COLD-INDUCED UNCOUPLING OF ENERGY TRANSFER BETWEEN PHYCOBILINS AND CHLOROPHYLL IN *ANACYSTIS NIDULANS*

Antagonistic effects of monovalent and divalent cations, and of high and low pH

U. SCHREIBER

Department of Biophysics, Huygens Laboratory of the State University, PO Box 9504, 2300 RA Leiden, The Netherlands

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1. Introduction

The phycobilin-containing phycobilisomes constitute the major light-harvesting pigment system in blue–green and red algae (reviewed [1,2]). Energy absorbed within the phycobilisomes, which appear to be located at the surface of the thylakoid membrane [3], is transferred with high efficiency to chl *a* which is integrated in the form of protein complexes within the membrane. Not much is known about the nature of the phycobilisome attachment in vivo. For an investigation of this aspect it is important to find ways to modify the degree of attachment. Treatments reported to give a reversible change of energy transfer efficiency between phycocyanin and chl *a* are application of hydrostatic pressure [4], preillumination [5] and cooling to $\lesssim 5^{\circ}C$ [6,7]. In a recent low-temperature spectrofluorimetric study [7] evidence was given that cooling induces the disconnection of allophycocyanin B, which is the last component of the energy transfer chain within the phycobilisome [8] and chl *a*. This cold-induced phycobilisome detachment is accompanied by a several fold increase in phycobilin fluorescence (peaking around 655 nm in the +30 to $-30^{\circ}C$ temperature region). The fluorescence change is practically fully reversible, which indicates that the phenomenon is not accompanied by gross structural damage.

Here, the effect of ionic parameters on the cold-induced phycobilin fluorescence increase has been investigated. It was found that the cold-induced phycobilin fluorescence increase was suppressed by increasing concentrations of divalent cations, while monovalent cations gave the opposite effect. At fixed K^+ and Mg^{2+} concentrations, the cold-induced fluorescence increase was stimulated by increasing pH in the range of pH 5–11. These data are discussed in a qualitative manner on the basis of the Goüy-Chapman theory of the diffuse electrical double layer [9] at the surface of the thylakoid membrane. It is concluded that a negative surface charge favors cold-induced phycobilisome detachment. Ideas of Träuble and Eibl [10] relating to electrostatic effects on fluid → ordered lipid phase transitions in model membranes are applied to explain the role of negative surface charges.

2. Material and methods

Anacystis nidulans was grown at 28°C in C medium of [11], 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 mV/cm²).

Cold-induced fluorescence changes were measured in a system which allowed rapid cooling (e.g., from 25–5°C in <2 s; T-jump curves) or slow cooling (at ~0.7°C/min; F–T curves), as in [12]. Phycobilin fluorescence was excited with 560 nm (2 nm half-bandwidth; ~1 μW/cm²) and measured at 640 nm (10 nm half-bandwidth).

To make the cell envelope permeable to ions, a 60 min lysozyme treatment (0.5 mg/ml) at 28°C was

given. Thereafter the cells were washed twice with 50 mM sucrose, 10 mM Tris (pH 7.5). If not stated otherwise, the washing medium also contained 10 mM EDTA-Na (pH 7.5) in order to remove bound Mg^{2+}. After another washing in the same medium without EDTA, the cells were resuspended in this medium with the appropriate additions of monovalent and divalent cations. Samples were incubated for ≥60 min before measurements. Chlorophyll was ~10 μg/ml.

3. Results

Figure 1 shows the effects of K^+ and Mg^{2+} on the cold-induced increase of phycobilin fluorescence. At time zero, temperature is rapidly switched from 25–5°C. This change in temperature induces an increase in phycobilin fluorescence which, if analyzed in terms of first order processes, is at least biphasic. The rapid phase displays a half-risetime of ~30 s, which is considerably slower than the change in temperature (~2 s). Presence of 50 mM Mg^{2+} diminishes the cold-induced fluorescence increase. On the other hand, in the presence of 100 mM K^+ the fluorescence increase is substantially stimulated. When temperature is switched back to 25°C, most of the cold-induced fluorescence increase is reversed in ≲30 s.

