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DECAY OF DELAYED LIGHT WITH THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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SUMMARY

Intact cells of the diatom *Phaeodactylum tricornutum* do not show a smooth afterglow decay curve. After a sharp decline (0—150 msec), a maximum may occur after 4 sec at 17° or after 22 sec at 2°. This maximum is present after excitation with far red light of low intensity, which is absorbed primarily by photosystem I. It appears to be structure-bound: cell disintegration causes its disappearance, while the 150 msec afterglow component is much less affected. The maximum also disappears after addition of 3-(3′4′-dichlorophenyl)-1,1-dimethylurea (DCMU) or ammonium chloride, but it remains unaffected by addition of phenazinemethosulphate (PMS) or pyocyanin. Addition of DCMU results in a strong increase in the 150 msec afterglow when the cells are excited with far red light.

The experiments suggest that a diffusion-limited energy transfer mechanism, depending on the physiological state of the cell, is responsible for the maximum.

INTRODUCTION

Irradiated photosynthetic unicellular organisms or leaves emit an afterglow during a time ranging from several nsec to minutes after the end of illumination [1—3]. In the "long term" delayed light (assumed here to range from 200 msec to several minutes), a maximum in the decay curve is sometimes observed with intact cells or leaves [4—7]. This maximum was found to occur only after illumination with far red light. The experimental results diverge with respect to the time of its occurrence: it ranged from a few seconds [4] to a minute or more [7]. As also the intensity of incident light was different in the various experiments, maxima showing up at various moments during afterglow decay may be due to more than one mechanism. Addition of some artificial cofactors for photophosphorylation resulted in an increase in the "long term" afterglow and a shift of the maximum towards shorter times [6,7].

Abbreviations: DCMU, 3-(3′4′-dichlorophenyl)-1,1-dimethylurea; PMS, phenazinemethosulphate.

From the very low concentration of the PMS needed to affect the "long wave" afterglow, Björn suggested that an "afterglow unit" the size of a thylakoid is present in the cell.

With isolated preilluminated spinach chloroplasts or chloroplast fragments the emission of a "light flash" could be brought about by the creation of an artificial proton gradient across the thylakoids by an acid-base transition [8,9]. This procedure can lead to phosphorylation. Also salt addition may stimulate luminescence with illuminated isolated chloroplasts [10,11]. Barber and Kraan [10] concluded from the maximum possible electrical gradient in their experiments that both pH and salt-induced luminescence should be independent of photophosphorylation.

With intact cells, however, the luminescence increase may be due to a different or a more complex mechanism, as suggested by the prolonged time elapsing between illumination and afterglow increase. In the present study the conditions for occurrence of the afterglow increase with Phaeodactylum are studied.

METHODS

Phaeodactylum was grown in diffuse daylight at 17° in artificial seawater enriched with sodium silicate. These diatoms grow slowly but steadily over a period of several months, and their photosynthetic capacity, as measured with the oxygen polarograph, changes little. The percentage of long wave chlorophyll *a*, characteristic for fluorescence and absorption spectra of this organism, increases gradually during growth [12,13].

Afterglow was measured with a circulating flow system. A cooling device enabled measurements to −4°. The average time between illumination and measurement was 0.15 sec. Decay before 0.20 sec is considered here to represent "fast" afterglow. Corrections were applied for the change in circulating velocity with changes in temperature. Illumination occurred with light isolated from a 150 W -24 V halogen lamp with a Bausch and Lomb grating monochromator (500 mm), with slit width between 1 and 2 mm, depending on the intensity needed. Intensities exceeding 1 W m^{-2} were obtained with a slide projector and interference filters. The volume of the illuminated circuit was about 3 times that of the dark circuit. The average time needed by a cell to circulate the whole system was about 8 sec.

