

CHLOROPHYLL *a* FLUORESCENCE OF *GONYAULAX POLYEDRA* GROWN ON A LIGHT-DARK CYCLE AND AFTER TRANSFER TO CONSTANT LIGHT

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Abstract—The chlorophyll *a* fluorescence properties of *Gonyaulax polyedra* cells before and after transfer from a light-dark cycle (LD) to constant dim light (LL) were investigated. The latter display a faster fluorescence transient from the level 'T' (intermediary peak) to 'D' (dip) to 'P' (peak) than the former (3 s as compared to 10 s), and a different pattern of decline in fluorescence from 'T' to 'D' and from 'P' to the steady state level with no clearly separable second wave of slow fluorescence change, referred to as 'S' (quasi steady state) → 'M' (maximum) → 'T' (terminal steady state). The above differences are constant features of cells in LD and LL, and are not dependent on the time of day. They are interpreted as evidence for a greater ratio of photosystem II/photosystem I activity in cells in LL.

After an initial photoadaptive response following transfer from LD to LL, the cell absorbance at room temperature and fluorescence emission spectra at 77 K for cells in LL and LD are comparable. The major emission peak is at 685–688 nm (from an antenna Chl *a* 680, perhaps Chl *a-c* complex), but, unlike higher plants and other algae, the emission bands at 696–698 nm (from Chl *a_{II}* complex, Chl *a* 685, close to reaction center II) and 710–720 nm (from Chl *a_I* complexes, Chl *a* 695, close to reaction center I) are very minor and could be observed only in the fluorescence emission difference spectra of LL minus LD cells and in the ratio spectra of DCMU-treated to non-treated cells.

Comparison of emission spectra of cells in LL and LD suggested that, in LL, there is a slightly greater net excitation energy transfer from the light-harvesting peridinin-Chl *a* (Chl *a* 670) complex, fluorescing at 675 nm, to the other antenna chlorophyll *a* complex fluorescing at 685–688 nm, and from the Chl *a_{II}* complex to the reaction center II. Comparison of excitation spectra of fluorescence of LL and LD cells, in the presence of DCMU, confirmed that cells in LL transfer energy more extensively from the peridinin-Chl *a* complex to other Chl *a* complexes than do cells in LD.

INTRODUCTION

Photosynthesis in the dinoflagellate *Gonyaulax polyedra* is under the control of a circadian oscillator (Hastings *et al.*, 1961; Prézélin and Sweeney, 1977). Photosynthesis in other dinoflagellates (Prézélin *et al.*, 1977) and in naturally occurring phytoplankton assemblages (Prézélin, 1978) is also rhythmic; hence, the phenomenon is of general importance (see Sweeney and Prézélin, 1978). Prézélin and Sweeney (1977) demonstrated that the room temperature chlorophyll (Chl) *a* fluorescence of *Gonyaulax*, plotted as the ratio of fluorescence intensity measured 30 s to 1 min after the beginning of irradiation to that in the presence of 3-(3,4 dichlorophenyl) 1,1, dimethylurea (DCMU), is rhythmic with a maximum value in the middle of the night phase of the circadian cycle. This finding

sparked our interest in exploring other rhythmic characteristics of fluorescence in the hope of explaining the manner in which the photosynthetic apparatus is linked to the circadian oscillator. The rhythmic fluorescence parameters are described in another paper (Sweeney *et al.*, 1979). To our knowledge, there are no previous measurements of the fluorescence characteristics of the dinoflagellates, in which the photosynthetic pigments include the peridinin-chlorophyll-protein (not found in other algal groups, Prézélin and Haxo, 1976). Thus, we present here several fluorescence characteristics of the dinoflagellate *Gonyaulax* under two conditions, an entraining light-dark cycle (LD) and constant light and temperature (LL), used to detect the presence of a circadian oscillation as distinct from a process responsive to light or darkness (Sweeney, 1977): (1) *fluorescence induction* that provides information on the activity of photosystem II of photosynthesis and its interaction with photosystem I (see Govindjee and Papageorgiou, 1971; Mohanty and Govindjee, 1974; Papageorgiou, 1975; Lavorel and Etienne, 1977); (2) *fluorescence emission spectra* that provide information on the presence of

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Abbreviations: Chl *a*, chlorophyll *a*; DCMU, 3-(3,4 dichlorophenyl) 1,1-dimethylurea; PS I, photosystem I; PS II, photosystem II; LD, light-dark cycle; LL, continuous light.

different fluorescent species and on the excitation energy transfer among them (see Goedheer, 1972; Govindjee *et al.*, 1973); and (3) *fluorescence excitation spectra* that provide information on the different absorbing species that fluoresce or transfer energy to the fluorescent species.

