

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

I. ANACYSTIS NIDULANS

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ABSTRACT The fluorescence yield of chlorophyll *a* in dark adapted *Anacystis nidulans* undergoes a slow change with continuous illumination. After the completion of the initial fast transient, the fluorescence yield rises from the level *S* to a plateau *M* within a minute, declining only after prolonged illumination. Both normal and 1,1-dimethyl-3-(3'4'-dichloro)-phenylurea (DCMU)-poisoned *Anacystis* are capable of these changes. In normal *Anacystis*, the slow increase in the fluorescence yield (*S* → *M*) requires light absorbed in system II while light absorbed in system I is ineffective. In DCMU-poisoned *Anacystis*, however, these changes are also promoted by light absorbed in system I. Addition of carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone (FCCP), a photophosphorylation uncoupler acting near the photosynthetic electron transport chain, abolishes the rise from *S* to *M* in normal but has no effect in the DCMU-poisoned system. Phlorizin, a phosphorylase inhibitor, has very little effect. These results suggest that the light-induced variation in the fluorescence yield is related to the conformational changes which accompany photophosphorylation. The fluorescence yield of the auxiliary pigment phycocyanin remains constant throughout the interval of the light-induced changes in the fluorescence yield of chlorophyll *a*. Consequently, the fluorescence spectrum of the alga is variable on continuous illumination.

INTRODUCTION

The fluorescence yield of chlorophyll *a* (Chl *a*), in dark adapted photosynthetic organisms, is variable during a period of continuous illumination. This phenomenon was first observed by Kautsky (1) in 1931, and it is known either as the "Kautsky Effect" or as the "fluorescence induction." The fluorescence change proceeds until a steady level of the fluorescence yield is attained and can be fully reversed during an adequately long dark period.

In algae, the fluorescence induction occurs in two distinct time intervals during the illumination period. On excitation, the fluorescence rises instantaneously to an

initial level 0 , proceeds to a maximum P , and then declines at a slower rate to a semistationary level S (2). The 0 - P - S transient, known also as the first wave of fluorescence induction, lasts approximately 2–3 sec. The first wave, recently the subject to intensive investigation, appears to reflect changes in the rate of the photosynthetic electron transport (3–10).

The fast initial fluorescence transient is succeeded by a much slower light induced change in the fluorescence yield referred to as the “second wave” or “long-term fluorescence induction.” A typical slow induction change in *Anacystis nidulans* consists of an increase of the Chl a fluorescence yield from the semistationary level S (at the end of the first wave) to a higher stationary level M . The Chl a fluorescence yield remains at this level for several minutes, declining only after prolonged illumination (11). In this report we shall discuss only fluorescence yield changes which succeed the first wave transient.

The long-term fluorescence induction phenomena have been investigated extensively and reviewed in detail in the past (12, 13). Early theories accounting for these effects invoked either quenching of the Chl a fluorescence by molecular oxygen (14) and hypothetical oxidized metabolic intermediates (15, 16) or variations in the rate of photochemistry because of enzymatic limitations (17). The validity of these theories is highly questionable since fluorescence induction is relatively insensitive to oxygen within wide limits of partial pressure (18, 19) and since the hypothetical oxidized metabolic intermediates have never been identified. In addition, the fluorescence and CO_2 uptake kinetics do not always show an inverse relationship (20) as would be expected in a competition between the chemical and radiative processes.

An alternate origin of the light induced fluorescence changes can be inferred from the work of Kandler (21) who demonstrated that the inorganic phosphate content (and inversely, the content in esterified phosphate) in *Chlorella* experiences a negative induction during a light period. In a subsequent dark interval, the change is reversed and the inorganic phosphate content exhibits a positive induction wave. The negative induction lasted for approximately 5 min, a duration typical for the second wave of fluorescence in *Chlorella*. Employing a more sensitive technique (luminescence of the firefly enzyme) Strehler (22) directly showed an induction wave in the adenosine triphosphate (ATP) content in *Chlorella*, which he correlated with the fluorescence induction. While these results suggest a link between the photophosphorylation and the fluorescence induction, they do not indicate the nature of this link.

It is now known, from experiments with isolated higher plant chloroplasts, that the photosynthetic electron transport is coupled to a translocation of cations across the plastid membranes. On illumination a proton influx (23) into membrane bound spaces and an efflux of mono- and divalent cations (24) accompanied by osmotic water movement are observed. The electrochemical ion gradients formed provide, according to Mitchell's chemiosmotic mechanism (25), the energy for the phosphorylation of adenosine diphosphate (ADP). Indeed, it has been shown that an

ATP-forming potential (X_E) accumulates during illumination of chloroplast preparations, and can be converted to ATP in the dark (26). The light induced ion displacements and the concurrent shift of the osmotic equilibrium are regarded as the cause of the gross deformation in the volume, shape, and internal structure of the chloroplast (27). Similar phenomena have been observed also with algal chloroplasts *in situ* (28). Such changes will, in general, alter the spatial relationships among the interacting Chl *a* molecules, the rate of transfer of electronic excitation to the reaction centers, and the fluorescence yield. In this paper we present observations which indicate that an indirect relation (as suggested above) exists between light-induced conformational changes and the fluorescence induction.

EXPERIMENTAL

Anacystis nidulans was cultured over low intensity white light in inorganic medium (29). 3–6 day old cultures were transferred into a trishydroxymethyl-aminomethane hydrochloride 0.02 M, NaCl 0.04 M, pH 8 (Tris-NaCl) buffer and the optical density at the red Chl *a* absorption maximum was adjusted to 0.5 for a 1 cm light path through the sample. Various additions were made at least 15 min prior to the measurements and an additional 15 min dark adaptation period was provided. The surface area of the sample was 19.6 cm² and its thickness 0.1 cm. The large surface area allows a fast equilibration with the atmospheric air and the alkalinity of the suspension medium is favorable for CO₂ absorption. The Chl *a* content of the samples, determined according to the method described by McKinney (30), was 8 μg/ml (approximately 8×10^{-6} M). All experiments were carried out at room temperature.