RESULTS

Temperature

The curves of afterglow decay (0.15 sec $\leqslant t \leqslant$ 1 min) emitted by a 2-month-old culture of Phaeodactylum suspended in or diluted with natural seawater and resulting from a 2-min illumination with light of 685 nm ($\sim$ 0.5 W m^{-2}) are given in Fig. 1 for various temperatures of the suspension. At temperatures below 20° a pronounced maximum in the decay curve is

observed. At 17° it occurs at about 4 sec after the end of illumination, while it is shifted towards longer times (12 sec at 7° and 22 sec at 2°) when the suspension is cooled. At temperatures above 20° the maximum is observed as a shoulder in the decay curve of the "fast" luminescence component. The influence of temperature is reversible. At very low temperature (−4°) the maximum is low as compared to the "fast" afterglow. With other organisms (the green alga *Chlorella vulgaris*, the blue-green alga *Synechococcus cedrorum* and the red alga *Porphyridium cruentum*) the maximum can be measured but disappears when the cells are cooled below 7°, and reappears after warming.

Wavelengths of excitation

Fig.2 shows that no maximum in the afterglow decay curve is found upon preillumination in green light (545 nm), which is mainly absorbed by the carotenoid fucoxanthin, an accessory pigment of photosystem II. In this figure also the induction curves of afterglow, measured with light of 685 or 545 nm "on", are given. The shape of these curves depends on the wavelength of exciting light, as well as on light intensity and temperature. It is reproducible only if light of the desired wavelength is preceded by at least 1 min 545 nm light followed by 2 min dark adaptation.

From the afterglow values at the maximum in the decay curve a tentative

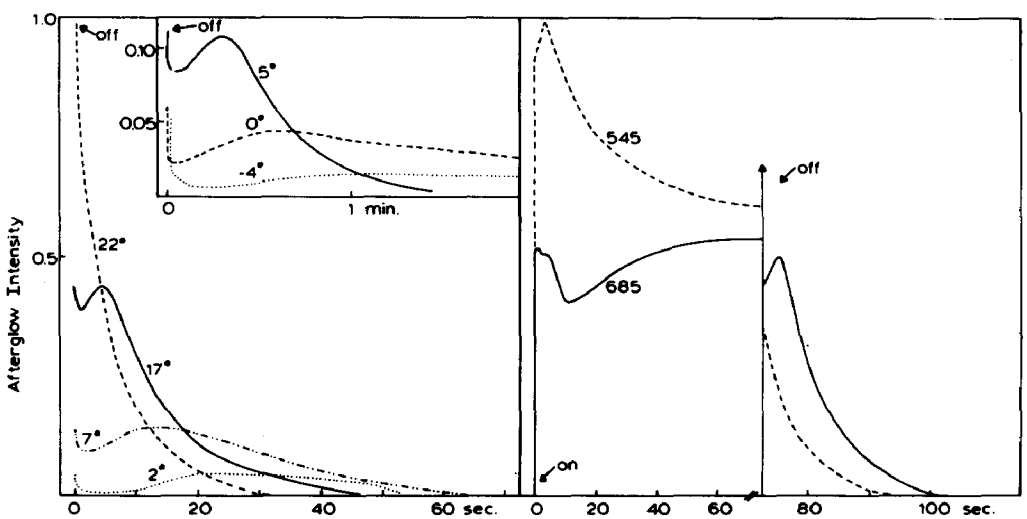

Fig.1. Afterglow decay curves of Phaeodactylum cells, resulting from a 2-min illumination with 685 nm light (0.5 W m^{-2}) isolated with a monochromator (2 mm slit width), and measured at various temperatures. The insert gives the enlarged curves at some low temperatures.

Fig.2. Afterglow induction (with light "on") and decay curves (with light "off") resulting from a 1-min illumination with 685 (——) and 545 (— — —)nm light (0.5 W m^{-2}) and measured at 13°.

