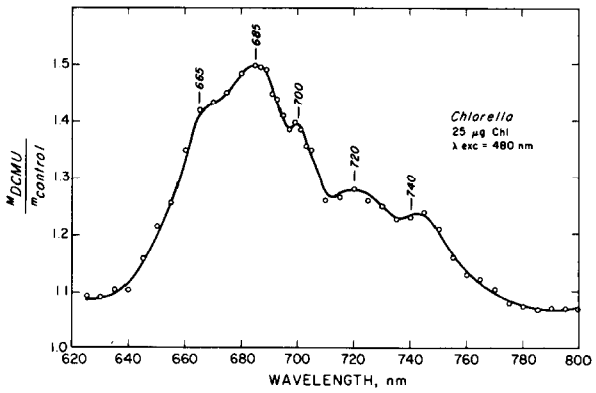

Fig. 3.

Experiments with Chlorella reported above were made at the laboratory in Gif-Sur-Yvette, and then again confirmed in two experiments with *Chlorella pyrenoidosa* grown at Urbana (as described in [10]).

Three experiments with Chlamydomonas (20 μg Chl ml^{-1} suspension) also confirmed all the features of the above findings.

The band at 660–665 nm, observed here for the first time, is, in all likelihood, due to Chl *b* absorbing at about 650 nm. We suggest that this arises because of a slight decrease in the efficiency of energy transfer from Chl *b* to Chl *a* when the traps of photosynthesis are closed. This fluorescence is not due to the "uphill" transfer [11] of energy from

Chl *a* to Chl *b*, as all attempts to observe any trace of Chl *b* fluorescence by exciting Chl *a* (675–695 nm) have failed.

The band at 699–700 nm is, in all likelihood, due to pigment molecules associated with, or close to, the trap II of system II as suggested earlier [4, 5]. The closure of the "traps" does not allow the excitation arriving at the trap to be used for photochemistry, and thus, it comes out, instead, as fluorescence.

Acknowledgement

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