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Fluorescence Induction in Whole Leaves: Differentiation between the Two Leaf Sides and Adaptation to Different Light Regimes

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Abstract. In a variety of plants, the induction kinetics of chlorophyll fluorescence vary substantially depending on whether measured on the upper or lower side of the same leaf. The responses are comparable to those of plants grown under sun and shade conditions. Leaf morphology appears not to be the primary cause of the differences since inversion of the leaves can lead to reversed fluorescence responses. Fluorescence induction was analyzed in control and inverted leaves, and in one case, in chloroplasts from sun and shade leaves. It is concluded from the data that the major differences between the chloroplasts of the upper and lower leaf side reflect ionic and thylakoid-membrane conformational factors, rather than structural differences. Mg^{2+} flux probably plays a significant role in the adjustment of the thylakoid membrane to high or low light conditions.

Key words: Energy distribution – Fluorescence induction – Light adaptation – Photosynthesis – Photosynthetic unit.

Introduction

After a period of darkness all photosynthetic plants show characteristic changes of chlorophyll *a* fluorescence (Kautsky effect) (Kautsky and Hirsch, 1931). This induction of chlorophyll fluorescence is a sensitive indicator of the various reactions in photosynthesis (for a review see Papageorgiou, 1975).

Substantial progress has been made in correlating fluorescence transients to the underlying photosynthetic reactions in unicellular algae or isolated chloroplasts, but little attention has been given to leaves of higher plants. It appears reasonable to apply the Kautsky method to natural, if more complex, systems like whole leaves, so that photosynthetic activity can be monitored quantitatively in situ. But for whole leaves the methodology for interpreting fluorescence induction both on a qualitative and a quantitative basis is still limited. In contrast to the homogeneity of the responses in unicellular algae or isolated chloroplasts, those from whole leaves are heterogeneous. Fluorescence-induction curves obtained under identical conditions can differ appreciably, even if taken from the same plant or the same leaf, depending for instance on the developmental stage of the leaf, local light intensity, and in particular, the side of the leaf. In this paper the differences in the fluorescence-induction characteristics of the upper and lower leaf surfaces are compared and analysed in relation to differences in the underlying photosynthetic apparatus. It is found that the fluorescence responses of the upper and lower leaf sides are closely comparable to those of high-light and low-light adapted plants, respectively, as extensively studied by Anderson et al. (1973) Björkman et al. (1973), and Boardman et al. (1974). We have discussed our data in terms of a dynamic photosynthetic unit, based on recent evidence that the two photosynthetic photosystems are connected via a common light-harvesting pigment system (for reviews see Thornber, 1975, and Anderson, 1975), and that Mg^{2+} concentration regulates energy distribution between the two systems (Butler and Kitajima, 1975) as well as Calvin-cycle activity (Walker, 1972).

Materials and Methods

Plants were collected from greenhouses (Simon Fraser Biology Department and Carnegie Institution, Stanford, Cal., USA) and the field (Burnaby Mountain, B.C.). If not otherwise stated measurements were on whole attached leaves. Chloroplasts were isolat-

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Table 1. Biological materials

Species names	Common names	Fig. no.
Spinacia oleraceae L.	Spinach	1, 2, 6
Phaseolus vulgaris L.	Bean	1, 2, 4, 7, 9
Dieffenbachia amoena Hort. ex Gentil		1, 2
Alocasia sp.		1
Lycopersicum sp.	Tomato	1
Citrus grandis Hassk.	Grapefruit	1
Hedera helix L.	Ivy	3
Fraxinus latifolia Benth.	Ash	5
Rubus sp.	Blackberry	5
Marah jabeceus Greene	Wild cucumber	8
Typha sp.	Cattail	10

ed in 0.05 M phosphate buffer, pH 7.2, containing 0.3 M sucrose and 0.01 M KCl. The plant material used is listed in Table 1.

