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TIME COURSE OF MICROSECOND-DELAYED LIGHT EMISSION FROM *SCENEDESMUS OBLIQUUS*

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SUMMARY

The time course of microsecond-delayed light emission and that of prompt fluorescence was measured during the period of light adaptation for the wild-type strain and mutants Nos 8 and 11 of *Scenedesmus obliquus*, Strain D_3, exposed to stimulus light in a photon-counting apparatus. As compared to prompt fluorescence, the delayed light emission was generally more susceptible to change in experimental conditions.

1. Illuminating normally grown wild-type cells with higher light intensities (100 mW/cm^2 or higher), the intensity of delayed light increases to a maximum in the first few minutes, then decreases to a steady-state level after about 1 h. The maximum is missing at lower exciting light intensities.

2. Illuminating dark-adapted wild-type cells with high light intensities, the time course of delayed light emission is similar to that one described above, apart from a lag period within the very first minutes of light adaptation. That lag period is characterized by a relative minimum of the intensity lying higher than the intensity of the steady-state level. The length and the profile of the lag period depends on the time of dark adaptation. With increasing dark adaptation time the maximum of delayed light intensity appears later.

3. This maximum of delayed light intensity is only present for times shorter than 100 μs after turning off the stimulus light.

4. For mutant No. 8 the intensity of delayed light shows a steady decrease during the time course.

5. For light-adapted wild-type cells the intensity of delayed light and that of prompt fluorescence increases linearly with increasing intensity of exciting light below the saturation point in the light curve (about 10 mW/cm^2). The increase is less than linear above the saturation point.

The results are discussed in terms of two photosystems coupled by an electron transfer chain.

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INTRODUCTION

In the early processes of photosynthesis the absorbed energy is either trapped photochemically, or dissipated by non-radiative mechanisms, or it is re-emitted as prompt fluorescence or delayed light. Fluorescence measurements have been extensively used to probe into the primary steps of photosynthesis. For algae and higher plants it is generally accepted that the major part of light emission arises from the water-oxidizing Photosystem II [1, 2]. The intensity of fluorescence is of the order of a few percent compared to that of absorbed light [1]. To explore primary mechanisms of photosynthesis, additional information is obtained by studying delayed light emission. Its intensity is much weaker than that of prompt fluorescence and it lasts many minutes after the exciting light is turned off. On a time scale from nanoseconds up to minutes, at least four different processes are probably involved in the generation of delayed light [3].

In order to get some insight into early processes, we report in this article about the use of delayed light as a probe for microsecond transients. As far as we know, this report is the first of its kind to deal with microsecond transients of delayed light, in contrast to previous work in the millisecond region [4–7]. Parallel to delayed light, prompt fluorescence will be also recorded since little quantitative research has been performed to relate these two types of emissions [8–12].

MATERIALS AND METHODS

Growth of the wild-type and mutants of *Scenedesmus obliquus* as well as the photon-counting apparatus have been described [8, 13]. The wild-type algae were grown at a light intensity of about 0.2 mW/cm^2 provided by white fluorescence tubes. The cells were either used directly or were further dark adapted over a certain period of time before starting the experiment. The argon laser beam was chopped with a frequency of 500 Hz, so that the algae sample was illuminated for a period of 1 ms followed by a dark period of 1 ms. The light distribution across the beam in the cuvette had a half-width of 0.6 cm. The light intensities given in the figures represent the time-averaged maximum values of this distribution measured in the absence of algae. The delayed light emitted between 2 and 22 μs after switching off the exciting light was detected with a photomultiplier through a 690-nm interference filter, and the signal was further processed with a multichannel analyzer operated in the multiscaling mode [13]. The time course of the delayed light emitted from the algae was followed over a time ranging from a few minutes up to 80 min, beginning at the onset of exposure to the laser light. In order to record the time course of prompt fluorescence, its intensity was measured in a similar time interval before the stimulus light was cut off. Samples were only used once for one type of experiment.