MATERIALS AND METHODS

Culture conditions. *Gonyaulax polyedra* Stein, clone 70 A, was grown in unialgal batch cultures in 2-l Erlenmeyer flasks containing 1 l of f/2 medium (Guillard and Ryther, 1962). Overhead illumination was provided by banks of cool-white fluorescence lamps (1 mWcm^{-2}). Cells were cultured on an alternating 12 h light-dark schedule (LD) at 22°C. Half of the cultures were transferred from LD at the beginning of the light period to continuous light (LL) at an illumination of 0.5 mWcm^{-2} . Experimental measurements were begun after 2 days in LL.

Sampling methods. At each experimental measurement time, a 50-ml suspension of late exponential cells (7×10^3 cells/ml) was harvested by gentle centrifugation (room temperature, 3 min, $\sim 400 \times g$) and resuspended in 3.0 ml of fresh f/2 medium. A 0.2-ml aliquot was taken for cell counts. All experimental results are expressed on a per cell basis. This was an acceptable method because there was no significant change in the gross pigment content of the cells. The remaining sample was incubated in the dark for 10 min and the room temperature fluorescence was measured. The sample was then divided, 1.0 ml being dark-adapted, adsorbed on cheesecloth, and cooled quickly to liquid nitrogen temperature (77 K) for low temperature fluorescence measurements. The remaining 1.8 ml of cells were used to establish that a circadian rhythm indeed existed in the rates of photosynthetic oxygen evolution in both LD and LL cells used in the present study (see Sweeney *et al.*, 1979).

Pigment determination. Estimates of the chlorophyll content of cultures in LD and LL were made by harvesting 50 ml of each culture by gentle membrane filtration, extracting overnight in cold 90% acetone with MgCO_3 , and determining the absorption spectra of the acetone supernatants. Chlorophyll *a* and *c* concentrations were calculated from the equations of Jeffrey *et al.* (1975). For pigments *in vivo*, absorption spectra of cells suspended in 40% ficoll (to reduce settling) were measured with a Cary 14 R spectrophotometer by scanning the suspensions against adjusted scattering samples of freshly prepared BaSO_4 in 40% ficoll.

Fluorescence measurements. Low temperature (77 K) fluorescence emission spectra were measured with the spectrofluorometer described by Shimony *et al.* (1967) and by the methods described by Cho (1969). Front surface fluorescence was detected by an EMI 9558B photomultiplier (S-20 response) through a Corning CS 2-61 sharp cut-off glass filter (thickness, 2.5 mm) and a Bausch and Lomb monochromator (blazed at 750 nm; band pass, 1.0 nm). This narrow band width (± 0.5 nm) permitted resolution of peaks separated by 1.0 nm. Fluorescence was excited with a Radiant Lamp Corporation (Newark, N.J.) DDY 750 W tungsten filament projection lamp through heat filters and a combination of Corning CS 3-73 and CS 4-96 glass filters (maximum, ~ 480 nm; half-maximum band-width, ~ 150 nm). The light flux at the sample measured with a Yellow Springs radiometer (Model No. 63) was 200 W m^{-2} . The photocurrent from the photomultiplier was converted to voltage (using an operational amplifier and a feedback resistor of 10^8 ohms), passed through a low pass filter (time constant, 100 ms), and displayed on a strip-chart Esterline Angus recorder (Model, E 1101S). The wavelength scan speed was 100 nm/90 s. Emission spectra were corrected, point by point, for photomultiplier sensitivity and transmission characteristics of the mono-

