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Light-induced changes in the fluorescence yield of chlorophyll *a* in *Anacystis nidulans* II. The fast changes and the effect of photosynthetic inhibitors on both the fast and slow fluorescence induction [1]

Prasanna Mohanty and Govindjee

Departments of Botany and Physiology and Biophysics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61081, U.S.A.

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(1) The intensity dependence and spectral variations during the fast transient of chlorophyll *a* (Chl *a*) fluorescence have been analyzed in the blue-green alga *Anacystis nidulans*. (Unlike the case of eukaryotic unicellular green or red algae, the fast fluorescence induction characteristics of the prokaryotic blue-green algae had not been documented before.)

(2) Dark adapted cells of *Anacystis* exhibit two types of fluctuations in the fluorescence yield when excited with bright orange light (absorbed mainly in phycocyanin). The first kinetic pattern called the fast (sec) fluorescence transient exhibits a characteristic original level O, intermediary hump I, a pronounced dip D, peak P and a transitory small decline to a quasi steady state S. After attaining S, fluorescence yield slowly rises to a maximum level M. From M, the decline in fluorescence yield to a terminal T level is extremely slow as shown earlier by Papageorgiou and Govindjee (*8*). As compared with green and red algae, blue-green algae seem to have a small PS decline and a very characteristic slow SM rise, with a M level much higher than the peak P.

(3) A prolonged dark adaptation and relatively high intensity of exciting illumination are required to evoke DPS type yield fluctuations in *Anacystis*. At low to moderate intensities of exciting light, the time for the development of P depends on light doses, but for M, this remains constant at these intensities.

(4) Fluorescence emission was heterogeneous during the induction period in *Anacystis*; the P and the M levels were relatively enriched in short-wavelength system II Chl *a* emission as compared to D and S levels.

(5) The fast DPS transient was found to be affected by electron transport cofactor (methyl viologen), and inhibitors (e.g., DCMU, NH_2OH) in a manner suggesting that these changes are mostly related to the oxido-reduction level of intermediates between the two photosystems. On the other hand, the slow SM changes in fluorescence yield, as reported earlier (*5*, *15*), parallel oxygen evolution. These changes were found to be resistant to a variety of electron transport inhibitors (*O*-phenanthroline, HOQNO, salicylaldoxime, DCMU, NH_2OH and Antimycin *a*). It is suggested that, in *Anacystis*,

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even in the presence of so-called inhibitors of cyclic electron flow, a "high energy state" is still produced.

(6) Measurements of Chlorophyll *a* fluorescence and delayed light emission in the presence of both DCMU and NH_2OH indicate that the slow SM changes are not due to the recovery of the reaction center II in darkness preceeding illumination.

(7) Our results, thus, suggest that in *Anacystis* a net electron transport supported oxidation-reduction state of the quencher Q regulates only partially the development of the DPS transient, but the development of the slow fluorescence yield changes seems not to be regulated by these reactions. It appears, from data presented elsewhere, that the slow rise in the yield results due to a structural modification of the thylakoid membrane.

The fluorescence yield of chlorophyll *a* (Chl *a*) in the intact cells of several photosynthetic tissues undergoes two types of changes during illumination (see review, *1*). The first, a rapid transient, lasting a few seconds, is called fast fluorescence induction. This kinetic pattern is characterized by a rapid rise of fluorescence yield to a low initial (or Original) level (O), from where fluorescence yield rises to an Intermediary maximum level (I), then declines to a Dip (D) and then finally rises to a Peak level of fluorescence (P). From P the yield of Chl *a* fluorescence declines to a quasi-Steady state 'S' (*2–4*). The second kinetic pattern of changes in fluorescence yield, called the slow or long-term fluorescence induction, begins with a rise of yield from S to a Maximum level M and then gradually declines to a Terminal T level (*5–8*). The SMT transient requires several minutes for completion (*5*, *7*, *8*). It has been generally agreed that the fast fluorescence induction (except for the rapid OI rise (*9*)), particularly the IDP part, can be explained on the basis of redox state of the primary electron acceptor of photoreaction II (*9–12*). The slow long-term changes in fluorescence yield cannot, however, be easily interpreted in terms of the oxido-reduction state of the primary acceptor, Q, of photoreaction II, which in its oxidized state but not in the reduced state quenches the Chl *a* fluorescence (*11*).

Several hypotheses have been recently advanced to explain the slow changes in fluorescence yield: Bonnaventura and Myers (*13*) and Murata (*14*) have suggested a change in the mode of excitation transfer between the two photosystems as the probable cause of slow changes in fluorescence yield. Bannister and Rice (*6*) proposed an activation of photosystem II centers and Papageorgiou and Govindjee (*5*, *15*) and Mohanty et al. (*7*) suggested that a light-dependent conformational change of the Chl *a* containing membrane structure brings about the slow changes in yield of Chl *a* fluorescence. Duysens (*16*) proposed a mutual movement of two photosystems to control the fluorescence yield by way of electronic excitation transfer between them.

Blue-green algae — the most primitive of all algae — are unique as they are prokaryotes (like photosynthetic bacteria), but they evolve O_2 (like green plants). In these algae, addition of DCMU causes an enhancement of photophosphorylation (*17*) and a gradual slow increase in fluorescence level, whereas in green algae, a decrease in ATP level and no fluorescence yield changes are observed; oligomycin does not affect the light-ATP level in green algae, but it decreases this level in blue-green algae (*17*). Since it has been suggested that energy conservation

processes (*1*, *5*, *15*) influence the slow fluorescence yield changes, we felt that a detailed investigation of the complete fluorescence induction phenomenon should be made in this algae to understand its relationship to photosynthetic processes. We chose *Anacystis nidulans*. The slow changes in fluorescence yield have recently been characterized partially by Papageorgiou and Govindjee (*1*, *15*), and by Mohanty and Govindjee (*18*) in *Anacystis nidulans* and by Duysens and Talens (*19*) in *Schizothrix calcicola*. However, the fast fluorescence transient in blue-green algae has not been well documented. In spite of preliminary observations of a fast transient in *Anacystis montana* by Clayton (*20*) and in *Schizothrix* by Sybesma and Duysens (*21*), it is generally believed that blue-green algae do not show OIDPS type kinetic pattern of fluorescence induction. In this paper, we report experimental results characterizing in detail, for the first time, the fast fluorescence transient in *Anacystis nidulans*. We have also used a variety of inhibitors (salicylaldoxime, HOQNO, *O*-phenanthroline, antimycin *a* and NH_2OH) to further characterize the nature of the fluorescence yield changes in blue-green algae. The above experiments, and those on intensity dependence, on O_2 evolution, and on delayed light emission show that the fast, but not the slow, changes are related to non-cyclic electron flow in *Anacystis*.

