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UNCOUPLER EFFECTS ON CHLOROPLAST FLUORESCENCE[1]

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Abstract

There are two major models proposed in the literature to explain the fluorescence stimulation effect of uncoupler. In this report analytic methods are suggested to analyse experimental results quantitatively. The methods are tested by empirical data. The two models are a Q model and a high energy state model. Q model assumes that there are two uncoupler sensitive limiting sites, one on the water splitting side and the other on the reducing side of photosystem II; under certain conditions, it is possible to observe simultaneous stimulation of both fluorescence (by stimulating Q reduction) and electron transport. High energy state model assumes that high internal proton concentration lowers fluorescence quantum yield.

Introduction

Under some conditions uncouplers stimulate chloroplast fluorescence* (Wraight and Crofts, 1970; Krause, 1973, 1974, 1975; Li, 1973; Barber *et al.*, 1975). Two models have been proposed to explain the phenomenon.

The effect may be explained in terms of modification of fluorescence yield by proton gradient changes as a result of uncoupler addition (referred as high energy state model, Wraight and Crofts, 1970; Krause, 1973, 1974, 1975; Barber *et al.*, 1975; see also Govindjee *et al.*, 1966; Bannister, 1967; Murata and Sugahara, 1969; Hoch and Randles, 1972). Alternatively (Gimmler, 1973; Li, 1973, 1975a; see also Cheniae, 1970), it may be attributed to an increase of the concentration of Q^- [Q model; Q, the primary electron acceptor of photosystem II, is a fluorescence quencher, but Q^- is not (Duysens and Sweers, 1963)]. The Q model assumes that there is an uncoupler sensitive limiting site (or energy conserving site) on the water side of Q (Site II, Site I being the coupling site existed between plastoquinone and cytochrome *f*, Avron and

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* See Jennings *et al.* (1976), and Sokolove and Marsho (1977) for uncoupler effect on fluorescence under high light illumination.

Chance, 1966; Böhme and Cramer, 1972).

In this report, I present two equations to analyse the empirical results, one in terms of Q model, and one in terms of high energy model. Both equations are tested with experimental observations on fluorescence and rate of electron transport as functions of the concentration of methyl viologen either in the presence or in the absence of an uncoupler. The theoretical basis of these experiments are described as follows:

According to the Q model of uncoupler effect, electron transport and fluorescence are controlled, among other things, by two rate limiting sites. Uncoupler releases these rate limiting factors, thereby altering fluorescence and electron transport. The uncoupler effect on fluorescence is complicated by the fact that the two limiting sites are on the opposite side of Q. But the uncoupler effect on limiting Site I may be minimized by adjusting the concentration of Hill oxidant so that the rate of Q^- oxidation is limited by electron acceptors whether there is uncoupler or not, thereby jutting out the uncoupler effect on Site II.

Materials and Methods

Field-grown lettuce leaves were harvested immediately before each experiment, rinsed, prechilled in dark in cold water (ca. 4°C) for ten minutes, and then were deveined and hand ground, in a prechilled mortar, with cold buffer which consisted of sucrose, 400 mM, $MgCl_2$, 2–5 mM, and tricine-NaOH, 20–40 mM (pH 8). After removal of cell debris by straining through eight layers of cheesecloth and 1 layer of fine nylon cloth; the filtrate was centrifuged at 4340 xg for five minutes, and pellet was resuspended in the same buffer. All the procedures, except the centrifugation, were carried out at room temperature (ranging approximately from 15°C to 25°C). The resuspended chloroplasts (ca. 1 mg chl/ml) were kept on ice for immediate experimentation.

Methyl viologen reduction was measured as O_2 uptake with a membrane covered Clark type electrode (Rank Brothers, Cambridge, England). A thermostate was set at 25°C. Incandescent light filtered with 12 cm 0.38% $CuSO_4 \cdot 5H_2O$ solution and GG 14 filter (500 nm cutoff, Schott & Gen., Mainz) was used. The intensity at the center of the empty cuvette was 2×10^5 erg/ cm^2 sec.

Fluorescence was excited with green light (broad-band interference filter, 500 to 640 nm, Corning glass CS 4–96, plus 8 cm 2% $CuSO_4 \cdot 5H_2O$), the intensity was 6×10^5 erg/$cm^2 \cdot$sec. Emitting light was observed 90 degree from the exciting beam at 681 nm (681 nm interference filter, monochrometor plus auxillary red filters).

