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77°K FLUORESCENCE SPECTRA OF DARK-GROWN *EUGLENA GRACILIS* SUBJECTED TO SHORT LIGHT FLASHES

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SUMMARY

The first steps of chlorophyll synthesis in dark-grown *Euglena gracilis* have been studied by giving light flashes of short duration to etiolated cells and measuring their fluorescence emission spectra at 77°K. In dark-grown cells the maximum of emission lies at 636 nm with a secondary band at 657 nm. After a single actinic flash the 657 nm emission decreases and a new peak appears at 675 nm. From flash to flash, given every 3 min, the emission at 675 nm increases and shifts progressively to 685 nm, while the emission at 636 nm decreases. After about 60 flashes a band centered at 715 nm appears which thereafter shifts toward 730 nm. By about 1000 flashes the 77°K emission spectrum was found typical of normal light-grown Euglena, with a maximum at 720—730 nm and secondary emission bands at 685 and 695 nm.

INTRODUCTION

Dark-grown cells of *Euglena gracilis* have been used to follow the formation of the plastid membranes in the light [3,6,7]. Low temperature fluorescence emission spectra of cells and changes of these spectra during the first 4 h of continuous illumination have been reported [2]. This paper is concerned with changes in the 77°K fluorescence emission of etiolated Euglena cells illuminated with repeated light flashes of short duration.

MATERIAL AND METHODS

Cells of *Euglena gracilis* Klebs 6 ar variety bacillaris Pringsheim were grown for more than twenty generations in darkness at 22° on the medium described by Bach [1]. In other experiments the medium devised by Greenblatt and Schiff [5] was used.

The cells were cultivated in a semi-automatic fermentor allowing periodic withdrawal of a portion of the culture and the injection of fresh medium in

the culture vessel all under aseptic conditions. The culture device allowed us to maintain cells for up to 3 months in the logarithmic phase of growth. After withdrawal from the fermentor, the cell suspension was poured into a test tube fitted with a hypodermic needle connected to an air pump in order to keep the cells under aerobiosis during the experiment. The normal life cycle was continued in this way for as long as 50 h (1 division per day).

The cells were handled under a dim green safelight obtained by passing the light of a 4.5 V, 0.8 W tungsten lamp through two sheets of coloured plexiglas each 2 mm thick (Rohm and Haas, W. Germany, No. 303 and No. 701). This filter combination gave a transmission spectrum centered at 540 nm with a half band width of 35 nm; the transmission between 595 and 690 nm was less than 0.5%.

The actinic illumination was provided by a photographic-type electronic flash (type Multiblitz Report from Gesellschaft für Multiblitzgeräte, Dr. Ing. Mannesmann, Porz-Westhoven, Germany). This device has a flash duration of 0.56 msec at half peak with an electrical output of 125 joules. The flash tube was placed at a distance of 20 cm from the tube containing the sample.

The cultures were bubbled with normal air for at least 10 min before the illumination. As soon as the last flash of a given series of flashes was fired an aliquot of the culture was taken and was put on a sample holder. The sample holder was precooled in liquid nitrogen. The time elapsing between the last flash of a series and the time of cooling the sample at liquid nitrogen temperature was approximately 10 sec.

The fluorescence spectra of the frozen samples were measured at 77°K with a recording spectrofluorimeter similar to the one described by Sironval et al. [8]; the excitation wavelength was the 436 nm line of a high pressure mercury vapour lamp isolated by a suitable filter (Lamp Zeiss type: St 41; filter Zeiss M436). The spectra in Figs. 1 to 4 are not corrected for the transmission of the monochromator, nor for the spectral response curve of the photomultiplier. The pigment concentration in the sample was extremely low (in the order of 2.4 μg per g fresh weight); there was no distortion due to reabsorption. Due to the low amount of pigments we increased the sensitivity of the spectrofluorimeter to a rather high value. Under these conditions there appears a parasitic emission as a peak at 615 nm; this distorts the spectrum. A same parasitic light was observed when a suspension of microcristalline cellulose or of silicagel G (Merck) was examined at the same high sensitivity of the fluorimeter in the same geometrical conditions. To avoid this artefact a high-pass-filter made of red coloured glass with a low-wavelength cut off at 590 nm and a 50% transmission value at 632 nm was placed in front of the entrance slit of the monochromator. Due to the spectral transmission properties of that filter, the maximum of the fluorescence band in the 635 nm region was slightly shifted to the red by some 3—5 nm. This was not the case for higher wavelengths.