chromator. For the 77 K excitation spectra of fluorescence at 750 nm (band pass, 6.6 nm), the samples were excited with a microscope illumination lamp (tungsten ribbon filament, General Electric Type 18A/T10/1P-6V) through a Bausch and Lomb monochromator (blazed at 300 nm; band pass, 9.9 nm). The excitation spectra were corrected for the numbers of incident quanta. A fast camera shutter between the projection lamp and the sample was used for measurements of fluorescence transients (for details, see Munday and Govindjee, 1969). For samples at room temperature, the photomultiplier signal was amplified with a Tektronix 502 oscilloscope and displayed on a strip-chart recorder. At 77 K, a Biomation Waveform recorder (Model 805) was used instead of the oscilloscope.

RESULTS

Pigment content

Chlorophyll *a* content, from the pigment extraction data, was within a narrow range (3 to 3.5×10^{-14} mol cell $^{-1}$) for LD cells and cells in LL after 2 days of transfer, cells in LL containing only slightly less chlorophyll than cells in LD. Chlorophyll *c*₂ content was also within a narrow range (2 to 2.3×10^{-14} mol cell), cells in LL again containing only slightly less than cells in LD. Although LL cells had presumably undergone an initial photoadaptive response upon transfer to continuous illumination, the pigment analysis and the absorption spectra (not shown) confirm that total pigmentation and gross absorbance of LL and LD cells are not significantly different at the time of comparison as noted earlier by Prézelin *et al.* (1977) and Prézelin and Sweeney (1977). We, however, noted small but important differences in the absorption spectra: a slight (1–2 nm) shift

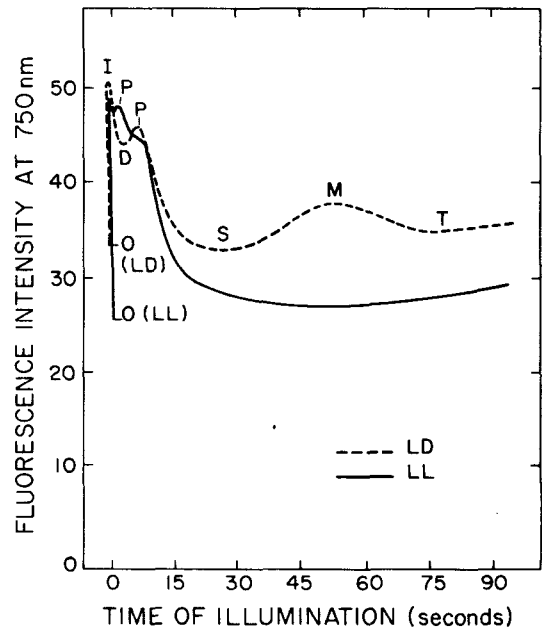


Figure 1. Chlorophyll *a* fluorescence transients of *G. polyedra* cultured on a light-dark cycle (LD, dashed line) and transferred to continuous dim illumination (LL, solid line). See text for definition of symbols. Temperature, 22°C. Dark adaptation, 10 min.

of the red peak to the long wavelength side and a decreased ratio of absorbance by long wavelength to short wavelength spectral forms of Chl *a* in LL (~ 0.05) than in LD (~ 0.10) cells.

Fluorescence transients

Features of chlorophyll *a* fluorescence induction curves (Fig. 1) that characterize LL cells as being distinct from LD cells are: (1) the ratio of intermediary peak 'T' to dip 'D' is smaller; (2) peak 'P' is almost as high as 'T'; (3) 'P' is reached earlier, within 3 s, as opposed to 10 s in LD cells; (4) the 'P' to 'T' (terminal steady state) transient goes through a hump at about 10 s that may represent the maximum 'M'; (5) the usual S (quasi-steady state) \rightarrow M \rightarrow T transient is obscured due to the presumably early appearance of 'M'; these 'SMT' changes were not observed in LL at any intensity used; (6) the ratio of the maximum variable (ΔF ; P-O) to the so-called "constant" (O) fluorescence is higher in LL cells, and finally, (7) the 'O' level is lower in LL than in LD cells.

