TEMPERATURE-DEPENDENT REVERSIBLE CHANGES IN PHYCOBILISOME–THYLAKOID MEMBRANE ATTACHMENT IN *ANACYSTIS NIDULANS*

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1. Introduction

In blue–green and red algae phycobiliproteins constitute a major part of the light harvesting pigments. They are aggregated in discrete structures, called phycobilisomes [1], on the surface of the chlorophyll-containing photosynthetic lamellae. Energy absorbed by the phycobilins is transferred with high efficiency to chlorophyll by inductive resonance [2,3]. The efficiency of energy transfer between the various phycobiliproteins, and between the phycobilisome and the various chlorophyll species within the thylakoid membrane is most conveniently studied by measurements of the relative fluorescence yields. From such studies a picture evolved, in which energy absorbed by phycocyanin ($A_{max} \sim 620$ nm; $F_{max} \sim 645$ nm) is transferred via allophycocyanin ($A_{max} \sim 650$ nm; $F_{max} \sim 660$ nm) and allophycocyanin B ($A_{max} \sim 670$ nm; $F_{max} \sim 680$ nm) to chlorophyll *a* ($A_{max} \sim 675$ nm; $F_{max} \sim 685$ nm) (see, e.g., reviews [4,5]).

Not much is known about the nature of the phycobilisome attachment to the thylakoid membrane. For an investigation of this aspect it is important to find ways to modify the degree of attachment. Treatments to give a reversible change of energy transfer efficiency from phycocyanin to chlorophyll include application of hydrostatic pressure [6] and preillumination [7]. An increase of allophycocyanin fluorescence in *Anacystis* was observed at < 5°C [8] and it was suggested that a block of energy transfer from the phycobilins to chlorophyll may be involved.

Abbreviation: chl *a*, chlorophyll *a*

Here the efficiency of energy transfer between the phycobilins and chl *a* in *Anacystis nidulans* was investigated by measurements of fluorescence emission spectra at –223°C. At this temperature, emission of the various phycobilins and chl *a* species can be clearly distinguished, and hence cold-induced changes in energy transfer can be located. It was found, that incubation of *Anacystis* at < 5°C induces a decrease in energy transfer efficiency within the phycobilisome, as well as between the phycobilisome and chl *a*. Following cold-pretreatment, it was possible to distinguish a stimulation of F680, attributed to allophycocyanin B [4], paralleled by a loss in chlorophyll emission. On the basis of these findings a cold-induced detachment of the phycobilisomes from the thylakoid membrane is discussed.

2. Materials and methods

Anacystis nidulans was grown at 25°C in C medium of [9], supplemented with 0.85 g $NaHCO_3$/l. The algae were supplied with air enriched with 5% CO_2, and continuously illuminated with daylight fluorescent light (~ 1 mW/cm^2).

Samples were contained in a perspex cuvette 1 mm thick, at 10 μg chl/ml. Each sample was pretreated at a given temperature in the dark by 10 min submersion in a constant temperature bath. Samples were rapidly frozen by dipping into liquid nitrogen before being cooled to –223°C in the helium-cooled continuous flow cryostat [10].

Fluorescence emission spectra were measured from the sample surface in an apparatus described [10,11].