Effects similar to those with K^+ and Mg^{2+} were found with a number of other monovalent and divalent cations. While ≤10% difference was detected in the relative effectiveness of monovalent cations (K^+, Na^+, Li^+, Cs^+, choline$^+$), among the divalent cations (Mg^{2+}, Ba^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}), Mn^{2+} proved particularly effective (~2-times the effectiveness of Mg^{2+}).

With a T-jump to 5°C, as applied in the experiment of fig. 1, a maximal fluorescence increase is not induced. In untreated *Anacystis* cells a T-jump to –5°C gives a close to maximal effect, associated with a fluorescence increase that is 5–6-times higher than with a T-jump to 5°C (not shown in the figures, but see fig. 1 of [8]). In fig. 2 the temperature dependency of the cold-induced fluorescence increase is given for the various ionic conditions. The figure shows F–T curves, which were continuously recorded with a slow cooling rate (~0.7°C/min). This cooling rate is slow enough to allow almost complete equilibration of the cold-induced change which causes the fluores-

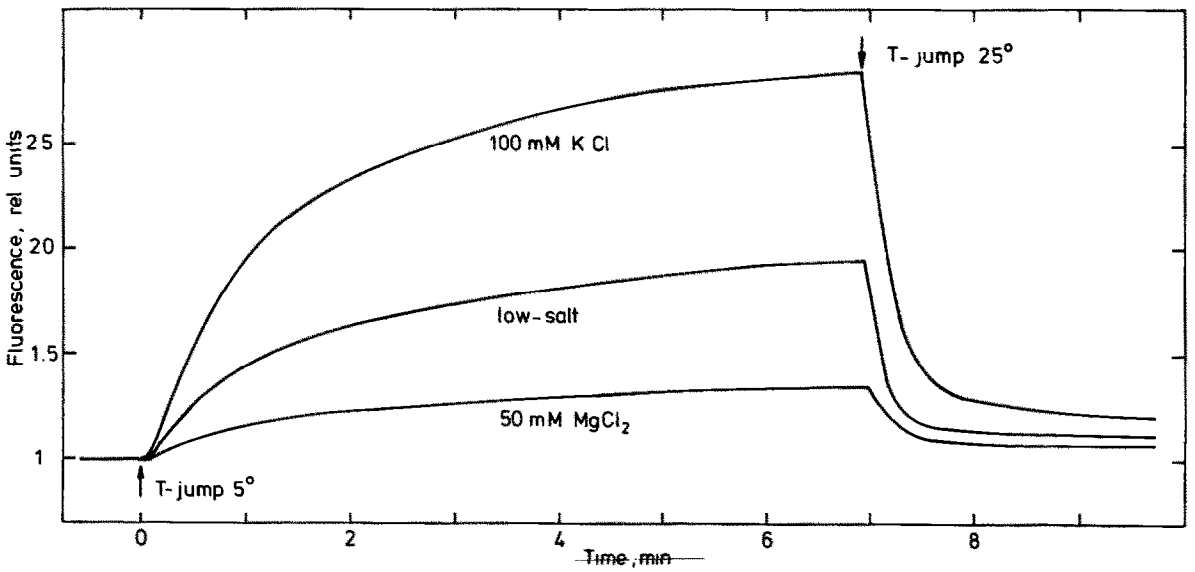

Fig. 1. Kinetics of cold-induced phycobilin fluorescence increase upon rapid decrease of temperature from 25–5°C. Samples were kept for 2 min in the cuvette at 25°C before the T-jump. The fluorescence signals of the three samples were normalized to one relative unit at 25°C. The relative fluorescence yield of the high-salt samples was ~10% higher than in the low-salt sample (see also fig. a).

