

Effect of Phenazine Methosulfate and Uncouplers on Light Induced Chlorophyll *a* Fluorescence Yield Changes in Intact Algal Cells*

PRASANNA MOHANTY** and GOVINDJEE***

Departments of Botany and Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801, U.S.A.

Abstract

The effects of uncouplers of photophosphorylation and of the cofactor (PMS) of system I mediated cyclic reaction on the fast chlorophyll *a* (Chl *a*) fluorescence transient, on the fluorescence yield (ϕ_f) and on the emission characteristics of Chl *a* fluorescence of intact algal cells have been analyzed. Proton permeating uncouplers like FCCP and DNP were found to have multiple modes of action. At relatively low concentrations, they seem to inhibit a recombination between the oxidized donor and the reduced acceptor of photosystem II as well as hasten the rate of electron flow. These agents, at relatively high concentrations, quench the Chl *a* fluorescence even in the presence of dichlorophenyl dimethyl urea (DCMU), a potent inhibitor of photosynthesis. This decrease in the yield of Chl *a* fluorescence also causes an increase in the ratio of system 1 (long wavelength) to system 2 (short wavelength) emission bands. Such a spectral shift is also shown to occur during the slow decline (*PS*) of fluorescence yield during fluorescence transient and is interpreted to be due to a shift in the extent of energy transfer in favor of weakly fluorescent photosystem I. Phenazine methosulfate (PMS)-induced cyclic electron flow also causes a slow decline in the yield of fluorescence in intact algal cells in the presence of DCMU. As in the case of isolated chloroplasts this reversible decline of fluorescence yield is suppressed by uncouplers. The emission characteristics of Chl *a* fluorescence at 77 K is not altered by PMS. It appears that cyclic cofactors like PMS increase the rate of loss of excitation as heat. Finally, it is suggested that some specific alteration in the orientation of the membranes containing the two photosystems governs the yield and emission by way of a controlled distribution of absorbed quanta.

* Received 19 December 1972.

** Present address: Photochemistry Unit, Department of Chemistry, University of Western Ontario, London, 72, Ontario, Canada.

*** Address all correspondence to: Dr. GOVINDJEE, 289 Morrill Hall, Department of Botany, University of Illinois, Urbana, Illinois 61801, U.S.A.

Two types of chlorophyll *a* (Chl *a*)* fluorescence yield changes have been observed in intact algal cells: one reflects the redox state of the primary** acceptor of photosystem II, *Q* (the quencher of Chl *a* fluorescence), the other not. The latter type of yield changes are usually manifested as slow or long-term changes.

Dark adapted algal cells exhibit a rapid transient of Chl *a* fluorescence; the characteristic points in the transient are a base level *O*, an intermediate level, *I*, a dip *D* and then a rise to a peak level *P* (see GOVINDJEE and PAPAGEORGIOU 1971). The *OIDP* transient is usually explained by the reduction of the primary electron acceptor *Q* to *QH* which in its reduced state does not quench chlorophyll fluorescence (DUYSENS and SWEERS 1963). From *P*, the fluorescence yield declines to a quasi steady state *S* and then rises to a second peak level *M*. After *M*, the yield gradually declines to a terminal *T* level. (See BANNISTER and RICE 1968, VREDENBERG 1970 and GOVINDJEE and PAPAGEORGIOU 1971 for different nomenclature of these points in the transient.) DUYSENS and SWEERS (1963) demonstrated that *PS* decline does not correspond to the oxidation of *QH*. Parallel studies of Chl *a* fluorescence and O_2 evolution very clearly indicate that the *SMT* type change in fluorescence is not directly linked to electron flow in photosynthesis (BANNISTER and RICE 1968; PAPAGEORGIOU and GOVINDJEE 1968a, b; MOHANTY *et al.* 1971) and the redox state of *Q* (MOHANTY *et al.* 1970). PAPAGEORGIOU and GOVINDJEE (1968a, b) established an indirect correlation between the energy conservation and the slow *SMT* yield changes in algae and suggested that alteration of the thylakoid structure regulates the yield of Chl *a* fluorescence. BONAVENTURA and MYERS (1969) and MURATA (1969a, 1970, 1971) proposed a change in the excitation energy transfer between the two photosystems that, in turn, regulates the slow fluorescence yield changes. In "state 1" more of the absorbed quanta are available to strongly fluorescent pigment system 2 than in "state 2". Extending this hypothesis, DUYSENS (1972) discussed the possibility of the movement of the two pigment systems closer to and away from each other causing the transition of "state 1" to "state 2" and state 2 to state 1, respectively. VREDENBERG (1970) suggested that such state transitions are induced by intercellular flow of ions or protons.

PAPAGEORGIOU and GOVINDJEE (1968a, b) have shown that slow fluorescence yield changes are susceptible to uncouplers like FCCP and atebirin. Earlier BANNISTER (1967) reported that FCCP can quench the Chl *a* fluorescence yield of intact algal cells in the presence of DCMU, a potent inhibitor of photosynthesis. Recently HOMANN (1971) and KIMIMURA *et al.* (1971) have investigated the effect of the uncoupler CCCP on the electron transport and Chl *a* fluorescence of isolated broken chloroplasts. Similarly, it has been shown that cyclic electron transport mediators (*e.g.*, PMS or DAD) can quench the Chl *a* fluorescence yield of isolated chloroplasts in the presence of DCMU (GOVINDJEE and YANG in GOVINDJEE *et al.* 1967; MURATA and SUGAHARA 1969; WRAIGHT and CROFTS 1970; MOHANTY *et al.*, unpublished). However, the effect of uncouplers of photophosphorylation and the cyclic electron transport cofactor on the Chl *a* fluorescence yield, particularly on the *DPS* transient in intact algae have not been well documented. Also, the quenching of Chl *a* fluorescence of DCMU-poisoned algal cells by cofactors like PMS has not been well

* *Abbreviations*: Chl *a*, chlorophyll *a*; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; DAD, diaminodurene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP, dinitrophenol; DPPI, 2,6-dichlorophenol indophenol; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone; PMS, phenazine methosulfate.

** Recently MAUZERALL (1972) has challenged the view that "*Q*" is the primary electron acceptor of photosystem II because fluorescence rise is too slow ($t_{1/2}$, 3 μ s) when *Chlorella* is irradiated with ns flashes. BUTLER (1972) has, however, suggested that this may be so because chlorophyll fluorescence yield rise is governed also by the dark reduction of the primary electron donor of photosystem II, only *Z.Q*⁻ being the high fluorescent state. (One could also suggest that the cause of the slow rise of chlorophyll fluorescence, observed by MAUZERALL, is that reduction of a chlorophyll molecule in the reaction center II is a primary event, *Q* being reduced with a slight delay.)