Since the 'O' level originates in both PS I and PS II and the variable fluorescence (ΔF) only in PS II (Govindjee and Papageorgiou, 1971), both the lower 'O' level and higher $\Delta F/O$ ratio indicate the greater ratio of PS II/PS I activity in LL cells. This conclusion is supported by the kinetic differences described above as a higher ratio of PS II to PS I activity leads to faster removal of the quencher Q (see Duysens and Sweers, 1963) and, thus, of an earlier appearance of 'P'. Differences in 'SMT' changes are, however, due to changes in the thylakoid membrane (see Discussion).

The above characteristic differences in the fluorescence induction curves were constant features of LD and LL cells and were arrhythmic.

Fluorescence emission

The fluorescence emission properties of cultures in LD and LL were compared to see if the initial photoadaptive response of LL cells had resulted in a change in the relative proportion of different Chl *a* forms and/or in their energy transfer distribution leading to changes in PS II/PS I activity ratios noted above. A low-temperature (77 K) emission spectrum of LD night phase cells of *G. polyedra* (Fig. 2, dashed curve) shows the major Chl *a* emission maximum at about 688 nm and small fluorescence inflections at about 670–675, 705–710 and 725–735 nm. The emission band of Chl *a* in the peridinin-Chl *a*-protein (PCP) is at 672–675 nm (Prézelin and Haxo, 1976; Song *et al.*, 1976; Koka and Song, 1977) and, thus, accounts for the 675 nm emission band seen in the whole cell fluorescence emission spectrum of *Gonyaulax*. The 688 nm emission band is ascribed to the remaining light-harvesting Chl *a*-proteins (Thorner *et al.*, 1976; Prézelin and Alberte, 1978). By analogy to other systems (see Govindjee *et al.*, 1973), the long wavelength bands (705–735 nm) are suggested to originate in Chl *a* in PS I.

The 77 K emission spectrum for LL cells (Fig. 2, solid curve) appeared qualitatively similar to that of LD cells, except that the major emission maximum was blue-shifted by 1 nm to 687 nm, accompanied by a narrowing of the band on the longwave side. The difference spectrum of the 77 K fluorescence emission of LL and LD cells [$F(LL) - F(LD)$] (Fig. 2, inset) provides resolution of an emission band at 690–695 nm (from Chl a_{II}), and suggests that a difference exists in its relative intensity in LL and LD cells. This is not due to a shift in the absorbance bands as that shift is to the red end of the spectrum, in contrast to the blue shift in the emission spectra for

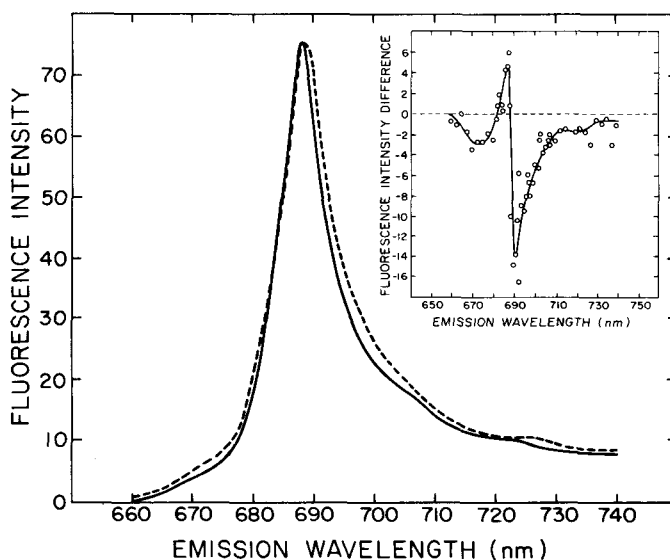


Figure 2. Fluorescence emission spectra of *G. polyedra* cultured on a light-dark cycle (LD, dashed line) and transferred to continuous dim illumination (LL, solid line). Temperature, 77 K. Band pass, 1.0 nm. Excitation, broad band blue light. *Inset*: Difference spectrum, $F(LL) - F(LD)$, of low temperature (77 K) fluorescence emission spectra of *G. polyedra*.