Absorption measurements were performed with a Spectronic 505 Bausch and Lomb spectrophotometer (Bausch & Lomb Incorporated, Rochester, N.Y.), equipped with an Ulbricht integrating sphere. A constant half bandwidth of 5 nm was used. The fluorescence measurements were carried out with a recording spectrofluorometer (31, 32). The optics of the instrument provide for a front-face collection of the fluorescence. This arrangement, as well as the low optical density of the samples (0.05 at the red Chl *a* absorption maximum for 1 mm sample thickness) are advantageous since they minimize the reabsorption of fluorescence. Absorption of fluorescence within the algal cell, possible because of the high Chl *a* concentration, was not corrected.

The fluorescence spectra are presented as relative fluorescence intensity (quanta) per spectral interval and have been corrected for the photomultiplier sensitivity and the transmission efficiency of the analyzing monochromator. The fluorescence time course data are plotted as the ratio $f = F_t/F_3''$, where F_t and F_3'' correspond to the fluorescence intensity values at t and 3 sec, respectively. F_3'' corresponds to the "S level" at the end of the first wave of fluorescence induction. Since the absorbance of the sample does not change during the experiments, f is the relative fluorescence yield (*i.e.*, ϕ_t/ϕ_3''). Incident actinic illuminations were measured with a Bi/Ag thermopile (The Eppley Laboratory, Inc., Newport, R.I.). Additional experimental details are given in the legends of the figures.

Oxygen evolution rates were measured with the Pt-electrode polarograph of Bannister and Vrooman (33). The instrument consists of a flat Pt cathode, on which the cell paste is deposited, and a large area Ag/AgCl anode. The electrodes were immersed in the Tris-NaCl buffer, which was stirred constantly by a stream of 5% CO₂ in air. The sample was illuminated with monochromatic light from a large Bausch and Lomb monochromator. The wavelength of excitation, half bandwidth, and intensity of light was adjusted to correspond to that used

for fluorescence measurements. The current, generated by a negative potential of 0.55 v applied to the cathode and amplified by model 150A Keithley microvolt ammeter (Keithley Instruments, Cleveland, Ohio) was recorded by a Varian G-10 chart recorder (Varian Associates, Palo Alto, Calif.). The current is proportional to the rate of oxygen delivery to the cathode, where it is reduced to H_2O . Because of the immediate contact of the sample with the electrode, the diffusion delay is minimal. The rate of the photosynthetic oxygen evolution is proportional to the difference of the recorded signal in light and in darkness, the latter being taken as a correction for the respiratory oxygen uptake.

All measurements were done at room temperature, 22–25°C.

EXPERIMENTAL RESULTS

Fluorescence Induction in Continuous and Flashing Light

On exposing dark adapted *Anacystis nidulans* to continuous system II (590 nm) excitation, a gradual increase in the relative fluorescence yield ($f = F_t:F_0$) of Chl *a* to a higher stationary level is observed (Fig. 1, curve A). Removal of the excitation at this stage results in a slow decay of the fluorescence yield toward the initially recorded level *S* (curve B). This decay was followed with brief (2–3 sec) flashes given during the dark period. In a series of experiments we varied both the intensity and the spacing between flashes to establish whether they have an influence on the dark decay kinetics. The nearly identical decay curves, obtained with widely differing total light doses given during the dark period, indicate that the brief light interruptions

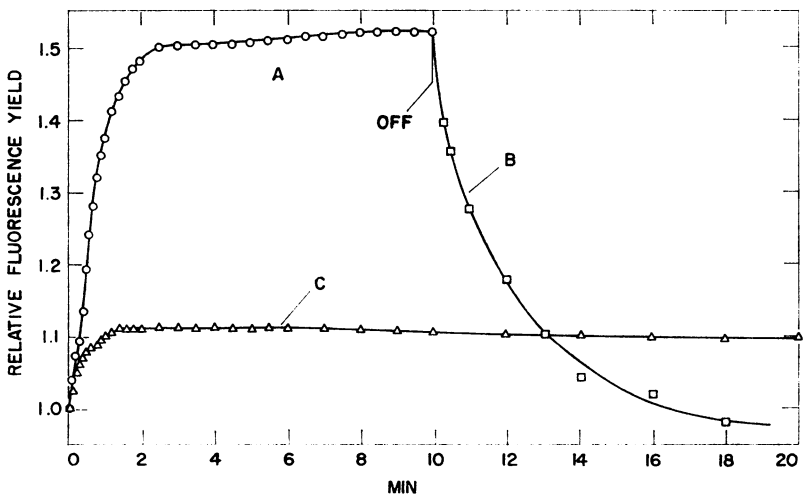


FIGURE 1 Time course of the relative fluorescence yield ($f = F_t:F_0$) in normal *Anacystis nidulans* (A). Excitation: $\lambda = 590$ nm; half bandwidth, 16.5 nm; incident intensity, 4.1×10^8 ergs $cm^{-2}sec^{-1}$. Observation: $\lambda = 685$ nm; half bandwidth, 13.2 nm; Corning filter, C.S. 2–60. Dark decay (measured with 2–3 sec light flashes) of the relative fluorescence yield (B). Time course of the relative fluorescence yield with blue excitation (C). Excitation: blue band, Corning filter, C.S. 5–60; half bandwidth, 100 nm; incident intensity, 9.4×10^8 ergs $cm^{-2}sec^{-1}$. Observation as in (A).

introduce no significant distortions. The fluorescence time course represented by the curves *A* and *B* of Fig. 1 is reproducible in consecutive dark-light cycles.

