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FACTORS AFFECTING ENERGY TRANSFER FROM PHYCOBILISOMES TO THYLAKOIDS IN *ANACYSTIS NIDULANS*

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Summary

Short illumination with white light of dark-maintained *Anacystis nidulans* prior to immersion in liquid nitrogen resulted in a marked change of fluorescence emission characteristics at 77 K. The fluorescence of Photosystem II-associated membrane bound pigments increases, while the emission due to phycobilins decreases. This effect seems to be due to a light-dependent alteration in the extent of contact between phycobilisomes and thylakoids, since the effect is reversible in the dark and is abolished by short glutaraldehyde fixation. The preillumination effect is not inhibited by DCMU. Emission spectra obtained with actively growing and CO_2-starved cells indicate that the light-dependent increase in energy transfer from phycobilins to chlorophyll depends upon the physiological state of the cells.

Introduction

Previous studies on the fluorescence characteristics of four species of blue-green algae, taken at liquid nitrogen temperature, led to the observation that short periods of white light given prior to cooling resulted in marked changes in the emission spectra obtained subsequently [1]. Principally, a decrease in phycobilin fluorescence was accompanied by an increase in chlorophyll emission, dependent on the time of illumination. This type of change was observed with *Anacystis nidulans* and *Chlorogloea fritschii*, while the opposite response was noted with *Nostoc muscorum* and *Anabaena cylindrica*.

Although this effect appeared reminiscent of the observations of Murata [2], that green preillumination (567 nm) given to *Porphyridium cruentum*

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Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

results in a decrease in Photosystem II and an increase in Photosystem I emission, our previously shown and the subsequently extended data of this paper argue for a second, additional and probably superimposed role of light. Besides inducing a rearrangement of pigment systems within the thylakoid membrane [2], we propose from our observations that light also mediates the degree of contact between phycobilisomes and thylakoids in *A. nidulans*.

Materials and Methods

A. nidulans, type 1402-1 of the Göttingen algae collection, was grown in batch culture under the same conditions as detailed before [1] using the Kratz and Myers medium. Cells were harvested in the logarithmic growth phase, washed twice and resuspended in 0.1 M potassium phosphate buffer, pH 8, before sample preparation.

The fluorescence measurements were made at liquid nitrogen temperature using essentially the same instrumental arrangement as described before [1]. The exciting light was obtained by a combination of a heat absorbing KG-1 (Schott) with an broad band interference K-6 (Balzers) and a line interference 600-nm filter (Spectral Systems Inc.) giving an excitation at 600 nm. Another set of filters (KG-1 in combination with a 450—500 nm special interference filter) was used to provide excitation of the photosystems proper without appreciable absorption by the phycobilins.

The samples were prepared by soaking cheesecloth in a cell suspension, gently squeezing it for the removal of excess liquid and mouting a double layer of the muslin-adsorbed algae on a frame of interlocking polypropylene rings. Chlorophyll per exposed area never exceeded 1 $\mu g/cm^2$ and was normally about 0.8 $\mu g/cm^2$.

Preillumination with white light was given prior to cooling for the times indicated in the figures. The same conditions and techniques were used for this as described previously [3]. The frozen samples were exposed for at least 2 min to the exciting light before measuring the emission spectra.

All spectra shown are averages of up to four individual experiments, corrected for residual exciting light and photomultiplier sensitivity but not for transmission efficiency of the monochromator. Further experimental details are given in the legends to the individual figures.

Results

The *A. nidulans* emission spectra, depicted in the upper part of Fig. 1, show five principal emission bands and shoulders upon excitation at 600 nm. The emission in the region of 645 and 660 nm is due to phycobilin fluorescence, while that at 690, 705 and 727 nm originates in the chlorophyll of the photosystems. The spectrum resembles those published before [4—7], although in our case the bands are shifted by 5—10 nm towards the longer wavelength. The lower curve of Fig. 1 shows the emission spectrum upon excitation with blue light. The bands appear at 690 and 705 nm and between 725—730 nm. Interpreting the spectra according to conventional lines, we assign the 690-nm and 705-nm bands to Photosystem II and the long wavelength band to Photosystem I.

