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LIGHT-INDUCED CHANGES IN THE FLUORESCENCE YIELD OF CHLOROPHYLL *a* IN *ANACYSTIS NIDULANS*

I. RELATIONSHIP OF SLOW FLUORESCENCE CHANGES WITH STRUCTURAL CHANGES

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SUMMARY

1. Both normal and 3-(3,4-dichlorophenyl)-1,1-dimethylurea-poisoned cells of the blue-green alga *Anacystis nidulans* show an extensive slow rise in the chlorophyll *a* fluorescence yield upon illumination (Papageorgiou and Govindjee, 1968). It was observed here that uncouplers of phosphorylation notably 5-chloro-3-(*p*-chlorophenyl)-4′-chlorosalicylanilide and 5-chloro-3-*tert*-butyl-2′-chloro-4′-nitrosalicylanilide suppress this slow rise in the fluorescence yield both in normal and poisoned samples. In comparison to these salicylanilides, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone seems to be less effective in this respect.

2. Emission spectra measured with the salicylanilides in 3-(3,4-dichlorophenyl)-1,1-dimethylurea-treated cells show that the uncouplers prevent a shift in the excitation transfer in favor of pigment system II, *i.e.* the transformation to the so-called "State 1". In other words, in the presence of the uncoupler, the pigment systems remain arrested in "State 2". According to this view, the slow fluorescence rise represents a shift from State 2 to State 1.

3. Furthermore, the uncouplers suppress as well the light-induced structural alterations (as measured by a change in absorbance at 540 nm) in both normal and poisoned *Anacystis* cells. Fixation of cells with aldehydes, that immobilizes the structural alterations, also abolishes slow fluorescence yield changes.

4. It was observed that the time course of the light-induced macroscopic structural alteration was slower as compared to slow fluorescence yield changes. It, therefore, seems that the microscopic conformational organization, that precedes the macroscopic volume changes, induces the slow changes in fluorescence yield by shifting the mode of excitation energy transfer.

Abbreviations: DCCD, *N,N′*-dicyclohexylcarbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, dichlorophenolindophenol; FCCP, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone; S_6, 5-chloro-3-(*p*-chlorophenyl)-4′-chlorosalicylanilide; S_{13}, 5-chloro-3-*tert*-butyl-2′-chloro-4′-nitrosalicylanilide.

5. In conclusion, we suggest that an energy-dependent specific alteration of the organization between the two photosystems controls and regulates the mode of excitation energy transfer between the two photosystems, the efficiency of such a transfer in *Anacystis* is approximately 10%.

INTRODUCTION

Blue-green algae exhibit extensive slow changes in the yield of chlorophyll *a* fluorescence upon illumination. In these algae one observes a slow rise in fluorescence yield attaining a maximal level (M) after 1–2 min of illumination[1,2]. From the level M, fluorescence yield declines very slowly to a terminal level (T) (ref. 3). During this time-dependent variations of chlorophyll *a* fluorescence, the yield of the fluorescence of the accessory pigments (phycobilins) remain essentially constant[1]. Secondly, blue-green algae show pronounced slow changes in fluorescence yield even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (refs 1, 2 and 4) and other inhibitors of O_2 evolution[2]. These photosynthetic inhibitor-resistant slow changes in the yield of chlorophyll *a* fluorescence could not be directly linked to the redox changes of the electron carrier "Q". Furthermore, Papageorgiou and Govindjee[1] have shown that uncouplers of photophosphorylation (carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP) and atebrin) suppress the slow changes in the fluorescence yield. Because of their sensitivity to uncouplers and resistance to inhibitors of electron transport (*e.g.* DCMU), it was suggested that some sort of "high energy state" participates in regulating these Q-independent slow changes in chlorophyll *a* fluorescence yield. Currently, it is believed that microscopic structural alterations of chlorophyll *a*-bearing thylakoid membranes lead to the observed fluorescence yield changes owing to the changes in the mode of spill-over of energy between the two pigment systems of photosynthesis[5–7]. In isolated broken chloroplasts, a correlation between the slow changes in fluorescence yield and changes in chloroplast structure could be made under certain conditions[8]. The present study was undertaken to ascertain if intact cells of blue-green alga *Anacystis nidulans* exhibit structural alterations that could be correlated with slow changes in the yield of chlorophyll *a* fluorescence. In this paper, we report the effect of various uncouplers of phosphorylation (5-chloro-3-(*p*-chlorophenyl)-4′-chlorosalicylanilide (S_6), 5-chloro-3-*tert*-butyl-2′-chloro-4′-nitrosalicylanilide (S_{13}), FCCP) on the slow changes of fluorescence yield and 540 nm absorbance change in DCMU-poisoned cells of *Anacystis*. It was observed that the loss of structural alterations is accompanied by a loss or suppression of fluorescence yield changes.