Continuous illumination of dark adapted *Anacystis* with system I light (broad blue band; Corning filter C.S. 5-60) results in a much smaller rise in the Chl *a* fluorescence yield (Fig. 1, curve *C*). Since blue light is primarily absorbed by Chl *a* we conclude that the direct excitation of this pigment does not support the long-term fluorescence induction in normal *Anacystis*. The small rise observed under these conditions can be partly attributed to the weak absorption in the blue by the system II pigment phycocyanin. Apparently, system I excitation is ineffective in promoting significant fluorescence induction in normal *Anacystis*.

The light-induced rise and the dark decay of the Chl *a* fluorescence yield are also observed with *Anacystis* cells, whose photosynthesis has been inhibited with DCMU or *o*-phenanthroline. Indeed, poisoned *Anacystis* exhibits even greater changes than normal cells do (Fig. 2, curves *A* and *B*). When the fluorescence is measured at 645 nm (mainly phycocyanin emission) only a slight increase in the fluorescence yield is observed (Fig. 2, curve *C*). This result precludes the possibility that the Chl *a* fluorescence induction originates from a change in the efficiency of the excitation energy transfer from phycocyanin to Chl *a*. If that was the case we would expect the variations of the fluorescence yields of Chl *a* and phycocyanin to be antiparallel.

Brody and Brody (34) have shown that prolonged light adaptation of *Porphy-*

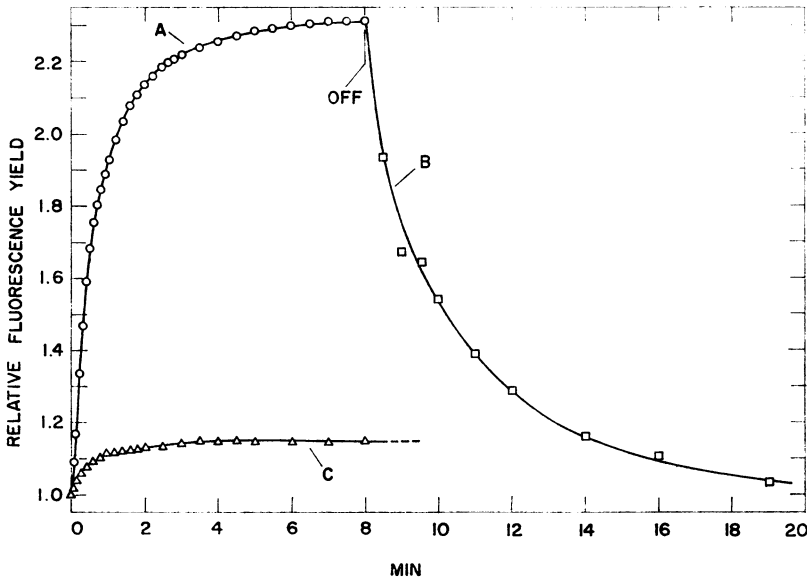


FIGURE 2 Time course of the relative fluorescence yield ($f = F_t:F_0''$) in DCMU (5×10^{-5} M) poisoned *Anacystis* (*A*). Excitation as in Fig. 1 (*A*). Observation: $\lambda = 685$ nm; half bandwidth, 6.6 nm; Corning filter, C.S. 2-58. Dark decay of the relative fluorescence yield (*B*). Time course of phycocyanin fluorescence (*C*). Excitation as in Fig. 1 (*A*). Observation: $\lambda = 645$ nm; half bandwidth, 8.3 nm; Corning filter, C.S. 2-63.

ridium cruentum to system I or system II light causes changes in the energy transfer efficiency from phycoerythrin to Chl *a*, which were ascribed to a rearrangement of the molecules. Since these effects persisted for hours after the removal of the adapting illumination they cannot be correlated with the fluorescence induction phenomena described in this paper. Also, Ghosh and Govindjee (35) have shown that changes in the energy transfer efficiency from phycocyanin to Chl *a* are responsible for the different emission spectra of *Anacystis* cultured with light varying either in intensity or spectral quality. Under these conditions, *Anacystis* cultures differing in the phycocyanin to Chl *a* ratio were obtained. Since our samples are characterized by an approximately constant ratio of phycocyanin to Chl *a*, the results presented here cannot be correlated with those of Ghosh and Govindjee. Consequently, in view of the constancy of the fluorescence yield of phycocyanin, we maintain that the efficiency of the excitation energy transfer is invariant during the slow induction phenomena.

It has been shown previously (11) that unlike normal *Anacystis*, DCMU-poisoned samples are capable of significant fluorescence induction with blue (system I) excitation as well. Thus, in spite of the qualitative similarity of the Chl *a* fluorescence time courses in normal and in poisoned *Anacystis*, different mechanisms must function in each case.

Changes in the Emission Spectrum During the Fluorescence Induction

Extensive changes in the emission spectrum of *Anacystis* take place during the fluorescence induction. The high-yield fluorescence (after several minutes of illumination) was sufficiently steady so that the spectrum could be scanned automatically during continuous illumination. In the case of the initial "S-level" fluorescence the spectrum was obtained "point-by-point" using short light flashes and long interposed dark intervals.

The emission spectra of normal *Anacystis*, excited at 590 nm and observed at the beginning (*S*) and at the stationary state (*M*) of the fluorescence induction time course are shown in Fig. 3. Prior to the initiation of the fluorescence rise, the Chl *a* emission band is relatively weak in our samples and appears as a shoulder on the long wavelength side of the phycocyanin emission band. At the end of induction the Chl *a* fluorescence is much stronger. The difference emission spectrum, obtained by subtracting curve *S* from *M*, is a typical Chl *a* *in vivo* emission band, peaked at 684 nm and having a half bandwidth of 22–23 nm. Similar results were also obtained with DCMU-poisoned *Anacystis* (Fig. 4).

