

# PHOTON TRAPPING IN PHOTOSYSTEM II OF PHOTOSYNTHESIS

## THE FLUORESCENCE RISE CURVE IN THE PRESENCE OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA

WARDELLA W. DOSCHEK *and* BESSEL KOK

*From the Research Institute for Advanced Studies, Martin Marietta Corporation,  
Baltimore, Maryland 21227*

**ABSTRACT** Using isolated chloroplasts in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an analysis was made of the rise of the fluorescence yield effected by weak light. Depending on the pretreatment, the time-course of the rapid photochemical part of the rise varied between nearly first-order and quadratic kinetics, i.e., reflected either a one-quantum or a two-quantum conversion. We consider the occurrence of two photoreductants per system II unit, which are reoxidized in different dark reactions. The data further showed that the "first-order process" is also inhomogeneous.

### INTRODUCTION

According to present concepts a photon collected in a pigment unit of photosystem II migrates to a trapping center where it causes the transfer of an electron from the primary electron donor  $Z$  to primary acceptor  $Q$  (1). In dark reactions the system is restored to the photochemically active state which has a low fluorescence yield ( $F_0$ ). With either  $Z$  or  $Q$  in the wrong state no photochemical trapping can occur, and the probability is enhanced that quanta absorbed by the sensitizing pigment are reemitted as fluorescence. The poison DCMU presumably isolates the primary reductant  $Q$  from its secondary electron acceptors  $A$ , a pool of plastoquinone (2-4). Consequently, illumination of a dark-restored system can induce only a single photoevent in a system II unit, which brings the fluorescence from its minimum value  $F_0$  to its maximum value  $F_{max}$ .

Kautsky et al. (5) observed that in the presence of *o*-phenanthroline, which acts similarly to DCMU, the fluorescence rise was first order and revealed a photochemical cross section of 400 chlorophylls, the Emerson-Arnold photosynthetic unit. Morin (6) could observe similar rise curves in the absence of any poison by inducing the rise with very strong light so that the photochemical rate was much

greater than that of the dark restoration reactions. Morin and Joliot and Joliot (7) found that the rise curve was not first order; i.e., the increase of the fluorescence yield above the  $F_0$  level was not proportional to the number of converted photo-centers. The latter authors observed a similar nonlinear relation between the flash yield of  $O_2$  (which presumably reflects the number of open traps in the system) and the rate of  $O_2$  evolution in weak light. They could quantitatively explain both phenomena by assuming photon transfer between the photosynthetic units of system II.

Like Morin (who used *o*-phenanthroline), we have noticed that the shape of the DCMU fluorescence rise depended upon the experimental conditions. Also, as will be described elsewhere, our  $O_2$  measurements showed the relation between flash yield and rate to be variable. This raised the question of whether energy migration between photosynthetic units is the correct or the exclusive explanation for the phenomena. This paper describes some further observations of the fluorescence behavior in the presence of DCMU.

## MATERIALS AND METHODS

Spinach chloroplasts isolated as described in reference 8 were resuspended in a concentration of 5  $\mu\text{g}$  chlorophyll/ml in grinding medium. No electron acceptor was added. DCMU was present in a concentration of  $10^{-5}$  M, tested to be saturating. Fluorescence rise curves were induced by illuminating the sample with a beam of blue light ( $<500$  nm) admitted through a rapid shutter ( $<1$  msec). The fluorescence emitted from the irradiated surface was collected through the same shutter and lenses which focused the exciting beam on the vessel via a beam splitter and a Schott RG-8 filter. It was detected by an S-20 photomultiplier and recorded on a Brush recorder (Gould Inc., Brush Div., Cleveland, Ohio) with a time resolution of  $\leq 50$  msec.