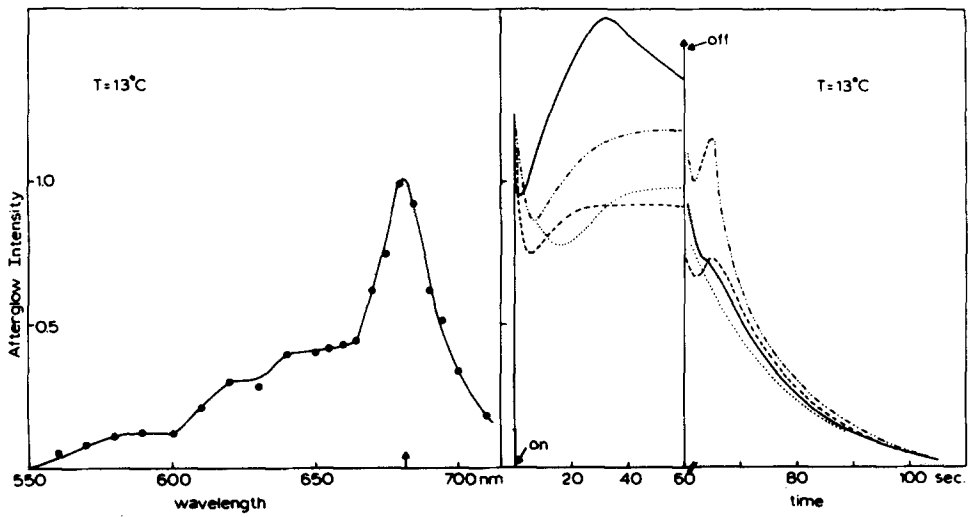

Fig.3. Tentative action spectra of the contribution of the maximum to the afterglow decay curve of Phaeodactylum.

Fig.4. Induction and decay curves of afterglow different light intensities (——, 5.5 W m^{-2} ; — — —, 4 W m^{-2} ; — · · —, 0.5 W m^{-2} , · · ·, 0.06 W m^{-2}) resulting from 680 nm light (interference filter, $\lambda_{1/2}$ = 11 nm). Between 2 W m^{-2} and 0.2 W m^{-2} the curves are similar.

action spectrum of its occurrence can be derived (Fig.3). As no maximum is observed with green incident light, it is assumed that this light does not participate in reactions giving rise to the maximum. Therefore, the afterglow after 4 sec resulting from illumination with light of 545 nm, is subtracted from that at other wavelengths.

Wavelength of emission

Insertion of interference filters (maximum transmission at 680, 695 and 714 nm) between the detection cuvette and photomultiplier did not affect the shape of the decay curves. This indicates that the light emitted during the whole time of afterglow had, probably, the same spectral distribution.

Light intensity

The influence of the intensity of the exciting light on the shape of the decay curves is given in Fig.4, when afterglow is excited with far red light. At intensities above 5.5 W m^{-2} the maximum in the decay curve disappears abruptly, while the 0.15 sec component increases sharply. In a wide range of intensities little change is seen in the afterglow decay, whereas at low intensities (< 0.1 W m^{-2}) the maximum disappears.

Illumination periods

The maximum in the decay curve is not observed after short periods of

illumination. After 4 sec excitation with 685 nm light of 0.5 W m^{-2} at 15° only a "fast" component is measured. After 16 sec of illumination with this light the maximum in the decay curve is at its full intensity. No correlation between the sharp induction peak measurable during illumination and the maximum in the decay curve, was found.

Cell structure and growth conditions

After grinding, French press treatment, ultrasonic vibration or "osmotic shock" treatment of the cells no maximum in the decay curve could be measured. Fig.5 shows the effect of "osmotic shock" treatment on a 6-week-old Phaeodactylum culture. Steady afterglow value at least with 545 and 685 nm excitation with illumination "on" are decreased. The maximum in the decay curve with illumination "off" disappears and the decay is similar for all wavelengths.

In younger cultures of Phaeodactylum (2—4 weeks) the maximum is generally less pronounced than in older ones (1—6 months). No further maxima at later stages in the decay curve were detected with cultures of different age grown under different light conditions, and measured at different light intensities and temperatures.

Inhibitors, uncouplers and cofactors

To obtain information about a possible influence of uncouplers of photophosphorylation, ammonium chloride was added. This treatment resulted in an 80% disappearance of the maximum in the afterglow decay curve at an ammonium chloride concentration of $10^{-2}M$, while at $10^{-3}M$ the shape of the decay curve was not affected, Ammonium chloride ($10^{-2}M$) increases afterglow, when light is on,by 30%. Potassium chloride ($10^{-2}M$) did not have any effect on the shape or intensity of afterglow curves.