The invariance of the phycobilin fluorescence yield with continuous illumination was first reported by French and Young (36) for marine red algae. With prolonged illumination (up to 2 hr), a slow decline of the Chl *a* fluorescence yield in normal and DCMU-poisoned *Anacystis nidulans* has been observed (11). During this

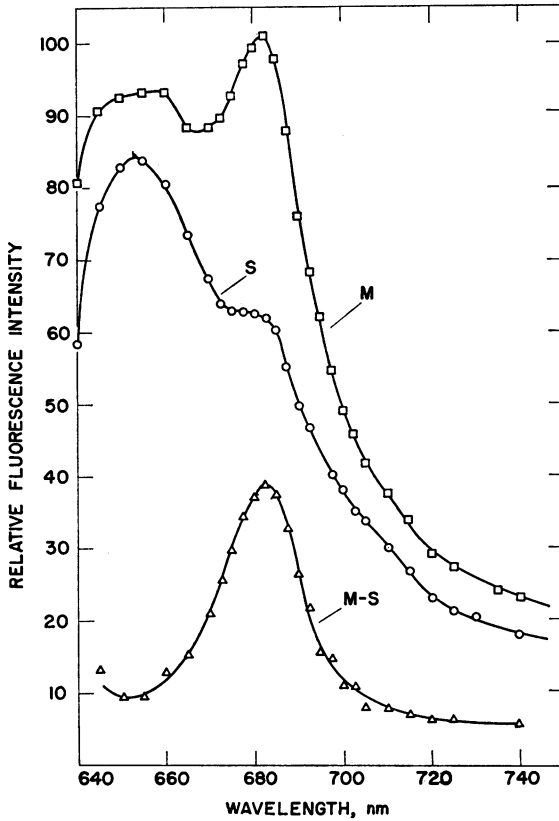


FIGURE 3 Emission spectra of normal *Anacystis* at 3 sec (*S*) and 10 min (*M*) of light exposure; *M-S*, the difference spectrum. Excitation as in Fig. 1 (*A*). Observation: half bandwidth, 8.3 nm; Corning filter, C.S. 2-63.

decline, changes in the spectral distribution within the Chl *a* emission band occur, which have been interpreted as indicative of the multiplicity of the in vivo Chl *a* forms.

Fluorescence Induction and Intensity of Excitation

Both the rate of increase of fluorescence and the maximum yield reached when induction is completed are functions which increase with illumination. The time courses of the relative fluorescence yield of unpoisoned *Anacystis* cells for several illumination intensities at 590 nm (phycocyanin absorption band) are shown in Fig. 5. Very weak light increases the yield slightly and the increase starts after a lag phase.

As Fig. 6 (curve *B*) indicates, the final fluorescence yield (f_M) reached during induction is approximately proportional to the intensity of illumination for low values of

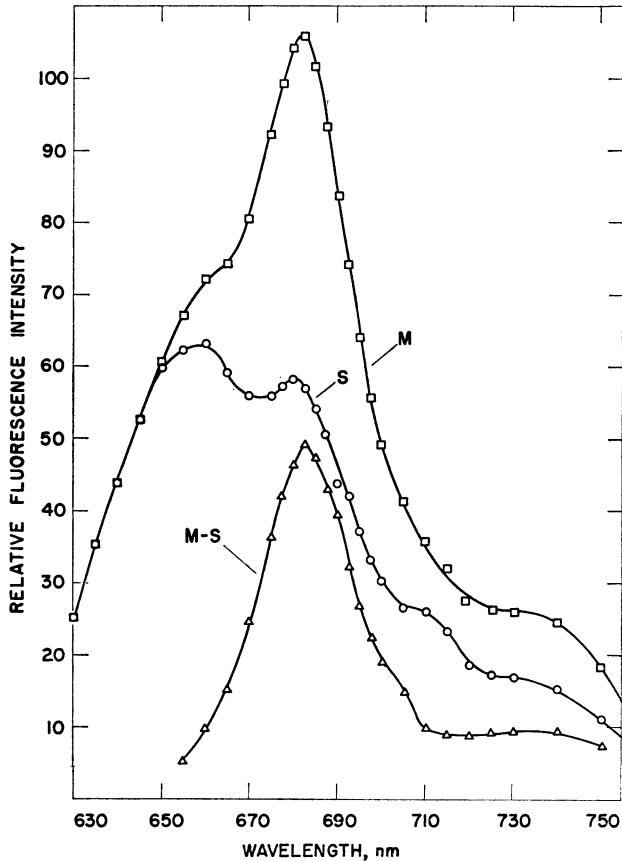


FIGURE 4 Emission spectra of DCMU ($5 \times 10^{-6} M$) poisoned *Anacystis* at 3 sec (*S*) and 10 min (*M*) of light exposure; *M-S* the difference spectrum. Excitation as in Fig. 1 (*A*). Observation: Half bandwidth, 7.6 nm; Corning filter, C.S. 2-63.

the latter. At high intensities, however, f_M saturates. A light curve for stationary state fluorescence is also shown (Fig. 6, curve *A*). The break in the light curve of fluorescence is a well-known feature and coincides approximately with the beginning of light saturation of the photosynthetic electron transport (see reference 37 for a similar curve in another phycobilin-containing alga, *Porphyridium*, and for references to earlier literature). It is apparent from Fig. 6 that the fluorescence yield increase (curve *B*) saturates at a considerably higher intensity than does photosynthesis itself. (In order to attain saturation of the fluorescence induction change the excitation was provided at the phycocyanin absorption maximum at 620 nm in this particular experiment.)

