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RESEARCH NOTE

IN VIVO CHLOROPHYLL *a* FLUORESCENCE TRANSIENTS AND THE CIRCADIAN RHYTHM OF PHOTOSYNTHESIS IN *GONYAULAX POLYEDRA*

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Abstract—The intensity of chlorophyll *a* fluorescence during the early part of fluorescence induction at *O*, initial fluorescence, and *P*, peak fluorescence, was higher during the day phase of the circadian cycle than during the night phase in continuous light (LL) conditions and was positively correlated with the rate of oxygen evolution. The circadian rhythm in fluorescence in LL persisted in the presence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which blocks electron flow from photosystem (PS) II in photosynthesis. The rhythmic changes in fluorescence intensity are consistent with a lower rate constant for radiationless transitions during the day phase than during the night phase of the circadian rhythmicity. The circadian changes in the intensity of fluorescence were abolished at 77K, which may indicate the importance of structural changes in membranes in circadian oscillations.

INTRODUCTION

The marine dinoflagellate, *Gonyaulax polyedra*, has been extensively used in studies of circadian rhythms because its bioluminescence, photosynthesis and cell division display very distinct circadian variations that continue for many days under constant conditions of light and temperature (Sweeney and Hastings, 1957, 1958; Hastings *et al.*, 1961). There is good evidence that this cell possesses a single oscillating mechanism that controls the timing of all these rhythms (McMurry, 1971). Its nature is still a matter of debate (Sweeney, 1976; Sweeney and Prézelin, 1978). The rhythmicity at low irradiances could be due to changes in the number of reaction center Chl *a* molecules but could also result from changes in primary photochemical events in photosynthesis, such as light absorption, excitation energy transfer, trapping of energy in the reaction center or the primary oxidation–reduction reaction at the reaction center. A similar conclusion was reached in the case of the photosynthetic rhythm in *Euglena* (Lonergan and Sargent, 1978). The investigation of the room temperature Chl *a* fluorescence transients in living cells has yielded valuable information regarding the organization of the photosynthetic apparatus and the timecourse of electron flow in a number of algae and higher plants (Govindjee and Papageorgiou, 1971; Mohanty and Govindjee, 1973; Papageorgiou, 1975; Lavorel and Etienne, 1977).

In the present paper, we discuss the constant and variable features of Chl *a* fluorescence induction in cells in both a light–dark cycle (LD) and constant light (LL) from the point of view of circadian rhythmicity. A clear correlation between fluorescence intensity and photosynthesis can be observed.

MATERIALS AND METHODS

Culture conditions. Gonyaulax polyedra Stein, clone 70A, was grown at 22°C in duplicate 1-*l* cultures in 2-*l* Erlenmeyer flasks in a light–dark cycle with 12 h each illumination (4000 lux) and darkness. Several days before the beginning of the experiments, one culture was moved to continuous illumination at the same temperature and one-half the irradiance. Samples were removed from these cultures at 2–4 h intervals over 2 days (50-m*l* samples concentrated by centrifugation to 3 m*l*) and measurements of cell number, room temperature Chl *a* fluorescence induction over a time-scale of ms to s, low temperature fluorescence transients at 77 K and oxygen evolution were made at each sampling time.

Fluorescence measurements. Fluorescence was excited with broad-band blue light and detected at 685 nm (bandpass, 20 nm) (for details, see Shimony *et al.*, 1967; Munday and Govindjee, 1969). Using a Dewar flask with a transparent bottom, the sample (1 m*l*), adsorbed on cheese cloth, was quickly frozen when low temperature fluorescence was to be measured (for details, see Cho, 1969). All cell samples were dark-adapted for at least 10 min before use. The exciting irradiance was about 200 $W \cdot m^{-2}$. Fluorescence was measured with a Biomation Waveform Recorder (model 805) at a fast time resolution (5 μs and