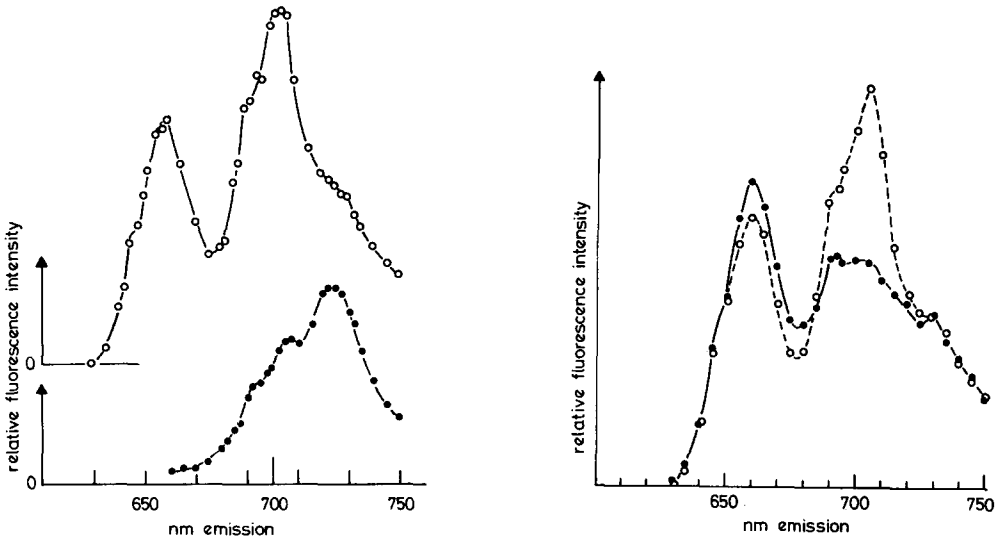


Fig. 1. Fluorescence emission spectra of *A. nidulans* excited at either 600 nm (○———○) or 450—500 nm (●———●). The same sample, which was preilluminated 4 min before cooling, was used in either case.

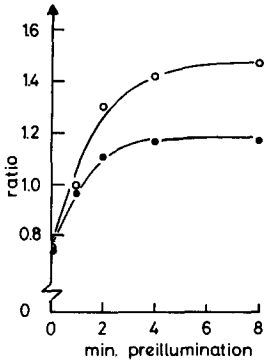
Fig. 2. Preillumination effect in the fluorescence emission of *A. nidulans*. The emission of the control samples, cooled down to liquid N_2 temperature in complete darkness, is given by filled points, that of preilluminated probes (8 min white light before cooling) is given by open circles. Excitation at 600 nm. Note the difference when compared to the spectrum of 4 min preillumination given in Fig. 1.

The preillumination effect on the fluorescence emission spectra of *A. nidulans* is shown in Fig. 2. While in our previous paper [1] the measurements were made using fully synchronized cultures this figure shows that the effect is observable also in batch cultures. Basically, three spectral changes occur upon illumination of the sample before cooling: first, a decrease in emission originating in the phycobilins, centering around 660 nm, secondly, an increase in chlorophyll emission in the 680—710 nm region, and thirdly a specific rise of the fluorescence peak around 705 nm. It should be noted that no (or only little) change is observed at 725 nm, the presumed emission maximum of Photosystem I.

Both the ratio F_{705}/F_{660} and the ratio F_{695}/F_{660} can serve as convenient indicators of the extent of the light-induced spectral changes. Fig. 3 shows that their values increase within 2 min after the onset of illumination to almost maximum level.

The effect on the emission spectra of preillumination before cooling is readily reversible in the dark. As shown in Fig. 4, it decreases with a half-time of approx. 1—1.5 min to, or even below, the level of the dark-maintained control samples. The observed spectral changes thus occur rather slowly, which is indicative of membrane-connected structural changes (compare the time-course for swelling and shrinking in isolated chloroplasts [8]).