RESULTS

The first series of experiments demonstrate the influence of the stimulus light intensity and of dark adaptation on delayed light emission and on prompt fluorescence. The time course of delayed light emission during the first 20 min is shown in Fig. 1 for several stimulus light intensities. At higher light intensities (about 100 mW/

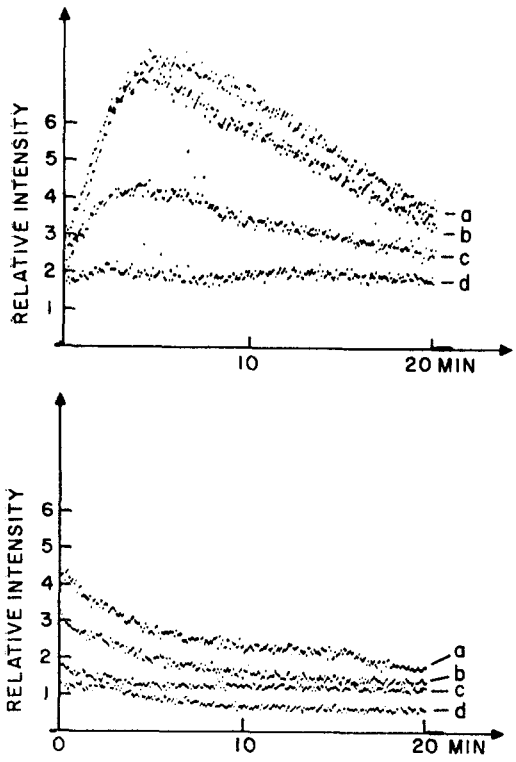

Fig. 1. The time course of delayed light emission (upper part) and prompt fluorescence (lower part) from *Scenedesmus obliquus*, wild-type Strain D_3, at several stimulus light intensities. a, 265 mW/cm^2; b, 220 mW/cm^2; c, 100 mW/cm^2 and d, 70 mW/cm^2. The algae were dark-adapted for 3–5 h prior to the experiments. The strong solid line at the abscissa represents the photomultiplier dark signal.

cm^2 and higher), the intensity of delayed light increases to a maximum in the first few minutes of light adaptation in the laser beam, then the intensity decreases. At lower exciting light intensities this maximum is absent, the intensity of delayed light gradually decreases to a steady-state level. Since there was not enough light intensity, the time course could not be recorded for times shorter than about 10 s.

The time course of prompt fluorescence (Fig. 1) does not exhibit any maximum, it tapers off until it reaches a steady-state level within a few minutes.

The influence of dark adaptation is presented in Fig. 2. For the dark-adapted algae the maximum of delayed light intensity appears later as compared to the non-dark-adapted ones. Furthermore, at the onset of the time course a lag period appears in which the delayed light intensity shows a relative minimum followed by an increase in intensity. Enhancing the time of dark adaptation, the intensity of delayed light in that lag period increases up to a certain value, and the lag period itself gets longer.

Following the time course of delayed light emission for dark-adapted cells over 80 min, it is seen that the stationary value of the emission intensity is lower than the minimum value in the lag period. Delayed light collected from 2–22 μs, or from 2–12 μs, or from 12–22 μs, yields a similar profile of the time course with the exception of the intensity. The maximum discussed above is absent in the time course of delayed