## RESULTS AND DISCUSSION

Fig. 1 schematically illustrates the rise curves that were observed after various pretreatments. Examples of actual observations are shown in other figures. In the first experiment ( $t_d \infty$  in Fig. 1, 1 in Fig. 2), chloroplasts were preilluminated for  $\sim 2$  min with far red light (720–740 m $\mu$ ) before the DCMU was added. This pretreatment brings the fluorescence yield to the lowest obtainable level (initial yield  $F_i = F_0$ ). The blue exciting light causes the yield to rise slowly initially, then more rapidly, and then sluggishly to approach the maximum fluorescence yield  $F_{\text{max}}$ . The two features, colloquially defined as “belly” and “tail,” imply severe deviations from the first-order kinetics which one would expect for a single photochemical event.

If the same experiment is repeated but a brief, saturating flash is given  $\ll 1$  sec before admittance of the blue light, the initial rapid part of the fluorescence rise is abolished and one observes only the tail (dotted trace 2 in Fig. 2). Obviously, the rapid rise can be effected in  $<10$   $\mu\text{sec}$  but the slow part, corresponding to the tail, cannot.

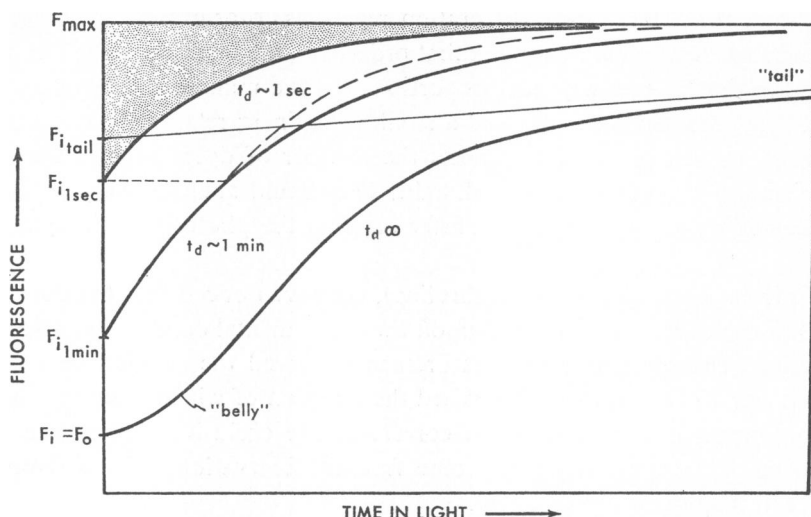


FIGURE 1 Schematic illustration of fluorescence rise curves observed in the presence of DCMU. The dashed curve is the rise seen after  $\sim 1$  sec dark, shifted along the time axis so that its  $F_i$  falls on the curve seen after  $\sim 1$  min dark. (For other details, see text.)

