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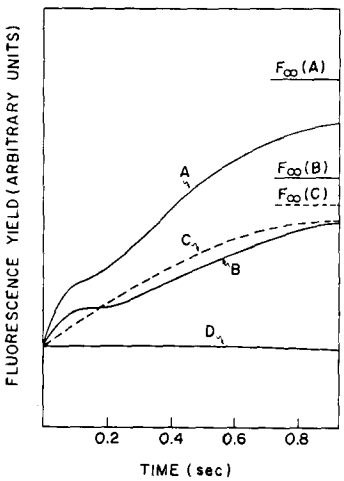
Photoinhibition and excitation quenching in Photosystem II of photosynthesis, from fluorescence induction measurements

Strong ultraviolet or visible irradiation inhibits photosynthesis and Hill reaction[1,2] (*cf.* references cited in refs. 1 and 2). The ultraviolet photoinhibition affects mainly the steps which lead to oxygen evolution (Photosystem II)[2]. Since at room temperature most of the chlorophyll *a* fluorescence originates from Photosystem II, fluorescence kinetic studies may give a clue to the mechanism.

In isolated chloroplasts, the fluorescence yield in continuous short-wavelength light rises from a low value, F_0, at the start of illumination, reaching a steady-state value, F_∞ (refs. 3, 4; Fig. 1A). This change probably reflects[4] the photoreduction of a postulated primary electron acceptor, Q, of Photosystem II:

$$Q \xrightarrow{h\nu_{II}} Q^- \text{ (or QH)} \qquad (1)$$

Q serves as a quencher to the excitation energy absorbed by the pigment system while Q^- does not, and therefore the probability of fluorescence from the pigment system increases with increasing Q^-. The electron donor to Q, D, is not written explicitly in (1). The assumption was made that D, in the oxidized form, D^+, reacts



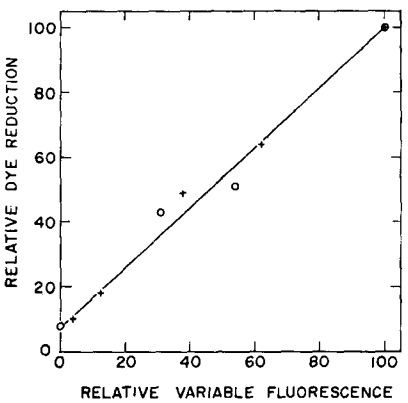


Fig. 1. The effect of photoinhibition on the fluorescence induction and steady-state value. Spinach chloroplasts after pre-illumination with 740 mμ light. (The same curve is obtained after long (10 min) dark period.) A. Control. B. Chloroplasts which were exposed to ultraviolet light (254 mμ, obtained from germicidal ultraviolet lamp) for 1 min. C. Chloroplasts which were exposed to strong red light (570 mμ, obtained from 2000-W Xenon Arc Lamp) for 2 min. D. Chloroplasts which were exposed to ultraviolet, or strong red light for 10 min. The concentration of chlorophyll was about $2 \cdot 10^{-5}$ M. For other methodical details *cf.* ref. 1 for chloroplasts preparation and photoinhibition and ref. 3 for fluorescence measurements.

Fig. 2. Relation between rate of 2,6-dichlorophenolindophenol photoreduction (under limiting light conditions) to the steady-state fluorescence level, for various degrees of photoinhibition. +, ultraviolet photoinhibition (254 mμ); O, red light photoinhibition ($\lambda > 570$ mμ). Concentration of chlorophyll approx. $2 \cdot 10^{-5}$ M. Methodical details in ref. 1.

Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

very fast with H_2O to give off O_2 and is not important in limiting the rate of (1)[3].

Additional support for the concept of Q came from light-titration measurements[3] which defined and measured Q quantitatively (in electron-equivalents).

Fig. 1 shows the effect of increasing doses of inhibiting irradiation on the fluorescence induction curves in isolated chloroplasts. The steady-state fluorescence level (F_∞) decreases, until at complete inhibition $F_\infty = F_0$, and the induction phenomenon disappears. With very large doses of inhibiting radiation there is also a slight decrease of the fluorescence below F_0, which is probably a secondary effect (not shown). The induction time is not influenced markedly, although there is a tendency for an increase in the ultraviolet inhibition case. Ultraviolet and visible photoinhibition results in different rise kinetics; an increase of the inflection for the case of ultraviolet photoinhibition, compared with its disappearance for the case of visible photoinhibition. Very likely the effects of the two wavelength regions are dissimilar on a molecular level.