148 characterized. We have, therefore, studied the effect of this cyclic electron transport cofactor (PMS) and the uncouplers of phosphorylation (FCCP and DNP) on the Chl *a* fluorescence yield and emission characteristics of intact algal cells both in the presence and the absence of DCMU and have compared the role of these two types of chemicals in modifying the Chl *a* fluorescence yield *in vivo*.

MATERIALS AND METHODS

The cells of the green alga *Chlorella pyrenoidosa* CHICK. and the red alga *Porphyridium cruentum* NÄG. were used. Occasionally, a blue-green alga *Anacystis nidulans* or the acidophilic alga *Cyanidium caldarium* was used. These cells were grown autotrophically in continuous culture as outlined earlier (GOVINDJEE and RABINOWITCH 1960). Low irradiance cells were cultivated at 3 W m^{-2} , high irradiance cells at 8 W m^{-2} . Five to six day old cells were harvested, washed, and then suspended in a buffer or in fresh growth medium. All additions were made in dark and the samples were allowed to equilibrate for 5–10 min before use.

The details of the spectrofluorometer (GOVINDJEE 1966; SHIMONY *et al.* 1967; MOHANTY *et al.* 1972), the procedures for measuring fast and slow fluorescence transient (PAPAGEORGIOU and GOVINDJEE 1968a; MUNDAY and GOVINDJEE 1969a, b; MOHANTY *et al.* 1971), and the methods for the measurement of the emission spectra (CHO *et al.* 1966; GOVINDJEE 1972) have already been described. These spectra were corrected for the spectral variation of the spectrofluorometer. Absorption spectra were measured in a *Bausch and Lomb Spectronic 505* spectrophotometer equipped with an integrating sphere.

Acknowledgements: Collaboration and/or help, at one stage or the other, of Drs. S. C. Sybesma, T. T. Bannister and R. Delosme is gratefully acknowledged. We thank the National Science Foundation and the University of Illinois for financial support. We also thank Dr. G. Heytler, E. I. duPont deNemours, for a generous gift of FCCP.

RESULTS

Fluorescence yield changes induced by uncouplers of photophosphorylation

In green and red algae, the poisons of electron transport, like CMU, DCMU and orthophenanthroline cause a very rapid rise in fluorescence yield (*OI* rise) to a maximal level and then the yield remains constant throughout the period of irradiation (see MOHANTY *et al.* 1971) although BANNISTER and RICE (1968) reported DCMU resistant fluorescence changes in the green alga, *Chlamydomonas*, at a relatively high intensity of excitation.

It has, however, been shown that Chl *a* fluorescence yield of DCMU-poisoned cells and chloroplasts could be altered by a variety of agents which uncouple phosphorylation (BANNISTER 1967; PAPAGEORGIOU and GOVINDJEE 1968a, b; HOMANN 1971; KIMIMURA *et al.* 1971). We have investigated the nature of this alteration in fluorescence yield by uncouplers in both poisoned and normal algal cells. Our data with CCCP (Table 1) confirm the findings of BANNISTER (1967) who first reported that FCCP depresses Chl *a* fluorescence yield of DCMU-poisoned cells. Upon the addition of FCCP, the fluorescence yield declined slowly to a lower value over a period of 2 to 3 min. The extent of quenching increased with an increase in the concentration of FCCP. The amount of depression of Chl *a* fluorescence yield by FCCP or CCCP also varied depending on the culture conditions, the age of the algal samples and the time of incubation. FCCP causes depression of

Table 1

Quenching of Chl *a* fluorescence yield of DCMU poisoned *Chlorella* by CCCP. Fluorescence was measured at 685 nm (half band width, 6.6 nm); cells were washed and suspended in fresh culture medium, with 15 μM DCMU; excitation blue radiation (C.S. 4-72 plus 3-73); ca. 10 W m^{-2} ; C.S. 2-61 filter before the photomultiplier.

Concentration of CCCP [μM]	None	10	30	50	100
Steady state fluorescence yield in relative units	50.0	45.5	39.0	29.0	27.0
Fraction of control	1.00	0.91	0.78	0.58	0.54

Chl *a* fluorescence both in aerobic and anaerobic cells (GOVINDJEE and BANNISTER, unpublished). The room temperature emission spectra of DCMU and DCMU plus FCCP treated (aerobic and anaerobic) *Chlorella pyrenoidosa* (Fig. 1) show that DCMU causes a preferential increase of the main band at 685 nm and FCCP suppresses mainly this band. In other words, the ratio of emission

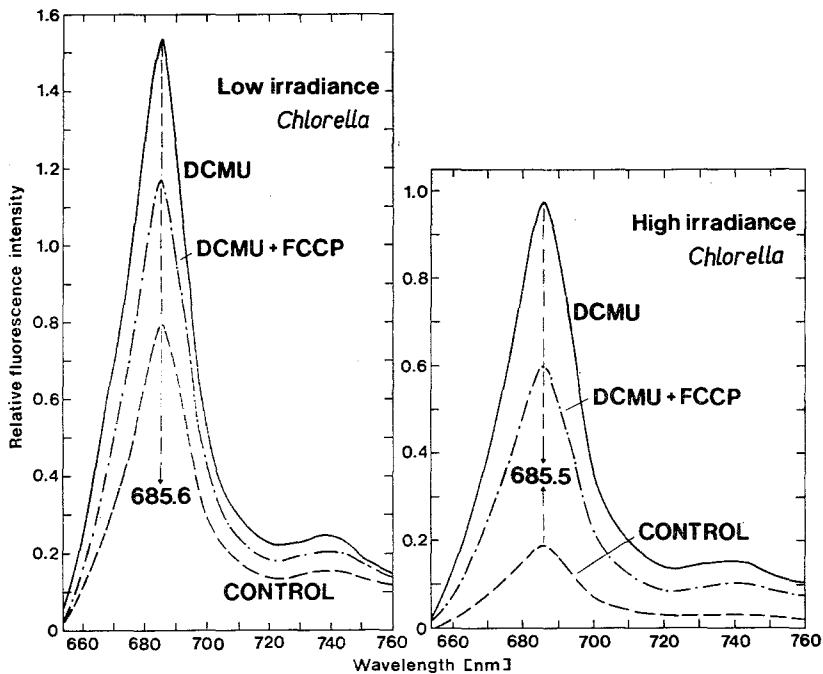


Fig. 1. Room temperature emission spectra of low and high irradiance aerobic cells of DCMU (30 μM) treated *Chlorella* with and without FCCP (80 μM). Cells were suspended in Warburg's buffer No. 9 (pH 9.2); absorbance of the sample was adjusted to have 5% absorption at the red band. Samples were excited with bright blue irradiation (C.S. 4-96 plus 3-73) of ca. 10 W m^{-2} . Observation monochromator was guarded by a C.S. filter 2-64; slit width of the monochromator = 5 nm. (These results confirm the unpublished results of GOVINDJEE and T. T. BANNISTER.)