Materials and methods

Anacystis nidulans was grown at room temperature under white light of approximately 2×10^6 ergs cm^{-2} sec^{-1}. The procedure for culturing *Anacystis* was as described earlier (*22*). For some experiments, the cells were grown at 35°C under red light for one to two days before harvesting. After 4–6 days of growth, the cells were harvested by centrifugation, washed with a fresh growth medium or with a buffer and then suspended in either carbonate-bicarbonate or phosphate buffer as described in the text or in the legends of the figures.

The absorption spectra were recorded with a Bausch and Lomb (Spectronic 505) spectrophotometer (half-band width, 5 nm) equipped with an integrating sphere. The optical density at the red peak of Chl *a* of samples used for fluorescence measurements usually varied from 0.025 to 0.04 for 1 mm path length. The ratio of the heights of phycocyanin to Chl *a* absorbance band varied from 1.2 to 1.4.

Fluorescence was measured with the spectrofluorometer as described in **Reference** (*23*). For the measurement of fluorescence transient the procedures of Munday and Govindjee (*24*) and Papageorgiou and Govindjee (*15*) were followed. Fluorescence was excited by a narrow 633 nm orange light beam (half-band width, 8 nm; transmittance, 85%). The excitation intensity was varied by inserting calibrated neutral density filters. For transient studies, fluorescence was observed at 685 nm (half-band width, usually 6.6 nm). The analyzing monochromator was guarded with appropriate (C.S. 2-58 and C.S. 2-61 or RG 645) filters. Fluorescence spectra were corrected for the spectral sensitivity of the spectrofluorometer.

Oxygen exchange was monitored with a Haxo-Blinks type of rate electrode (*7*, *25*). The cell sample was deposited on the bare platinum electrode and was covered with a dialysis membrane. The electrode was immersed in a buffered electrolyte and uniformly gassed with either 2% CO_2 in air or argon (temperature,

20±1°C). The samples were illuminated from top as described in the legends of the figures.

The slow component of delayed light emission was measured as described by Jursinic and Govindjee (*26*).

Chlorophyll *a* was estimated in 80% acetone using an extinction coefficient E'^{1cm}_{663} of 82 cm^2/mmole. All chemical additions preceeded the measurement at least for 10 min, unless otherwise stated. Dark adaptation lasted usually 15 min for fluorescence and O_2 measurements. All measurements were done at room temperature, 22–25°C, under aerobic conditions.

Results

1. *Time course of Chl* a *fluorescence and the rate of* O_2 *evolution*

Fig. 1 shows the oscilloscope tracing of the time course of Chl *a* fluorescence in *Anacystis nidulans*. Like the green alga *Chlorella* (*24*), *Anacystis* shows a pronounced ID decline and from the dip, D, fluorescence rises to its peak level P. PS decline is clearly seen in this picture. From S, fluorescence monotonously rises to the M level. The ratio of (P—O)/O is 1.7. We note that in the blue-green alga *Anacystis*, unlike the green alga *Chlorella* (*3*) or the red alga *Porphyridium* (*7*), the peak fluorescence, P, is not the maximum yield during the entire fluorescence induction period. The large S-M slow rise in the fluorescence yield usually masks the IDPS fast transient in blue-green algae.

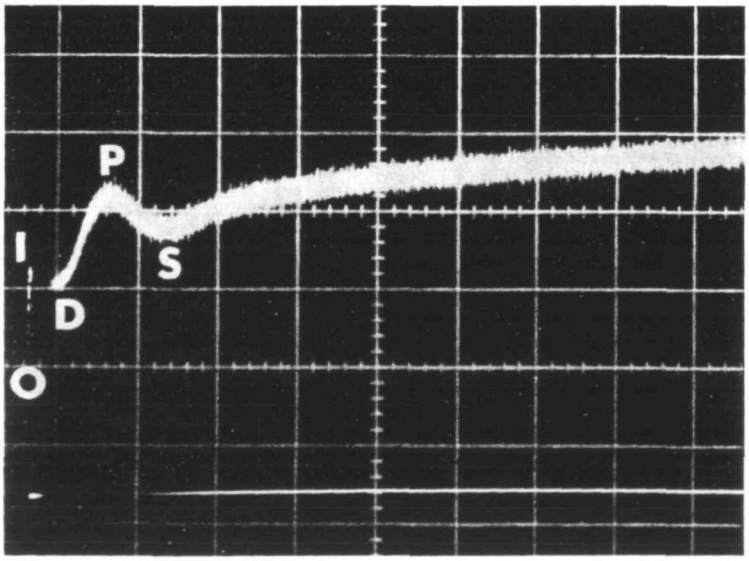

Fig. 1. *Oscilloscope tracing of a typical Chl* a *fluorescence transient in* Anacystis nidulans. λ observation, 685 nm (half band width, 6.6 nm); C.S. 2-58 filter before the photomultiplier; λ excitation, 633 nm (half band width, 8 nm; peak transmittance, 85%); intensity, 26 Kergs $cm^{-2}\,sec^{-1}$. A 7 day old culture of *Anacystis* was suspended in fresh culture medium after washing of the cells in the same medium; 15 min dark period preceeded illumination. Ordinate, 1 volt/division; abscissa, 1 sec/division.

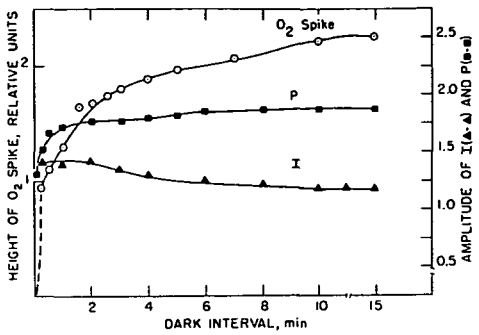

Fig. 2. *Dark recovery of the fast transient of Chl* a *fluorescence (I and P levels) and oxygen spike in* Anacystis. Fluorescence was measured as in Fig. 1, but the cells were suspended in 50 mM phosphate plus 0.01 M KCl, pH 7.8; amplitude of I (solid triangles) and of P (solid squares); ordinate scale to the right. Open circles, height of the O_2 spike measured on the same sample with a Haxo-Blinks type rate electrode; illumination, orange light (K-5 Balzers filter), intensity, ~ 20 Kergs cm^{-2} sec^{-1}. O_2 measurements with 5 sec duration flashes; ordinate scale to the left.