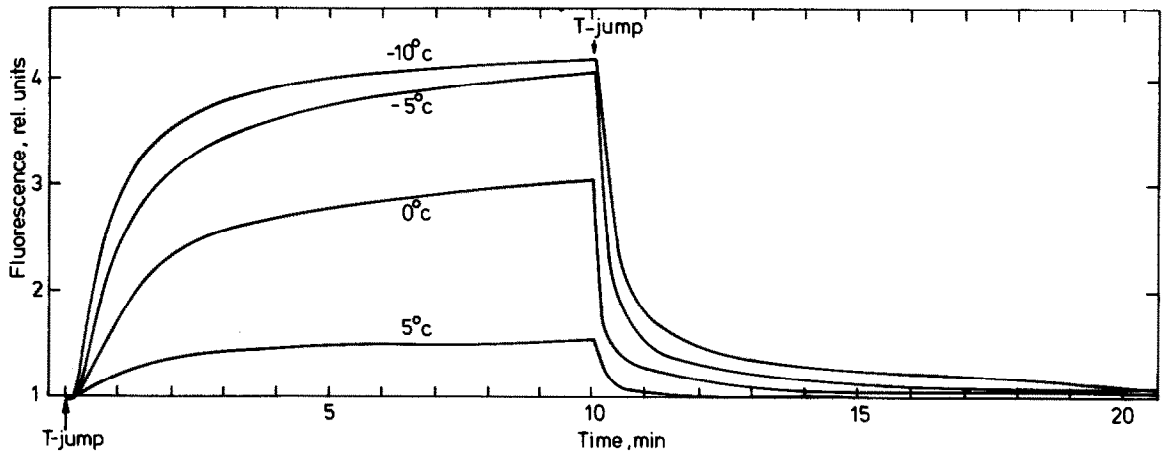

Fig.1. Temperature jump-induced changes in phycobilin fluorescence intensity around 643 nm. Fluorescence excitation, 560 nm. 95% of a 30°C temperature jump occurred in 10 s. One relative ordinate unit is equivalent to the fluorescence of a dark-adapted sample, which is not affected by the weak excitation beam. The arrows (T-jump) indicate the switch from 25°C to the indicated temperatures and vice versa.

Computer programs were used to correct the spectra for apparatus spectral sensitivity, to calculate difference spectra, and for graphic display. The fluorescence changes induced during the cold-pretreatment were monitored by a weak excitation beam (560 nm; 1 $\mu W/cm^2$), similarly to that in [12].

3. Results

Figure 1 shows the changes of phycobilin (phycocyanin and allophycocyanin) fluorescence during cooling and rewarming of *Anacystis* at the indicated temperatures. Excitation is at 560 nm where absorption is almost exclusively by the phycobilins. Fluorescence is measured at the short-wavelength side ($\sim$643 nm) of the main fluorescence peak (at 655 nm) to minimize overlap of chl *a* fluorescence. Cooling induces a large increase ($\leqslant$ 400%) of the phycobilin fluorescence. The major increase of fluorescence already occurs with cold-pretreatment around 0°C. Treatment at −10°C does not produce significantly more of a fluorescence increase than treatment at −5°C. As the temperature change was completed in $\lesssim$ 10 s, the curves represent the kinetics of the cold-induced changes, which are faster, the lower the applied temperature. With the relatively high salt concentration of the medium, freezing did not occur even in the − 10°C experiment. Upon rapid rewarming of the samples, there was a rapid decay of the fluorescence yield back to the original level.

These data suggest that relatively mild cold-treatment of *Anacystis* leads to rather vast, reversible changes of energy transfer parameters either within the phycobilisome, or between the phycobilisome and the chlorophylls. To distinguish between these two possibilities, it is important to measure the spectral changes of fluorescence emission, preferentially at very low temperature.

A problematic aspect with low temperature spectral measurements is the question of possible changes of energy transfer parameters upon cooling, e.g., in liquid nitrogen. In principle one might suspect that the changes indicated in fig.1 upon a temperature change to, e.g., −10°C could as well occur upon rapid cooling to −196°C. However, as already apparent in fig.1, the cold-induced changes of energy transfer parameters are far from instantaneous, displaying half-times in the order of 1 min. Therefore, it appeared likely that one can 'freeze in' a given state, by rapidly dipping the sample into liquid nitrogen.