MATERIAL AND METHODS

Details of the preparation of the samples, absorption and fluorescence measurements are described in another paper[2] and in the legends of the figures.

The 540-nm absorbance change was measured with the difference (absorption) spectrophotometer of Sybesma and Fowler[9]. The measuring monochromator was set at 540 nm (half band width, 6.6 nm): a Corning C.S. 4-72 filter was placed before the photomultiplier to eliminate the red actinic light (Schott RG 665: intensity, $3.0 \cdot 10^4$ $ergs \cdot cm^{-2} \cdot s^{-1}$).

Anacystis cells were fixed with 2% paraformaldehyde in 0.05 M phosphate buffer, pH 7.4, as described by Ludlow and Park[10]. Dichlorophenolindophenol (DCIP) Hill reaction was measured as described elsewhere[11]. ATP levels were measured by fire-fly luminiscence method as described by Bedell[12]: these data are not reported here, but are briefly discussed in the Discussion.

RESULTS

Effect of uncouplers of phosphorylation

Papageorgiou and Govindjee[1] have shown that the photophosphorylation uncouplers like (30 μM) FCCP and atebrin, but not the so-called energy transfer inhibitor (1.0 mM) Phloridzin, abolish the slow (SM) rise in *Anacystis.* These authors also documented a differential sensitivity of these uncouplers in the presence or the absence of DCMU: it was observed that FCCP was less effective in suppressing the yield in the poisoned than in the normal cells of *Anacystis.* We investigated the effect of uncouplers, the salicylanilides S_6 and S_{13}, as well as FCCP, on slow fluorescence yield changes both in normal and DCMU-poisoned cells. (It is known that salicylanilides S_6 and S_{13} are quite effective for photosynthetic[13,14] and oxidative phosphorylation[14,15].) Fig. 1 shows the typical inhibitory effect of the uncouplers S_{13} (0.5 and 1.5 μM) and FCCP (1 and 10 μM). Both the uncouplers abolish the fast fluorescence transient at very low concentrations and suppress the slow (SM) rise: S_{13} brought about quite a large suppression of the rise in fluorescence yield at a very low concentration compared to FCCP.

Unlike untreated samples, the DCMU-poisoned *Anacystis* is relatively resistant to the uncoupler FCCP or CCCP: only at very high concentrations these uncouplers