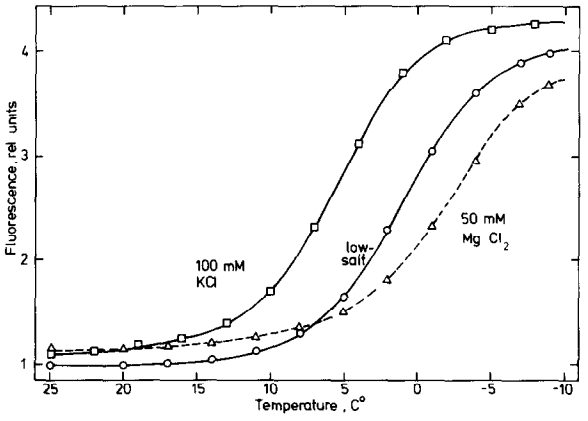

Fig. 2. F–T curves dependent on ionic environment. Samples were continuously cooled from 25–10°C at ~0.7°C/min. Pigment concentration was adjusted to be identical for the three samples. One relative unit corresponds to the 25°C fluorescence signal of the low-salt sample.

cence rise at any given temperature. It is apparent that differences in ionic conditions result primarily in shifts of the F–T curves along the T-axis, and to a much less extent in differences in the maximal cold-induced fluorescence increase. Temperatures where the slopes of the F–T curves are maximal are around 7°C, 2°C and −1.5°C in the 100 mM KCl, the low-salt and the 50 mM $MgCl_2$ samples, respectively. In this context, it may be important to note that normal *Anacystis* cells (not lysozyme-treated and not EDTA-washed) show a F–T curve with a maximal slope around −1.5°C (not in the figures). Hence, physiologically healthy cells appear to correspond most closely to the Mg^{2+}-samples, at a Mg^{2+}-concentration which is saturating in providing protection against the cold-induced changes (see fig. 3).

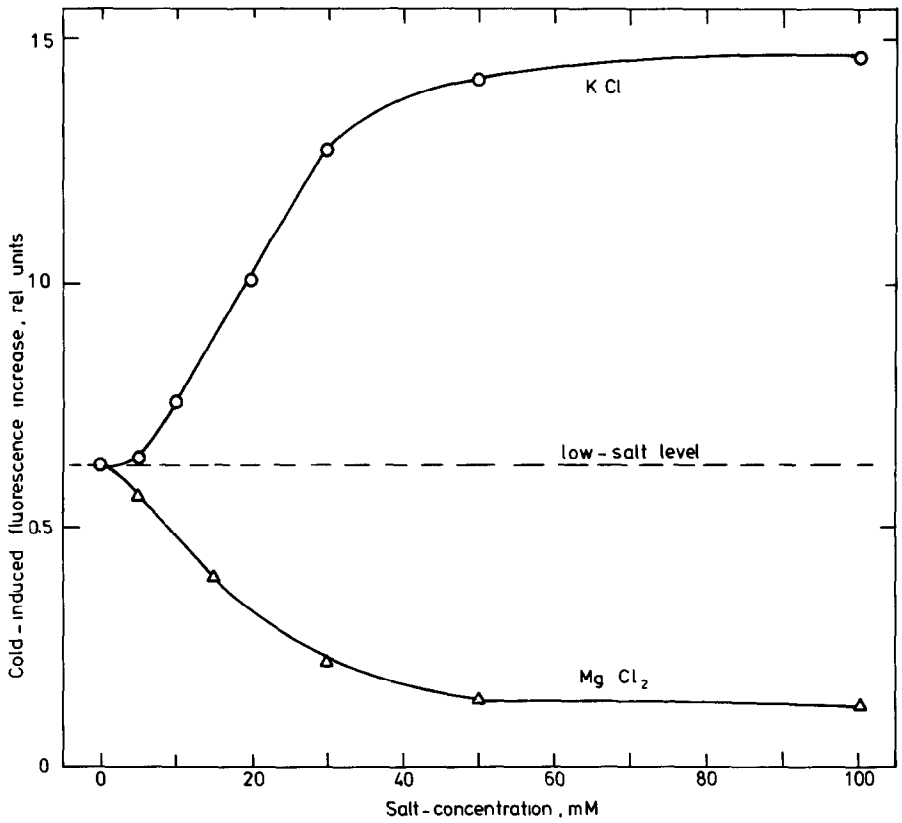

Fig. 3. Cold-induced increase of phycobilin fluorescence dependent on concentrations of added KCl and $MgCl_2$. A T-jump from 25–5°C was applied. Fluorescence levels reached after 2 min at 5°C were plotted. Concentrations were adjusted by addition of concentrated salt solutions to low-salt aliquots. Pigment concentrations were identical for all samples by appropriate additions of low-salt buffer. One relative unit corresponds to the amplitude of the normalized fluorescence at 25°C.