Figure 2 shows fluorescence emission spectra at −223°C dependent on the pretreatment temperature. Samples were cold-pretreated in the dark for 10 min, under conditions very similar to those of fig.1, then frozen as rapidly as possible in liquid

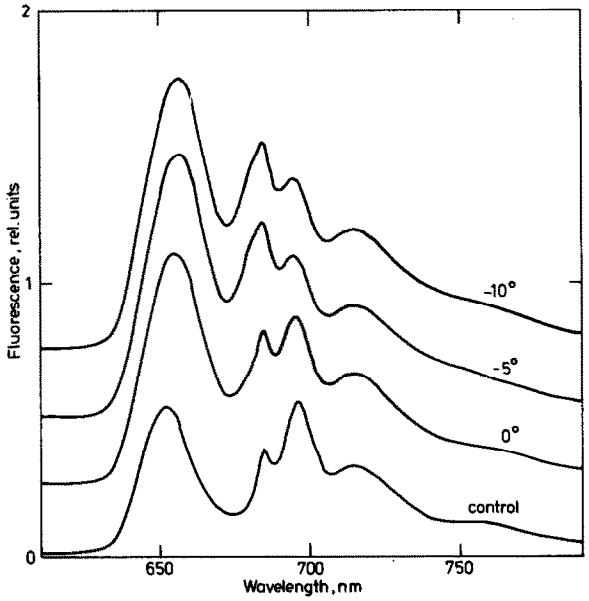

Fig.2. Effect of cold-pretreatment on emission spectra at −223°C. Excitation at 560 nm. Samples were cold-pretreated in the dark at the indicated temperatures for 10 min, contained in the same 1 mm cuvette which was used in the spectral measurement. The time between removal from the cold-bath and freezing in liquid nitrogen was ~5 s. For clarity of presentation, the spectra were vertically shifted relative to each other.

nitrogen, from where they were transferred into the cryostat. With the 560 nm excitation light, absorption is primarily within the phycobilisome and only to a very small extent by chl *a*. Without cold-pretreatment the low-temperature emission spectrum displays a broad peak around 652 nm, representative of phycocyanin fluorescence. There is efficient energy transfer from the phycobilins to chl *a*, as evidenced primarily by a dominant chl *a* peak around 695 nm. Smaller chl *a* emission peaks are around 685 nm, 715 nm and 760 nm.

Cold-pretreatment induces large changes in the emission spectrum. Phycobilin fluorescence is substantially increased and the emission peak is shifted from 652 nm to 656 nm. The overall shape of the spectrum is modified particularly in the wavelength region around 680 nm, where a broad shoulder is induced overlapping the narrow F685 band. The cold-induced spectral changes are reversible, i.e., the spectrum of a sample, which was pretreated for 10 min at −10°C and brought back to 25°C before freezing in liquid nitrogen , is almost identical to the spectrum of a sample immediately frozen in liquid nitrogen (not shown in the figures).

A more detailed evaluation of the spectral changes induced by cold-pretreatment is possible by analysis of the difference spectra (rapidly cooled to −196°C from given temperature minus rapidly cooled to 196°C from 25°C). Such difference spectra, presented in fig.3, reveal the complexity of fluorescence stimulation within the phycobilisome. Peaks can be distinguished around 645 nm, 660 nm and 680 nm, which may be correlated with phycocyanin, allophycocyanin and allophycocyanin B, respectively. The stimulation of allophycocyanin B fluorescence is even more clearly demonstrated in the spectra of fig.4A. These spectra are recorded in the presence of 1 mM 1,4-naphthoquinone which quenches preferentially chl *a* fluorescence, leaving the emission from the phycobilisome essentially unaffected (C.P.R., U.S., J.A., in preparation). Without cold-pretreatment near 680 nm only a shoulder is present, and despite the presence of the quencher, there is some remaining peak around 685 nm. Following cold-pretreatment the 680 nm emission band is substantially stimulated, while the 685 nm peak is suppressed.