150 intensities at 685 nm to that of 720 nm is higher in DCMU-treated cells than in the cells treated with both DCMU and FCCP. It seems that in intact algal cells DCMU keeps the two photosystems in state 1 and the addition of uncoupler induces interconversion of state 1 to state 2.

Table 2

Quenching of Chl *a* fluorescence yield by DNP in DCMU poisoned *Chlorella*. DCMU, 8 μM ; buffer 50 mM phosphate, pH 6.8; ethanolic stock solution of DNP was diluted in H_2O before use; all additions were made in the dark. [Chl], 45 $\mu\text{g ml}^{-1}$; excitation, 633 nm; irradiance ca. 25 $\text{W} \cdot \text{m}^{-2}$.

Concentration of DNP [μM]	None	40	80	120	160	200
Relative fluorescence yield	1.0	1.0	0.83	0.75	0.59	0.55

Like FCCP, DNP causes a quenching of the fluorescence yield (Table 2), although unlike FCCP, DNP is a poor uncoupler of photophosphorylation. The percent-quenching remained the same at the low as well as at the high irradiance. These results appear to be contradictory to those of HOCH and RANDLES (1971), who observed an enhancement of fluorescence yield upon the addition of DNP at weak irradiance. However, the irradiance used by them was extremely weak, such that the fluorescence yield was low even in the presence of DCMU. It appears that DNP inhibits the back recombination between Z^+ and Q^- and thus causes an increased yield of fluorescence in their experiments.

Room temperature emission spectra of *Chlorella* in the presence of DCMU and DCMU plus DNP (Fig. 2) show that, as in the case of FCCP, the DNP causes a preferential lowering at the

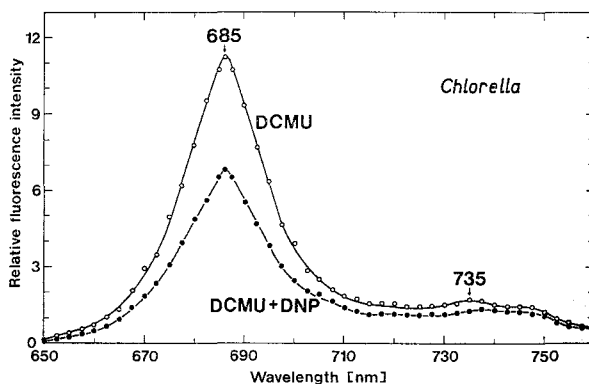


Fig. 2. Room temperature emission spectra of DCMU (15 μM) poisoned aerobic cells of *Chlorella* with and without DNP (1 mM). Low irradiance cells were suspended in fresh growth medium (pH 6.7); excitation λ , 633 nm; irradiance 20 W m^{-2} . Absorbance of the sample was adjusted to have 5% absorption at the red Chl *a* peak. Observation monochromator was guarded by a C.S. filter 2-64; half band width of the monochromator, 6 nm.

main 685 nm band without a significant effect on the system 1 emission. The ratio of F685 to F710 was decreased from 6.2 to 4.9 by the addition of 1 mM DNP. The quenching of fluorescence by DNP and FCCP seems to be very similar and both cause a similar change in the emission characteristics of *Chlorella*.

The above-described changes in the Chl *a* fluorescence yield by uncouplers like CCCP, FCCP or DNP can not be due to an alteration in the electron transport, as these changes occur even in the presence of DCMU. On the other hand, the changes in Chl *a* emission characteristics seem to be related to the shift in the distribution of quanta between the two photosystems (BONAVENTURA and MYERS 1969; MURATA 1970).

Effect on DPS transient

KIMIMURA *et al.* (1971) supported the view of ITOH *et al.* (1969) that low steady state yield of fluorescence in the presence of CCCP is mainly due to an inhibition of electron transport from water to photosystem II reaction centers, while HOMANN (1971) has shown that CCCP accelerates a Meh-

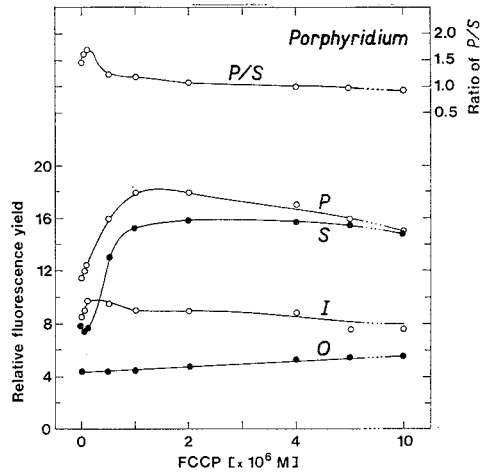


Fig. 3. Changes in the relative fluorescence yield at various characteristic points (and the ratio P/S) of the fast transient in *Porphyridium cruentum* as a function of concentration of FCCP. Excitation blue-green irradiation (C.S. 4—96 plus 3—73) of ca. 18 W m^{-2} ; observation λ , 685 nm (half band width, 10 nm); Corning C.S. 2—58 filter before the analyzing monochromator. Cells were suspended in carbonate—bicarbonate plus 0.25 M NaCl buffer, pH 8.5. FCCP was added in the dark and equilibrated for at least 5 min before the measurements. (These results confirm the unpublished results of GOVINDJEE and R. DELOSME in *Chlorella*.)

ler type photoreduction of oxygen even in previously uncoupled chloroplasts and causes a lowering of fluorescence yield. Thus, the quenching of fluorescence by CCCP in normal chloroplasts is due to a rapid rate of electron transport. We have, therefore, studied the effect of varying concentration of FCCP and CCCP mainly on the fast fluorescence yield changes in intact algal cells of *Porphyridium* and *Chlorella* as the fast DPS transient [s] reflects mainly the changes due to electron flow between the two photosystems (see GOVINDJEE and PAPAGEORGIOU 1971). (Earlier, PAPAGEORGIOU and GOVINDJEE 1968a, b, reported the suppressing effect of FCCP in *Anacystis* and *Chlorella* on the slow *SM* rise and on *MT* decline, respectively.)

GOVINDJEE and R. DELOSME (unpublished) analyzed the effect of FCCP on the fast transient of *Chlorella*; our results on the red alga *Porphyridium* are very similar to theirs on *Chlorella*. At very low concentrations of FCCP there is an increase in *I* and *P*, but the fluorescence yield at *O* and *S* remains relatively unchanged (Fig. 3; *Porphyridium*). On increasing the concentration to $0.5 \mu\text{M}$, the fluorescence yield at *I* declines while the yield at *P* and *S* increases. At still higher concentrations of FCCP ($4 \mu\text{M}$) both *P* and *S* decline and we observe an increase in the *O* level fluorescence. The P/S ratio decreases at a relatively lower ($1 \mu\text{M}$) concentration of FCCP when the yield at *P* is still high. We confirm these results in *Chlorella* using FCCP and CCCP; the latter

152 is known to be comparatively less potent than FCCP and thus a relatively higher concentration of CCCP was needed to cause a similar effect. (In these experiments we have used relatively higher concentrations of both CCCP and FCCP as compared to what is routinely used in the case of isolated chloroplasts or mitochondria (KRAAYENHOF 1971). Such high external concentrations of uncouplers are necessary in studies with intact cells; we do not know the concentration of the compound that permeates to the functional site of the chloroplasts *in vivo*.)