The development of IDPS transient is quite dependent on growth conditions. Cells grown at high temperature (30 to 35°C) or at low light intensity exhibit a more pronounced IDPS transient than cells grown at high light intensity. This is opposite to that observed in *Chlorella* (*27*). We have also observed fast fluorescence transient in filamentous blue-green algae, *Plectonema* and *Phormidium*, in unicellular *Microcystis* and in thermophilic *Synechococcus*. Thus, it seems that all blue-green algae exhibit the characteristic fast transient, with the P level smaller than the M level; the ratio of M to P varied from 1.3 to 2.0.

A prolonged dark adaptation is necessary to restore the development of complete OIDPS transient. Fig. 2 shows the amplitude of I and P as a function of dark time between illumination. At shorter dark times, there is a rapid OI rise

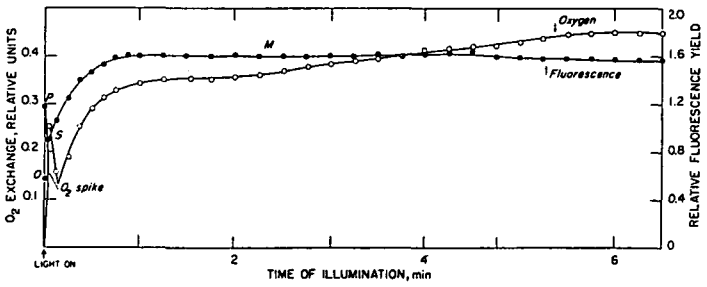

Fig. 3. *Time course of the fluorescence yield of Chl* a *and the rate of oxygen exchange (evolution) in* Anacystis. Replotted recorder trace of the fluorescence transient (solid circles); cells were suspended in 50 mM phosphate plus 10 mM KCl, pH 7.8; intensity of excitation, 26 Kergs cm^{-2} sec^{-1}; the fast OI phase is not shown. Oxygen exchange (open circles) was measured with a Haxo-Blinks type rate electrode; samples were illuminated from the top with saturating red light (C.S. 2-58); intensity, ~ 15 Kergs cm^{-2} sec^{-1}; electrolyte, 50 mM phosphate plus 10 mM KCl, pH 7.8; 2% CO_2 in air in gas phase; a 15 min dark time preceded the measurements. The delay in the occurrence of O_2 spike is due to the slow response of the polargraph; note parallel rise in fluorescence yield (SM) and O_2 evolution.

and a small DP rise. As the dark period is increased, the level of fluorescence at I decreases slightly and the DP transient becomes prominent. When the dark interval between illumination is short, some of the electron carriers reduced by the previous illumination remain in the reduced state; this would cause a high level of fluorescence at I upon second illumination. If the dark period is, however, increased, the amplitude of fluorescence at P increases and attains a maximum value after 8–12 min of darkness. In the darkness, besides oxidation of Q, some other transition related to high fluorescence yield must occur (*1*, *11*, *28*). From Fig. 2 it is clear that rise of P in darkness is biphasic; there is a rapid phase occurring within 1 to 2 min of darkness and then a slow asymptotic rise in the yield at P with increase in dark time. On the other hand, the O_2 gush (O_2 spike) which has been shown to be complementary with the DP fluorescence (*9*, *10*) rise (and which, also like fast transient, requires darkness for its development) does not show indentical biphasic rise. This indicates that besides the redox state of the pool A, which affects the O_2 gush, there is some other factor that influences the development of fast (DPS) fluorescence transient. This supports the original suggestion of Duysens and Sweers (*11*) that the restoration of OIDPS transient is not linked to the oxidation of the primary acceptor Q of photosystem II (PS II).

It is now well known that S-M slow rise in fluorescence yield (in red and green algae) parallels the oxygen evolution (*5–7*). Papageorgiou and Govindjee (*15*) showed only an approximate parallel slow rise in the yield of Chl *a* fluorescence and in the rate of O_2 evolution in *Anacystis*; for some reason, they had oscillations in the O_2 evolution (not seen in fluorescence curve) and the parallelism was not clearly shown in their data.

Fig. 3 shows the time course of oxygen evolution and of fluorescence measured after a 15 min period of darkness. For both of these measurements a bright saturating orange-red light was used to excite the sample and the transients were recorded on identical samples. The lag in the appearance of O_2 spike with reference to the P level of fluorescence is, however, due to diffusional lag of the polarograph to oxygen. But, it is very clear (Fig. 3) that the S-M rise in the fluorescence induction is parallel to O_2 evolution; however, the latter increases even after fluorescence reaches the maximum intensity (M) and then slowly declines.

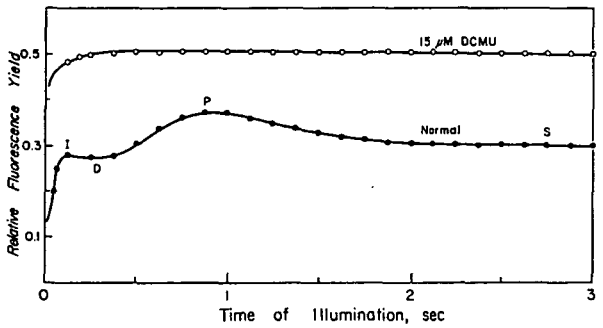

Fig. 4. *Time course of the yield of Chl* a *fluorescence in* Anacystis *with and without 15 μM DCMU.* λ observation, 685 nm (half band width, 5 nm); λ excitation, 633 nm; intensity, ~ 25 Kergs cm^{-2} sec^{-1}; Corning C.S. 2-61 before the photo multiplier; cells were suspended in Warburg buffer No. 9; 15 min dark period before illumination.

Fig. 4 shows that the DPS portion of the fast fluorescence transient is abolished by 10 μM DCMU. (In this figure the very fast OI rise has not been recorded due to the slow response time of the recorder used.) In *Anacystis*, 10 μM DCMU is enough to abolish all the "O_2 gush" and most of the oxygen evolution. These results indicate that the fast transient, particularly the DPS yield change are linked to the redox state of Q. However, the slow SM type changes in the fluorescence yield are independent of Q as these changes are not abolished by DCMU poisoning (see below).