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5 ms sampling rate) for the detection of the *O* level of fluorescence induction and measurements at 77 K and at a slower time resolution for the measurement of the remainder of the fluorescence transients using an Esterline Angus stripchart recorder (model E 1101S). All fluorescence and oxygen measurements were corrected for cell number. The following nomenclature see Govindjee and Papageorgiou, 1971; Papageorgiou, 1975) was adopted for the various phases of the curve for fluorescence as a function of time after the beginning of illumination, *O*, the initial level; *I*, the first maximum; *D*, the dip which may follow; *P*, the principal peak in the fluorescence induction curve; *S*, the lower level of fluorescence which immediately succeeds *P*; *M*, a late maximum, usually lower than *P*; and *T*, the terminal level of fluorescence. In LL, separate *S*, *M* and *T* levels cannot be distinguished (details to be published later).

Oxygen evolution. The oxygen evolved by samples of the cell suspension irradiated with saturating white light and maintained at 20°C by means of a circulating water bath was measured using a Yellow Springs Instruments Clark-type oxygen electrode. Photosynthetic rates were corrected for respiration during the subsequent dark period. The electrode sensitivity was standardized against the oxygen content of air.

All fluorescence data were graphed as a function of time of day and examined for the presence of rhythmicity. Only rhythms which appeared in LL were considered circadian and hence of interest here.

RESULTS AND DISCUSSION

During the course of measurements at frequent intervals for 2 days, we observed no differences in the *shape* of the fluorescence transients, which resembled those in higher plants and other algae. Thus, the time course of the fluorescence transients from the initial level, *O* to the fluorescence maximum at *P* and finally to the terminal level *T* do not show any circadian changes, either in LD or in LL. To reach this conclusion, a number of different measurements of fluorescence were made at different times after the beginning of the excitation of fluorescence and the half-times for the different phases ($O \rightarrow P$; $P \rightarrow S$; $S \rightarrow M$; $M \rightarrow T$) were determined. The data were examined for evidence of circadian rhythms both the same and different in phase from that of the photosynthetic rhythm. The absence of any differences in the temporal relationships between different stages in fluorescence induction lead us to the conclusion that the ratio of photosystem (PS)II to PSI electron flow and excitation energy distribution are constant over circadian time, at least during the time immediately after the beginning of illumination, and hence are not factors by which the circadian oscillator controls the rate of photosynthesis.

There is, however, a consistent difference in the fluorescence intensities at various points in the induction curves correlated with time in the circadian cycle, both in LD and in LL cells. A comparison of the *intensity* of the room temperature fluorescence of Chl *a* at different times along the curve for fluorescence induction indicates that there is a circadian variation in the intensity of the fluorescence levels at *O* (Table 1) and most clearly at *P* (Fig. 1). Each point in this figure is from a separate measurement of the fluorescence transient. It will be noted that adjacent points agree very closely in most cases. The rhythms in *P* and in oxygen evolution both show a period of less than 24 h, and the 2 rhythms are correlated in phase (correlation coefficient, 0.87). Rhythmicity in both the intensity of fluorescence at *P* and oxygen evolution in photosynthesis has persisted in LL for 3 days prior to the beginning of the measurements presented here.

Table 1. The relative magnitude of fluorescence immediately after the beginning of illumination, the *O* level in *Gonyaulax polyedra* in continuous light at room temperature, measured at different times of day and a comparison of these values with the circadian rhythm of photosynthesis measured as μmoles O_2 evolved by 10^6 cells per h. Fast recording. Day 2 of measurements in LL, 2/28/78.