Fig. 7 illustrates the time courses of the relative fluorescence yield at various exciting intensities in a DCMU-poisoned *Anacystis* samples. The final change in the relative fluorescence yield (f_M) saturates rapidly, while the fluorescence light curve

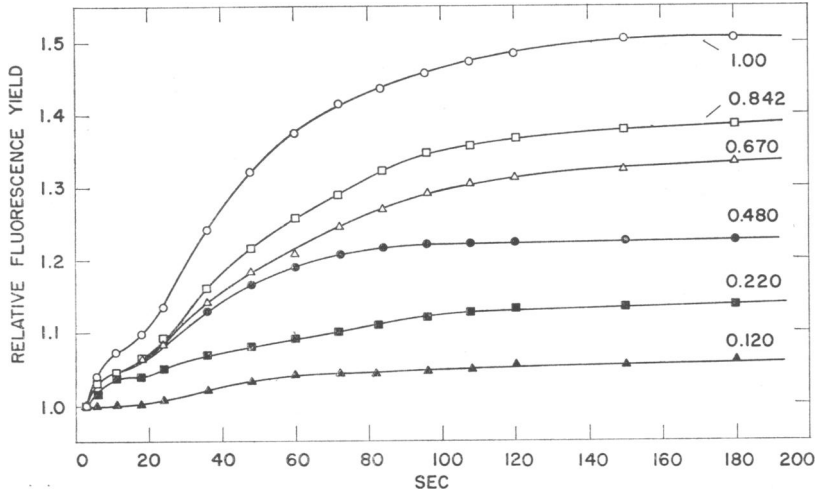


FIGURE 5 Time course of the relative fluorescence yield ($f = F_t:F_0$) in normal *Anacystis* at different exciting intensities. Excitation and observation as in Fig. 1 (A). Intensity 1.00 $\equiv 4.1 \times 10^8$ ergs $\text{cm}^{-2}\text{sec}^{-1}$.

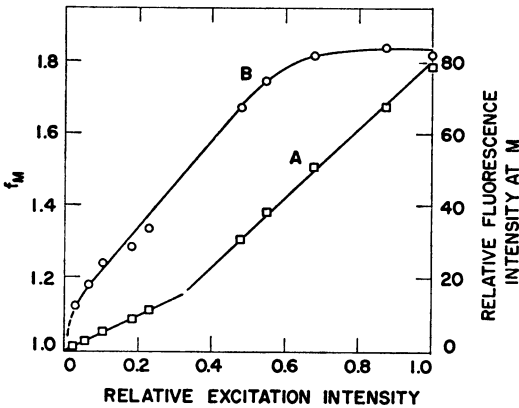


FIGURE 6 The fluorescence intensity (A) and the relative fluorescence yield (B) at M in normal *Anacystis*, as functions of the incident light intensity. Intensity 1.00 $\equiv 4 \times 10^8$ ergs $\text{cm}^{-2}\text{sec}^{-1}$. Excitation: $\lambda = 620$ nm; half bandwidth, 16.5 nm. Observation: $\lambda = 685$ nm; half bandwidth, 6.6 nm; Corning filter C.S. 2-64.

has a constant slope throughout the range of the excitation intensities of the experiment (Fig. 8, curves B and A, respectively). Fluorescence light curves of this type are typical of photosynthetic organisms with inhibited electron transport.

The fact that the rise rate of fluorescence yield increases with illumination (Figs. 5 and 7) indicates that induction originates from photochemical events. The establishment of steady state with a higher fluorescence yield may reflect a competition between a forward photochemical process and an opposing dark one. Since the maximal light-induced change will be determined by the rates of the competing processes, increased illumination would be expected to lead to higher stationary fluorescence yields. The light saturation of the maximal change suggests that the

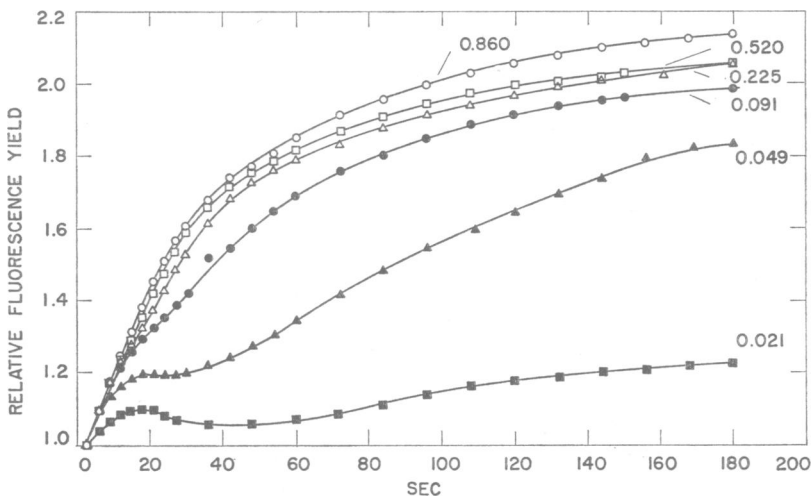


FIGURE 7 Time course of the relative fluorescence yield ($f = F_t:F_s$) in DCMU (5×10^{-5} M) poisoned *Anacystis* at different exciting intensities. Excitation as in Fig. 1 (A). Observation: $\lambda = 685$ nm; half bandwidth, 6.6 nm; Corning filter, C.S. 2-58.

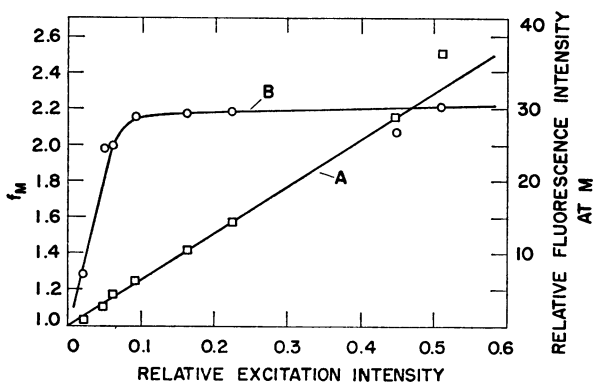


FIGURE 8 The fluorescence intensity (A) and the relative fluorescence yield (B) at M, in DCMU poisoned *Anacystis* as functions of the incident light intensity. Intensity 1.00 = 4.1×10^8 ergs $\text{cm}^{-2}\text{sec}^{-1}$. Details as in Fig. 7.

forward process may be limited enzymatically. At low excitation intensities, for example, the rate limiting factor is the photon flux while at high intensities the supply of the enzyme.