2. *Light intensity and the fast fluorescence transient*

The development of OIDPS transient is responsive to wavelength and intensity of illumination. Blue light, which preferentially excites Chl *a*, does not induce any measurable transient while the orange light, mainly absorbed by phycocyanin, evokes fluorescence transient due to excitation energy transfer to strongly fluorescent Chl *a* of pigment system II. This is so because in blue-green algae, a large proportion of the phycocilin pigments belongs to system II and a larger proportion of Chl *a* to system I. The time course of Chl *a* fluorescence, recorded at various intensities of illumination, is shown in Fig. 5. From this figure, it is evident that weak intensity of illumination fails to evoke the DP rise. On increasing the intensity of illumination, both OI (not shown) and DP rise are accelerated and a distinct P is observed. It is also clear that the dip (D) remains pronounced even when excitation intensity is lowered from 26 to 9 Kergs cm^{-2} sec^{-1}. Munday and Govindjee (*24*) suggested that ID decline in *Chlorella* is due to the antagonistic effect of two photosystems and, thus, one would expect to observe a pronounced D at moderate intensity of system II light. PS decline is pronounced at high

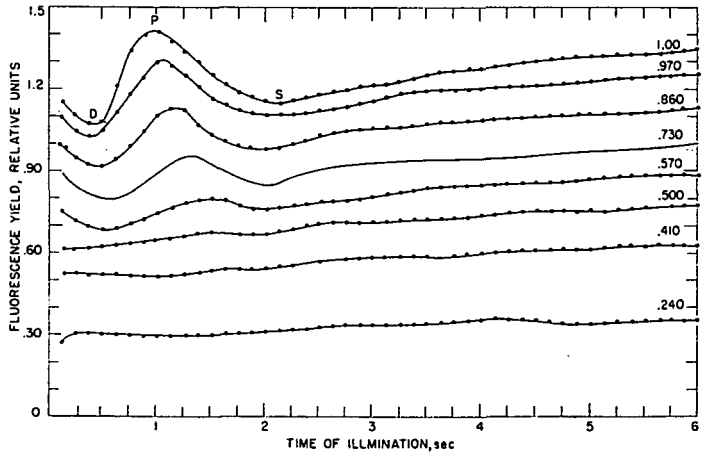

Fig. 5. *Fast transient of Chl a fluorescence yield at different intensities of excitation.* λ observation, 684 nm; C.S. 2-58 filter before the photomultiplier. The numbers on the curves are fractions of the maximum intensity used. Intensity, 1.0=26 Kergs cm^{-2} sec^{-1}; 15 min dark period between measurements; cells were centrifuged and suspended in Warburg buffer No. 9 (pH 9.2) plus 0.1 M NaCl; absorbance at the "red" Chl peak, 0.035; phycocyanin (PC)/Chl a=1.3 (as measured by the ratio of absorbance at 620 nm to that at 675 nm). Signals were recorded on an Easterline Angus recorder; OI not recorded.

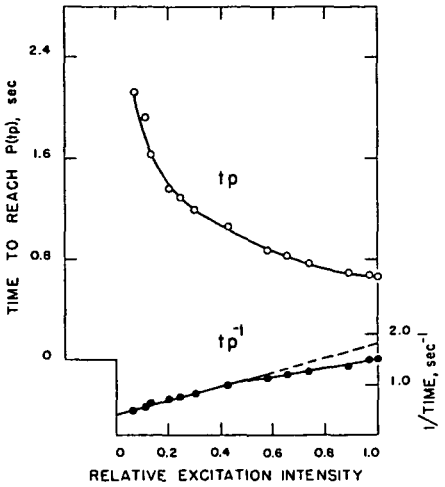

Fig. 6. *Time for the appearance of peak (P) versus intensity of exciting light.* Open circles, time to reach P (tp), scale to the left; solid circles, reciprocal plot (1/tp), scale to the right. Intensity, 1.0=26 Kergs cm^{-2} sec^{-1}. Other conditions for measurements as in Fig. 5 except that an oscillographic recorder was used for the determination of the time interval between illumination and the development of P.

intensity of illumination and the extent of the decline was lower as excitation intensity was lowered.

As shown in the case of red alga *Porphyridium* (*7*) and the green alga *Chlorella* (*24*), the time interval between the onset of illumination and the peak P (tp) depends on the exciting light intensity. A graph of this time interval (tp) versus exciting intensity for *Anacystis* is shown in Fig. 6. (These data were plotted from a separate experiment very similar to that shown in Fig. 5.) As intensity increases, the tp becomes gradually smaller. The plot of 1/tp versus I (intensity) shows that I×t is constant at low intensities of excitation, but this relationship breaks down at higher intensities of illumination. Under light limiting conditions, the formation of P depends solely on the dose of light which is proportional to the absorbed quanta suggesting a true photochemical origin of DP rise. At high light intensity, some other factors besides light become limiting and delay the development of P. The

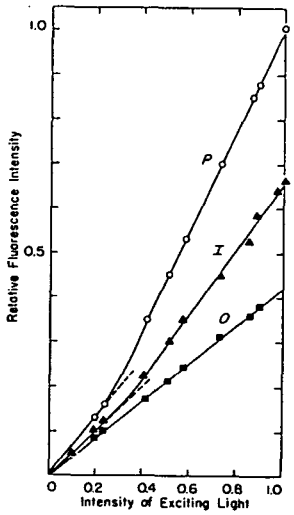

Fig. 7. *Amplitudes of fluorescence at O, I and P as a function of excitation intensity.* Intensity, 1.0=26 Kergs cm^{-2} sec^{-1}; other details as in Fig. 5 and 6 except that a different culture of *Anacystis* was used here.

time required to reach P at saturating light intensity seems to be quite characteristic of each organism. In *Chlorella* and *Porphyridium* P occurs within 300–500 msec of illumination (*7*, *24*, *25*) while in *Anacystis* it takes 700–800 msec of illumination to reach P level fluorescence. It seems that *Anacystis*, perhaps, has a larger pool of intermediate carriers. Fig. 7 shows the variation of the amplitude at I and P as compared to background O level as a function of exciting intensity. The O curve has a constant slope (as first documented by Lavorel (*29*) in *Chlorella*, also see **Ref.** *24*) and the fluorescence yield at I and P increases as light intensity is increased. The ratio of high intensity yield to the low intensity yield varied from 1.3 to 1.4 for I, and 2.0 to 2.2 for P. Thus, the fast OIDPS fluorescence transient in prokaryotic blue-green algae, although small in extent, is qualitatively similar to the fast yield changes described for other eukaryotic unicellular algae (see **Ref.** *1*).