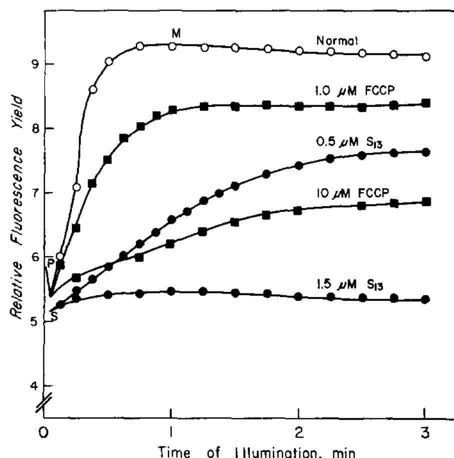

Fig. 1. Effect of uncouplers on the slow chlorophyll *a* fluorescence transient of normal *Anacystis.* λ observation, 685 nm (half band width, 6.6 nm); λ excitation, 633 nm; intensity, 23 kergs · cm^{-2} · s^{-1}; a 6-day-old culture, centrifuged and suspended in 50 mM Tris–HCl *plus* 10 mM NaCl buffer, pH 7.8, was used. Phycocyanin/chlorophyll *a* (peak ratio) = 1.3; ethanolic solution of S_{13} and FCCP were first added to the Dewar flask and then cells were added and incubated in the darkness for at least 10 min before measurements (final ethanol concentration, approx. 2%). M, maximal fluorescence intensity; P, peak in the fast fluorescence transient, discussed in ref. 2; S, semi-steady state of the fast fluorescence transient, discussed in ref. 2.

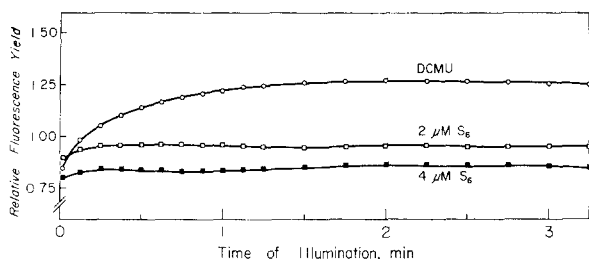

Fig. 2. Time course of the slow rise in chlorophyll *a* fluorescence yield measured at 685 nm in 15 μM DCMU-poisoned cells of *Anacystis* with and without uncoupler S_6. Other conditions for measurement as in Fig. 1.

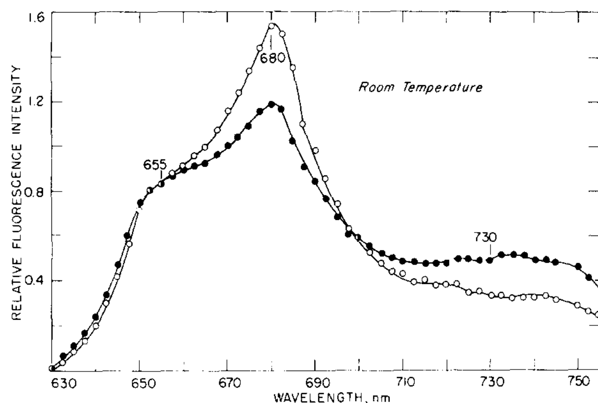

Fig. 3. Room-temperature emission spectra of DCMU-poisoned cells of *Anacystis* with and without uncoupler S_{13}. Cells were suspended in growth medium containing 15 μM DCMU; ○—○, without uncoupler; ●—●, with 5 μM S_{13}.

inhibit the slow rise in the yield of fluorescence[1]. On the other hand, with the uncoupler S_6 (Fig. 2) and also S_{13} most of the fluorescence transient was abolished. Thus, slow changes in the fluorescence yield in DCMU-poisoned *Anacystis* are quite sensitive to uncouplers.

Fig. 3 shows the room temperature emission spectra of DCMU-treated *Anacystis* in the presence and absence of uncoupler S_{13}. The samples were excited with a broad band 570-nm light (half-band width, 20 nm: intensity, approx. 10 kergs $\cdot$ cm$^{-2}\cdot$s^{-1}) for 5 min and then the spectra were recorded. The two spectra were arbitrarily adjusted at 655 nm with the assumption that phycocyanin fluorescence remains unchanged. A depression of System II fluorescence (680 nm peak) accompanied by an increase in the long wavelength fluorescence was caused by S_{13}. In other words, the uncoupler causes an increase of excitation energy transfer to pigment System I. Thus, the slow (S to M) fluorescence rise is due to an increase in the quanta residing in strongly fluorescent System II at the expense of those in weakly fluorescent System I, and the uncouplers arrest (or reverse) this transformation (*cf.* refs 5–7).