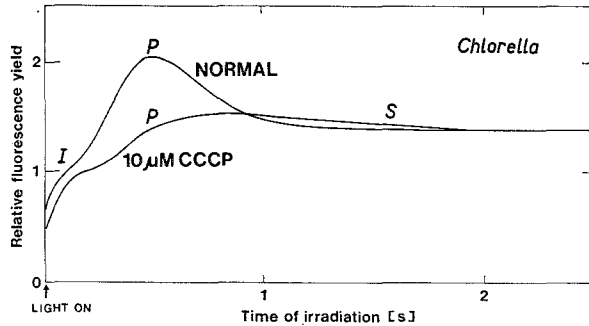


Fig. 4. Time course of Chl *a* fluorescence yield in *Chlorella* with and without CCCP. Cells were suspended in 50 mM Tris-Cl plus 0.01 sodium chloride buffer, pH 7.8. λ observation, 685 nm (half band width, 6.6 nm). A C.S. 2-62 filter (half band width, 6.6 nm) before the analyzing monochromator. 10 min dark incubation before irradiation. CCCP added in the dark; ethanol concentration, $\sim 1\%$.

In *Chlorella* CCCP (10 μ M) significantly suppresses the peak fluorescence of the fast transient *P* (up to 2 s of irradiation; Fig. 4). Besides this suppression in *P*, there was a delay in the time to reach *P* level (*tp*). In *Porphyridium* a different effect on *tp* was observed at the low concentrations of FCCP than at the high concentrations of FCCP (Fig. 5): At a low concentration of FCCP, the *tp* is slightly hastened but on increasing the concentration of FCCP, the *tp* is progressively delayed. In *Cyanidium* (Fig. 5) no clear hastening of *tp* at low concentrations of the uncoupler was noted, but a progressive delay of *tp* at higher concentrations was observed. Again, the amplitudes of fluorescence at various characteristic points of the fast transient, namely at *O*, *I*, *P*

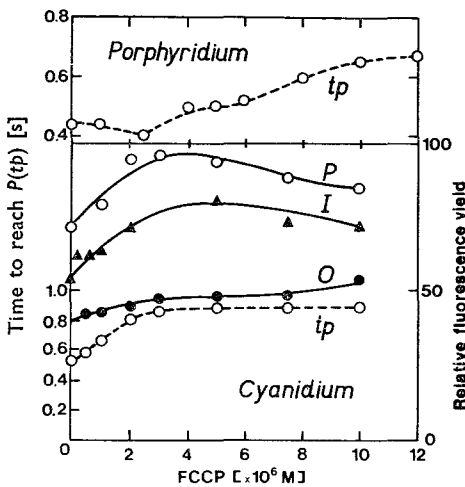


Fig. 5. Effect of varying concentrations of FCCP on the time for development of peak fluorescence yield (*P*) in *Porphyridium cruentum* and *Cyanidium caldarium* and on the fluorescence yield changes in *C. caldarium*. *Porphyridium* cells were suspended in bicarbonate carbonate buffer (pH 8.5) plus sodium chloride; *Cyanidium* cells were suspended in fresh growth medium (pH 2.0); other details of measurement as in Fig. 4.

and S , showed a similar variation with increasing doses of FCCP as it was observed in the case of *Porphyridium* and *Chlorella*. Thus, the results obtained with three different algae suggest that observed results are a general manifestation of the action of this uncoupler. It has at least two modes of action: at lower concentrations, it causes an enhancement of I , P and the rate of DP rise, and at a higher concentration, it suppresses them. Atebrin, another potent uncoupler of phosphorylation acts very similar to FCCP (GOVINDJEE and R. DELOSME, unpublished).

The enhancement of I and P , and of the rate of DP rise suggests that CCCP and FCCP at low concentrations bring about inhibition of the rate of back reoxidation of Q^- . This would cause a hastening of tp . (Indeed, HOMANN 1971 has observed that CCCP prevents back reoxidation of Q^- in isolated chloroplasts in the presence of DCMU.) On increasing the concentration, CCCP or FCCP presumably accelerates the flow of electrons either due to the uncoupling effect as suggested by ITOH *et al.* (1969) or increasing the O_2 uptake as suggested by HOMANN (1971). FCCP has been known to stimulate light induced O_2 uptake in *Chlamydomonas* (BANNISTER and RICE 1968) and also in *Anacystis* (MOHANTY 1972). Such stimulated O_2 uptake in whole cells would cause an accelerated flow of electrons and would bring about a depression of P and delay in its formation (Fig. 4).

Recovery of transient

VREDENBERG (1969) has shown that some proton permeating agents (CCCP, nigericine) shorten the requirement of dark period for the restoration of the $OIDPS$ transient in *Porphyra*. Fig. 6 confirms that $0.2 \mu M$ FCCP considerably shortens the requirement of dark time for the develop-

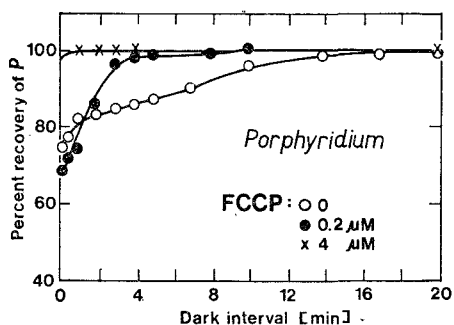


Fig. 6. Restoration of the fast changes in Chl a fluorescence yield in the dark in 6 d old *Porphyridium* cells suspended in growth medium. The amplitude of P after a long dark period (30 min) was taken as 100%.

ment of peak fluorescence in *Porphyridium cruentum*. At higher concentrations ($4 \mu M$), the transient is restored rapidly but the PS decline is suppressed (see also MOHANTY *et al.* 1971). We could not find any hastening of the dark requirement by FCCP in *Chlorella* or spinach chloroplasts. We do not know the cause of this negative result in *Chlorella*.