The loss in chl *a* fluorescence was analysed in more detail by comparison of the difference spectrum in fig.3 for −5°C pretreatment with the emission spectrum of the cold-pretreated sample in fig.4A. The rationale for such comparison is that the difference spectrum in fig.3 reflects stimulation of phycobilisome fluorescence as well as suppression of chl *a* fluorescence, while the spectrum of fig.4A (cold-pretreated

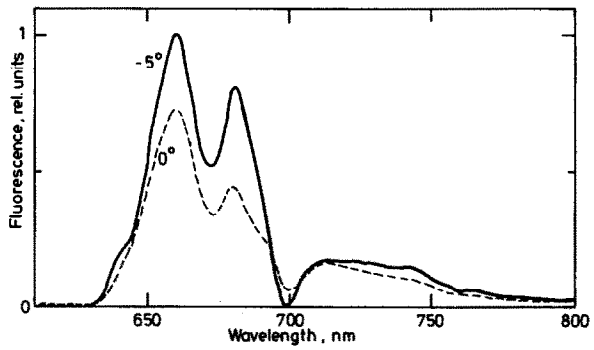

Fig.3. Difference spectra between cold-pretreated and control samples. The original spectra are those displayed in fig.2.

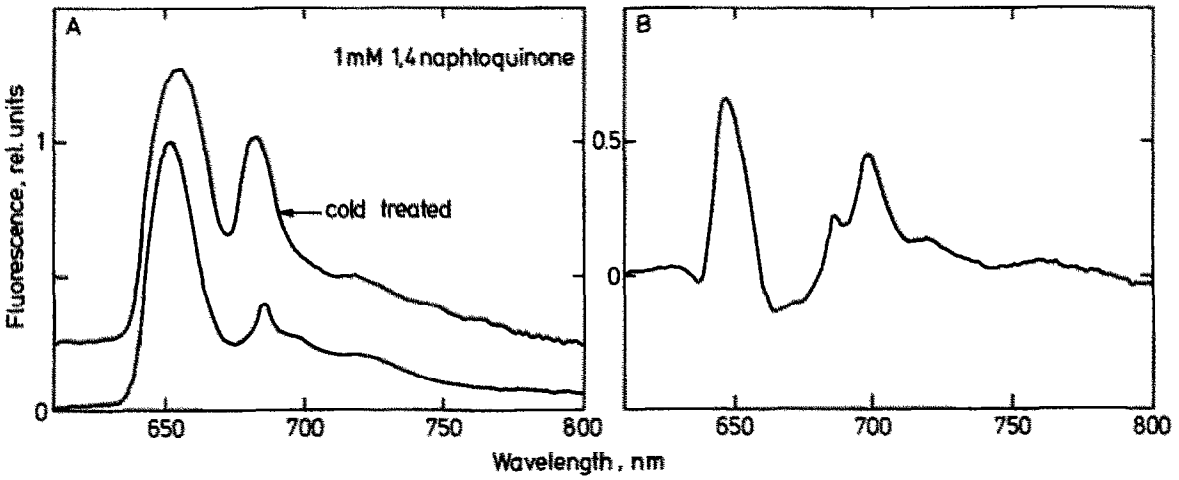

Fig.4. Analysis of the cold-induced changes. (A) Emission spectra at −223°C in the presence of 1 mM 1,4-naphtoquinone without (lower curve) and with cold-pretreatment (10 min at −5°C). The spectra are normalized at the 655 nm peak. (B) Difference between the spectrum of fig.4A (upper curve), representing mainly phycobilisome emission, and the spectrum of fig.3 (−5°C treatment), representing the overall cold-induced changes. The two curves were normalized at 680 nm.

sample) approximates the emission of the separated phycobilisome. Figure 4b shows a plot of the difference between these two spectra. At $\lambda > 680$ nm this plot clearly represents a chl *a* emission spectrum. The peaks around 685, 695, 715 and 760 nm occur in almost the same ratios as in the emission spectrum of a control sample with 560 nm excitation (for comparison see fig.2, bottom curve). At $\lambda < 680$ nm the dominant features are a peak around 645 nm and a minimum around 665 nm, which seem to be caused by an apparent cold-induced shift of the phycobilin emission peak towards longer wavelengths (see fig.2). In fact, it appears that the 655 nm fluorescence peak is composed of two overlapping emission bands at 645 nm and 665 nm, and cold-treatment leads to a relative stimulation of F665 at the expense of F645.