Time	μmoles O_2 h^{-1} 10^6 $cells^{-1}$	*O*
0010	4.1	112
0155	4.1	103
0540	5.5	122
1000	8.3	161
1300	8.5	179
2125	6.7	145

Although the correlation shown here is with oxygen evolution at saturating light intensity, previous measurements (Prézelin and Sweeney, 1977) have established the associated rhythm in relative quantum yield of photosynthesis. Furthermore, the differences in the intensity of fluorescence reported here are differences in the quantum yield of fluorescence, because the intensity of the exciting light was kept constant throughout the experiment and previous experiments

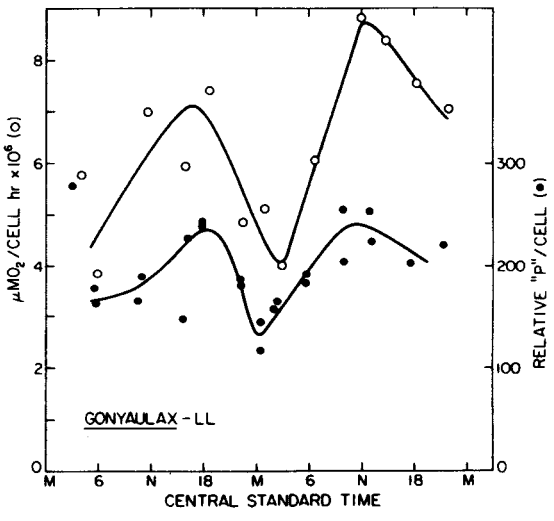

Figure 1. The circadian rhythms in photosynthetic oxygen evolution and the relative magnitude of the chlorophyll (Chl) *a* fluorescence at stage *P* in the same cell suspension of *Gonyaulax polyedra* from continuous light, (LL) 2000 lux and 22°C. Note the similarity in the shape of the two curves. Both oxygen evolution and fluorescence values have been corrected for cell number. Each point is a single determination of *P* or oxygen evolution.

have shown that there are no significant circadian changes in pigment content (Prézelin and Sweeney, 1977). Thus, the circadian rhythm in the quantum yield of photosynthesis parallels those in the quantum yield of Chl *a* fluorescence.

It was previously reported (Prézelin and Sweeney, 1977) that Chl *a* fluorescence, measured as the ratio of the fluorescence intensity some minutes after the beginning of illumination (F) to that when the cells had been exposed for some time to 10 μM DCMU before irradiation (F_{DCMU}), showed a circadian rhythm with a maximum in the middle of the night phase. Thus this rhythm in F/F_{DCMU} was opposite in phase to and of much smaller magnitude than both the rhythm in photosynthesis and in the intensity of fluorescence during induction at P. This ratio was remeasured using the same cell suspension and apparatus as that used for the measurements of fluorescence induction. The observation of a larger ratio at night (0.89) than during the day (0.73) was confirmed. The seeming contradiction can be resolved since the presence of DCMU increases the fluorescence more during the day than at night ($\times 1.4$ at noon as compared with $\times 1.1$ at midnight). Thus, the ratio F/F_{DCMU} is smaller during the day than at night although the level of fluorescence is higher.

One interesting observation made in the course of these studies which may have some bearing on the function of membranes in the circadian oscillator (Njus *et al.*, 1974; Sweeney, 1974) is that no circadian changes in the intensity of fluorescence can be detected at 77 K. This might be expected if the differences in the intensity of fluorescence over the course of a cycle were due to differences in membrane fluidity, since such membrane differences would be abolished by freezing to 77 K.

Since circadian changes in the fluorescence at P occur in the presence of the inhibitor DCMU, the cause is not a change in the net electron transport between PS II and PS I because this transport is blocked by DCMU. The observed temporal changes in fluorescence could also arise from differences in the efficiency of spillover of energy from the strongly fluorescent PS II to the weakly fluorescent PS I. Such spillover should take place unimpaired at 77 K. The absence of rhythmicity in P at this temperature argues against this explanation, as does the lack of changes in the shapes of the curves for fluorescence induction. Another possibility is that the efficiency of radiationless transitions changes. We favor this possibility because changes in the fluorescence yield at P, in the photosynthetic yield at low light intensity, in the yield of fluorescence in the presence of DCMU and in the ratio of fluorescence without to that with DCMU can all be explained by a single change in radiationless loss.

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