3. *Light intensity dependence of the slow fluorescence transient*

The time course of the slow SM rise of fluorescence yield, recorded at various intensities of excitation is shown in Fig. 8. Papageorgiou and Govindjee (*15*) had published such a curve for *Anacystis*, but they did not show the relationship of P and M levels, and they did not provide the absolute data as they had "normalised" their curves at 3 sec. It is clear from Fig. 8 that M is much higher than P. Low intensities of illumination evoke slight development of SM rise. At moderate to low intensity of excitation, the SM rise is biphasic and this biphasic rate of rise "disappears" at very high intensity of illumination. This biphasic

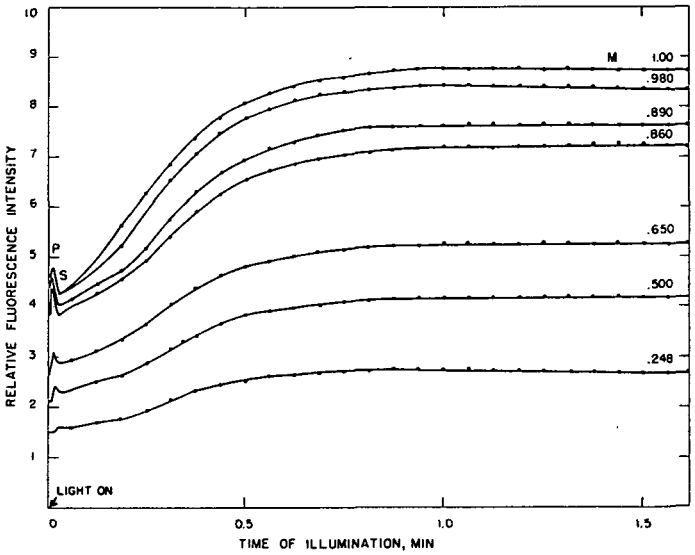

Fig. 8. *Slow transient of Chl a fluorescence at various intensities of excitation.* λ excitation, 633 nm; λ observation, 684 nm (half band width, 6.6 nm); C.S. 2-61 filter before the photomultiplier to guard the light leak. The numbers on the curves represent the fraction of the maximal intensity (1.0= 29 Kergs cm^{-2} sec^{-1}) used. Cells were centrifuged, washed, and suspended in Warburg buffer No. 9 (pH 9.2) with 0.01 M NaCl. [Chl] = 72 μg/3 ml; PC/Chl *a* (peak ratio) = 1.4; 15 min dark period between measurements. Transients were recorded with a Keithley Voltammeter and an Easterline Angus recorder.

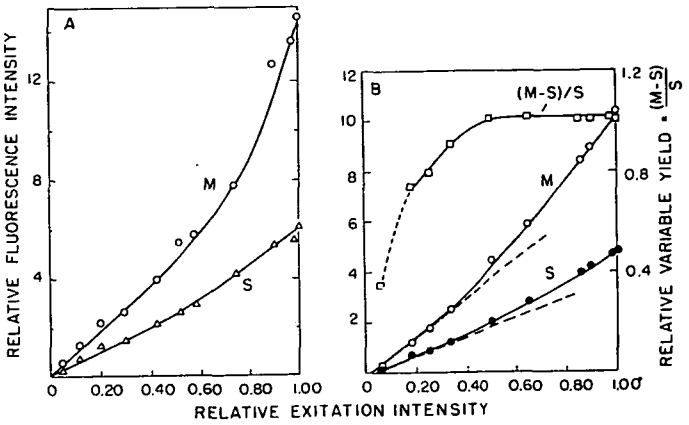

Fig. 9. *Variation of the amplitude of Chl a fluorescence at S and M as a function of exciting intensity.* λ observation, 685 nm (half band width, 4.8 nm). One week old cultures of *Anacystis* (A) and *Microcystis* (B) were suspended in Warburg buffer No. 9, plus 0.01 M NaCl. 10–15 min dark period was given before illumination.

SM rise indicates that light induced increase in the fluorescence yield is complex and, perhaps, involves two reactions. But from Fig. 8 it is clear that time to reach maximum M does not vary with the light doses and thus SM rise, unlike DP rise, does not seem to result from I × t type photochemical reaction. We have previously shown this to be true in *Porphyridium* (*7*).

Fig. 9A shows the variation of the amplitudes of fluorescence at S and M as a function of exciting light; the "separation" of SM occurs at relatively low light intensity. At M, the increased yield at high intensity is much larger than at the S level. Fig. 9B shows a similar plot of amplitudes at S and at M in another blue-green algae *Microcystis*; it illustrates that the variable yield at M with respect to S, represented as (M—S)/S, saturates at a relatively low light intensity. Similar low light saturation of SM rise of fluorescence has been shown to occur in the red alga *Porphyridium* (*7*) and in green alga *Chlorella* (*5*). It has been shown that SM rise saturates at an intensity considerably lower than what is needed to saturate photosynthesis (*5*, *15*) or the corresponding DP rise in the fast fluorescence transient (*7*). Thus, the low light saturation of the relative yield at M is in agreement with the suggestion that the increase in fluorescence yield is not directly correlated with electron flow.

4. *Slow fluorescence yield changes in DCMU poisoned cells*

The most striking evidence that the slow rise in fluorescence is not linked to the redox state of Q has been obtained from the observation that both system I and system II light can cause large variations in fluorescence yield even in the presence of DCMU. This was clearly shown by Papageorgiou and Govindjee (*15*) in *Anacystis* and Duysens and Talens (*19*) in *Schizothrix*. These investigators have shown that system I light is more effective in evoking the slow rise in yield than the equivalent dose of system II light particularly in the presence of DCMU. We have confirmed this to be true for *Anacystis* in the presence of 15 μM DCMU (not shown).

Papageorgiou and Govindjee (*15*) measured the time course of slow fluorescence yield changes as a function of light intensity in DCMU poisoned *Anacystis*. Here again, these authors had arbitrarily normalized their data at 3 sec. In DCMU-poisoned *Anacystis*, we have confirmed that an increase in excitation intensity increases the fluorescence yield changes which attain a maximal level after 2 to 3 min of illumination. Also, we observed that the relative variable fluorescence yield at M in DCMU poisoned level saturates at low light intensity like in normal cells (not shown).

In spite of the similarity in the slow changes in the fluorescence yield both in DCMU-treated and untreated cells, there is a difference in the dark decline in fluorescence yield in the two cases. As shown in Fig. 10, the dark decay, monitored with a very weak intensity (~80 ergs cm^{-2} sec^{-1}) of PS I illumination, of fluorescence yield is slower in normal cells (t 1/2, 20–40 sec) than in poisoned cells (t 1/2, 10–15 sec). Although the decrease in fluorescence yield occurs within several seconds, a dark adaptation of several minutes are required to restore the complete transient.

5. *Is there a role of back reaction between Z^+ and Q^- in causing the induced slow fluorescence yield changes in DCMU poisoned* Anacystis?