Results and Discussion

A. *Experiment*

Table 1 shows that in the absence of phosphorylation cofactors and uncoupler (referred as control condition) an increase of methyl viologen (MV, an autooxidizable Hill oxidant) from 50 to 5,000 nM changes not much the rate of electron transport. However, there is a conspicuous fluorescence drop when the concentration of methyl viologen is increased from 50 nM to 500 nM. These observations suggest that at 50 nM methyl viologen, electron transport is oxidant limiting, whereas above this concentration it is Site I limiting.

Gramicidin D, an uncoupler, in the absence or in the presence of low concentration of MV, has little effects on fluorescence (Table 1 and Fig. 1). Gramicidin D does not stimulate electron transport in the presence of 50 nM MV, it slows down** the rate sometimes (Table 1), and shows little effect at other times (results not shown). At 500 nM MV, uncoupler doubles the rate, and the relative fluorescence stimulation effect is maximal among the concentrations tested. As the Hill oxidant is increased to 5,000 nM, uncoupler quadruples the rate but the fluorescence effect is downed.

If experimental results of uncoupled chloroplasts are compared among

Table 1. *Chloroplast fluorescence and Hill reaction*

MV nM	Control			Gramicidin		
	R	F	*f*	R	F	*f*
0	0	64.5	44.5	0	64.5	44.5
50	41	56.5	36.5	29	59.5	39.5
500	47	46.5	26.5	97	60.5	40.5
5,000	50	46	26	203	49.5	29.5

Fluorescence data were taken from figure 1. All data were taken one minute after the onset of illumination. Hill rates were measured from samples containing chloroplasts with an equivalent chlorophyll concentration 25 μg chl/ml, assayed at 25°C in tricine 11 mM, pH 8.1; sucrose, 200 mM; $MgCl_2$, 3 mM, and varied amount of methyl viologen (MV); gramicidin D, 0.5 μM when added. R=rate in μmoles O_2/mg chl/hr; F=fluorescence in relative unit; *f*=F-20 (variable fluorescence, see text for explanation).

** Uncoupler may damage water splitting system (Ranger, 1969; Harth *et al.*, 1974; Homann, 1971) in the presence of low concentration of Hill oxidant when the oxidant equivalents generated by PS II are held on, for accumulation of 4 oxidant equivalents are delayed owing to a lack of Q. Uncoupler may accelerate the decay of a oxidation product of PS II (Renger, 1969; see also Homann, 1971). When electron transport rate is fast, i.e., in the presence of high methyl viologen concentration, the decay of the O_2 evolution precursor states in the presence of uncoupler can not complete with the forward reaction, therefore, one does not observe the rate inhibition effect of uncoupler in the presence of high methyl viologen concentration.