Effect of "energy transfer" inhibitors

Fig. 4 illustrates the effects of the so-called energy transfer inhibitors on the slow changes in fluorescence yield. Dio-9, an antibiotic, is known to inhibit ATP synthesis

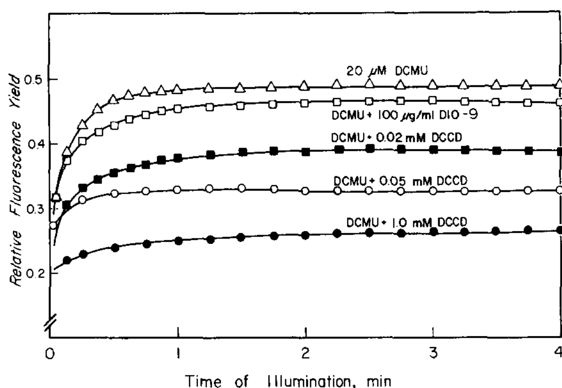

Fig. 4. Effect of Dio-9 and DCCD on the slow rise in chlorophyll *a* fluorescence in 20 μM DCMU-poisoned *Anacystis*. Additions as shown; other details as in Fig. 1.

in chloroplasts[16]. Dio-9, at a concentration of 100 μg/ml, slightly lowered the extent of slow changes in fluorescence yield in the presence of DCMU. Phloridzin, which is also a terminal inhibitor of phosphorylation[17], did not cause any inhibition of slow change in fluorescence yield even at 1 mM concentration (not shown) (also see ref. 1). *N,N'*-Dicyclohexylcarbodimide (DCCD), also considered as an energy transfer inhibitor of chloroplasts[18] and mitochondria[19], however, at a concentration of 0.1–1.0 mM, inhibited most of the slow changes in yield.

Structural alterations during illumination

It has been proposed that light induced variations in fluorescence yield, that are not directly linked to oxidation reduction reactions of photosynthesis, are related to the conformational changes of the thylakoid membrane which accompany photophosphorylation[1,11,20]. Light induced alterations of chloroplast structure which probably reflect the alteration of the conformation and configuration are usually measured by 90° scattering change or change in absorbance around 540 nm (in the valley region of chlorophyll *a* absorption spectrum)[21]. We have attempted, in the experiments described below, to ascertain if cells of *Anacystis* show gross structural alteration during illumination and if this alteration is resistant to the potent inhibitor of O_2 evolution (DCMU) and finally if these structural changes are affected by uncouplers of photophosphorylation.

If *Anacystis* cells suspended in 0.05 M phosphate buffer (pH 8.0) with 0.01 M NaCl or in fresh growth medium (pH 6.8) were illuminated by red light (Schott R.G. 665, R.G. 645 or C.S. 2-64), a decrease in absorbance at 540 nm was observed suggesting a swelling of the thylakoid membranes: this decrease in absorbance at 540 nm was observed both in the absence and in the presence of 15 μM DCMU. However, DCMU-treated samples exhibited slightly greater change than normal cells (not shown).

Fig. 5 illustrates the decrease in absorbance at 540 nm in *Anacystis* induced by three intensities of red illumination. For the bottom trace the actinic illumination was $1.8 \cdot 10^4$ ergs·cm^{-2}·s^{-1}, for the middle trace, $2.4 \cdot 10^4$ ergs·cm^{-2}·s^{-1}, and for the upper trace $3.0 \cdot 10^4$ ergs·cm^{-2}·s^{-1}. Upon illumination there was a lag for about 15–20

s and the saturation of the change was obtained after 4–5 min of illumination. On turning off the actinic illumination (not shown) there was again a lag of 15–20 s after which the absorbance increased and was restored to dark level in about 5–7 min of darkness. This sequence could be repeated. Decrease in the intensity of actinic illumination pronounced the lag period and lowered the rate and extent of decrease in absorbance at 540 nm. However, time to attain saturation did not alter very much.