Although slow changes in the fluorescence yield in the presence of DCMU suggests that it is independent of the redox state of Q, it is possible that a rapid back recombination between oxidized donor (Z^+) and the reduced acceptor (Q^-) of PS II may be sufficient to contribute to the slow fluorescence yield changes. A comparatively fast dark decrease of fluorescence yield (Fig. 10) is indicative of such a possibility. The following results, however, rule out such a possibility.

It has been recently shown that NH_2OH, in the presence of DCMU, inhibits the back recombination between Z^+ and Q^- (*30*, *31*). Such an inhibition of back recombination between Z^+ and Q^- results in a total loss of the slow component of delayed light emission. It has been proposed that in the presence of DCMU

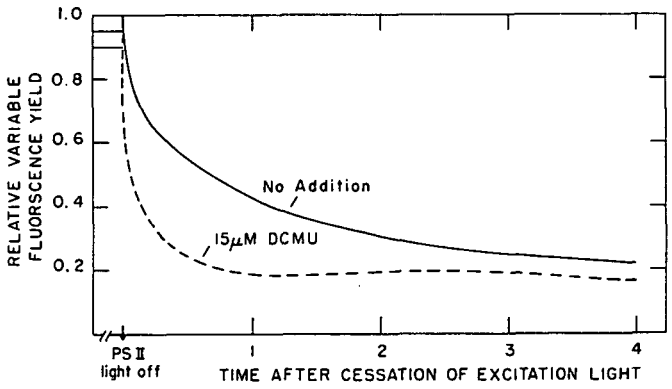

Fig. 10. *Dark decay of fluorescence yield at M in normal and (15 μM) DCMU poisoned samples of* Anacystis. Cells were suspended in fresh culture medium. After a dark period of 15 min, slow rise in fluorescence yield was measured at 685 nm (half band width, 6.6 nm) until M was reached (λ excitation, 633 nm; intensity, ~25 Kergs cm^{-2} sec^{-1}); then, excitation light was turned off and a weak (~ 80 ergs cm^{-2} sec^{-1}) system 1 illumination was quickly turned on. Decay of variable yield was recorded with an Easterline Angus recorder.

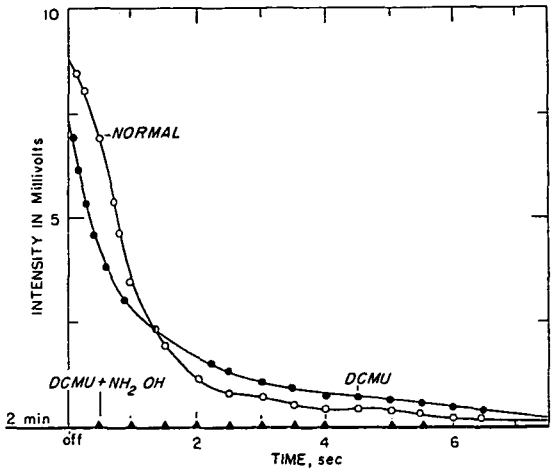

Fig. 11. *Slow delayed light (DLE) intensity in normal* Anacystis, *in the presence of DCMU and of DCMU plus hydroxylamine.* 1 ml algal sample was illuminated with a broad band blue light (C.S. 4-96 plus C.S. 3-73; intensity, 21 Kergs $cm^{-2} sec^{-1}$) for 2 min. DLE was measured 0.1 sec after illumination. Cells were suspended in carbonate-bicarbonate buffer (0.1 M), pH 9.2, plus 0.01 M NaCl; absorbance at the red peak; 0.03; PC/Chl *a* (peak ratio) = 1.4; 15 μM DCMU; 10 mM NH_2OH. Note that no measurable DLE in the presence of both DCMU and NH_2OH suggests a complete inhibition of back reaction between Z^+ and Q^- (see Stacy et al., *32* and Mohanty et al., *31* for details).

the slow component of DLE originates mostly from recombination of charges between Z^+ with Q^- to yield ZQ (*32*).

Fig. 11 shows that in the presence of both DCMU and hydroxylamine there is no measurable amount of delayed light, suggesting a complete elimination of back reaction. Fig. 12 shows that the slow rise of fluorescence yield, however, was not affected by these two inhibitors when added together. (It was also observed that NH_2OH suppressed the fast transient in the normal *Anacystis*, and brought about characteristic fluorescence changes in the transient very similar to what has been reported in *Porphyridium* (*31*).)

Thus these results, together with observations that DCMU inhibits fast fluorescence yield fluctuations (Fig. 4) but not the slow fluorescence yield changes

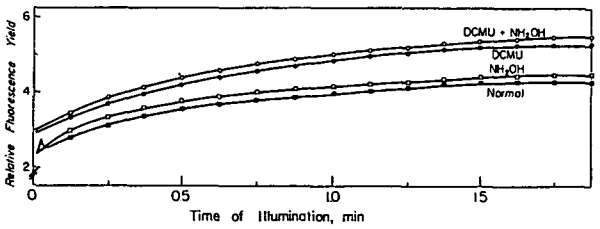

Fig. 12. *Time course of Chl a fluorescence measured at 685 nm in untreated, DCMU, NH_2OH, and DCMU plus NH_2OH treated cells of* Anacystis. Cells were suspended in Warburg buffer No. 9 with 0.01 M NaCl, pH 9.2; 15 μM DCMU; 10 mM NH_2OH; PC/Chl *a* (peak ratio) = 1.4; absorbance, 0.03 at the red Chl *a* peak; 15 min dark period before illumination; λ excitation, 633 nm; intensity, $\sim$ 26 Kergs $cm^{-2} sec^{-1}$. (The details of fast transient are not shown.)

and also the fact that this type of slow fluorescence yield change is evoked both by system I and system II illumination, very clearly show that these changes are not associated with electron transport mediated changes of the redox state of the primary acceptor of system II.

6. *Changes in emission spectra during fluorescence induction*

Papageorgiou and Govindjee (*8, 15*) showed that spectral variations accompany long term fluorescence induction in *Anacystis*. However, spectral changes during the first few seconds of illumination were not reported in this alga. Fig. 13 shows the room temperature emission spectra at three characteristic points (D, P and S) in the fast fluorescence transient in *Anacystis*. The fluorescence band peaking at 655 nm represents the emission from phycocyanin, and that at 683 nm from Chl a_2. The ratio of fluorescence intensity at 683 nm to that at 710 nm (Chl a_1) is 4.32 for "P" spectrum, 3.89 for "S" spectrum and 3.76 for "D" spectrum. It is clear that P is comparatively enriched in Chl a_2 (chlorophyll *a* excited by PS 2 light) fluorescence than S and conversely S is comparatively enriched in Chl a_1 fluorescence. These results are similar to those on other algae (*33*), in showing the heterogeneity in emission spectra. We also observe here an enrichment of system I fluorescence in the "D" spectrum.