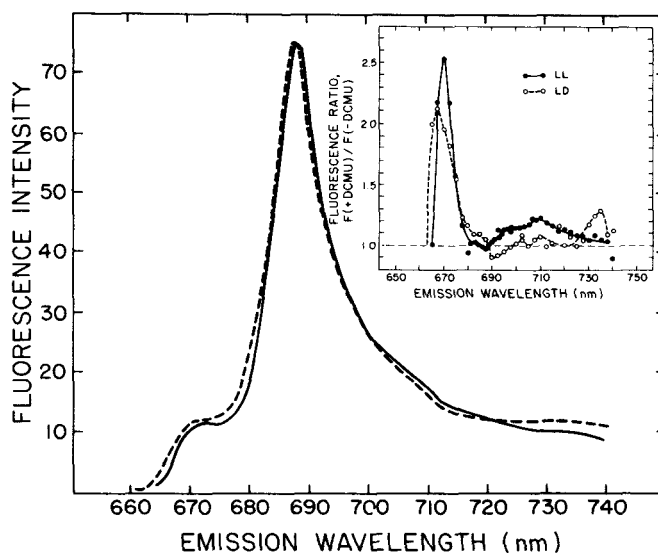


Figure 3. Fluorescence emission spectra of *G. polyedra* cultured on a light-dark cycle (LD, dashed line) and transferred to continuous dim illumination (LL, solid line). Measurements were made in the presence of $10 \mu\text{M}$ DCMU. Temperature, 77 K. Bandpass, 1.0 nm. Excitation, broad band blue light. Inset: Ratio spectra $F(+\text{DCMU})/F(-\text{DCMU})$ of low temperature (77 K) emission spectra of *G. polyedra*, measured in the presence (+) and absence (-) of $10 \mu\text{M}$ DCMU. Cells were cultured on a light-dark cycle (LD, dashed line) and transferred to continuous dim illumination (LL, solid line).

LL as compared to LD cells. The simplest interpretation of the difference bands (Fig. 2, inset) is an increased energy transfer from peridinin-Chl complex (fluorescing at 675 nm) to other complexes (fluorescing at 685 nm), and from Chl a_{II} (fluorescing at 690–695 nm) to the reaction center II in LL than in LD cells. Some of these conclusions were confirmed by results on excitation spectra (see later), and are consistent with the earlier interpretation of a greater ratio of PS II/PS I activities in LL than in LD cells.

Figure 3 shows the emission spectra of LL (solid line) and LD (dashed line) night phase cells in the presence of $10 \mu\text{M}$ DCMU, and the inset shows the effect of DCMU on the emission spectra, plotted as ratio spectra $F(+\text{DCMU})/F(-\text{DCMU})$, for cells in LD and LL. The main changes due to DCMU addition seem to be increases in emission at 670–675 (from Chl a in PCP), 695–700 (Chl a_{II}) and 710 nm (Chl a_1). Since reaction center II is closed in the presence of DCMU, the increases at 670–675 and 695–700 nm are interpreted as a net decrease in energy transfer from PCP and Chl a_{II} to reaction center II. This must increase the excited state lifetimes in PCP and Chl a_{II} leading to a small net increase in energy transfer to Chl a_1 giving the peak at ~ 710 nm in the inset. The increases in several Chl a emission bands upon closure of reaction center II, observed here, are consistent with the earlier observations at room temperature on the effects of DCMU on changes in excitation energy transfer, as deduced from changes in the degree of polarization of fluorescence (Mar and Govindjee, 1972; Govindjee and Wong, 1978; Van Rensen *et al.*, 1978) and in the emission spectra (Krey and Govindjee, 1966; Govindjee and Briantais, 1972; Van

Rensen *et al.*, 1978) in *Porphyridium*, *Chlorella* and in chloroplasts from higher plants. Differences between LL and LD cells are mainly due to slight changes in absorption spectra.