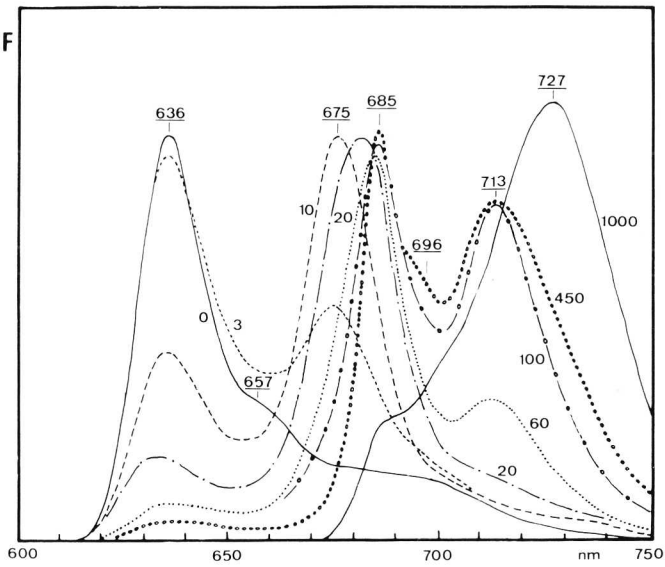

Fig.1. Changes in the form of the 77°K fluorescence emission of dark-grown Euglena (O) exposed to flashes given repeatedly every 3 min from 3 up to 1000 flashes (3, 10, 20 . . . , 450, 1000). Underlined figures represent wavelengths of characteristic emission bands. The cells were grown on the medium of Bach. The spectra refer to 0.6 ml samples from a same Euglena culture.

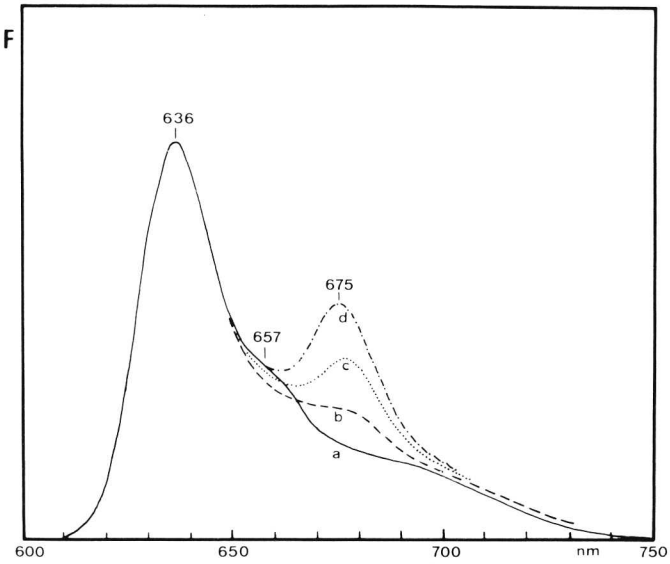

Fig.2. Changes in the form of the 77°K fluorescence emission of dark-grown Euglena after the first 3 flashes. The curves are fluorescence emission of etiolated cells (a) and cells given one (b), two (c) or three (d) flashes. Curves are adjusted to equal emissions at 636 nm. The cells are grown on the medium of Bach.