Since Papageorgiou and Govindjee (*15*) have made a study of the spectral changes during the slow fluorescence induction, we did not measure point by point spectra for the SM rise phase of the induction curve. But we have compared the ratio of intensity at 684 nm to that at 710 nm at S and at M levels in several experiments to establish the range of changes observed. This ratio for S varied from 3.0 to 3.5 and for P it ranged from 3.5 to 4.5 depending on the culture conditions. These variations in the ratio are mainly due to changes in the extent of

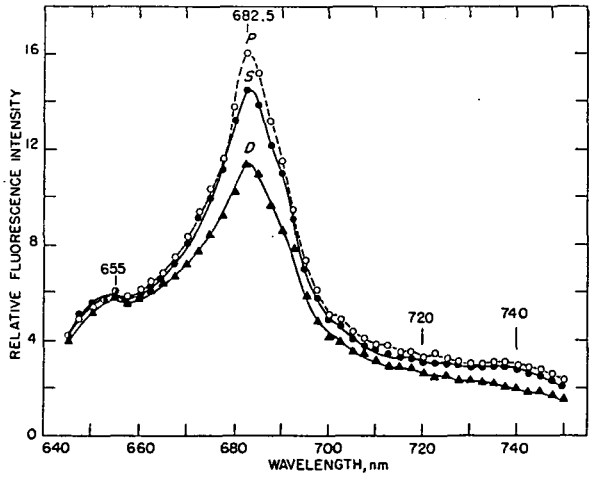

Fig. 13. *Room temperature emission spectra at various points of the fast transient D (solid triangles), S (solid circles), and P (open circles).* 15 min dark period between each measurement; λ excitation, 633 nm; intensity, 26 Kergs cm^{-2} sec^{-1}; Schott red filter (RG 645) was placed before the analyzing monochromator (half band width, 3.3 nm); 6 day old culture in carbonate-bicarbonate buffer (0.1 M) plus 0.01 M NaCl, pH 9.2; [Chl], 6.5 μg/ml; PC/Chl *a* (peak ratio) = 1.2.

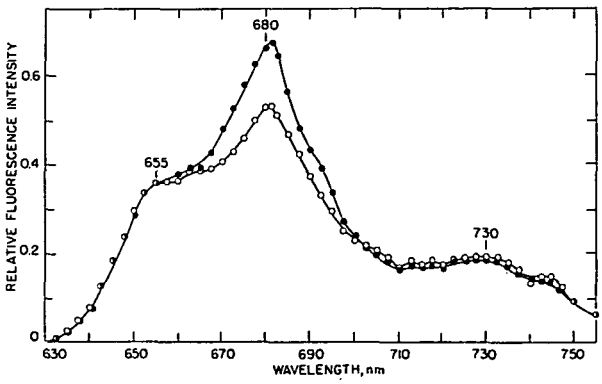

Fig. 14. *Room temperature emission spectra of* Anacystis *in the presence of DCMU.* Solid circles, excitation with high intensity of 570 nm light (half band width, 15 nm), intensity, 9.3 Kergs cm^{-2} sec^{-1}; open circles, with low intensity of 570 nm light, 0.11 Kergs cm^{-2} sec^{-1}. 15 μM DCMU in carbonate-bicarbonate buffer, pH 9.1, plus 0.01 M NaCl. Both spectra were adjusted at 650 nm to read equal intensity. Observation monochromator's half band width, 6.6 nm; C.S. 2-63 filter to guard the light leak.

evergy transfer from phycocyanin to Chl a_2. (See Ghosh and Govindjee (*34*).) In spite of these variations, we have always observed more of an enrichment of Chl a_2 fluorescence at M than at S. Similar enrichment of the main 685 nm fluorescence band in the M spectrum compared to the initial S level in the presence of 15 μM DCMU was also observed in our work. We compared the room temperature emission spectra of DCMU poisoned *Anacystis*, excited by low and high intensity of 570 nm light (Fig. 14). (The two curves were adjusted at the invariable phycocyanin fluorescence band for comparison.) At low intensity, used in the experiment, there is no significant change in fluorescence yield, and thus, the emission spectrum would be comparable to that of the yield at initial low level (F_1 level). At high intensity of illumination, the recorded spectrum would correspond to M level (F_m). It is clear from our data that an increase in fluorescence at high light intensity is due to enhancement in Chl a_2 fluorescence bands (F 683; F 695). As in the case of unpoisoned cells of *Anacystis*, the DCMU poisoned samples show an extensive change in the emission spectra suggesting an enhancement in fluorescence intensity in the Chl a_2 fluorescence band at the high emissive (F_m) state compared to low emissive initial (F_1) state. The higher fluorescence yield and parallel rise in the rate of oxygen evolution in unpoisoned cells are indicative of change in distribution of quanta in favor of PS 2 (*13*). Our results clearly show that this type of shift in energy distribution also occurs even when the PS 2 reactions are blocked by DCMU in *Anacystis*.

7. *Effect of inhibitors of electron transport on the slow fluorescence yield changes in* Anacystis

In contrast to the green alga *Chlorella* (*3, 5*) or the red alga *Porphyridium* (*7*), the blue-green algae exhibit a large variation in yield when net electron transport is blocked by poisons such as CMU or DCMU. A slow increase in the fluorescence yield in the presence of these poisons led many investigators to conclude that PS 1 mediated cyclic electron transport supported ATP or high energy intermediate

Table 1 *Effect of various electron transport inhibitors on the maximal steady state fluorescence yield increment in* Anacystis nidulans

Additions	Ratio of relative fluorescence yield change at the final (Fm) and the initial (Fi) level, Fm/Fi
None	1.60
10 μM DCMU	1.80
5 mM Salicylaldoxime	1.67
10 μM HOQNO	1.50
0.1 mM Orthophenanthroline	1.70
1 μM, 5 μM or 10 μM Antimycin A	1.65
30 μM Antimycin A	1.70
1 mM MeV	1.47
15 μM DCMU+1 mM MeV	1.75

Samples were incubated in 50 mM phosphate buffer with 10 mM NaCl, pH 8.0 with each inhibitor for 10 min. HOQNO and orthophenanthroline were obtained from Sigma Chemicals, St. Louis, Missouri; Salicylaldoxime from Kodak Chemicals, Rochester, New York; and Methyl viologen (Mev) from Mann Research Chemicals, Orangeburg, New York. HOQNO and orthophenanthroline were dissolved in ethanol, salicylaldoxime in methanol and MeV in distilled H_2O. Salicylaldoxime was added to the empty Dewar flask and was allowed to evaporate a few seconds before the addition of cells. All additions were made in dim room light.