Induction of slow changes in Chl a fluorescence yield of DCMU poisoned cells by cofactor(s) of electron transport

PMS (phenazine methosulfate) is an efficient cofactor for cyclic electron transport in isolated chloroplasts (AVRON 1967). This cyclic flow of electrons, generating ATP, is mediated by photosystem I. ARNON *et al.* (1965) and GOVINDJEE and YANG (see GOVINDJEE *et al.* 1967) observed that PMS could quench the fluorescence yield of normal and DCMU poisoned chloroplasts respectively. MURATA and SUGAHARA (1969) reported a detailed analysis of this quenching of Chl a fluorescence in DCMU poisoned spinach chloroplasts by reduced PMS. WRIGHT and CROFTS (1970) made similar studies with another cofactor DAD (diaminodurene) which was found to be more effective in bringing about quenching of Chl a fluorescence in chloroplasts than PMS. The quen-

ching was found to be sensitive to all uncouplers and ionophorous antibiotics like gramicidin and nigericine. SYBESMA and WILLIAMS (1967) and WILLIAMS *et al.* (1969) reported a similar type of quenching of fluorescence yield with another cofactor of electron transport (DCPIPH₂) in intact algal cells of *Chlorella*. Recently, MOHANTY *et al.* (1973) have reported that quenching of Chl *a* fluorescence by PMS in isolated chloroplasts is related to the structural modifications in the chloroplast. We report below the effect of the cofactor PMS on the fluorescence yield changes in intact cells of *Chlorella* (Fig. 7) and *Anacystis* (Fig. 8). In *Chlorella* the induction of lowering of Chl *a*

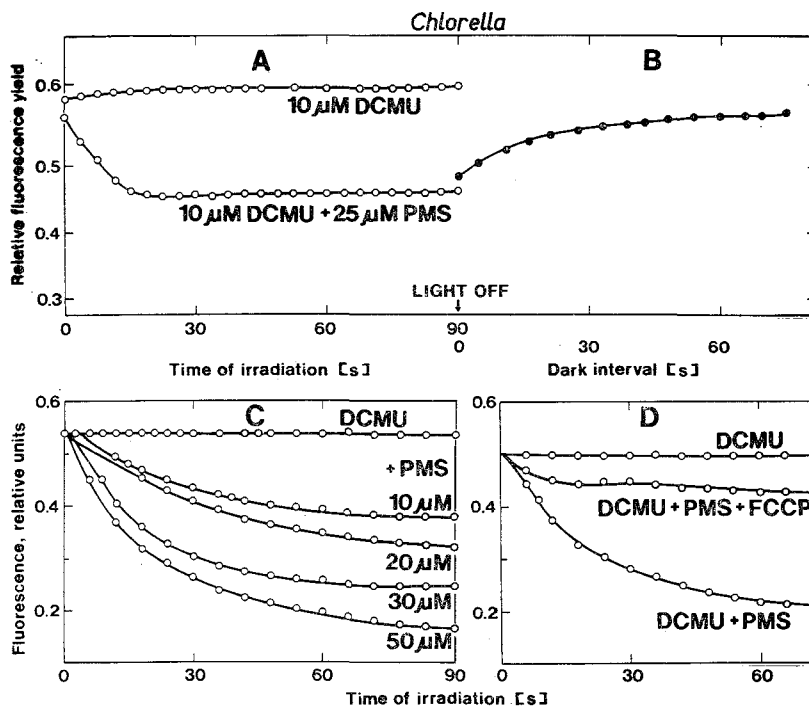


Fig. 7. Light induced quenching of Chl *a* fluorescence yield in *Chlorella* by PMS in the presence of DCMU. *A*: effect of adding PMS; *B*: dark recovery of the fluorescence yield as measured by weak intensity 1/10 s flash; *C*: various concentrations of PMS with 10 μ M DCMU; *D*: 10 μ M DCMU, 4 μ M FCCP and 30 μ M PMS, additions as shown. Buffer, 50 mM potassium phosphate, pH 8.0; [Chl], 60 μ g per 3 ml.

fluorescence yield in the presence of 10 μ M DCMU by 25 μ M oxidized PMS was slow, and the recovery of the original fluorescence level occurred within 40–45 s of darkness (Fig. 7). This restoration to the original level of fluorescence yield is slower than what has been seen in chloroplasts. The quenching of fluorescence with PMS saturates at about 50 μ M PMS (Fig. 7 *C*). The quenching requires a relatively high irradiance (*ca.* 14 W m⁻²). FCCP (4 μ M) slows down the rate of quenching of the fluorescence yield by PMS and decreases also the extent of the quenching (Fig. 7 *D*).

PMSH₂ brings about a quenching of fluorescence yield in DCMU poisoned *Anacystis*, while the same concentration of PMSH₂ does not inhibit the slow rise in the yield of Chl *a* fluorescence (Fig. 8). In some cases, as in Fig. 8, it stimulated the slow *SM* rise although the *DPS* transient was arrested. In this case, PMSH₂ seemed to inhibit the noncyclic electron flow and it is well known that blue-green algae exhibit a pronounced change in the yield of fluorescence in the presence of inhibitors of electron flow (PAPAGEORGIOU and GOVINDJEE 1968a).

The emission spectrum of *Chlorella* at liquid nitrogen temperature (77 K) in the presence of DCMU is not significantly altered by the addition of PMS (Fig. 9). The samples were preirradiated before cooling rapidly with liquid nitrogen. MURATA and SUGAHARA (1969) reported a total depression of yield of fluorescence in chloroplasts, without any alteration of emission character-

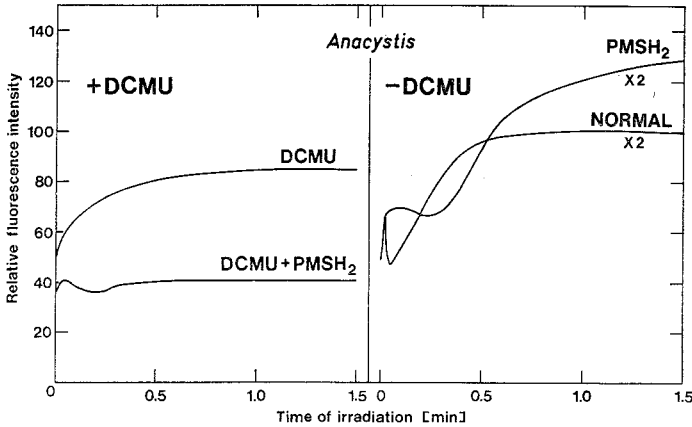


Fig. 8. Slow changes in the yield of Chl *a* fluorescence measured at 685 nm in the blue-green alga in normal and DCMU poisoned *Anacystis nidulans* with and without PMSH₂. λ excitation, 633 nm; irradiance *ca.* 26 W m⁻². 20 μ M PMS plus 0.1 mM ascorbate in 40 mM Tris-Cl buffer. All additions were made in the dark; 15 min dark time before irradiation.

istics by PMS at 77 K. As it is difficult to know the exact pathlength of irradiation after freezing with liquid nitrogen, we do not make any conclusion regarding the depression of yield at 77 K, but it is clear that PMS does not alter significantly the relative ratios of fluorescence bands. This suggests that the lowering of yield by PMS in intact cells, as in the case of isolated chloroplasts (MOHANTY *et al.* 1973), is probably not associated with a change in the spill-over of quanta from PS II to PS I.