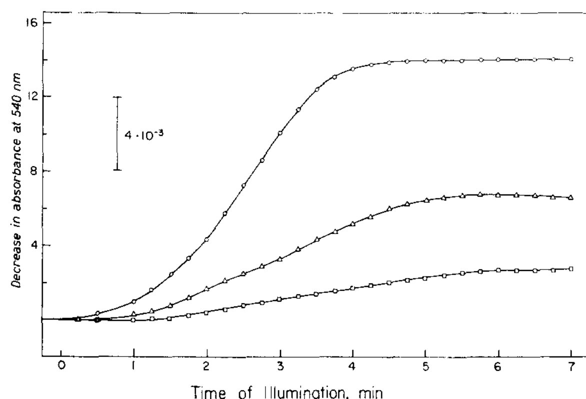

Fig. 5. Decrease in absorbance at 540 nm induced by red actinic illumination of three different intensities in normal *Anacystis*. (The numbers on the ordinate should be multiplied by 10^{-3}.) Slit width, 6.6 nm; C.S. 4-72 filter before the photomultiplier. Actinic filter, Schott RG 665. ○—○, intensity= 1.00; △—△, intensity= 0.80; □—□, intensity= 0.60 (1.00= $3.0 \cdot 10^4$ ergs·cm^{-2}·s^{-1}). Cells were suspended in phosphate-NaCl buffer; absorbance at the red peak of chlorophyll *a*= 0.035; phycocyanin/chlorophyll *a* (peak ratio)= 1.32.

The intensity of actinic light used for these measurements was slightly higher than that used for the fluorescence measurements. However, the time course of decrease in 540-nm absorbance is much slower (Fig. 5) than that of slow changes in fluorescence yield (see normal curve in Fig. 1, and Fig. 2). We do not expect a correspondence in time of these two events. The decrease in absorbance reflects the large macroscopic volume changes of the membraneous vesicles and changes in conformation of the individual thylakoid membrane should precede gross macroscopic changes in structure and we expect only the former to alter spatial relations or orientations of the two pigment systems. Also, the extent of swelling is not proportional to the intensity of illumination, although relative fluorescence yield remains constant at these intensities. This observation does not pose any restriction on the hypothesis that structural changes are related to chlorophyll *a* fluorescence yield changes. Macroscopic changes in volume may not be proportionately related to a photochemical event while fluorescence yield is dependent on the excitation intensity.

Fig. 6 shows the effects of uncouplers (10 μM FCCP, 5 μM S_{13} and 5 μM S_6) on the changes in transmission at 540 nm in DCMU-poisoned cells. The concentration of chlorophyll used here is slightly higher than the concentration used for fluorescence measurements. Interestingly, 10 μM FCCP did cause some 50–60% inhibition while S_{13} and S_6 suppressed 80–90% decrease in absorbance at 540 nm. In some measurements, a complete suppression of change in absorbance at 540 nm was observed.

FCCP did not cause as large an inhibition in fluorescence yield changes as it

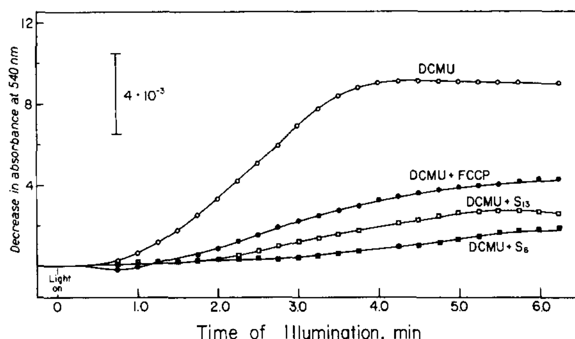

Fig. 6. Time course for the decrease in absorbance at 540 nm with and without uncouplers in DCMU-poisoned cells of *Anacystis*. (The numbers on the ordinate should be multiplied by 10^{-3}.) Slit width, 6.6 nm; C.S. 4-72 filter before the photomultiplier; sample was illuminated with red actinic light (Schott RG 665; intensity, $3.0 \cdot 10^4$ ergs·cm^{-2}·s^{-1}); cells were suspended in phosphate buffer with 15 μM DCMU. Uncouplers (10 μM FCCP, 5 μM S_6 or 5 μM S_{13}) were added to the sample and preincubated for 10 min in the dark before each measurement. Absorbance of the sample at the red chlorophyll *a* peak= 0.035; phycocyanin/chlorophyll *a* (peak ratio)= 1.2.