Fluorescence Induction and the Rate of Oxygen Evolution

The possibility that the fluorescence induction reflects concomitant changes in the rate of photosynthetic electron transport can be examined by comparing the kinetics of fluorescence and oxygen evolution. Spectroscopic and polarographic measurements were carried out with samples taken from the same stock culture and subjected

to identical illuminations. The sample in the polarograph, but not the one in the spectrofluorometer, was subjected to a stream of 5% CO₂ in air passed through the electrolyte medium. As shown in Fig. 9, the rate of oxygen evolution undergoes several oscillations before reaching the steady rate, while at the same time, the flu-

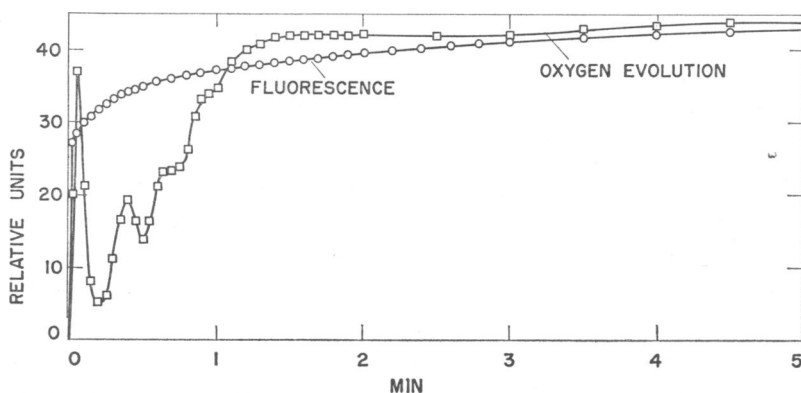


FIGURE 9 Time course of the fluorescence intensity and of the rate of oxygen evolution in normal *Anacystis*. Details as in Fig. 7.

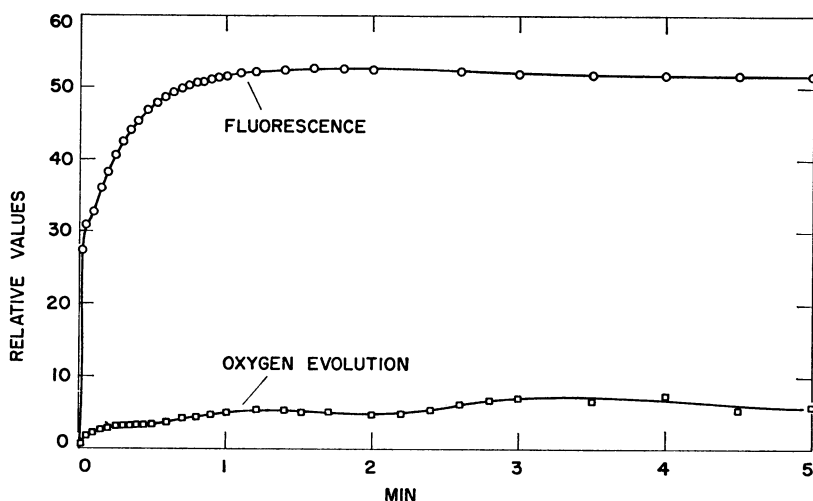


FIGURE 10 Time course of the fluorescence intensity and of the rate of oxygen evolution in DCMU ($5 \times 10^{-5}M$) poisoned *Anacystis*. Details as in Fig. 7.

orescence intensity (proportional to the fluorescence yield) increases without any oscillations whatsoever. The important feature is that the fluorescence yield generally increases together with the rate of oxygen evolution. On poisoning with DCMU (Fig. 10) the residual signal observed is probably due to photoinhibition of respiration (38). As already noted earlier, DCMU does not eliminate the fluorescence induction.

Effects of Uncouplers of Photophosphorylation on the Fluorescence Kinetics

The results presented in the previous sections confront us with a dilemma. Apparently, the fluorescence induction in normal *Anacystis* requires the operation of the noncyclic photosynthetic electron flow since it does not occur with system I excitation only. On the other hand, on the basis of the oxygen evolution kinetics, it appears that the rate of photosynthesis is not critical in determining the time course of fluorescence; indeed, when photosynthesis is inhibited, the fluorescence induction is enhanced. The effectiveness of light absorbed by system I in promoting the fluorescence induction in DCMU-poisoned samples may indicate a coupling of the phenomenon with the cyclic electron transport.

The possible relation of the fluorescence induction with the light-induced changes in the conformation of the lamella, of the type known to accompany phosphorylating electron flow, was examined by observing the effects of various uncoupling agents on the induction kinetics. Since the uncouplers employed act on both the photophosphorylation and the oxidative phosphorylation, they are expected to cause an overall ATP deficiency, affecting various cell functions. However, it appears that the photosynthetic electron transport was not critically impaired (with our experimental conditions) since a sharp increase in the fluorescence yield was elicited on addition of DCMU. In addition, the kinetics of the fluorescence induction modified by the uncouplers were reproducible in successive dark-light cycles. This is taken to indicate a direct influence on the fluorescence changes, distinguished from the effects due to the over-all ATP deficiency.