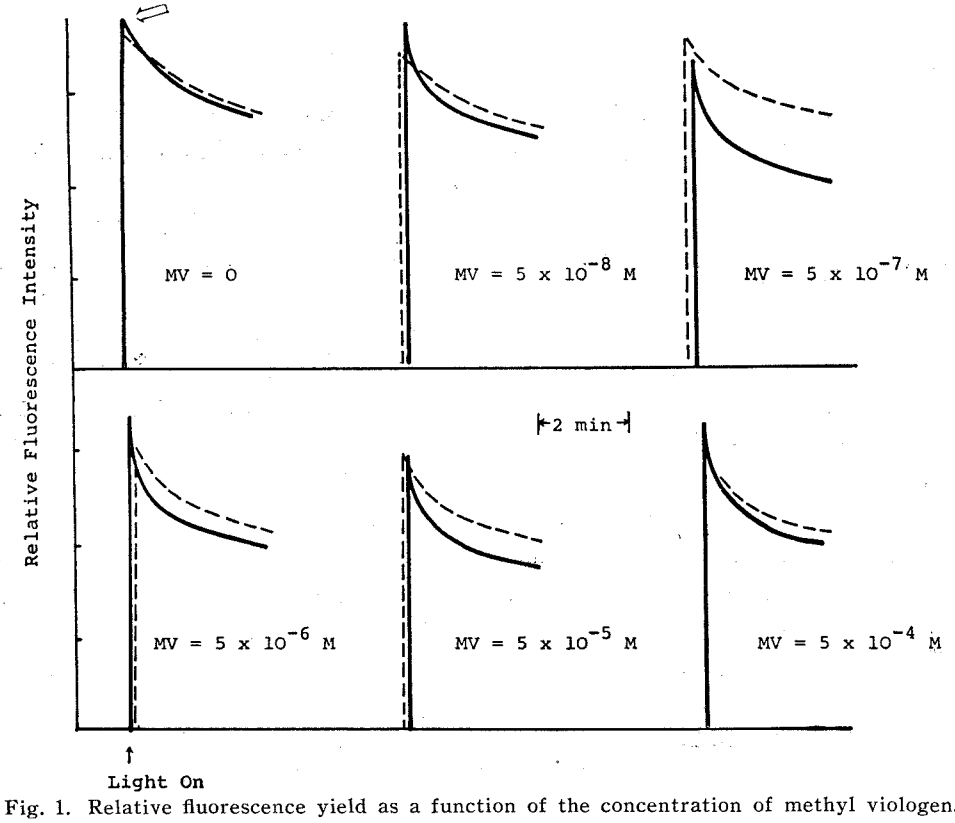

Fig. 1. Relative fluorescence yield as a function of the concentration of methyl viologen. Samples containing chloroplasts with an equivalent chlorophyll concentration 6 μg chl/ml, tricine 20 mM, pH 8; sucrose, 400 mM; $MgCl_2$, 5 mM; gramicidin D when added, 0.5 μM, and varied amount of methyl viologen as indicated in the figure. ---- plus gramicidin D; —— no gramicidin D added. Open arrow in the figure indicates the peak value of fluorescence having a relative value of about 80.

themselves, it shows that between 50 and 500 nM methyl viologen rate is tripled, while there is no change in fluorescence (see later this report for discussion).

Two values of fluorescence are listed in Table 1, one is the total fluorescence (F), the other is "variable fluorescence" (f, which are included for the purpose of analysis). The values of variable fluorescence are obtained by substracting from total fluorescence a constant part of the fluorescence of chloroplast in the absence of added oxidant (about one fourth of the peak value indicated by an open arrow in Fig. 1 is assumed to be constant fluorescence).

B. *Formulations*

The following analyses are independent of the model of photosynthetic unit chosen, because the effective concentration of Q, q, is used in all equations (Li, 1978a).

B-1. Variable fluorescence

Not all fluorescence is related to PS II photochemistry (Lavorel, 1962; Clayton, 1969), there is a constant part of fluorescence which is independent of the redox state of Q. The exact level of this constant fluorescence is not known (Lavorel and Joliot, 1972), estimation of it by various methods is possible. One can estimate it by a weak exciting beam with and without a time-separated actinic light (Li, 1975b) which shows that constant fluorescence is about one fourth of the total fluorescence (see also Malkin and Kok, 1966; Cramer and Böhme, 1972). This estimation is adopted in the present report. The difference between total and constant fluorescence is called variable fluorescence (Lavorel, 1962), its value depends on the redox state of Q.

B-2. Q model

One consequence of assigning a rate-limiting site on the oxidizing side of Q is that the rate of electron transport will sometimes be limited by activities on the water side of PS II. And Li (1978a) has introduced the following rate equation to describe such a situation

$$R = \lambda skq/(k + \lambda sq) \qquad (1)$$

where q, is the normalized effective concentration of Q; λ includes light intensity, absorbances and quantum yield for the photochemical conversion,

$$Q \xrightarrow{\lambda} Q^- \qquad (2)$$

and s is a parameter dealing with both the concept of fixity of intermediates (first order in space domin) and that of the independent fluctuation of the redox states of primary electron donor D and primary electron acceptor Q (second order in time domain), $s \geq 1$ normally; k is the rate constant for

$$D^+ \longrightarrow D \qquad (3)$$

The variable fluorescence intensity (f) can be expressed in terms of q (Malkin and Kok, 1966).

$$f = \alpha_2 I\phi_F q^- = \alpha_2 I\phi_F (1 - q) \qquad (4)$$

where α_2 is the fraction of light (I) absorbed by PS II; ϕ_F is the fluorescence quantum yield; and q is the normalized, effective concentration of Q.