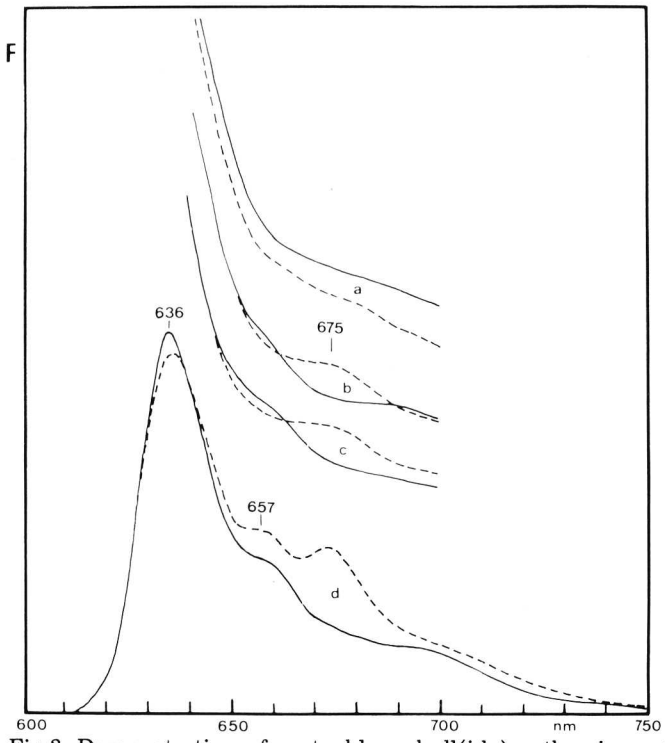

Fig.3. Demonstration of protochlorophyll(ide) as the pigment of dark-grown Euglena. (a) characteristic fluorescence emission of dark cells grown on Bach's medium. (b) fluorescence emission of an acetone extract (acetone—water 80:20 v/v) of these cells. The same spectrum also is obtained by exciting at 475 nm. Excitations of the emissions at 436 nm.

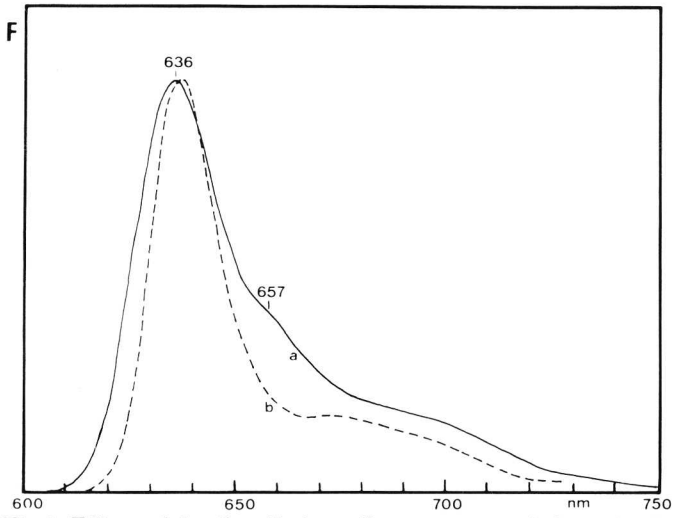

Fig.4. Effect of the first flash on fluorescence emission of cells with varied 657 nm emission. Full line, dark-grown Euglena; dashed line, the same cells 10 sec after the first flash. Cells with no 657 nm peak or shoulder (a) were grown on the medium of Greenblatt and Schiff. Cells with 657 nm shoulder or peak (b, c, d) were grown on the medium of Bach. In order to avoid curve superposition, the baseline was shifted upwards from d to a.

RESULTS

(1) Changes in the 77°K fluorescence during greening of etiolated Euglena under a flash regime

Representative 77°K fluorescence emission spectra of dark-grown Euglena which received from 3 up to 1000 flashes 3 min apart, under aerobiosis and at 22°, are shown in Fig. 1. The first 10 flashes produced a decrease of the 636 nm emission of the etiolated cells which was correlated with an increase of the emission at 674—675 nm. Additional flashes further decreased the fluorescence at 636 nm and the emission at 674—675 nm shifted to 685—686 nm. After some 60 flashes, the contribution of the 636 nm emission had become almost negligible; the emission at 685 nm did not shift further to longer wavelengths and a new emission band appeared around 713 nm which increased to about 450 flashes. Between 100 to 450 flashes a shoulder appeared at about 696 nm, while the peak at 685 nm became narrower. After flash No. 100 the 713 nm emission broadened and shifted to 727 nm. After 450 flashes the emission at 685 nm decreased. At some 1000 flashes the 77°K emission was found typical of normal light-grown Euglena with a single broad emission culminating at 720—730 nm, and with indications of bands around 695 and 685 nm.