Addition of FCCP to a normal *Anacystis* sample results in a suppression of the long-term fluorescence induction (Fig. 11). FCCP is known to prevent light-induced conformational changes in isolated chloroplasts apparently by making membranes leaky and thus preventing the establishment of cation gradients (39). On the basis of the almost identical initial rise rates of the fluorescence yield of the control and the FCCP-containing samples, it appears that the suppression of the fluorescence induction is indeed due to an enhancement of the dark decay processes. Addition of cysteine to the FCCP-inhibited sample removes the inhibition of the fluorescence induction (Fig. 11). It is known also that the ATP formation is restored when 1,2- and 1,3-aminothiols are added to FCCP-uncoupled mitochondria (40) and chloroplasts (41). We found that malonylnitrile and KCN were ineffective in modifying the fluorescence induction.

The effects of the photophosphorylation uncoupler atabrin (quinacrine) and the photophosphorylation inhibitor phlorizin on the fluorescence kinetics are shown in Fig. 12. Atabrin, like FCCP, obliterated the light-induced increase in the fluorescence yield. Atabrin is known to prevent the accumulation of X_E (39, 42); it is also known to enhance the light-induced absorbancy change in isolated chloroplasts (43, 44). On the other hand, the phosphorylase inhibitor phlorizin appears to have no inhibitory effect on the fluorescence time course. Since phlorizin acts only on the terminal

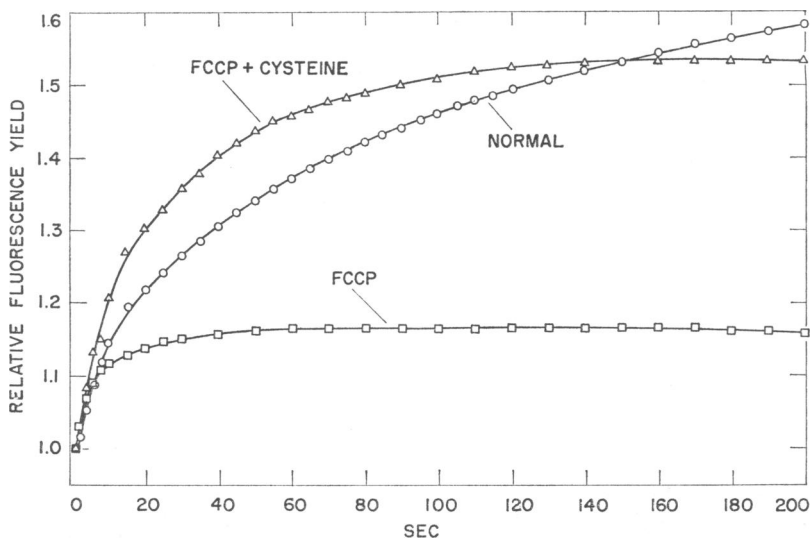


FIGURE 11 Time course of the relative fluorescence yield ($f = F_t:F_3''$) in normal *Anacystis*. Control; with $3 \times 10^{-5}M$ FCCP; with $3 \times 10^{-5}M$ FCCP and $10^{-3}M$ cysteine. Details as in Fig. 7.

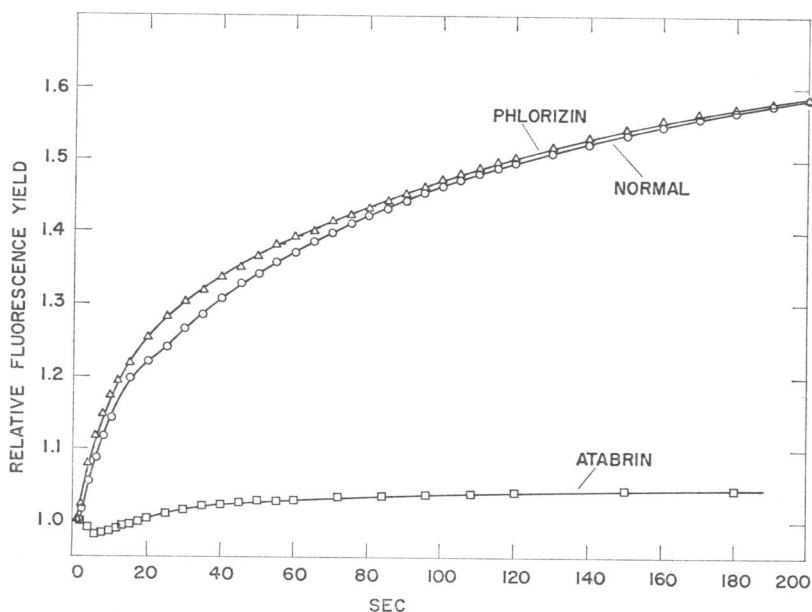


FIGURE 12 Time course of the relative fluorescence yield ($f = F_t:F_3''$) in normal *Anacystis*. Control; with $2 \times 10^{-3}M$ phlorizin; with $3 \times 10^{-5}M$ atabrin. Details as in Fig. 7.

TABLE I
EFFECTS OF VARIOUS ADDITIONS ON THE MAXIMAL
LIGHT-INDUCED INCREASE IN THE FLUORESCENCE
YIELD OF *ANACYSTIS NIDULANS**

Added chemical and concentration	Maximal change f_M
None	1.65
FCCP (3×10^{-5} M)	1.16
FCCP (3×10^{-5} M) + Cysteine (10^{-3} M)	1.61
‡ Malonylnitrile (3×10^{-4} M)	2.23
‡ KCN (3×10^{-3} M)	1.64
‡ NH_4Cl (10^{-2} M)	1.64
‡ $\text{CH}_3\text{NH}_2 \cdot \text{HCl}$ (10^{-2} M)	1.64
Atabrin (3×10^{-5} M)	1.04
Phlorizin (2×10^{-3} M)	1.61

* Figures given in the last column are the average of three separate samples, except those denoted with a double dagger, with which only one experiment was performed. Except for the additions, all samples were prepared identically and were subjected to identical light (590 nm) excitation.