From Eqns. (1) and (4), and let $\lambda = \alpha_2 I\phi_{2c}$ we have

$$f = \alpha_2 I\phi_F - \phi_F Rk/(k - R)\,\phi_{2c} \qquad (5)$$

B-3. High energy state model

The high energy state model states that aside from the Q effect on fluorescence, the latter can be decreased by the high energy state established

by the process of electron transport. Variable fluorescence (f) is then expressed as follow

$$f = \alpha_2 I(1 - q)\, \phi_F \cdot X(R) \qquad (6)$$
$$0 < X(R) \geq 1$$

X(R)=1 when R=0, or when electron transport is uncoupled; X(R) represents the effect of high energy state on the yield of fluorescence, it is a function of the electron transport rate (R). Since the high energy state model does not assume that rate is limited by Site II, we have.

$$R = \alpha_2 \phi_{2c}\, Iq \qquad (7)$$

i.e., the overall electron transport is limited by reactions on the reducing side of PS II (Malkin and Kok, 1966). From Eqns. (6) and (7), we have

$$f = \alpha_2 I(1 - R/\alpha_2 \phi_{2c}\, I)\, \phi_F\, X(R) \qquad (8)$$

C. *Analyses*

C-1. High energy state model

Eqn. (8) are used to analyse data listed in Table 1. An example of the calculation is shown as follows. In the absence of methyl viologen, assume

$$R = 0, \quad X(0) = 1,$$

We did not observe Mehler reaction during the period we performed the experiments reported here, and KCN did not stimulate O_2 uptake. The value of variable fluorescence (f) under this condition is,

$f = 44.5$*** (Table 1)

At a concentration of 50 nM methyl viologen,

$$R = 41$$

and

$$f = 36.5$$

There is a 18% fluorescence decrease relative to R = 0. At a concentration of 500 nM methyl viologen,

$$R = 47$$

and

$$f = 26.5$$

*** If the initial fluorescence is taken as the maximum fluorescence (q=0), the value is 80, substrating a constant part (about 20) from it, we have a value of 60 for f. We do not use the initial fluorescence for our calculation because fluorescence also decline in the presence of gramicidin, and one minute after the onset of light fluorescence values are approximately the same for both control and gramicidin sample.

There is a 41% fluorescence decrease relative to R=0. And at concentration of 5,000 nM methyl viologen,

$$R = 50$$

and

$$f = 26$$

There is a 42% fluorescence decrease relative to R=0.

The observed relationship between rate and fluorescence is non-linear. But, according to Li (1978a, and references quoted therein), fluorescence and q, the effective concentration of Q, are linearly related, which together with Eqn. (7) suggest that rate and fluorescence are linear. The non-linearity can therefore all be attributed to X(R) according to the high energy state model.

But the fact that a rate of 41 μmoles/mg chl·hr (from R=0 to R=41) induces a 18% fluorescence drop, whereas a slight increase of rate (from R=41 to R=47 μmoles/mg chl·hr) causes an additional 23% fluorescence drop seem to be peculiar. If one assumes X(R) and R are linearly related, and let $X(41) \doteqdot X(47)$, it turns out, by solving Eqn. (8) for X(R), that $X(47) > 1$, in contradiction to the assumption that $0 < X(R) \leq 1$.

In the presence of uncoupler, the relationship between fluorescence and rate in terms of both Q and high energy state models may be expressed by

$$f = \alpha_2 I \phi_F (1 - R/\alpha_2 \phi_{2c} I) \qquad (9)$$

for X(R) = 1 for any value of R, and $k \gg R$. It is then difficult to apprehend the fact that in uncoupled chloroplasts a ten fold increase of methyl viologen (from 50 to 500 nm) triples the rate of electron transport without affecting fluorescence whatsoever. One possible explanation is that in the absence or in the presence of low concentration of methyl viologen, electrons are cycling around PS II; and an increase of oxidant (but not to the extent to support a rate anywhere near its maximum) may simply intercept electrons from the mini-circle. It is also possible that a fast rate of Q^- oxidation stimulates electron transport on the water side of Q, thereby keeps the concentration of Q^- high. Another possibility is that when I is much bigger than R, a certain change in R may not affect fluorescence appreciably.