did for structural changes in DCMU-poisoned *Anacystis*, although 10–20 μM FCCP did suppress (10–20%) fluorescence yield. (We do not know the reason for this difference.) (Izawa and Good[22] have previously shown that FCCP suppresses structural alterations in chloroplasts.) But, qualitatively, our results indicate that both salicylanilides and FCCP suppress the light-induced volume changes as well as slow changes in fluorescence, S_6 and S_{13} more effectively than FCCP. (Kraayenhof[14] has shown that these two potent uncouplers (S_6, S_{13}) are very effective in inhibiting many energy linked reactions in chloroplasts and mitochondria.)

Fixation with formaldehyde

We have earlier shown that fixation of *Porphyridium* cells[11] causes a loss of slow fluorescence transient and loss of CO_2 fixation although a net electron transport capacity is retained in these preparations. Ludlow and Park[10] have shown that formaldehyde fixed cells can support both pigment System I and II activity. Fixed *Anacystis* cells reduced 30 μmoles of DCIP per mg chlorophyll per h with water as a donor. However, formaldehyde fixation caused a complete loss of slow changes in fluores-

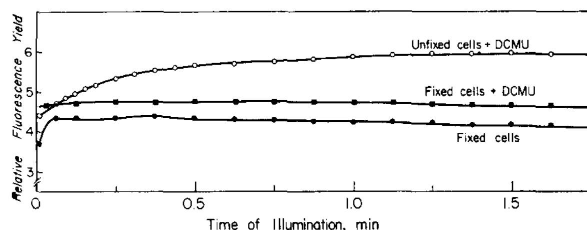

Fig. 7. Time course chlorophyll *a* fluorescence in formaldehyde-fixed cells of *Anacystis* in the presence of 15 μM DCMU. λ observation, 684 nm; C.S. 2-61 filter before the photomultiplier; λ excitation, 633 nm; intensity, approx. 25 kergs·cm^{-2}·s^{-1}. Cells were suspended in phosphate–NaCl buffer, pH 8.0. Fixation of cells was done as in ref. 10.

cence yield both in the presence and in the absence of DCMU (Fig. 7). Fixation with aldehydes changed the fast transient to look like that of isolated chloroplasts (not shown). This result was similar to that observed in *Porphyridium*[11]. From Fig. 7 it is clear that the addition of DCMU caused an increase in the fluorescence yield in fixed cells due to an inhibition of electron transport. However, the long term slow changes in yield were completely arrested by aldehyde fixation.

The results reported in this paper suggest that a correlation exists between light-induced structural changes and slow changes in fluorescence yield. Also, the probable cause of alteration in fluorescence yield must be associated with microscopic alteration in membrane structure and not with the gross swelling or shrinkage of the entire organelle. This latter phenomenon is the net terminal result of changes in membrane conformation, as suggested by Packer *et al.*[23] from their studies with isolated broken chloroplasts from higher plants. (The fixation of membrane proteins, however, suppresses most of the conformational and fluorescence yield changes.)