Fluorescence excitation

Differences in LL and LD fluorescence properties were observed in the 77 K excitation spectra of Chl a fluorescence measured at 750 ± 9 nm in *G. polyedra* cells poisoned with $10 \mu\text{M}$ DCMU (Fig. 4). In both LL (solid line) and LD (dashed line) cells, the excitation spectra corrected for numbers of incident photons showed a striking difference from the absorption spectra of the same cell suspensions. Between 400 and 500 nm, the excitation spectra are much lower, as compared to the Chl a band around 675 nm. This must be due to a very low average efficiency of excitation energy transfer from carotenoids (diadinoxanthin and dinoxanthin) and/or due to the fact that some carotenoids are not in the photosynthetic machinery and act as filters of incident light falling on the thylakoids. Such conclusions have been made earlier from other systems (Emerson and Lewis, 1942; Duysens, 1952; see also Goedheer, 1972; Boardman *et al.*, 1978).

That PCP (fluorescing at 670–675 nm) is more effective in LL cells in transferring energy to the antenna Chl a fluorescing at 685 nm, was suggested by the fluorescence emission difference spectrum (Fig. 2, inset). In the presence of DCMU, the ratio of effectiveness of PCP to Chl a , as shown in the difference excitation spectrum for 750 nm fluorescence of LL and LD cells (Fig. 4, bottom), further suggests a more effective energy transfer to Chl a forms fluor-

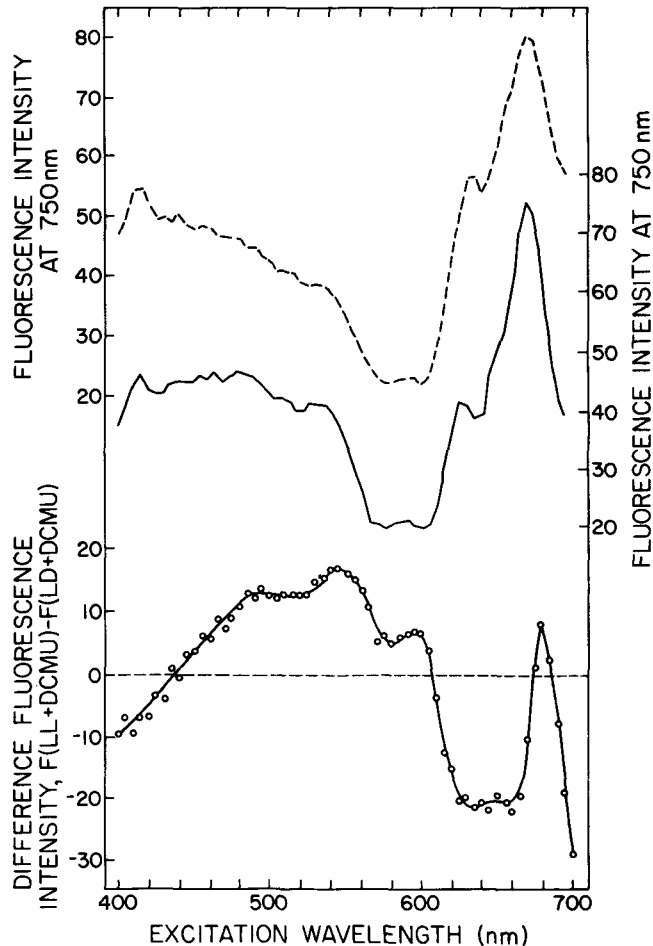


Figure 4. Fluorescence excitation spectrum of *G. polyedra* cultured on a light-dark cycle (LD, dashed line; top curve) and under continuous dim illumination (LL, solid line; middle curve) measured in the presence of $10 \mu\text{M}$ DCMU. Temperature, 77 K. Slitwidth, 10.0 nm. Measuring wavelength, $750 \pm 9 \text{ nm}$. Difference spectrum $[F(\text{LL} + \text{DCMU}) - F(\text{LD} + \text{DCMU})]$ of 77 K fluorescence excitation spectra (bottom curve).

escing at 750 nm (F_{750}) in LL cells. The excitation difference spectrum of F_{750} between LL and LD cells treated with DCMU resembles the absorption spectrum of PCP at low temperatures (Prézelin and Haxo, 1976).