somehow regulates the fluorescence (*5*, *6*, *15*, *16*, *18*). With an aim to further characterize the slow fluorescence yield changes we have thus employed a variety of inhibitors of both (supposedly) cyclic and noncyclic electron transport to study such resistant fluorescence yield changes in *Anacystis*. Table 1 shows the effect of some of the common electron transport inhibitors and cofactors we have tested. None of these compounds (*O*-phenanthroline, salicylaldoxime, HOQNO and antimycin *a*) cause any significant suppression of the slow increase in the yield of fluorescence, but the terminal electron acceptor of PS 1, methyl viologen (MeV), did suppress the slow SM rise in normal *Anacystis*. However, MeV did not cause comparable suppression when added together with DCMU. We have also observed that MeV in the range of 0.1 to 1 mM concentration eliminated DP rise in *Anacystis* in a manner similar to what has been observed in *Chlorella* (*12*). In many cultures, but not in all cases, we have observed that the extent of the slow rise is higher in DCMU poisoned cells than in untreated samples (see Table 1).

Discussion and conclusions

The induction of Chl a_2 fluorescence in prokaryotic blue-green alga, *Anacystis nidulans*, follows a kinetic pattern very similar to that observed in eukaryotic algae (*1*). The most distinctive feature of the fluorescence induction curve of the blue-green alga is the pronounced slow rise (SM) in the fluorescence yield, which develops after the fast OIDPS transient. The magnitudes of variation in the fast fluorescence wave are small but show all the characteristics (OIDPS) of fast transient. In blue-green algae the yield at P is not the maximum fluorescence yield attained during the induction curve, but the M level is. The ratio of M to S yields varied

from 1.3 to as high as 1.8. After attaining the M level, fluorescence yield declines very slowly to a T level only after prolonged illumination (*5, 8*). All the blue-green algae we have examined showed a slow increase in yield in the presence of DCMU, a potent inhibitor of photosynthesis, which confirms the earlier observations of other investigators. On the other hand, green alga, *Chlorella pyrenoidosa*, or the red alga, *Porphyridium cruentum*, do not show significant time dependent alteration of fluorescence yield in the presence of DCMU (*5, 7*) although a slow rise in the presence of DCMU has been observed in *Chlamydomonas* by Bannister and Rice (*6*).

The development of the fast OIDPS transient in *Anacystis* required a prolonged dark adaptation and a high intensity of system 2 illumination. This transient is not evoked by system 1 illumination. The ratio of variable to constaut fluorescence yields (P—O)/O varied, depending on the culture conditions, from 1.3 to 1.7. The fast transient shows a pronounced D and a small PS decline. During the OIDPS transient, the emission characteristics of Chl *a* fluorescence varied; the S and the D spectra showed a higher system 1 and lower system 2 emission compared to the P spectrum. A relative enrichment of system 2 fluorescence at P compared to S has been previously shown in many eukaryotic algae (*1, 7*) and is interpreted as an indication of a shift in the mode of energy transfer from PS 2 to PS 1 (*1, 7, 13, 14, 16*). The terms State 1 and State 2 have been used to describe the regulation in energy transfer between the two photosystems (*13, 14, 16*). In the state 1, more quanta are available to system 2 and less to system 1 than in State 2. The same interpretation appears to hold for blue-green algae.

We have observed that electron transport cofactor, methyl viologen, eliminates the DPS transient of *Anacystis*, suggesting that DP rise results due to a temporary accumulation of reduced intermediate between the two photosystems (*12*). Hydroxylamine, at a relatively high concentration (1 mM) also eliminates DPS transient very similar to that observed in *Porphyridium* (*31*). Addition of DCMU which blocks electron transport beyond Q (*11, 30*) evokes a rapid OI rise and suppresses the PS decline. But the slow SM rise in fluorescence yield persists; it is even stimulated with this poison. Anaerobic conditions (data not shown in the paper) like DCMU poisoning, eliminate PS decline. These two conditions are known to induced cyclic electron flow in vivo. Thus, it seems that slow PS fluorescence decline, although not directly linked to the redox state of Q (*11*), is, however, linked in directly to non-cyclic electron transport between the two photosystems. We are not sure of the nature of this relationship.

The slow long-term changes in fluorescence yield characterized by SM (fi → fm) rise is resistant to many inhibitors of electron transport. These inhibitors, as inferred from studies made with isolated chloroplasts, block at different sites in the electron transport chain (*35, 36*). Antimycin *a* and HOQNO, which are known to interfere with the cyclic electron flow, did not bring about any suppression in the slow changes in yield. It is likely that these inhibitors act like inhibitors of non-cyclic electron flow, and are not specific for cyclic flow in *Anacystis*. From the effects of these inhibitors on fast fluorescence transient, we know that these chemicals do enter the cells of *Anacysis*. That is, in their presence, a "high evergy state" may be produced in *Anacystis*.

Although the terminal electron transport acceptor (methyl viologen) eliminated

DPS fast transient and caused a suppression of SM rise in normal cells, it did not cause significant alterations in DCMU-poisoned *Anacystis*. It seems DCMU stimulates a very effective cyclic electron flow in *Anacystis*, and methyl viologen is unable to break the cyclic electron flow. On the other hand, methyl viologen competes effectively with ferredoxin or $NADP^+$ as a terminal electron acceptor in unpoisoned cells; here, it keeps most of the electron transport intermediates in the oxidized state. Thus, the quenching of fluorescence by methyl viologen, seen in normal *Anacystis*, seems to arise mostly from Q-dependent changes in the yield. Similarly, addition of 0.1 mM PMA (phenyl mercuric acetate) caused a comparatively greater (20–25%) inhibition in normal than in DCMU-treated cells (5–10%). Although this compound is expected to interfere with the cyclic electron flow, it also intercepts electron flow from H_2O to $NADP^+$ (*37*).

In summary, we have shown that blue-green algae exhibit the typical OIDPS transient, and the fast (sec) DPS transient is very susceptible to electron transport inhibitors. However, the slow rise in the fluorescence yield seems to be supported by the energy conserving processes associated with both non cyclic and cyclic electron flow, the latter appears to be induced by DCMU. Also, we have established clearly that the slow SM rise in fluorescence yield in *Anacystis* parallels a rise in the rate of oxygen evolution. During this fluorescence induction, the emission spectrum also varies, exhibiting an enrichment of system 2 (685 nm) fluorescence at M as compared to S. As described earlier, such a change in the emission characteristics together with change in the rate of O_2 evolution could be taken as an indication of a shift in the regulation of excitation transfer in favor of PS 2.

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