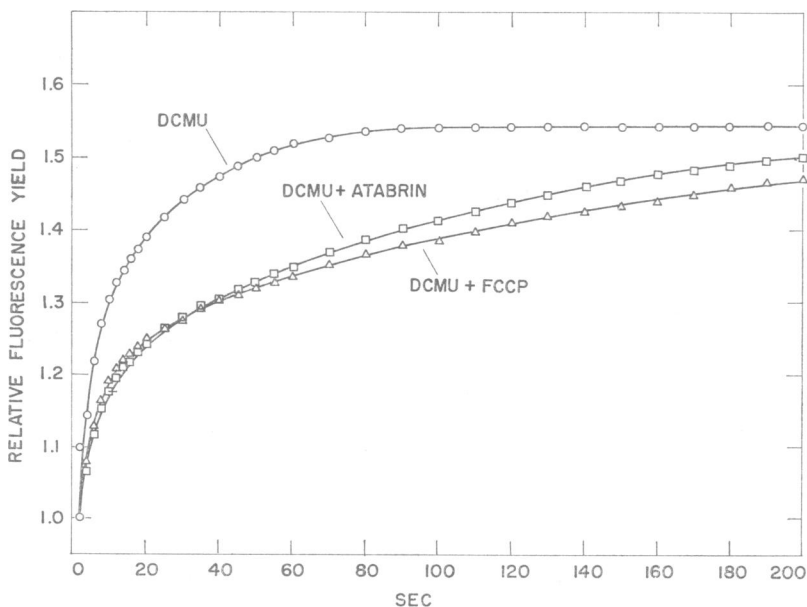


FIGURE 13 Time course of the relative fluorescence yield ($f = F_t/F_0$) in DCMU (1.8×10^{-5} M) poisoned *Anacystis*; with 1.8×10^{-5} M DCMU and 3×10^{-5} M atabrin; with 1.8×10^{-5} M DCMU and 3×10^{-5} M FCCP. Details as in Fig. 7.

phosphorylation step (45, 46) it should not prevent the light-requiring translocation of cations and the associated changes in the plastid and lamellar conformation. Accordingly, no inhibition of the fluorescence rise is expected. The effects of various additions on the maximal light-induced fluorescence yield increment are summarized in Table I.

Compared with normal *Anacystis*, addition of FCCP and atabrin in DCMU-poisoned samples has little effect on the steady-state level of the relative fluorescence yield. The fluorescence time courses are modified, however, the rise phase being slower in the presence of uncouplers (Fig. 13). The differences in the induction of normal and DCMU-poisoned *Anacystis* in the presence of the uncouplers suggests that different mechanisms may be involved.

DISCUSSION

In the preceding sections we described several dissimilarities in the fluorescence induction of normal and DCMU-poisoned *Anacystis nidulans*. A pronounced increase of the fluorescence yield, activated by both system II and system I excitation, characterizes the induction in poisoned *Anacystis*. System I excitation, however, does not support significant fluorescence induction in normal *Anacystis*. Differences in the induction of normal and poisoned *Anacystis* were observed also in respect to light saturation and the effects of added uncouplers.

The ineffectiveness of system I excitation to support the fluorescence induction in normal *Anacystis* suggests that the photosynthetic electron transport is essential for these phenomena. The time courses of fluorescence and the rate of oxygen evolution are not complementary, during the slow induction, indicating that the rate of photosynthesis is not the determinant of fluorescence kinetics. The parallel increase in fluorescence and in oxygen evolution can be interpreted phenomenologically as a light activation of Chl *a*.¹ The activation converts a nonphotoactive and nonfluorescent portion of the total Chl *a* into a photoactive and fluorescent form. Energetically, the light activation may be interpreted as a reduction of the rate of internal conversion, the excess excitation being channeled to photosynthesis and fluorescence (47).

We propose, as a working hypothesis, that the inactive-active Chl *a* transformation is related to the light-induced structural changes which accompany the phosphorylating electron transport. A modified spatial distribution of the Chl *a* molecules is expected to manifest itself in fluorescence magnitudes such as the quantum yield, the lifetime, and the degree of polarization of fluorescence. The rate with which these changes proceed is, generally, too slow to be compared with the rate by which an electron is transported in the photosynthetic pathway but of comparable magnitude with the rate of fluorescence induction. The effects of various uncouplers of photo-

¹ Bannister, T. T. 1968. Personal communication during the final preparation of this article.

phosphorylation show indeed that a correlation must exist between the fluorescence induction and the light-induced structural changes.

Uncouplers of photophosphorylation, operating near the electron transport chain (e.g. FCCP) inhibit the fluorescence induction in normal *Anacystis*. FCCP is known to prevent also the light-induced accumulation of electrochemical gradients and the associated conformation changes in chloroplast preparations. On the other hand, the phosphorylase inhibitor phlorizin had no effect on the light-induced change in the fluorescence yield. These results suggest that the fluorescence induction in normal *Anacystis* requires a phosphorylating electron transport and is, in all likelihood, related to the process which couples electron transport and formation of ATP.

The capacity of poisoned *Anacystis* to undergo fluorescence induction with system I light indicates that a phosphorylating electron transport of the cyclic type may be involved. The failure of the uncouplers of photophosphorylation to suppress the induction in poisoned systems is indicative of their reduced activity in the cyclic electron transport, as shown by Avron and Shavit (48). Bannister (49) observed a lowering of the fluorescence yield of poisoned *Anacystis* on the addition of FCCP and an elimination of the induction as well. Our results indicate the induction in such systems is not affected by either FCCP or atabrin. It is possible that the discrepancy is due to the widely different intensities of illumination used by Bannister and by us.

The fluorescence induction changes are localized in the Chl *a* emission band, with the fluorescence yield of the auxiliary system II pigment phycocyanin remaining constant. As a consequence, large changes in the spectrum of the total emitted fluorescence take place during the induction. The invariance of the phycocyanin fluorescence yield rules out light-induced changes in the rate of energy transfer from phycocyanin to Chl *a* as the cause of the induction.

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