Fluorescence was excited by a broad blue band from a movie projector lamp (Sylvania, Type DLG) with 10 mm Corning 9782 filter (Corning Glass Works, Corning, N.Y., USA) and Balzers K6 interference filter (Balzers, Vaduz, Liechtenstein) with the cut-off filter removed. If not otherwise stated, standard light intensity (I=1) was 1 mW cm^{-2}, as measured with a YSI-Kettering Radiometer (model 65, Yellows Springs Instrument Co., Yellow Springs, O., USA). Illumination and collection of surface fluorescence in long and short wavelengths was achieved by trifurcated fiberoptics (Schreiber and Vidaver, 1973). Photomultipliers EMI 9658 R (EMI Electronics & Industrial Operations, Hayes, Middlesex, U.K.) were protected by a 3-mm Corning 2600 filter for F > 715 nm (F = fluorescence) and by 3-mm Corning 2030 and Balzers K6 interference filters for F < 690 nm. In most cases only the long-wavelength fluorescence responses are reported. Fluorescence induction at F < 690 nm differed quantitatively from that at F > 715 nm (see also Schreiber and Vidaver, 1976a, b), but the qualitative differences between the responses of the upper and lower leaf sides were very similar to those with F > 715 nm. Signals were displayed on a Tektronix 5103 N storage oscilloscope (Tektronix, Beaverton, Ore., USA) and photographed. Line drawings are composed from original photographs and are the result of superimposing at least three responses in each case. Photographs of oscilloscope traces represent single responses taken from a series where differences between 3 or more samples could not be discerned. If not otherwise stated, the plants were kept in the dark for 4 to 8 h prior to measurements. After 4 h no appreciable change of the fluorescence induction was observed. For comparison all associated curves in one figure are standardised at their initial fluorescence levels. With the exception of the experiment dealing with isolated chloroplasts, all experiments were carried out at room temperature (22–24° C).

Results and Discussion

1. Comparison of Fluorescence Induction Curves from Upper and Lower Leaf Sides

Phenomenology: Curves of fluorescence induction measured from the upper leaf side differ substantially from those of the lower leaf side under identical measuring conditions. Figure 1 shows examples of this phenomenon, which was found to be general for all plants studied. The difference between the "upper" and "lower" responses extends both over the fast transients, reflecting primary electron-transport reactions, and over the slow transients, indicative of the rate-limiting step of the Calvin cycle and changes in the conformation of thylakoid membranes (see Papageorgiou, 1975). In general the rapid transients are faster for the lower sides, while the slow transients are faster for the upper sides. From the phenomenological point of view, the lower-side fluorescence response appears to be induced by a far higher light intensity than the upper-side response.

Aspects of Leaf Morphology: Upper and lower leaf sides can differ substantially in morphology, and these may result in differences of the effective light intensities, because of such factors as reflection, scattering and screening. The question arises whether the phenomenon reported here is based on such morphological differences, i.e. that the chloroplasts in the lower leaf side are exposed to the higher light intensities in the experiments of Figure 1. We believe this possibility can be excluded for three reasons: (1) In all examples of Figure the lower leaf sides were more reflective than the upper sides. Thus differences in reflectance would be expected to cause an effect opposite to the one observed. (2) While 2–5 times higher light intensities made the upper-side responses approach those of the lower-side (see next section), complete matching by increasing light intensity for the upper sides was not possible. (3) In addition it was shown that simply inverting a leaf for several days caused a reversal of the fluorescence behaviour (see section "Effect of Leaf Inversion"). Therefore, although morphological differences must have some effect on light absorption, the major differences in the fluorescence responses appear to be based on differences within the chloroplasts of the two leaf sides.

Upper-side Responses at Increased Light Intensities: By increasing the intensity of incident light for the upper leaf side, it is possible to speed up the rate of the fluorescence rise until it matches that in the lower leaf side. The factor by which light intensity must be increased for closest matching may be considered an approximate relative measure for the difference in effective light intensities. In the examples of Figure 1 we observed matching factors between 2 and 5. The extent of the intermediate step in the fluorescence rise and the slope of the decay prevented complete matching. Figure 2 shows three upper-side curves at increased intensities, which can be compared with the corresponding lower-side curves at standard intensities in Figure 1. Although intensities were