To check further the possibility of a structural change underlying the observed effect, dark-incubated algae were treated with 2% gluteraldehyde for a period of 5 min, then washed free of the crosslinking agent and examined for their response to illumination as before. Under these conditions there is neither



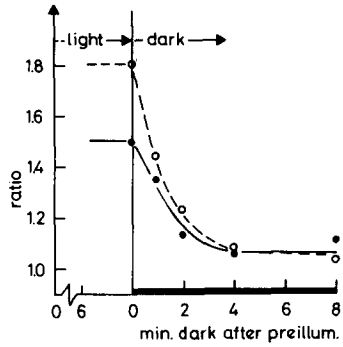


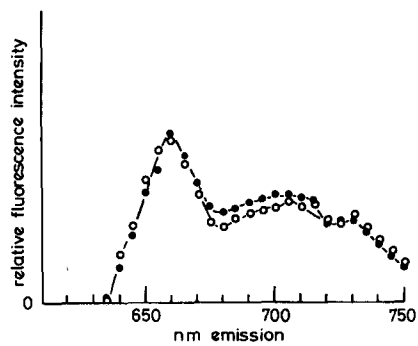
Fig. 3. Effect of preillumination on the fluorescence ratio F_{705}/F_{660} (○———○) and F_{695}/F_{660} (●———●) in *A. nidulans*. Excitation at 600 nm.

Fig. 4. Reversibility of preillumination effect. The samples, preilluminated 8 min, were coooled down after inserting the dark periods indicated. 600 nm excitation; (○———○) equals F_{705}/F_{660}; (●———●) represents F_{695}/F_{660}.

a significant effect of light on the emission ratios, nor on the spectra themselves (Fig. 5). Longer exposure to gluteraldehyde (2 h) gave the same results.

To investigate any connection of the spectral changes with electron transport, measurements were taken with algae incubated with $1 \cdot 10^{-4}$ M DCMU. This treatment abolishes oxygen evolution and, by inference, open-chain electron transport and its connected phosphorylation. However, no significant influence of this compound on the preillumination effect was observed (Table I) although the absolute values seem to vary somewhat.

The physiological state of the batch material used appears to be of importance for the appearance of the light-induced increase of F_{705}/F_{660} or F_{695}/F_{660}. Algae which were unspecifically altered in their metabolism, in our case either deprived of CO_2 for several days or illuminated as a thick suspension for 3—4 days without aeration, show the exact counterpart of the stan-



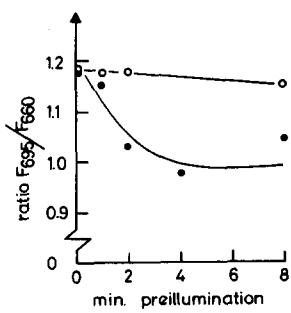


Fig. 5. Effect of preillumination on fluorescence emission of gluteraldehyde-fixed *A. nidulans*. (Closed circles represents control, open circles 8-min preilluminated samples.)

Fig. 6. Preillumination effect in physiologically altered *A. nidulans*. The ratio F_{695}/F_{660} of cells CO_2-starved for two days is given by open circles, that of 3-day cells by the black circles. Exitation at 600 nm.

TABLE I

THE INFLUENCE OF $1 \cdot 10^{-4}$ M DCMU ON THE LIGHT INDUCED SPECTRAL CHANGE OF FLUORESCENCE EMISSION IN *ANACYSTIS*

Preillumination (min)	F_{695}/F_{660}	F_{705}/F_{660}
0	0.88	0.86
1	1.08	1.15
4	1.10	1.22
8	1.22	1.30

dard preillumination effects. Fig. 6 shows that a decrease of the ratio of chlorophyll/phycobilin fluorescence takes place upon light exposure prior to cooling. It should be noted that the control value of the ratio F_{695}/F_{660} is in the same range as that obtained after 8 min preillumination of the healthy, standard algae (see Fig. 3). If *A. nidulans* is subjected, e.g., to CO_2 starvation for shorter periods, this reversal of spectral changes becomes less and less pronounced. The upper curve in Fig. 6 was obtained after two days of CO_2 starvation. It is an approximately straight line, with a ratio for the control sample of around 1.2 for F_{695}/F_{660}.