DISCUSSION

(1) Fluorescence transients: higher ratio of activity of PS II to PS I in LL cells

The present study of the fluorescence properties of *G. polyedra*, showing changes induced in some fluorescence parameters by the transfer of cells from LD and LL conditions, led to new information on the nature of the photosynthetic apparatus of dinoflagellates. The most important feature of *G. polyedra* fluorescence data is the difference between the fluorescence induction curves of LL and LD cells. The LD cells show the fast fluorescence ($O \rightarrow I \rightarrow D \rightarrow P$), followed by a decline to the 'S' level and the usual slow 'SMT' fluorescence change (Fig. 1). This compares well with

green algae (see Papageorgiou and Govindjee, 1968), except that here the 'P' level is lower than the 'I' level, perhaps because of a slightly greater ratio of PS I to PS II activity than in other algal cells. The most striking difference as a result of the LD to LL transfer is a change in the time course and nature of the fluorescence induction curves. Cells of *G. polyedra* in LL displayed a faster fluorescence rise to 'P', and a different pattern of fluorescence decline to the level 'T', without the usual slow 'SMT' fluorescence change, which is replaced by an indistinct SMT transient, 'M' appearing at 10 s in contrast to 45 s in LD cells. Since the gross pigment content per cell is not significantly different between LD and LL cells, the absorption per cell is almost constant for the same number of incident quanta. Thus, compared to LD cells, the shorter time needed to reach 'P' in LL cells cannot be related to the intensity-dependent nature of 'P' (Munday, 1968), but is due either to a more efficient use of absorbed quanta in the initial photochemical transformations of PS II or to a

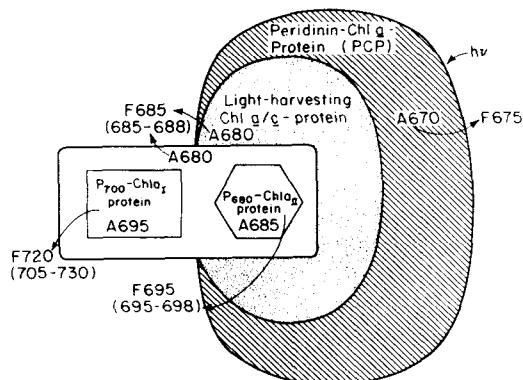


Figure 5. A working model for the possible assignment of absorption (A) and emission (F) maxima, in nm, to the various Chl *a* complexes in *G. polyedra* (cf. Prézélin and Alberte, 1978).

slower interaction with PS I (also, see Results). Support for this possibility comes from arguments that the efficiencies of energy transfer from Chl *a* in PCP to the other antenna Chl *a* and from PCP and Chl *a*₁₁ to the reaction center II is probably higher in LL cells (Figs. 2, 4).

The different characteristics of fluorescence decline from 'P' to 'T' in LL cells may be related to the differences in the distribution, between PS I and PS II, of electronic excitation of Chl *a* by the light-harvesting pigments or of changes in H⁺ (inside) which affect the state of the membrane (Papageorgiou, 1975). It has been suggested that the 'P' to 'S' decay is affected by the rate of photoconversion of the photosynthetic apparatus from state I (low 'spillover' state; more Mg²⁺ or less H⁺ in the thylakoid) to state II (high 'spillover' state; less Mg²⁺ or more H⁺ in the thylakoid); in state II more energy spills over from the strongly fluorescent PS II to the weakly fluorescent PS I (see Govindjee and Papageorgiou, 1971; Mohanty and Govindjee, 1974). If the initial distribution of absorbed light between the photosystems had been altered by the photo-adaptation to LL conditions, then it would be expected that the fluorescence kinetics would also be affected. There were several suggestions in this paper that excitation energy flow had indeed changed.

The usual 'SMT' transient was not seen in LL cells although measurements were made over a wide range of light intensities. These slow fluorescence changes were obscured since the entire transient occurred faster and the 'M' was at 10 s. Slow fluorescence changes are complicated phenomena, being responses to State I-State II photoconversion or to membrane alterations caused by changes in phosphorylating conditions (see above discussion). In any case it seems clear that 'SMT' transients are due to differences in the organization of the pigment complexes in the thylakoid membrane. It appears that a higher PS II/PS I activity in LL cells can explain most of the differences between LL and LD cells.