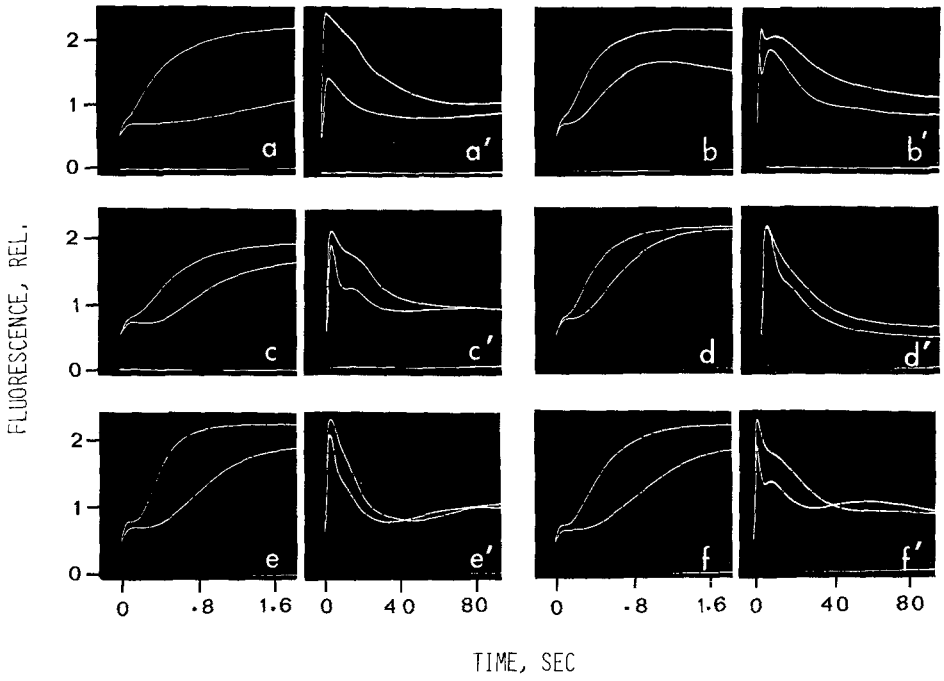

Fig. 1a–f. Fluorescence induction kinetics from upper and lower sides of a variety of leaves. **a** Spinach; **b** bean; **c** *Dieffenbachia amoena*; **d** *Alocasia* sp.; **e** tomato; **f** grapefruit. Curves a′–f′ are from the same samples at slower sweep rates on the oscilloscope. All top curves are from lower leaf sides. $I=1$, as described in Materials and Methods

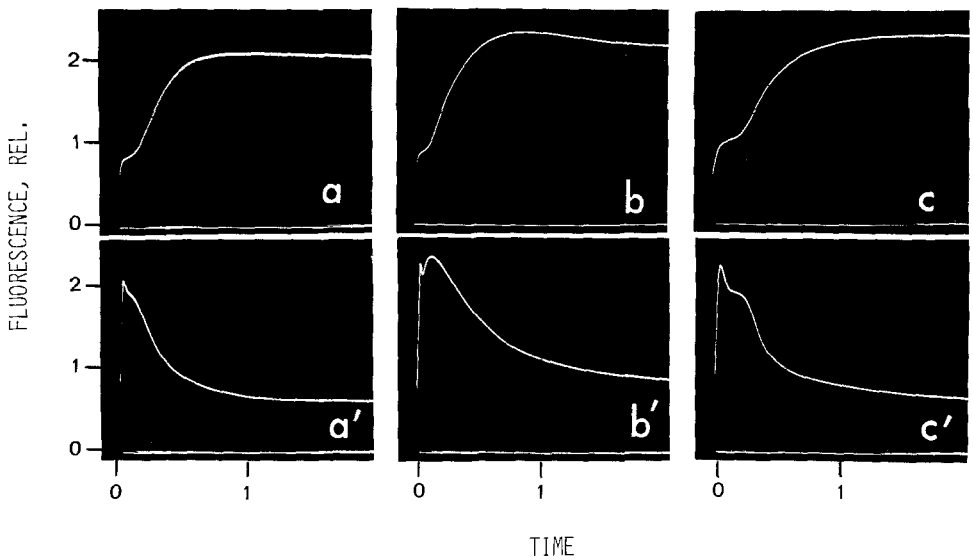

Fig. 2a–c. Fluorescence induction in upper-leaf-sides at increased light intensity. **a** Spinach, $I=10$; **b** bean, $I=2.5$; **c** *Dieffenbachia*, $I=3$. Curves a′–c′ from same leaves at slower recording rate. One time unit corresponds to 1 for the top curves (a–c) and 50 for the bottom curves (a′–c′). Compare with responses at $I=1$ in Figure 1

chosen higher than necessary to match the rise rates, the intermediate step is still more pronounced in the upper side. These results support our suggestion that the differences in the responses of the two leaf sides are not simply because of less light being absorbed in the chloroplasts in the upper side. Upper-side chloroplasts appear to differ primarily in properties related to the extent of the intermediate step in the fluorescence rise and the rate of the fluorescence decay.