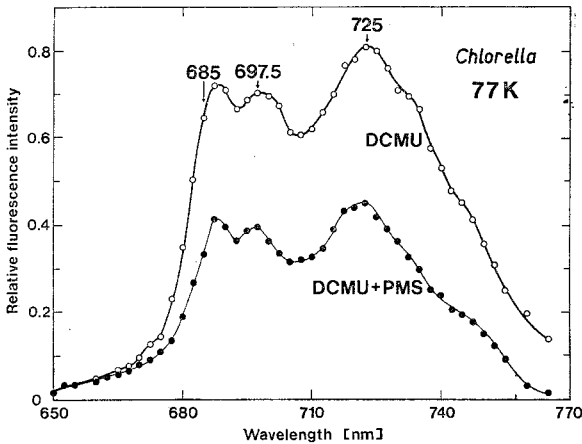


Fig. 9. Emission spectra of *Chlorella* at 77 K with DCMU (10 μ M) and with or without PMS (30 μ M). Buffer: 50 mM phosphate, pH 7.8 plus 10 mM NaCl; excitation λ , 435 nm (half band width, 6.6 nm); observation slit, 3.3 nm. The samples were preirradiated for 3 min with broad band blue radiation before cooling.

SYBESMA and MOHANTY (unpublished; see MOHANTY 1972) have observed a quenching of Chl *a* fluorescence by DCPIPH₂ which is reversed by an uncoupler, FCCP, and an "energy transfer" inhibitor, phloridzin in *Porphyridium cruentum*. The quenching caused by photosystem I cofactor, DCPIPH₂, is faster than that caused by PMSH₂. A low concentration of uncoupler FCCP was found to be very effective in reversing the lowering of Chl *a* fluorescence yield while energy transfer inhibitor phloridzin was less effective.

DISCUSSION

In this report, we have characterized two different types of Chl *a* fluorescence yield changes in intact algal cells that are not linked to the redox state of the quencher Q. In the one case (uncoupler induced), alteration of fluorescence yield is accompanied by a change in Chl *a* emission characteristics (Figs. 1 and 2) and in the other case (PMS induced) fluorescence yield fluctuations do not exhibit such an alteration in emission (Fig. 9). However, both types of fluorescence yield changes may be linked to an alteration of the thylakoid membrane structure.

Protonophorous uncouplers (FCCP, DNP), like cations (see MOHANTY and GOVINDJEE 1971; DE KOUCHKOVSKY 1972; MOHANTY and GOVINDJEE, unpublished), seem to induce similar changes in the fluorescence yield (quenching of fluorescence yield) and the emission (spectral) characteristics in intact algal cells. On the other hand, cations (like Mg⁺⁺ and Na⁺) enhance the fluorescence* yield (HOMANN 1969; MURATA 1969b; MURATA *et al.* 1970; MOHANTY *et al.* 1973) while an uncoupler like FCCP, at a relatively high concentration, quenches the fluorescence in isolated broken chloroplasts both in the absence and the presence of DCMU (Itoh *et al.* 1969; HOMANN 1971; KIMIMURA *et al.* 1971). From the above discussion, it appears that in intact algal cells both salts and uncouplers of phosphorylation (added to the external medium) cause similar structural alterations leading to spill-over of excitation energy in favor of pigment system 1. However, in isolated chloroplasts, salts seem to cause their shrinkage and suppress excitation energy transfer from pigment system II to system I (MURATA 1969b), while uncouplers (*e.g.*, high concentration of FCCP) seem to suppress such structural alterations (see GOOD *et al.* 1966). The differential results obtained in broken chloroplasts and intact algal cells may suggest that microscopic alterations in the orientation of the two pigment systems, and not the gross structural volume changes control the observed fluorescence changes.

We have shown, in this communication, that the potent uncoupler FCCP has multiple mode of action. It seems to cause an inhibition of back recombination reactions (between oxidized donor Z⁺ and reduced acceptor Q⁻) at a low concentration (Fig. 2) in normal cells, while at a moderate concentration it may increase the electron transport and bring about a quenching (Figs. 3 and 4) of fluorescence. Fluorescence yield changes induced by relatively high concentration of FCCP in DCMU poisoned cells seem to be caused predominantly by a structural modification (see GOOD *et al.* 1966) and consequently an alteration in the mode of excitation transfer (see PAPAGEORGIOU 1968; GOVINDJEE and PAPAGEORGIOU 1971; MOHANTY and GOVINDJEE 1973). DNP seems to exhibit similar mode of action at relatively high concentrations (Fig. 2; Table 2).

Cofactors that induce cyclic electron flow *via* photosystem 1 cause a lowering of Chl *a* fluorescence yield, both in broken chloroplasts (MURATA and SUGAHARA 1969; WRAIGHT and CROFTS 1971; MOHANTY *et al.* 1973) as well as in DCMU poisoned whole algal cells (Figs. 7 and 8). This type of decline in fluorescence yield in intact algal cells is reversed more effectively by uncouplers

* Recently E. GROSS (17th Annual Meeting of Biophysical Society, held in Columbus, Ohio, February 1973) has shown that low concentration of monovalent salts (1.2 mM) decrease the fluorescence yield, and it is this decreased yield that is reversed by divalent and higher concentrations of monovalent salts.

than by energy transfer inhibitors (SYBESMA and MOHANTY, unpublished; see MOHANTY 1972). It is known that PMS or DAD induced lowering of fluorescence yield in isolated chloroplasts is sensitive to a wide range of uncouplers and ionophorous antibiotics (MURATA and SUGAHARA 1969; WRAIGHT and CROFTS 1971; MOHANTY *et al.* 1973). This PMS-induced lowering of fluorescence yield, thus, seems to be related to energy-linked processes of photosynthesis. However, we believe that cyclic electron transport mediated quenching of fluorescence yield is also regulated by a modification of the membrane structure and is associated with the two photosystems in such a way that it causes an increase in non-radiative losses (heat).