C-2. Q model

Table 2 illustrates how, according to Eqn. (5), a change in the value of k can either decrease or increase fluorescence intensity. In Eqn. (5), the $\alpha_2 I \phi_F$ term is independent of the presence of an uncoupler, while the $\phi_F Rk/(k - R)\alpha_{2c}s$ term is. Of the $\phi_F Rk/(k - R)\alpha_{2c}s$ term, $\phi_F/\alpha_{2c}s$ is assumed to be not affected by uncoupler. We can then predict how uncoupler affects

Table 2. *Theoretical predictions of uncoupler effect on fluorescence from experimentally observed rate of electron transport*

A. In the presence of 500 nM methyl viologen

$R_{control} = 47$,	$R_{uncoupled} = 97$;	$k=55$,	$k_u=ak$,	$a>1$
$Rk/(k-R)$		Fluorescence effect		
Control	Uncoupled			
323	821 ($k_u=2k$)	fluorescence decreased		
	150 ($k_u=5k$)	fluorescence increased		
	118 ($k_u=10k$)	fluorescence increased		

B. In the presence of 5 μM methyl viologen

$R_{control} = 50$	$R_{uncoupled} = 203$	
550	−240 ($k_u=2k$)	physically unreasonable
	775 ($k_u=5k$)	fluorescence decreased
	322 ($k_u=10k$)	fluorescence increased

See text for details, briefly one predicts uncoupler effects on fluorescence by calculating $Rk/(k-R)$, R is the observed rate of electron transport and k is the uncoupler sensitive rate constant for a reaction on the water side of Q. When $[Rk/(k-R)]_{uncoupled} > [Rk/(k-R)]_{control}$, fluorescence is decreased, otherwise fluorescence is either increased or unchanged by uncoupler.

fluorescence by examining how uncoupler modifies $Rk/(k-R)$. If uncoupler increases the value of $Rk/(k-R)$, fluorescence will be decreased; otherwise, fluorescence will be either increased or unchanged. But, the value of $Rk/(k-R)$ must be within a range so that Eqn. (5) is physically meaningful, i.e., it must be positive, and it must not make f negative.

D. *General Discussion*

Upon illumination, broken chloroplasts supplemented with Mg^{2+} or intact chloroplasts show a slow fluorescence falling of large magnitude after the well known instananeous rise to a peak. For reasons of its Mg^{2+} dependency and its reversibility by uncoupler addition, the fluorescence falling has been attributed to Mg^{2+}—efflux from the thylakoids in response to light-induced proton-uptake (Krause, 1974; Barber and co-workers, 1975). Jennings *et al.* (1976), based on the observations that uncouplers stimulate slow fluorescence falling, whereas subjection of chloroplasts to an acid-base transition is inhibitory, suggest instead that the falling is due to a decrease in the probability of the back transfer of energy from PS II reaction centers to the fluorescent "light harvesting" chlorophyll system.

Sokolove and Marsho (1977) resolve the fluorescence falling into two distinct components, an ionophore-reversible and an ionophore-resistant components. The ionophore-reversible falling is attributed to Mg^{2+}— efflux from thylakoid driven by proton uptake, whereas the ionophore-resistant falling is attributed to photoinhibition.

Li (1978b), by showing that the initial fluorescence declining is a function not only of the coupling state but also of the concentration of Hill oxidant and of the presence of an artificial PS II donor, proves convincingly that the declining is related to the redox change of Q. The initial fluorescence changes, therefore, reflect the time-dependent changes in rates of Q^- oxidation and Q reduction; the reduction is a function, among other things, of the activity of H_2O splitting system.

The fluorescence decline is dark reversible (Barber *et al.*, 1975; Li, Y.S. unpublished observation), suggesting in the framework of Q model that when dark adapted chloroplasts are first exposed to light, the initial re-oxidation of Q^- is somehow retarded owing to, possibly, that Site I is limiting, for uncoupler greatly accelerating the rate of declining (note, the high energy state model does not anticipate that uncoupler stimulates fluorescence declining, note also that photoinhibition does not constitute a serious problem in the study of Li, 1978b). However, the Q model does not explain the observation of Barber *et al.*, (1975) that in whole chloroplasts, DCMU, in the light, induces a small prompt increase of fluorescence followed by a large slow increase of fluorescence, whereas ionophores initiate a large and fast fluorescence rise.

On the other hand, the high energy state model explains the observations presented in this article with difficulty. It does not explain Gimmler's observations that uncoupler accelerates greatly fluorescence induction without a large stimulation of steady state fluorescence in whole cells (Gimmler, 1973), nor a preliminary observation (Li, Y.S. unpublished) that uncoupler lowers the fluorescence intensity of chloroplasts with impaired**** water splitting system supplemented with PS II donor (the uncoupler effect, again, depends on the concentration of Hill oxidant, no effect at 500 nM MV and the effect saturated at 5 μM, unpublished observation). The Q model may explain some of the donor experiments, if one assumes that Site II may not control electron transport sometimes (see Gould and Izawa, 1973a, b), and that PS II donors may be able to bypass Site II partially (Li and Ueng, 1980). Yet, gramicidin lowers fluorescence even in the absence of a donor, when no electron transport is observed, at least not the non-cyclic one. I shall not discuss this problem

**** Treated with hydroxylamine and ethylenediaminotetraacetic acid (Ort and Izawa, 1973).

further at the present stage of my investigation of the donor experiment, for the uncoupler effects on the photosynthetic apparatus may be much more complicated (Bannister, 1967; Renger, 1969; Homann, 1971; Harth *et al.*, 1974) than what I can handle now.