The concentration dependency for the K^+ and Mg^{2+} effects on the cold-induced fluorescence change is shown in fig. 3. Half-maximal effects of K^+ and Mg^{2+} were 20 mM and 15 mM, respectively. Particularly in the case of Mg^{2+}, the concentration dependency was relatively variable with different batches of lysozyme-treated and EDTA-washed cells. It may be concluded that complete removal of Mg^{2+} from the thylakoids and complete equilibration of externally added Mg^{2+} may not have been achieved by the applied procedure. More drastic treatments, like cell rupture by ultrasonication, resulted in release of phycobiliproteins from the cell fragments.

In the experiments of fig. 1–3 the pH was kept constant at pH 7.5 by Tris buffer. In fig. 4 the effect of different H^+-concentrations is shown on the cold-induced phycobilin fluorescence rise with a T-jump from 25–5°C. The samples used in this experiment were lysozyme-treated, but were not washed with EDTA. Furthermore they contained 20 mM KCl and 1 mM $MgCl_2$, to minimize relative changes in ion-concentrations upon adjustment of the pH with minimal amounts of KOH or HCl. Low pH is found

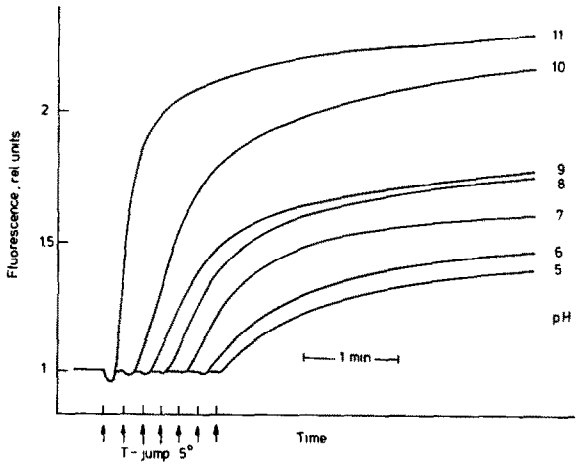

Fig. 4. Kinetics of cold-induced increase in phycobilin fluorescence dependent on pH. Cells were not EDTA-washed and were suspended in 50 mM sucrose, 20 mM KCl and 1 mM $MgCl_2$. The pH was adjusted by addition of minimal amounts of KOH or HCl. The resulting changes in K^+- and Cl^--concentrations did not exceed 3 mM. Samples were incubated for ≥1 h at the given pH, which was re-adjusted every 15 min, and 2 min before T-jump experiment. Fluorescence signals were normalized. One relative unit corresponds to the fluorescence signal at 25°C.

to suppress the cold-induced fluorescence increase, while high pH causes considerable stimulation. The effects were largely reversible at pH 5 and pH 11. At more extreme pH values irreversible denaturation sets in. Comparison of the data of fig. 1 and 4 suggests that for the cold-induced fluorescence changes, a low pH is equivalent to presence of divalent cations, while a high pH is equivalent to the presence of high monovalent cation concentrations. It is apparent from the data in fig. 4 that mainly two types of dissociable groups are involved, with $pK \simeq 6.5$ and 9.5.