CRAMER and BÖHME (1972), from their studies on the effect of inhibitors (*e.g.*, antimycin A, NH_2OH and FCCP) on the Chl *a* fluorescence and the redox state of Cyt *b* 559, propose an alternative explanation to these observed changes in fluorescence yield in the presence of DCMU. They believe that a shift of the midpoint potential of Cyt *b* 559 from a high to a low potential and *vice versa* regulates the fluorescence yield through the redox state of Q. If Cyt *b* 559 remains primarily in the high potential state, it can accept the electron in a cyclic reaction mediated through PS II. Such a cyclic flow around photosystem II will keep Q in a predominantly oxidized state and fluorescence at a low level. If Cyt *b* 559 remains in a low potential (more negative) state, an accumulation of reduced Q results due to DCMU blocking. It is conceivable that a change in the ionic environment or an alteration in the structural conformation may shift the redox potential of Cyt *b* 559. But it seems that if high potential Cyt *b* 559 mediated cyclic electron flow were the sole cause of fluorescence yield (ϕ_f) changes in the presence of DCMU, then these changes would be most predominant at low irradiance and would be minimal at high irradiance. This does not seem to be the case of ion induced alteration in the yield or PMS induced quenching of Chl *a* fluorescence. Low concentration of uncouplers (like FCCP, DNP) may shift high potential Cyt *b* to a low potential form and increase the yield of Chl *a* fluorescence but at a high concentration these uncouplers alter fluorescence yield by alteration of structure and in the mode of excitation transfer. It, therefore, seems that some specific alteration of the thylakoid structure promotes de-excitation of Chl *a* by a change in the rate constant of energy transfer between the two photosystems or in the rate of heat losses. (For a discussion of the light-induced structural changes see CROFTS *et al.* 1967 and PACKER and DEAMER 1968.) We do not know how the microscopic alteration of structure induced by uncouplers and cofactors differ. It may be related to the redox state of the carriers of photosystem I.

(After the present work was completed, the authors became aware of the following papers on Chl *a* fluorescence yield changes presented at the IV International Biophysics Congress held in Moscow, U.S.S.R. (Aug. 7–14, 1972). For the sake of completeness, reference to these papers is given below; they can be found in the book of abstracts of contributed papers, volume 1: (1) G. H. KRAUSE: Effects of oxygen on chloroplast shrinkage and steady state chlorophyll fluorescence in intact leaves of higher plants, paper EIVa 2/2, p. 323; (2) N. V. KARAPETYAN: Fluorescence induction of chloroplasts under reductive conditions, paper EIVa 2/3, p. 324; (3) O. ELGERSMA: Reaction kinetics of the primary acceptor Q of pigment system II in photosynthesis, paper EIVa 2/4, p. 325; (4) V. M. GOLD, YU. S. GRIGOREV and N. A. GAEVSKY: The induction transfer of chlorophyll fluorescence in native and model systems, paper EIVa 2/5, p. 326.)

REFERENCES

- ARNON, D. I., TSUJIMOTO, H. Y., McSWAIN, B. D.: Quenching of chloroplast fluorescence by photosynthetic phosphorylation and electron transfer. — Proc. nat. Acad. Sci. U.S.A. **54**: 927–934, 1965.
- AVRON, M.: Mechanism of photoinduced electron transport in isolated chloroplasts. — Curr. Topics Bioenerg. **2**: 1–22, 1967.

- 158 BANNISTER, T. T.: Effect of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone on chlorophyll fluorescence and photosynthesis. — *Biochim. biophys. Acta* **143**: 275—278, 1967.
- BANNISTER, T. T., RICE, G.: Parallel time courses of oxygen evolution and chlorophyll fluorescence. — *Biochim. biophys. Acta* **162**: 555—580, 1968.
- BONAVENTURA, C., MYERS, J.: Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. — *Biochim. biophys. Acta* **189**: 366—383, 1969.
- BUTLER, W. L.: On the primary nature of fluorescence yield changes associated with photosynthesis. — *Proc. nat. Acad. Sci. U.S.A.* **69**: 3420—3422, 1972.
- CHO, F., SPENCER, J., GOVINDJEE: Emission spectra of *Chlorella* at very low temperatures (-269° to -196°). — *Biochim. biophys. Acta* **126**: 174—176, 1966.
- CRAMER, W. A., BÖHME, H.: High potential cytochrome *b* 559 as a secondary quencher of chloroplast fluorescence in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. — *Biochim. biophys. Acta* **256**: 358—369, 1972.
- CROFTS, A. R., DEAMER, D. W., PACKER, L.: Mechanisms of light-induced structural change in chloroplasts. II. The role of ion movements in volume changes. — *Biochim. biophys. Acta* **131**: 97—118, 1967.
- DUYSENS, L. N. M.: 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) inhibition of system II and light-induced regulatory changes in energy transfer efficiency. — *Biophys. J.* **12**: 858—863, 1972.
- DUYSENS, L. N. M., SWEERS, H. E.: Mechanism of two photochemical reactions in algae as studied by means of fluorescence. — In: ASHIDA, J. (ed.): *Studies on Microalgae and Photosynthetic Bacteria*. Pp. 353—372. Univ. Tokyo Press, Tokyo 1963.
- GOOD, N., IZAWA, S., HIND, G.: Uncoupling and energy transfer inhibition in photophosphorylation. — *Curr. Topics Bioenerg.* **1**: 75—112, 1966.
- GOVINDJEE: Fluorescence studies on algae, chloroplasts and chloroplast fragments. — In: THOMAS, J. B., GOEDHEER, J. C. (ed.): *Currents in Photosynthesis*. Pp. 93—103. Donker, Rotterdam 1966.
- GOVINDJEE: Discussions on chlorophyll fluorescence *in vivo*: Techniques and applications. — In: *Chloroplast Fragments*. Pp. 17—45. Univ. Göttingen, Göttingen 1972.
- GOVINDJEE, PAPAGEORGIOU, G.: Chlorophyll fluorescence and photosynthesis: Fluorescence transients. — In: GIESE, A. C. (ed.): *Photophysiology*. Vol. 6. Pp. 1—46. Academic Press, New York—London 1971.
- GOVINDJEE, PAPAGEORGIOU, G., RABINOWITCH, E.: Chlorophyll fluorescence and photosynthesis. — In: GUILBAULT, G. G. (ed.): *Fluorescence Theory, Instrumentation, and Practice*. Pp. 511—564. Marcel Dekker, Inc., New York 1967.
- GOVINDJEE, RABINOWITCH, E.: Action spectrum of the “second Emerson effect”. — *Biophys. J.* **1**: 73—89, 1960.
- HOCH, G. E., RANDLES, J.: On the interaction of Photosystems I and II in algal cells. — *Photochem. Photobiol.* **14**: 435—449, 1971.
- HOMANN, P. H.: Cation effect on the fluorescence of isolated chloroplasts. — *Plant Physiol.* **44**: 932—936, 1969.
- HOMANN, P. H.: Action of carbonylcyanide *m*-chlorophenylhydrazone on electron transport and fluorescence of isolated chloroplasts. — *Biochim. biophys. Acta* **245**: 129—143, 1971.
- ITOH, M., YAMASHITA, K., NISHI, T., KONISHI, K., SHIBATA, K.: The site of manganese function in photosynthetic electron transport system. — *Biochim. biophys. Acta* **180**: 509—519, 1969.
- KIMIMURA, M., KATOH, S., IKEGAMI, I., TAKAMIYA, A.: Inhibitory site of carbonyl cyanide *m*-chlorophenylhydrazone in the electron transfer system of the chloroplasts. — *Biochim. biophys. Acta* **234**: 92—102, 1971.
- DE KOUCHKOVSKY, Y.: Effect of ionic environment on chlorophyll fluorescence, absorption spectrum and photochemical activity of photosynthesizing cells. — In: QUAGLIARIELLO, E., PAPA, S., ROSSI, C. S. (ed.): *Energy Transduction in Respiration and Photosynthesis*. Pp. 269—281. Adriatica Editrice, Bari 1971.