Now back to the main point. With all the pros and cons for both the Q and the high energy state model, and in view of the fact that these two models are not mutually exclusive, one may postulate that under the present experimental condition it is the Q effect which dominates, whereas the high energy state effect may dominate under some other conditions. To what an extent each effect is responsible for the rise of fluorescence may be studied by simultaneous measurements of both fluorescence and rate, by DCMU experiment, and by fluorescence induction, but a knowledge of q is most desireable. Knowing q one can calculate λs and k by measuring R in the presence of different concentrations of methyl viologen. Knowing q one can also test the assumption that q and f are linearly related, an assumption derived from (see Li, 1978a) the Q hypothesis of Duysens and Sweers (1963). A strictly linear relationship between q and f independent of the coupling state may discourage the high energy state model. In any case, Eqn. (8) provides a means to test the relationship between fluorescence and proton gradient.

To establish the Q or the high energy state model is in no way to disprove other models, especially in view of the donor experiments just mentioned, minor changes of other properties of chloroplasts which modify the fluorescence yield can not be ruled out. However, it is not likely, considering the fluorescence responses to the oxidant concentration, that uncoupler induces a change of spillover between photosystem II and photosystem I to such an extent that it causes a major change of fluorescence. Nor is the fluorescence change a result of transforming high potential cytochrome$_{b-559}$ to low potential one (Cramer and Böhme, 1972) for the exciting light intensity is saturating and also for reason mentioned just above.

Literature Cited

Avron, M. and B. Chance. 1966. Relation of phosphorylation to electron transport in isolated chloroplasts. Brookhaven Symp. Biol. **19**: 149-160.

Bannister, T.T. 1967. Effects of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone on chlorophyll fluorescence and photosynthesis. Biochim. Biophys. Acta **143**: 275-278.

Barber, J., A. Telfer, J. Mills and J. Nicolson. 1975. Slow chlorophyll fluorescence changes in isolated intact chloroplasts: Evidence for cation control. pp. 1021-1030, In M. Avron (ed). Proc. 3rd Int. Congr. Photosyn. Elsevier, the Netherlands.

Böhme, H. and W.A. Cramer. 1972. Localization of a site of energy coupling between plastoquinone and cytochrome *f* in the electron-transport chain of spinach chloroplasts. Biochem. **11**: 1155-1160.

Cheniae, G. M. 1970. Photosystem II and O_2 evolution. Ann. Rev. Plant Physiol. **21**: 467–498.

Clayton, R. 1969. Characteristics of prompt and delayed fluorescence from spinach chloroplasts. Biophys. J. **9**: 60–76.

Cramer, W. A. and H. Böhme. 1972. 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.

Duysens, L. N. M. and H. E. Sweers. 1963. Mechanism of two photochemical reactions in algae as studied by means of fluorescence. pp. 353–372. In S. Miyachi (ed). Studies on Microalgae and Photosynthetic Bacteria. Univ. of Tokyo Press. Tokyo.

Gimmler, H. 1973. The effect of FCCP on the fluorescence induction of chlorophyll in DBMIB-treated intact cells of the unicellular green alga *Dunaliella parva*. Z. Pflanzenphysiol. **68**: 385–390.

Gould, J. M. and S. Izawa. 1973a. Studies on the energy coupling sites of photophosphorylation. I. Seperation of site I and site II by partial reactions of the chloroplasts electron transport chain. Biochim. Biophys. Acta **314**: 211–223.

Gould, J. M. and S. Izawa. 1973b. Photosystem-II electron transport and phosphorylation with dibromothymoquinone as the electron acceptor. Eur. J. Biochem. **37**: 185–192.

Govindjee, J. C. Munday and G. Papageorious. 1966. Fluorescence studies with algae: Changes with time and preillumination. Brookhaven Symp. Biol. **19**: 434–445.