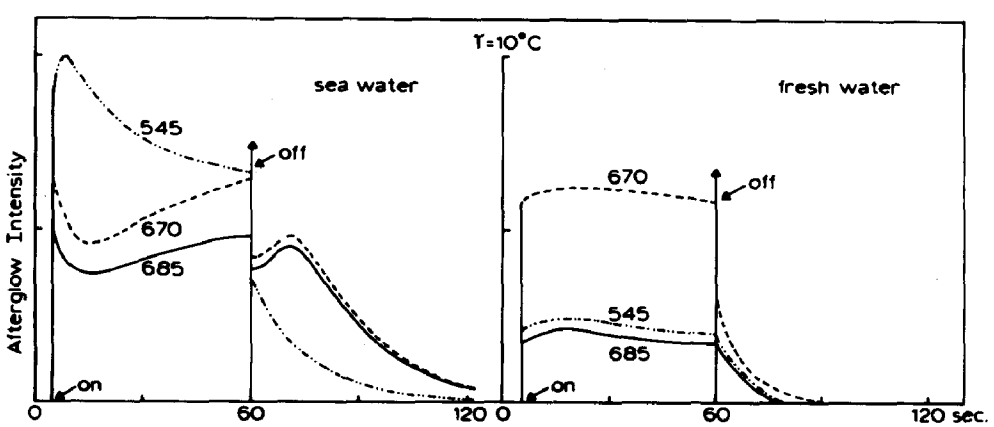

Fig.5. Decay and induction curves of Phaeodactylum diluted 15 times with seawater (left) or 0.02 *M* tris buffer pH 7.6 (right) and resulting from illumination at 685, 670 and 545 nm (0.5 W m^{-2} (right).

Addition of the artificial cofactor for photophosphorylation PMS or pyocyanin (10^{-7} to 10^{-4} *M*) did not have any effect on the afterglow curves in Phaeodactylum. A pronounced effect on the afterglow resulted from the addition of the photosynthetic inhibitor DCMU (10^{-7} to 10^{-5} *M*). The afterglow intensity measured during 685 nm illumination increased about 5-fold, while the "long term" afterglow decreased and no maxima remained. The shape of the decay curves with 685 nm and with 545 nm now are similar and strongly resemble those obtained with disintegrated cells.

DISCUSSION

Light absorbed at 545 nm by the carotenoid fucoxanthin is transferred with high efficiency to chlorophyll of photosystem II, while at this wavelength, absorption by photosystem I pigments (long wave chlorophyll *a* and β-carotene) is very low [14]. At 685 nm, however, the absorption bands of chlorophyll *a* from photosystem I and II overlap. As a result, less energy will be available for back reactions leading to light emission, resulting in a low yield of the 150 msec component at this wavelength [15,16]. The experiments show that with intact cells, this "quenching" of the "fast" afterglow is abolished by addition of DCMU. After this treatment the ratio of afterglow excited with 685 nm light to that excited with 545 nm one is increased markedly and is similar to that observed with disintegrated cells. The "long term" emission, however, decreases strongly due to DCMU addition, while it also disappears due to structural changes, which leave the "fast" emission relatively unaffected. Several mechanisms seem possible to account for the increase in afterglow 4 to 60 sec after illumination. The maximum could either be caused by an increase in fluorescence yield of the excited chlorophyll molecules, or by an increase in energy flow to photosynthetic intermediates, resulting in a larger number of excited chlorophyll molecules.

The afterglow value at the time of the maximum due to far red irradiation entirely to increase is about ten times higher than that due to irradiation with green light. It seems unlikely that the fluorescence yield also varies with this value, although the possibility of a change in fluorescence yield cannot be neglected.

An increase in energy flow towards intermediates remaining after illumination with system II light by products resulting from system I illumination seems more plausible. In view of the low intensities of exciting light needed to produce the maximum, the high temperature coefficient of the time of occurrence of the maximum (4 sec at 17° and 22 sec at 2°) and its dependence on the structure of the cell, a diffusion-limited process seems compatible with the results reported here.

Whether this process is coupled with the pH and salt-induced afterglow increase observed with isolated chloroplasts and assumed to be caused by stimulation of an electrical gradient over the thylakoids [15,16] remains to be investigated.

The experiments show that with intact cells afterglow decay is markedly dependent upon physiological and environmental conditions. Hence determination of this parameter, especially when measured in combination with photosynthetic activity, can give valuable information about in vivo properties.

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