- KRAAYENHOF, R.: Uncoupling of Energy Conservation in Chloroplast and Mitochondrion. — Ph. D. Thesis, Univ. Amsterdam 1971.
- MAUZERALL, D.: Light-induced fluorescence changes in *Chlorella*, and the primary photoreactions for the production of oxygen. — Proc. nat. Acad. Sci. U.S.A. **69**: 1358—1362, 1972.
- MOHANTY, P.: Regulation of Chlorophyll Fluorescence during Photosynthesis: A Study of the Factors Affecting Changes in Yield and Emission of Chlorophyll Fluorescence in Intact Algal Cells and Isolated Chloroplasts. — Ph. D. Thesis, Univ. Illinois, Urbana—Champaign 1972.
- MOHANTY, P., BRAUN, B. Z., GOVINDJEE: Light-induced slow changes in chlorophyll *a* fluorescence in isolated chloroplasts: effects of magnesium and phenazine methosulfate. — Biochim. biophys. Acta **292**: 459—476, 1973.
- MOHANTY, P., BRAUN, B. Z., GOVINDJEE, THORNER, J. P.: Chlorophyll fluorescence characteristics of system I chlorophyll *a* protein complex and system II particles at room and liquid nitrogen temperatures. — Plant Cell Physiol. **13**: 81—91, 1972.
- MOHANTY, P., GOVINDJEE: Salt induced changes in (relative) fluorescence yield in intact algal cells. — In: Abstract: Conference on Primary Photochemistry of Photosynthesis, Argonne National Laboratory and Northwestern University. P. 31. Argonne, Illinois 1971.
- MOHANTY, P., GOVINDJEE: Light induced changes in the fluorescence yield of chlorophyll *a* in *Anacystis nidulans*: I. Relationship of slow fluorescence changes with structural changes.— Biochim. biophys. Acta **305**: 95—104, 1973.
- MOHANTY, P., MUNDAY, J. C. JR., GOVINDJEE: Time-dependent quenching of chlorophyll *a* fluorescence from (pigment) system II by (pigment) system I of photosynthesis in *Chlorella*. — Biochim. biophys. Acta **223**: 198—200, 1970.
- MOHANTY, P., PAPAGEORGIOU, G., GOVINDJEE: Fluorescence induction in the red alga *Porphyridium cruentum*. — Photochem. Photobiol. **14**: 667—682, 1971.
- MUNDAY, J. C. JR., GOVINDJEE: Light-induced changes in the fluorescence yield of chlorophyll *a* *in vivo*. III. The dip and the peak in the fluorescence transient of *Chlorella pyrenoidosa*. — Biophys. J. **9**: 1—21, 1969a.
- MUNDAY, J. C. JR., GOVINDJEE: Light-induced changes in the fluorescence yield of chlorophyll *a* *in vivo*. IV. The effect of preillumination on the fluorescence transient of *Chlorella pyrenoidosa*. — Biophys. J. **9**: 22—35, 1969b.
- MURATA, N.: Control of excitation transfer in photosynthesis. I. Light-induced changes of chlorophyll *a* fluorescence in *Porphyridium cruentum*. — Biochim. biophys. Acta **172**: 242—251, 1969a.
- MURATA, N.: Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in spinach chloroplasts. — Biochim. biophys. Acta **189**: 171—181, 1969b.
- MURATA, N.: Control of excitation transfer in photosynthesis. IV. Kinetics of chlorophyll *a* fluorescence in *Porphyra yezoensis*. — Biochim. biophys. Acta **205**: 379—389, 1970.
- MURATA, N.: Effects of monovalent cations on light energy distribution between two pigment systems of photosynthesis on isolated spinach chloroplasts. — Biochim. biophys. Acta **226**: 422—432, 1971.
- MURATA, N., SUGAHARA, K.: Control of excitation transfer in photosynthesis. III. Light-induced decrease of chlorophyll *a* fluorescence related to photophosphorylation system in spinach chloroplasts. — Biochim. biophys. Acta **189**: 182—192, 1969.
- MURATA, N., TASHIRO, H., TAKAMIYA, A.: Effects of divalent metal ions on chlorophyll *a* fluorescence in isolated spinach chloroplasts. — Biochim. biophys. Acta **197**: 250—256, 1970.
- PACKER, L., DEAMER, D.: Studies on the effect of light on chloroplast structure. — In: GIESE, A. C. (ed.): Photophysiology. Vol. 3. Pp. 91—122. Academic Press, New York—London 1968.
- PAPAGEORGIOU, G.: Fluorescence Induction in *Chlorella pyrenoidosa* and *Anacystis nidulans* and its Relation to Photophosphorylation. — Ph. D. Thesis. Univ. Illinois, Urbana—Champaign 1968.
- PAPAGEORGIOU, G., GOVINDJEE: Light-induced changes in the fluorescence yield of chlorophyll *a* *in vivo*. I. *Anacystis nidulans*. — Biophys. J. **8**: 1299—1315, 1968a.

- 160 PAPAGEORGIOU, G., GOVINDJEE: Light-induced changes in the fluorescence yield of chlorophyll *a* *in vivo*. II. *Chlorella pyrenoidosa*. — *Biophys. J.* **8**: 1316—1328, 1968b.
- SHIMONY, C., SPENCER, J., GOVINDJEE: Spectral characteristics of *Anacystis* particles. — *Photosynthetica* **1**: 113—125, 1967.
- SYBESMA, C., WILLIAMS, W. P.: Chlorophyll *a* fluorescence studies in poisoned algae. — In: Abstracts Biophysical Society, 11th Annual Meeting. P. 36. Houston, Texas 1967.
- VREDENBERG, W. J.: Enhancement of the dark reconstitution of photosynthetic reaction center 2 by nigericin and CCCP. — *Biochim. biophys. Acta* **189**: 129—132, 1969.
- VREDENBERG, W. J.: Chlorophyll *a* fluorescence induction and changes in the electrical potential of the cellular membranes of green plant cells. — *Biochim. biophys. Acta* **223**: 230—239, 1970.
- WILLIAMS, W. P., MURTY, N. R., RABINOWITCH, E.: The complexity of the fluorescence of *Chlorella pyrenoidosa*. — *Photochem. Photobiol.* **9**: 455—469, 1969.
- WRAIGHT, C. A., CROFTS, A. R.: Energy dependent quenching of chlorophyll *a* fluorescence in isolated chloroplasts. — *Europe. J. Biochem.* **17**: 319—327, 1971