Harth, E., S. Reimer and A. Trebst. 1974. Control of photosynthetic oxygen evolution by the internal pH of the chloroplast thylakoid. FEBS Letters **42**: 165–168.

Hoch, G. E. and J. Randles. 1972. On the interaction of photosystems I and II in algae cells. Int. Conf. on the Photosynthetic Unit, Gatlinburg, 1970, Photochem. Photobiol. **14**: 435–449.

Homann, P. H. 1971. Actions of carbonylcyanide *m*-chlorophenyl-hydrazone on electron transport and fluorescence of isolated chloroplasts. Biochim. Biophys. Acta **245**: 129–143.

Jennings, R. C., F. M. Garlaschi and G. Forti. 1976. Studies on the slow fluorescence decline in isolated chloroplasts. Biochim. Biophys. Acta **423**: 264–274.

Krause, G. H. 1973. The high-energy state of thylakoid system as indicated by chlorophyll fluorescene and chloroplast shrinkage. Biochim. Biophys. Acta **292**: 715–728.

Krause, G. H. 1974. Changes in chlorophyll fluorescence in relation to light dependent cation transfer across thylakoid membranes. Biochim. Biophys. Acta **333**: 301–313.

Krause, G. H. 1975. Light induced movement of metal cations in chloroplasts: Evidence from chlorophyll fluorescence and light scattering. pp. 1021–1030. In M. Avron (ed). Proc. 3rd Int. Cong. Photosynthesis, 1974, Rehovot. Elsevier, Amsterdam.

Lavorel, J. 1962. Heterogeneite de la chlorophylle in vivo. 1. Sepectres d'emission de fluorescence. Biochim. Biophys. Acta **60**: 510–523.

Lavorel, J. and P. Joliot. 1972. A connected model of the photosynthetic unit. Biophys. J. **12**: 815–831.

Li, Y. S. 1973. Gramicidin D enhanced ferricyanide Hill reaction and fluorescence intensity during electron transport—an indication of a possible coupling site on the water side of photosystem II. Bot. Bull. Academia Sinica **14**: 65–69.

Li, Y. S. 1975a. The sites of induced limited steps of the electron transport reactions in chloroplasts isolated with low-salt medium. pp. 665–670. In M. Avron (ed). Proc. 3rd Int. Cong. Photosynthesis, 1974, Rehovot. Elsevier, Amsterdam.

Li, Y. S. 1975b. Salts and chloroplast fluorescence. Biochim. Biophys. Acta **376**: 180–188.

Li, Y. S. 1978a. Derivation of an electron-transport rate equation, energy-conservation equations and a luminescence-flux equation of algal and plant photosynthesis. Biochem. J. **174**: 569–577.

Li, Y. S. 1978b. Redox of Q regulated slow fluorescence induction of isolated chloroplast. Bot. Bull. Academia Sinica **19**: 33–40.

Li, Y. S. and S. H. Ueng. 1980. Effects of ascorbate on O_2 consumption mediated by

methyl-viologen in chloroplasts. Bot. Bull. Academia Sinica **21**: 135-139.
Murata, N. and K. Sugahara. 1969. 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.
Malkin, S. and B. Kok. 1966. Fluorescence induction studies in isolated chloroplasts. I. Number of components involved in the reaction and quantum yields. Biochim. Biophys. Acta **126**: 412-432.
Ort, D. R. and S. Izawa. 1973. Studies on the energy-coupling sites of photophosphorylation. II. Treatment of chloroplasts with NH_2OH plus ethylenediaminetetraacetate to inhibit water oxidation while maintaining energy-coupling efficiences. Plant physiol. **52**: 595-600.
Renger, G. 1969. Reaction of CCCP in photosynthesis on an intermediate between chlorophyll a_{II} and water. Naturwissenschaften, **56**: 370.
Sokolove, P. M. and T. V. Marsho. 1977. Slow fluorescence quenching of type A chloroplasts. Resolution into two components. Biochim. Biophys. Acta **549**: 27-35.
Wraight, C. A. and A. R. Crofts. 1970. Energy-dependent quenching of chlorophyll a fluorescence in isolated chloroplasts. Eur. J. Biochem. **17**: 319-327.

Uncoupler 對葉綠體螢光的影響

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由 uncoupler 對葉綠體螢光和電子傳送率的影響之實驗及理論分析得知，在第二光合反應中心與水分解系統之間有一受 uncoupler 影響並限制電子傳送的反應。