Discussion

In our previous communication we proposed a variation in the degree of attachment of the phycobilisome to the thylakoid as the basic cause of the observed effects [1]. It was suggested that light induces an increase in contact between the phycobilisome and the thylakoid membrane, resulting in a more efficient energy transfer from the terminal phycobilins to chlorophyll and Photosystem II. The present data are consistent with this model. They show clearly that energy is directed specifically to the reaction centre of Photosystem II, if we interpret the bands as stated before.

The emission spectrum, excited at 450—500 nm (Fig. 1), shows that the emission peak at 705 nm belongs definitely to Photosystem II. Thus, we can exclude the possibility that the differential increase of this band upon preillumination is due to a rearrangement between Photosystem I and Photosystem II [2]. The basically unchanged size of the 725—730 nm emission of Photosystem I upon preillumination argues also against an involvement of this rearrangement effect.

The preillumination effect reported here is probably related to, or even a different expression of, data represented earlier [9—12]. These observations showed that the fluorescence kinetics of *Anacystis* differed between dark-adapted cells and a specimen which had been exposed previously to the orange exciting light at room temperature. They showed further that, during fluorescence induction of dark-adapted *A. nidulans*, there was a change in the emission spectrum leading to an enrichment of the Photosystem II signal, and the investigators interpreted this effect as an energy-dependent structural change of the thylakoid membrane.

The dependence of the preillumination effect in *A. nidulans* on the structure

of the thylakoid is strongly implied by the time course and the gluteraldehyde experiments. It seems likely that the membrane state for efficient energy transfer has to be actively maintained. This is indicated by the reversibility in the dark.

The DCMU data do not contradict this argument, since cyclic photophosphorylation could still provide sufficient energy. This is in agreement with the observation of Bedell and Govindjee [13], that cyclic phosphorylation of *Anacystis* increases rather than decreases in the presence of DCMU. It should also be noted that *P*-700 turnover in *Anacystis nidulans* increases with higher concentrations of DCMU, until it reaches a plateau at $1 \cdot 10^{-7}$ M. It does not decrease up to $1 \cdot 10^{-4}$ M concentration [14].

An independent assessment for structural changes can in theory be obtained by comparing dark-maintained and preilluminated algae under the electron microscope. We have attempted such measurements, but have not succeeded so far in overcoming the difficulty of unequivocally discriminating between thylakoid and the minute phycobilisomes compared to *Porphyridium*. We can report only a general tendency of these experiments in line with our interpretation.

The data obtained with algae in different physiological states are difficult to interpret. However, while we previously thought the reversal of the effect to be possibly species-specific, the above data strongly indicate that, at least in *A. nidulans*, the culture conditions and thus by implication the physiological state of the cells, are of primary importance. The light-mediated changes in the cyanophyte phycobilisome-thylakoid structural association, leading to changes in energy transfer efficiency, are indicated to be under complex control.

Besides the above mentioned there are a number of questions still open to further investigation. The apparent absence of the Murata effect [2] in our observatons, concluded from the invariability of the emission at 725 nm, needs to be studied in more detail. We believe that the rearrangement between Photosystem I and Photosystem II described by Murata [2] is an initial response of the algae to adjust to monochromatic illumination in order to maintain a minimum of photosynthesis. Since we used white light for preillumination, this effect seems to be suppressed. Nevertheless, a detailed study of action-, excitation- and emission spectra is needed to resolve the circumstances.

Another problem still to be resolved concerns the mechanism of energy transfer between the phycobilins and chlorophyll. The system of *A. nidulans* should allow to distinguish between exciton and resonance transfer by determinating the temperature dependence of energy distribution after preillumination.

The experiments discussed here give only a rough description of the preillumination effect. A full assessment of its scope, especially its role in the control of photosysthesis in blue-green algae, requires considerably further experimentation.

Acknowledgement

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