DISCUSSION

The Q (redox)-independent* slow changes in chlorophyll *a* fluorescence yield observed in intact cells or in isolated chloroplasts seem to be linked to conformational changes of the membrane, associated with energy conservation and ion transport processes; thus, these slow chlorophyll *a* fluorescence yield changes are quite sensitive to uncouplers of phosphorylation. The Q-independent slow fluorescence yield changes, described in this paper, seem to be associated with energy conservation processes linked predominantly to cyclic electron flow, although noncyclic pathway may also contribute to slow changes in unpoisoned cells. From the measurements of total ATP content[12] the simplest interpretation would be that DCMU poisoning induces a cyclic electron flow mediated phosphorylation. It is, therefore, very likely that this energy conserving event indirectly regulates the slow changes in fluorescence yield. Hence, these fluorescence yield changes are suppressed by uncouplers (Figs 1 and 2). This was first observed by Papageorgiou and Govindjee in their studies with FCCP and atebrin on *Chlorella*[24] and *Anacystis*[1]. It is inferred that the slow (SMT) fluorescence changes are supported by a cyclic electron transport mediated photophosphorylation. In blue-green algae, the extent of this latter process seems to be quite high, unlike the case of *Chlorella*[24] and maybe of *Porphyridium*[11].

In spite of similarities in the slow fluorescence induction between normal (unpoisoned) and DCMU-poisoned cells of *Anacystis*, it is to be noted that the latter is comparatively more resistant, although not totally immune to uncouplers. S_{13} and S_6 abolished the slow changes in fluorescence yield (Fig. 2) and these uncouplers also lowered the ATP contents of the cells (data not shown). The dark decay of fluorescence yield in poisoned samples is faster than in normal untreated cells[2]. Also, System I light is more effective in the DCMU-poisoned cells as compared to unpoisoned cells (1, 2 and 4). These differences probably arise due to specific changes in conformation of the two photosystems which control and regulate the extent of change in excitation transfer.

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* It is important to note that the concentration of DCMU used in this investigation was enough to block all electron flow, in the presence or the absence of uncouplers, as checked by O_2 evolution measurements (data not shown).

We have presented evidence that a change in structure, probably a separation of the two photosystems, occurs during illumination in *Anacystis*, and uncouplers affect this separation (Fig. 3). Although the time course of macroscopic volume change (Figs 5 and 6) does not directly correlate with the time course of slow change in in fluorescence yield (Figs 1 and 2), both are suppressed by uncouplers to almost similar extent. Furthermore, fixation of thylakoid membranes, that immobilizes structural change, arrests all fluorescence yield changes (Fig. 7). Such a fixation of cells does not suppress electron transport[8,11]. Our results with the so-called energy transfer inhibitors appear to be somewhat contradictory to the observation of Papageorgiou and Govindjee[1]. However, we confirmed that phloridzin exerted no effect on DCMU-resistant slow changes in fluorescence yield. Dio-9, unlike phloridzin, slightly suppresses the slow rise in fluorescence yield. DCCD, which also interferes with energy transfer in mitochondria and chloroplasts, inhibited the slow changes in fluorescence yield (Fig. 4). It has been observed that DCCD affects a number of energy dependent processes (*e.g.* K^+, Na^+ exchange) in intact bacterial cells of *Streptococcus faecalis*[19,25,26]. These studies also reveal that this inhibitor interacts with membrane bound ATPase in intact cells[25]. We, therefore, do not consider our results with DCCD or Dio-9 to be inconsistent with the suggestion that a high energy intermediate rather than ATP is the source of energy for light-induced changes in lamellar conformation. Chemicals like DCCD or Dio-9, besides being energy transfer inhibitor, may also directly interfere with energy-dependent ion uptake and other osmotic changes in lamellar conformation. We have recently observed that membrane-bound ATPase indirectly influences the cation-induced chlorophyll a_2 fluorescence enhancement in broken chloroplasts[27]. Conceivably, if compounds like DCCD bind with ATPase[26] they will affect the regulatory control of excitation transfer between the two photosystems.