All the fluorescence transient differences described here were constant features of LD and LL cells, not dependent either on the preparation procedure or the time of the day.

(2) Fluorescence emission spectra: four Chl *a* complexes

The fluorescence emission curves of *G. polyedra* at 77 K show an obvious lack of dominant bands at 695–698 nm (Chl *a*₁₁) and 720–740 nm (F730) (Figs. 2 and 3). This is in sharp contrast to 3- or 4-peaked emission curves described for most other algae and chloroplasts from higher plants (Govindjee, 1966; Cederstrand and Govindjee, 1966; Govindjee and Yang, 1966; Krey and Govindjee, 1966; Gasanov and Govindjee, 1974; Rijgersberg *et al.*, 1979). However, a similar situation is observed in the diatoms (Goedheer, 1972).

In spite of the simplicity of the emission spectra presented for *G. polyedra*, a careful examination of the absorption spectra, emission spectra (Figs 2 and 3), difference emission spectrum between LL and LD cells (Fig. 2, inset), ratio spectrum between DCMU-treated and control cells (Fig. 3 inset), the excitation spectra of Chl *a* fluorescence (Fig. 4) and the difference excitation spectrum (Fig. 4) together reveal that *G. polyedra* contains at least four Chl *a* complexes (in addition to those in the two reaction center I and II which are, perhaps, so weakly fluorescent that they cannot be observed) (Fig. 5). Based on approximate Stoke's shifts of 5–15 nm, we use their possible absorption peaks as subscripts and their possible emission peaks as superscripts, the following Chl *a* spectral forms must be present in *G. polyedra*: Chl a_{670}^{675} (antenna Chl *a* in peridinin-Chl *a* complex, PCP); Chl $a_{678-681}^{686-687}$ (antenna Chl *a*, possibly in Chl *a*-Chl *c* complexes; Chl *c* is non-fluorescent because it transfers energy with 100% efficiency to this Chl *a*); Chl $a_{11685}^{690-695}$ (associated with Chl *a* 685 close to reaction center II); and Chl $a_{1690-705}^{705-720}$ (associated with Chl *a* 695, close to reaction center I) (see Prézélin and Alberte, 1978, for a model of organization of these complexes). A fifth minor band fluorescing around 730 nm is inferred from a comparison of the 77 K emission spectra of LL and LD cells treated with DCMU: this emission in LD - LL difference spectra (not shown) could not be from Chl $a_{1690-705}^{705-720}$ because the difference emission at 730 nm is positive but that at 710 nm is negative; it could, however, be from another minor long-wavelength-emitting complex.

(3) Energy distribution and transfer in PS I and PS II

The inability to observe PS I activity through P₇₀₀ changes (Prézélin, *et al.*, 1976) or the intense F730 band in intact cells of *G. polyedra* (Figs. 2 and 3) and a *Glenodinium* sp. suggest something new about this group of algae. It could be that the spectral differences between PS I and II are minimal in these algae, and the balanced excitation of the two

pigment systems does not allow net oxidation or reduction of P700 *in vivo*. That PCP is 100% efficient in transferring absorbed light energy to other Chl *a* complexes and in promoting whole cell oxygen evolution, has been previously demonstrated (Prézélin and Haxo, 1976; Prézélin *et al.*, 1976; Song *et al.*, 1976). Interestingly, the transfer of cells from LD to LL seems to alter slightly the energy distribution pattern (see Results) in the photosynthetic apparatus.

It is clear that the changes brought on by the LD to LL transfer reflect adaptive processes, independent of regulation by a biological clock, in which *G. polyedra* adjust chlorophyll organization and excitation energy distributions to deal, perhaps, with

physiological changes imposed by lowering incident light levels and daylength prolonged to 24 h. In another paper (Sweeney *et al.*, 1979), we have presented our data on the circadian rhythm in fluorescence intensities at various points on the fluorescence transient curves.

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