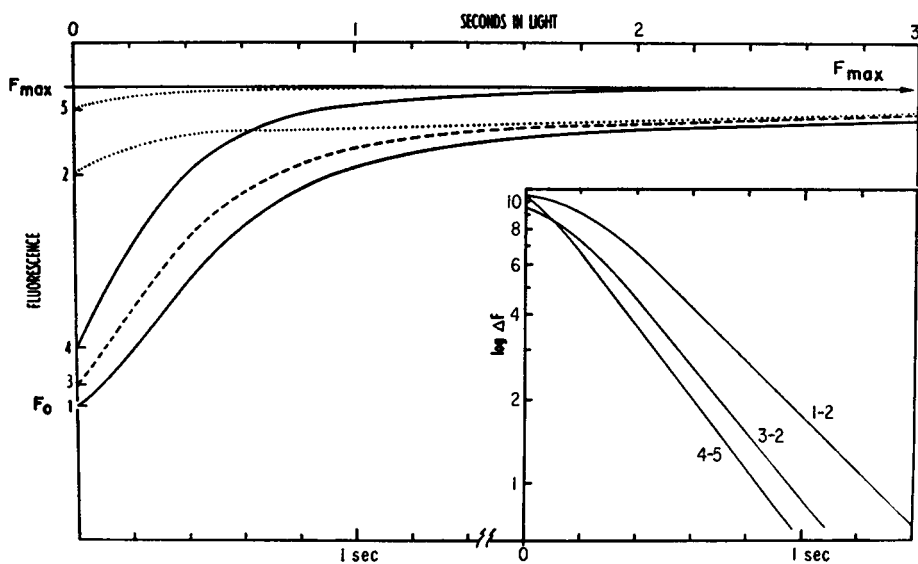


FIGURE 2 Fluorescence rise curves observed with the same actinic blue light beam after various pretreatments: 1, 2 min far red light, addition of DCMU,  $\sim 30$  sec dark; 2, as 1, except that a  $5 \mu\text{sec}$  saturating flash was given 0.1 sec before the blue light; 3, as 2, but the dark time between the flash and the blue light was 30 sec; 4, as 1, then blue light to bring the fluorescence to  $F_{\text{max}}$ , then 30 sec dark; 5, as 4, but a  $5 \mu\text{sec}$  flash was given 0.1 sec before the blue light. Insert: Semilog plots of curves 1, 3, and 4 after correction for the respective fluorescence rises which were not removed by a preilluminating flash.

The time-course of the restoration of the system in darkness, after it has been brought to  $F_{\max}$  by the actinic beam, is biphasic (9–11). In an initial rapid phase with a half-time of  $\sim 1$  sec some 80% of the fluorescence is restored. In a second, very much slower phase (first half-time  $\sim 5$  min),  $F_i$  returns to  $F_0$  and the tail of the rise curve is restored. We have assumed that 2 min far red light before the addition of DCMU was equivalent to "infinite dark" in the presence of the poison. Actually, in the presence of DCMU complete restoration may take hours. The time-course of restoration in the dark, and its acceleration by far red light (in the absence of DCMU) and by ferricyanide (which causes return of the tail in  $\sim 30$  sec) suggest that this residual quenching somehow reflects the oxidized state of the plastoquinone pool ("*A* pool") (12). Presently we have no adequate explanation for the tail and we will only consider the rapid part of the rise, which is removable by a flash.

The insert of Fig. 2 (curve 1–2) shows a semilog plot of this rapid rise, corrected for the tail. After a pronounced delay, corresponding to the belly, the time-course approaches first order.

In the next experiment ( $t_d \sim 1$  min in Fig. 1 and 4 in Fig. 2), blue light was given until the fluorescence reached  $F_{\max}$ . This was followed by a dark period of 30 sec, long enough for completion of the rapid restoration phase. The actinic light now induces a smooth rise from  $F_i$  to  $F_{\max}$  with no obvious belly or tail. A brief preilluminating flash practically abolishes this rise (trace 5 in Fig. 2). As shown in the insert of 2 (curve 4–5), the time-course is close to first order; however, a small initial deviation remains which is reminiscent of the belly. Compared with the rapid rise in the far red light-restored system, the total change of the fluorescence yield ( $\Delta F$ ) is generally 10% smaller.

Curve 3 in Fig. 2 (dashed) was obtained as follows. After far red light restoration and DCMU addition a flash was given followed by 30 sec dark. The fast rise was again restored and now showed an intermediate and somewhat variable time-course (insert curve 3–2). This observation correlates the occurrence of the belly with the oxidized state of the system.

Because of its variability, the non-first-order nature of the rapid phase of the rise curve is difficult to explain on the basis of photon transfer between units. The amount of transfer should depend upon the state of  $Q(F)$  and not on its prehistory. The more obvious interpretation of the variability is that under different conditions different amounts of photochemical substrate are converted. The areas bounded by the rise curves or parts thereof are a measure of these amounts (shaded in Fig. 1). For Fig. 3 we analyzed two rise curves and computed the relation between the momentary fluorescence yield ( $F$ , see legend) and the fraction of the area which was removed at that moment. One curve was observed after 45 sec dark restoration, the other after far red light restoration. The rise after 45 sec dark shows a nearly linear relation between  $F$  and area removed; the rise after far red restoration is quadratic and reflects the conversion of about twice the amount of photochemical