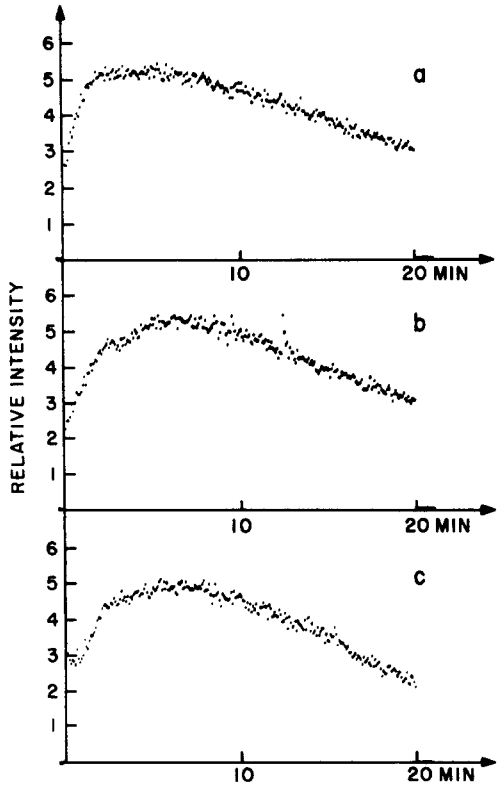

Fig. 2. The time course of delayed light emission from *Scenedesmus obliquus*, wild-type Strain D_3, at 200 mW/cm^2 stimulus light intensity. a, non-dark-adapted algae; b, time of dark adaptation (1 h) and c, time of dark adaptation (6 h).

light emission if the delayed light is collected later than 100 μs after switching off the exciting light.

The time course of delayed light emission and prompt fluorescence was also studied for mutants Nos 8 and 11 from *Scenedesmus obliquus* [14, 15]. Both mutants show fluorescence; however, mutant No. 11 which lacks a functional Photosystem II does not exhibit any measurable intensity of delayed light [2, 8]. The time course of delayed light emission from mutant No. 8, which lacks a functional Photosystem I [16], differs drastically from that of the wild-type (Fig. 3).

For the wild-type cells the intensity of delayed light and prompt fluorescence was measured as a function of the intensity of the stimulus light. Prior to recording these curves, the cells were first adapted to the highest laser light intensity (250 mW/cm^2). Generally speaking, the intensity of the emission increases linearly with increasing intensity of the stimulus light below 10 mW/cm^2, which is about the saturation point in the light curve [15]. The increase is less than linear at higher exciting light intensities.

Dividing the measured intensity of prompt fluorescence at a particular time in the steady state range of the time course by that of delayed light at the same time, this ratio is lower below the saturation point than above this point (Fig. 4). That ratio

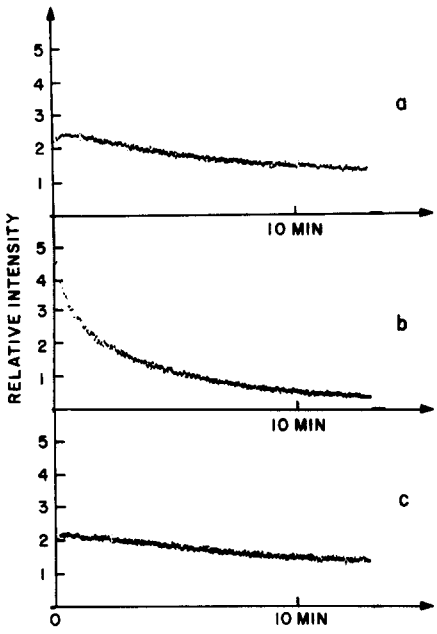

Fig. 3. The time course of light emission from *Scenedesmus obliquus*, at 175 mW/cm^2. a, prompt fluorescence, mutant No. 8; b, delayed light (mutant No. 8) and c, prompt fluorescence (mutant No. 11).

remains constant within two regions of exciting light intensities. This behavior may indicate that there exist two types of coupling between prompt fluorescence and delayed light. At higher exciting light intensities more fluorescence light is emitted relative to delayed light. The reason for this phenomenon may be the closing of traps above the saturation point. For the preceding non-stationary part of the time course such a straightforward two-level diagram (Fig. 4) cannot be drawn. The time course