We conclude that a light-induced change in structure alters the mode of excitation transfer between the two pigment systems and brings about changes in chlorophyll *a* fluorescence yield that are not controlled by the redox state of "Q". The slow (SM) fluorescence rise observed in the presence of DCMU (also, other similar inhibitors) is entirely due to such a shift in excitation transfer. In other words, during the slow (SM) fluorescence rise there is either a suppression of energy transfer from pigment System II to I (ref. 4) or an increased transfer from pigment System I to II as suggested by Sun and Sauer[28]. Unlike the situation in isolated chloroplasts, where an enhancement in yield is associated with a shrinkage in volume, we observe a swelling (Figs 5 and 6) during illumination. We, however, note that these macroscopic changes in volume reflect the final configuration, not the specific orientation and conformation of the two photosystems. We assume that it is the latter which regulates the yield. Assuming the slow rise in fluorescence yield is due to a total suppression of energy transfer from pigment System II to I, we estimate, as we have done in chloroplasts[27], the rate constant of energy spillover, k_t, to be $3.6 \cdot 10^8$ s and the efficiency of such a transfer to be approximately 11% in *Anacystis*.

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REFERENCES

1 Papageorgiou, G. and Govindjee (1968) *Biophys. J.* 8, 1299–1315
2 Mohanty, P. K. and Govindjee (1972) *Plant Cell Physiol.*, submitted for publication
3 Papageorgiou, G. and Govindjee (1967) *Biophys. J.* 7, 375–390
4 Duysens, L. N. M. and Talens, A. (1969) *Prog. Photosynth. Res.* 2, 1073–1081
5 Murata, N. (1969) *Biochim. Biophys. Acta* 189, 171–181
6 Bonaventura, C. and Myers, J. (1969) *Biochim. Biophys. Acta* 189, 366–383
7 Duysens, L. N. M. (1970) *Abstr. Int. Congr. on Photosynthetic Unit, Gatlinburg, Tenn., 1970*, Abstr. No. A4, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.
8 Murata, N. (1971) *Biochim. Biophys. Acta* 245, 365–372
9 Sybesma, C. and Fowler, C. F. (1968) *Proc. Natl. Acad. Sci. U.S.* 61, 1343–1348
10 Ludlow, J. C. and Park, R. (1969) *Plant Physiol.* 44, 540–543
11 Mohanty, P., Papageorgiou, G. and Govindjee (1971) *Photochem. Photobiol.* 14, 667–682
12 Bedell, G. W. (1972) Ph. D. Thesis, University of Illinois at Urbana-Champaign
13 Black, C. C. (1968) *Biochim. Biophys. Acta* 162, 294–296
14 Kraayenhof, R. (1971) Ph. D. Thesis, University of Amsterdam, The Netherlands
15 Williamson, R. L. and Metcalf, R. L. (1967) *Science* 158, 1694–1695
16 McCarty, R. E., Guillory, R. J. and Racker, E. (1965) *J. Biol. Chem.* 240, 4822–4823
17 Izawa, S., Winget, G. D. and Good, N. E. (1966) *Biochem. Biophys. Res. Commun.* 22, 223–226
18 McCarty, R. E. and Racker E. (1967) *J. Biol. Chem.* 242, 3435–3439
19 Harold, F. M. (1970) *Adv. Microbiol. Physiol.* 4, 46–96
20 Govindjee and Papageorgiou, G. (1971) in *Photophysiology* (Giese, A. C., ed.), Vol. 6, pp. 1–46, Academic Press, New York
21 Packer, L. and Deamer, D. (1968) in *Photophysiology* (Giese, A. C., ed.), Vol. 3, pp. 91–122 Academic Press, New York
22 Izawa, S. and Good, N. (1965) *Biochim. Biophys. Acta* 109, 372–381
23 Packer, L., Murakami, S., and Mehrad, C. (1970) *Annu. Rev. Plant Physiol.* 21, 271–304
24 Papageorgiou, G. and Govindjee (1968) *Biophys. J.* 8, 1316–1328
25 Harold, F. M., Baarda, J. R., Baron, C. and Abrams, A. (1969) *J. Biol. Chem.* 244, 2261–2268
26 Harold, F. M., Pavalsová, E. and Baarda, J. R. (1970) *Biochim. Biophys. Acta* 196, 235–244
27 Mohanty, P., Braun, B. Z. and Govindjee (1972) *Biochim. Biophys. Acta* 292, 459–476
28 Sun, A. K. and Sauer, K. (1972) *Biochim. Biophys. Acta* 256, 409–427