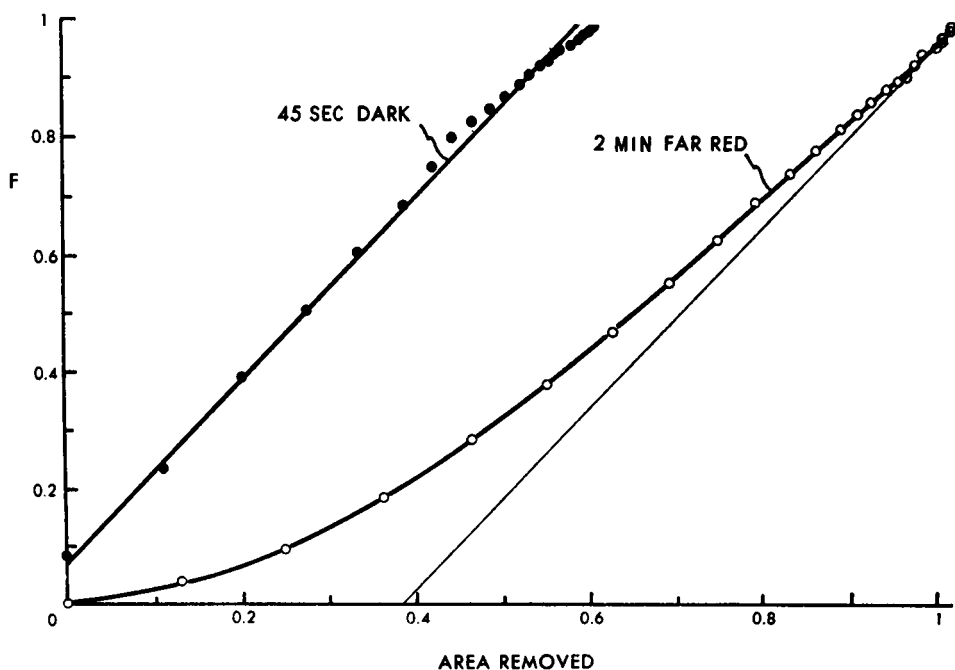
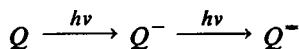


FIGURE 3 Relation between the momentary fluorescence yield (corrected for  $F_0$ ) and the area removed until that moment, computed for two fluorescence rise curves. One was observed after 45 sec dark restoration, the other after restoration in far red light and corrected for the tail (see 1-2 in Fig. 2). Yield and area values were normalized to the maximal values of the second curve.

substrate. Apparently, while the first curve reflects the removal of a single quenching molecule per photosynthetic unit, the second one reflects the removal of two.

In either case the photochemical conversion follows  $I \times t$  over a wide range ( $\sim 1$  sec-5  $\mu$ sec). This is illustrated in the two series of experiments shown in Fig. 4. In the first series we used far red light-restored chloroplasts; in the other, 30 sec darkness was given before each observation. In each experiment the actinic light was preceded (0.1 sec) by a flash. The intensity of the flash was varied by the use of different discharge capacitances (13). With increasing quantum content of the flash (abscissa in Fig. 4) an increasing fraction of the subsequent fluorescence rise is removed. The resulting rise curves (of  $F_t$ ) closely resemble the corresponding rise curves (of  $F$ ) observed in weak light. This implies that the events are photochemical, or at least that they are completed in a few microseconds.

The data thus support the hypothesis that in a fully restored system the rise curve reflects a two-quantum process, a possibility which was originally considered by Morin (6). Of several possible mechanisms, a series sequence like



might be mentioned. In this case one assumes that after full restoration the system