4. Discussion

It shown in [7] the cold-induced increase in phycobilin fluorescence correlates with a partial uncoupling of excitation energy transfer from the phycobilins to chlorophyll. By this cold-induced change, the function of the phycobilisome as a light-harvesting pigment complex for the reaction centers is substantially diminished. Therefore, the cold-induced increase of phycobilin fluorescence can be taken as indicator of a 'functional detachment' of the phycobilisome from the chlorophyll pigment complexes within the thylakoid membrane. This functional detachment of the phycobilisome could be caused by some conformational change of the phycobilisome or of the chlorophyll complex it is associated with. According to the Förster theory of resonance energy transfer [13], the efficiency of energy transfer will decrease rapidly with the distance between the two pigment complexes, resulting in an appreciable increase of phycobilin fluorescence.

The data presented above show that the cold-induced functional detachment of the phycobilisome is favored by monovalent cations and by high pH, while it is suppressed by divalent cations and by low pH. These findings suggest that an increase of the net negative charge on the surface of the thylakoid membrane will stimulate the cold-induced detachment. The negative surface charge increases with pH, as dissociation of acidic groups on membrane proteins or lipids is favored. Divalent cations are effective in screening negative surface charges (see, e.g., [14]), which explains that divalent cations work in the same direction as low pH, where dissociation is less. Mono-

valent cations are known to be much less effective than divalent cations in screening negative surface charges [14,15]. However, this fact does not explain yet their antagonistic effect. A possible explanation is offered by the Goüy-Chapman theory, as applied by Träuble and Eibl [10] for related phenomena in lipid vesicles. These authors conclude that an increase in ionic strength of the medium causes increased dissociation of acidic groups, and furthermore favors release of divalent cations from the diffuse electrical double layer. Thus it may be expected that high concentrations of salts, where the cation is monovalent, will result in exposure of negatively charged groups. The pH-dependency depicted in fig. 4 suggests that mainly two types of ionic surface groups are involved with p$K \simeq$6.5 and 9.5. It appears possible that $-NH_3^+$ groups on phospholipids or proteins are involved, which have pK 6–10. Neutralization of $-NH_3^+$ at high pH tends to expose the negative charge on carboxyl or phosphatidyl groups.

The question arises how the negative surface charge can effect the cold-induced increase of phycobilin fluorescence, i.e., the functional detachment of the phycobilisome. One possible effect of the surface charge density is on the fluid → ordered transition of membrane lipids [10]. According to [10] the transition occurs at higher temperatures when the charge density is higher. From the work in [6,15] it is known that in *Anacystis*, grown at 28°C, the fluidity change occurs at ~13°C. These aspects can be linked up with the above results by adopting the following working hypothesis:

> The functional detachment of the phycobilisomes from the chlorophyll–protein complexes, which results in the increase of phycobilin fluorescence, is caused by a cold-induced movement of the phycobilisomes away from the chlorophyll–protein complexes or vice versa. This movement is fluidity-dependent, i.e., it is hindered by a fluid → ordered transition of matrix lipids which is favored by a low surface charge density, as observed at low pH or presence of Mg^{2+}. At high pH and presence of high monovalent cation concentrations the unscreened surface charge is high and the fluid → ordered transition occurs at substantially lower temperatures. Then the cold-induced particle movement and accompanying functional detachment of the phycobilisomes can occur more rapidly.

This interpretation is consistent with recent ultrastructural observations [17,18] which suggest cold-induced displacement of integral membrane proteins.

The above working hypothesis invokes two separate cold-induced phenomena:

(i) The cold-induced particle movement which leads to phycobilisome detachment;
(ii) The membrane fluid → ordered transition.

These two phenomena have opposing effects, which may explain the apparent biphasic character of the cold-induced phycobilin fluorescence rise curves (see fig. 1,4). It appears that process (ii) is somewhat retarded relative to process (i).

The opposing effects of monovalent and divalent cations reported in this study bear some resemblance, although the concentrations required in our experiments are larger, to antagonistic monovalent and divalent cation effects observed in isolated chloroplasts (reviewed [19]). Barber et al. [19,20] discuss the ionic effects applying the Goüy-Chapman equation to the diffuse electrical double layer at the surface of the thylakoid membrane. It may be worthwhile investigating whether also in the green system, cation effects may be correlated with local fluidity changes, e.g., in the boundary lipids of the chlorophyll–protein complexes, which can be expected to be less fluid than the matrix lipids [21].

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