4. Discussion and conclusions

The data presented here show that in *Anacystis* the fluorescence emission characteristics are considerably changed by cold-treatment below 5°C. In principle, these cold-induced change of fluorescence properties could make low temperature fluorescence studies in blue–green and red algae problematic. However, if freezing occurs rapidly, e.g., by dipping the sample into liquid nitrogen, the symptoms of cold-treatment are minimized. We conclude this from the fact that the −223°C emission spectra readily reflect those changes which are induced by prolonged treatment at different temperatures (fig.2). On the other hand, it is apparent from the data in fig.1, that any cooling rate, which allows a sample to stay for more than ~10 s in the 5°C to −10°C region, will result in substantial changes in fluorescence properties. It has been claimed before [13] that the rate of cooling affects the emission spectra of isolated chloroplasts. However, it was concluded [14] that no effect of the cooling rate persists, if fluorescence reabsorption is minimized. In turn, our results now suggest that such conclusions should not be extrapolated to *Anacystis*, and presumably also not to other phycobilisome containing algae.

The data in fig.2–4 allow some conclusions concerning the sites of cold-induced energy transfer changes, and relating to the nature of the phycobilisome–thylakoid membrane attachment. They can be discussed within the frame of current phycobilisome models [4,5], according to which energy absorbed by phycocyanin is transferred via allophycocyanin and allophycocyanin B to chl *a* of photosystem II. Cold-pretreatment induces a general increase of phycobilin emission (F645, F660, F680 and the long-wavelength fluorescence) and a general decrease of chlorophyll emission (F685, F695, F715

and F760). The phycobilin fluorescence changes are considerably larger than the chlorophyll fluorescence changes. This feature is readily explained by the normally high efficiency of energy transfer from phycobilins to chlorophyll, a change of which will cause much larger relative fluorescence changes in the 'donor molecules' than in the 'acceptor molecules'. Within the phycobilins the stimulation of fluorescence is particularly pronounced in the region of allophycocyanin (F665) and allophycocyanin B (F680). Within the chlorophylls suppression of F685, F695, F715 and F760 occurs to about the same extent.

As stated above the stimulation of F665 probably occurs at the expense of F645 emission, i.e., this spectral change appears to reflect changes within the phycobilisome. Presumably cold-induced changes in the aggregation state of phycocyanin are involved. The stimulation of F680, on the other hand, reflects changes in the interaction between the phycobilisome and the chlorophyll within the thylakoid membrane. As allophycocyanin B is the last component within the phycobilisome, transferring energy to chl *a*, stimulation of F680 strongly suggests that cold-treatment indeed induces a partial detachment of the phycobilisomes. Most importantly this detachment is practically fully reversible, i.e., the phenomenon is not paralleled by gross structural damage. Earlier data which showed reversible changes in overall phycobilin emission, and which were interpreted to indicate changes in phycobilisome attachment [6–8], did not differentiate changes in allophycocyanin B fluorescence (F680), and could therefore as well be explained by structural changes within the phycobilisomes. With the present approach it was not possible to decide whether the observed 'partial detachment' involves complete detachment of part of the phycobilisomes or limited detachment of all phycobilisomes. In this context, a very recent report of Diner [15] is relevant, who observed that in *Cyanidium caldarium* (grown at 38°C) only 60% of the photosystem II centers were connected to phycobilisomes when measurements were done between 25°C and 5°C. Possibly, also in this organism phycobilisome attachment is temperature-dependent.

Acknowledgements

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