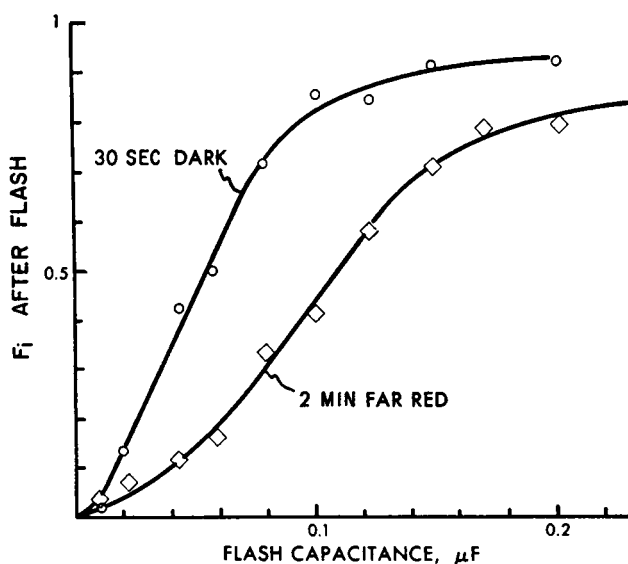
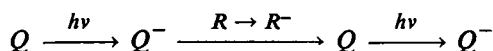


FIGURE 4 Ordinate, initial fluorescence yield ( $F_i$ , relative units) observed 0.1 sec after a flash. Abscissa, discharge capacitance, calibrated in terms of the relative amount of light emitted in the flash (13). Various flash intensities were given to chloroplasts which were (a) preilluminated with far red light before addition of DCMU (squares) or (b) kept in the dark during 30 sec after a preillumination with blue light (circles).

reverts to state  $Q$  while the rapid restoration phase only involves the return to  $Q^-$ . Precise analysis shows that the fully restored curve is not quite quadratic, and that  $\Delta F$  after 30 sec dark is  $\sim 10\%$  less than  $\Delta F$  after far red. To quantitatively describe the observations, it suffices to assume that in the  $Q^-$  state the fluorescence is quenched somewhat less than in the  $Q$  state, i.e., the photochemical trapping by  $Q^-$  is somewhat slower.

Other mechanisms are conceivable: the two traps in each unit might be photo-reduced "in parallel" but reoxidized in darkness by different pathways. Alternatively, one could assume a single electron acceptor per unit, which is restored very rapidly ( $\ll 10 \mu\text{sec}$ ) by a secondary acceptor ( $R$ ) of equal abundance:



(see also Delosme [12]). The question can be raised whether such a two-quantum conversion is the normal system II event, occurring also in the absence of DCMU and in flashes separated by short dark periods, i.e., under conditions of sustained electron transport. It could also reflect a priming or activating event which occurs only in the first flash after a substantial recovery period.

One possible consequence of the latter assumption is that, after a dark period long enough to allow deactivation of  $O_2$  evolution, the first flash carries out two system II photoacts, whereas later flashes in a rapid sequence carry out only one.

This would offer a ready explanation for the observation that in a sequence of flashes the yield of the third flash ( $Y_3$ ) and not that of the fourth is the highest (13).

B. Forbush (unpublished data) tested this hypothesis; he varied the intensity of the first flash (all other ones saturating) and analyzed the effect of this upon the yield of the third and subsequent flashes. In control experiments the intensity of the second flash was varied. In both cases, he found the same dependence between  $O_2$  yield and flash energy. This implied that the first flash was no more effective than the second in producing  $O_2$  precursor equivalents.

Our explanation of the DCMU fluorescence rise curve in terms of more than one quencher would leave a restricted role, if any, to photon transfer between units of system II. For example, if the deviation in curve 4 of Fig. 2 were due to photon transfer, rather than to a residual extra quenching, it would correspond to a transfer probability  $P \leq 0.1$  (as defined in reference 7).

Interpretation is further complicated by an additional anomaly in the fluorescence kinetics in the presence of DCMU: rise curves recorded after different (short) dark times after a preillumination to attain  $F_{\max}$  cannot be superposed (illustrated in Fig. 1 for 1 min dark and 1 sec dark). This reflects an apparent change of the photochemical rate constant during the dark restoration, due to either a change of the state of the system or an additional quenching process. Our presently available data seem to favor the latter explanation, but a more precise analysis will be required. Presently, therefore, it is also difficult to decide which of the two fluorescence anomalies might correlate with the nonlinearity between  $O_2$  rate and flash yield.

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