Fig. 2 shows that the effects on the fluorescence parallel the inhibition of the Hill-reaction activity. The linear correspondence between the rate and the variable fluorescence is probably due to the linear relation between either kind of activity to the fraction of intact photosynthetic units. This parallelism is common to the ultraviolet and visible photoinhibitions. It appears that although one inhibition site of visible photoinhibition is close to Photosystem I (ref. 2) it must also have another site close to Photosystem II, similar to the ultraviolet photoinhibition.

We have to explain why the fluorescence decreases as the inhibition progresses. The usual expectation is that the fluorescence will be higher when the chemistry is inhibited, since both are competitive processes. We rewrite (1) in a more detailed way as follows:

$$\text{Chl} + h\nu_{II} \longrightarrow \text{Chl}^* \quad (2)$$

$$\text{Chl}^* + \text{Q} \longrightarrow \text{Chl} + \text{Q}^* \quad (3)$$

$$\text{Q}^* + \text{D} \longrightarrow \text{Q}^- + \text{D}^+ \quad (4)$$

Two explanations are apparent for the effect of photoinhibition: (a) Photoinhibition brings about a fast, back-reaction between Q^- and its electron donor, D^+, by inhibiting the consecutive reaction of D^+ with water. The fluorescence yield is low after photoinhibition because the steady-state concentration of Q is strongly toward the oxidized (quenching) form.

One argument against this explanation is a kinetic one. In general, if the steady-state extent decreases, as a result of an increase in the rate constant of a back-reaction, the time to reach the steady-state must decrease (except for a zero-order back-reaction which is not likely here). Inspection of the induction time did not show such a tendency, and therefore explanation (a) does not have a good kinetic basis.

(b) Photoinhibition causes a separation of the quenching process (3) and the photochemistry (4) by an alternate reaction in which excitation energy is degraded to heat without photochemistry:

$$\text{Q}^* \longrightarrow \text{Q} + \text{heat} \quad (5)$$

The quenching of Chl* according to this view, depends only on the molecular properties of Q as an energy acceptor, and is independent of the fact that the system is capable or incapable of performing photochemistry.

The effect of adding dithionite and 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a strong inhibitor of O_2 evolution to photoinhibited chloroplasts is interesting. Table I shows that the addition causes the increase from the low steady fluorescence of photoinhibited chloroplasts approximately to the maximal level of the intact samples. There is no difference in this respect between intact and photoinhibited chloroplasts. Addition to intact chloroplasts brings an almost immediate rise to the maximal level (most of the fluorescence induction disappears).

TABLE I

STEADY FLUORESCENCE LEVEL FROM ISOLATED CHLOROPLASTS, AS FUNCTION OF THE TYPE OF INHIBITION

Control 100%.

Addition	*Intact chloroplasts*	*After ultraviolet* inhibition*	*Intact chloroplasts plus e^- acceptor ($K_3Fe(CN)_6$ concn., approx. 10^{-4} M)*
None	100	30	42
Sodium dithionite**	110	100	110
DCMU (10^{-5} M)	90	85	95

* In a minimal dose sufficient to bring F_∞ to F_0, about 10 min.
** Addition of a few small crystals.

The effect of sodium dithionite is explained by an immediate chemical reduction of Q to Q^-. The effect of DCMU shows that its inhibition point must be within the steps which are not photoinhibited. According to explanation (a) one must postulate that the back-reaction between Q^- and D^+ does not occur directly; it therefore must involve a cyclic path which includes the step susceptible to DCMU. According to model (b) the DCMU effect is explained by an inhibition of the quenching step (3). This seems intriguing, since hitherto it was usually assumed that DCMU interferes directly with the electron transport.

An investigation of all the possibilities described above is in progress.

Research Institute for Advanced Studies, Baltimore, Md. (U.S.A.), and Department of Chemistry, The Weizmann Institute of Science, Rehovoth (Israel) SHMUEL MALKIN

Research Institute for Advanced Studies, Baltimore, Md. (U.S.A.), and Department of Botany, The University of Tennessee, Knoxville, Tenn. (U.S.A.) LARRY W. JONES

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