(2) Changes observed at the first three flashes

Fig. 2, curves b, c and d reproduces the 77°K fluorescence emission spectra of initially etiolated cells which have been cooled after having received, under aerobiosis and at 22° one, two or three flashes respectively, the dark interval between the flashes being 3 min. Each spectrum is that of a sample frozen 10 sec after the last flash. The spectra have been adjusted to the same emission intensity at 636 nm in order to show the relative increase of the intensity of the emission at 675 nm from flash to flash. Each flash produced an increase at 675 nm. The first flash, but not the second and third flash produced a decrease in the region around 657 nm (Fig. 2, curves a and b; and Fig. 4).

(3) 77°K fluorescence of etiolated Euglena; effect of the first flash

The fluorescence emission of etiolated cells grown on Bach medium [1] had a main band at 636—637 nm and a shoulder at around 657 nm (Fig. 3, curve a). The intensity at 636—637 nm was always much higher than the intensity at 657 nm, but the ratio of the two varied from one batch of cells to another. In some cases a distinct band was seen at 657 nm (Fig. 4, curve d) and in other cases only a small shoulder (Fig. 4, curves b, c).

However, acetone extract (acetone—water 80:20 v/v) of the etiolated cells always had maximum fluorescence emission at 636 nm, without any indication of another emission peak between 636 and 750 nm. This result was independent of the ratio of the emission intensity of the living cells at 657 nm and 636 nm (Fig. 3, curve b). The emission of the extract was the same when exciting the fluorescence at 436 nm as when exciting it at 475 nm.

When the cells were grown in darkness at 22° on the medium used by Greenblatt and Schiff [5], they never exhibited, any shoulder at 657 nm (Fig. 4, curve a). The first flash produced no increase of the 675 nm emission of these cells (Fig. 4, curve a). However, when cultures with more or less pronounced 657 nm shoulder were flashed, an increase in emission at 675 nm was observed which was more pronounced in samples with a more prominent 657 nm band (Fig. 4, curves b,c,d).

DISCUSSION AND CONCLUSION

The observed sequence of changes af the 77°K fluorescence of etiolated Euglena cells subjected to light flashes given every 3 min (Figs. 1 and 2) resembles the sequence of changes observed in bean leaves submitted to a flash regime [8—10]. Etiolated Euglena show the emission of normal green cells after having received light for a total duration in the order of 1 sec in a series of some 1000 flashes.

The formation of chlorophyll by the flashes involves a photoreduction of protochlorophyllide since only protochlorophyllide is found at the start in the etiolated cells (Fig. 3, curve b). Egan and Schiff [4] have given evidence that protochlorophyll(ide) is the light detector for chlorophyll synthesis in Euglena.

In the etiolated cells, two forms of protochlorophyllide are seen with main emissions respectively around 635 and 655—660 nm, when the etiolated cells are grown on a Bach medium. The 655—660 nm emitting form is lacking when the culture is on the medium of Greenblatt and Schiff (Fig. 4). The main difference between the two media is the presence of saccharose in the former. On a Bach medium the 655—660 nm emission decreases or disappears almost completely at the first flash while a 675 nm emission appears. For technical reasons our observation of the effect of the flash refers to the situation 10 sec after the flash; we did not observe any shift of the emission at 675 nm after that time. The amount of 675 emission produced by the first flash depends on the amount of the 655—660 nm emission in the etiolated cells (Fig. 4, curves b, c and d). On the other hand, the first flash does not produce any 675 nm emission band in cells etiolated on the Greenblatt and Schiff medium hwich exhibit a relatively strong 636 nm, but no 657 nm emission band (Fig. 4, curve a). This indicates that: (*a*) the 636 nm emitting form is photoinactive in etiolated Euglena as it is in etiolated higher plants; (*b*) a same photoreaction as in higher plants occurs when etiolated Euglena are illuminated for the first time, i.e. a reaction involving the protochlorophyllide form emitting at 657 nm.

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