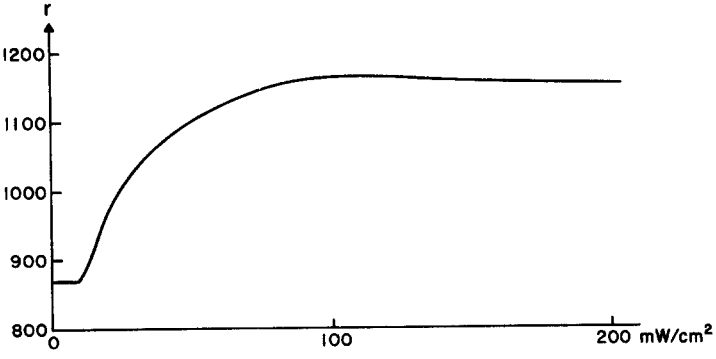

Fig. 4. The ratio *r*, defined by the intensity of prompt fluorescence/intensity of delayed light, as a function of the exciting light intensity. The stimulus light intensity was applied to normally grown wild-type cells of *Scenedesmus obliquus*, Strain D_3, which were adapted to laser light for about 2 h prior to the measurements.

of prompt fluorescence is apparently not affected by the long times of dark adaptation employed in our experiments.

DISCUSSION

Keeping photosynthetic organisms in the dark for a sufficiently long time, fluorescence transients are generated when those cells are suddenly excited with light [17–22]. The fluorescence induction curves thus obtained are characterized by a fast portion lasting a few seconds followed by a slow portion lasting for minutes [23]. The slow change shows a Minimum S followed by a Maximum M after about 1 min and a slow decrease to a steady-state Level T within 5–10 min. It is believed that this S–M–T transition is accompanied by light-induced structural changes of the membrane system coupled with photophosphorylation [23]. For normally grown wild-type cells the time course covered by our experiments (Fig. 1) corresponds to the M–T part of the induction curves of fluorescence.

In contrast to fluorescence experiments, only few experiments have been carried out dealing with induction processes of delayed light emission [4–7]. The intensity of delayed light measured in the time interval from 2–22 μs after switching off the exciting light reflects the status of transient states at that time, which become, however, modified during the time of adaptation of the cells in the laser beam. Essential transient states are the redox level of the primary electron acceptor, Q, of Photosytem II and that of at least one intermediate pool, generally known as Pool A, which is probably plastoquinone or Component X-335 [24, 25], lying between Photosystem II and Photosystem I. Flash experiments demonstrate that the characteristic time of electron transfer from photoreduced Q to plastoquinone is of the order of 0.6 ms and of the order of nanoseconds for Component X-335 [25, 26]. Under our conditions of light excitation it is reasonable to expect that Pool A becomes photoreduced within the light period of 1 ms. Therefore, the yield of delayed light emission measured in the microsecond region is a monitor for energy storage in Pool A. The following portion of the electron transfer chain acts more slowly [26] and is characterized by stationary redox levels. Upon changing the illumination conditions, slow processes cause a pile-up or drainage of electrons in the time-averaged redox levels of the pool. On the basis of that model, the increase in intensity of delayed light (Fig. 1) seems to indicate that the average redox level of Pool A is strongly shifted towards the reduced side due to the high electron influx from Photosystem II which was illuminated with high intensity stimulus light. At later times the drainage of electrons increases, concomitant with a decrease in delayed light intensity until a steady state level is established. The maximun of delayed light emission appears later than the M-state of the slow fluorescence induction curve.

The maximum at high exciting light intensities is only present for delayed light emitted at times shorter than 100 μs, after turning off the stimulus light. This fact seems to indicate that Pool A is characterized by a decay-time of about 100 μs. Therefore, Pool A can perhaps be identified with Component X-335 which has a half-life of 600 μs [25]. This maximum in the time course is not be confused with the maximum in the dacay curve of delayed light emission described recently [27]. The latter maximum appears only when Photosystem I is excited with far-red light.

In dark-adapted cells, Pool A is appreciably oxidized because of the absence

of an electron flow from Photosystem II. In non-dark-adapted cells, however, the incoming electrons shift the equilibrium value of the redox level towards the reduced side. The lag period present in the time course of delayed light emission from dark adapted cells (Fig. 2) represents perhaps the time required to reduce the Pool A from the level of dark adaptation to the level present in non-dark-adapted cells.

Further studies are necessary to interpret the time course of delayed emission from mutant No. 8. It may be concluded that the difference in the time course does not result from quantitative changes in the pigment system [14].

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