2. *Adaptation to Different Light Regimes*

Sun- and Shade-grown Plants: Apart from morphology, the major difference between the two leaf sides

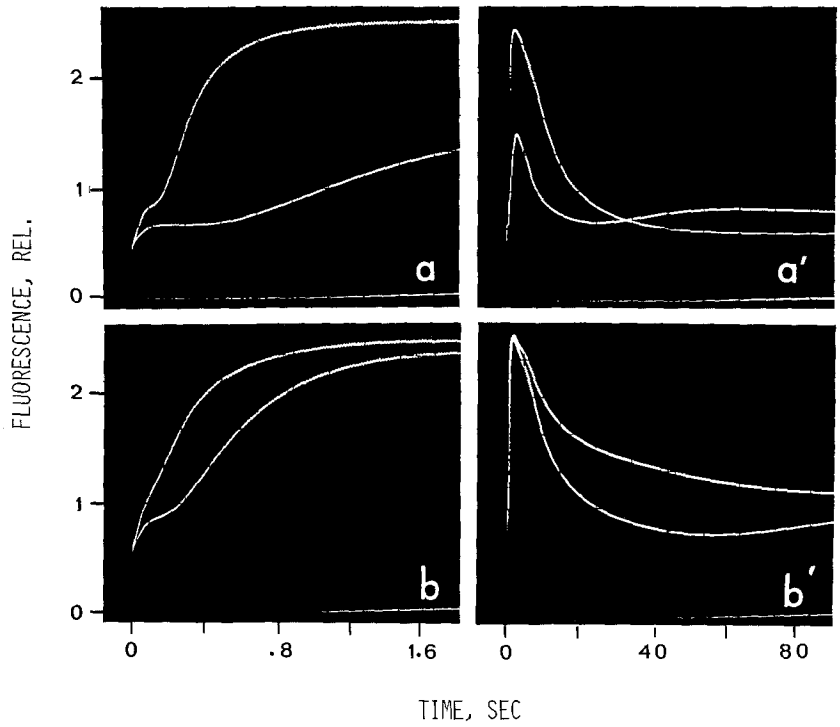

Fig. 3a and b. Fluorescence responses from sun- and shade-grown leaves from the same ivy plant. **a** a′: sun leaf, light intensity of growth ca. 10 MW cm^{-2} at $\lambda < 700$ nm. **b** b′: shade leaf; light intensity of growth ca. 0.1 MW cm^{-2} at $\lambda < 700$ nm. The top curves are from lower leaf sides, the bottom curves from upper leaf sides. $I = 1$

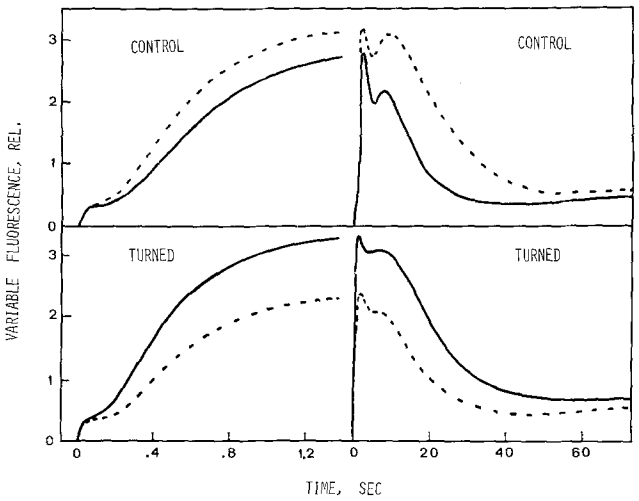

Fig. 4. Effect of leaf inversion relative to the light source. Fully expanded primary bean leaves (17th day after sowing) were kept for 48 h in the normal or inverted position in an incubator (light from top, intensity ca. 1 mW cm^{-2}). Fluorescence responses were measured after 4 h in the dark. Solid lines: responses from upper leaf side; broken lines: from lower leaf sides. $I = 1$

is environmental, in that the lower leaf sides receive substantially less light than the upper ones. It is well known that the photosynthetic apparatus can adapt to different light regimes (for a review see Björkman, 1973), and consequently such adaptations should apply to "sun" and "shade" chloroplasts within one and the same leaf. In fact the difference in fluorescence responses between sun and shade plants is similar to that found between upper and lower leaf sides. In Figure 3 responses are shown from an ivy plant, part of which was grown in the sun (a) and another part in the shade (b), with an approximate ration of 100 in light intensities. It is apparent that the rapid transients are faster in the shade leaf. This is true for both sides of the leaves. In particular the response from the lower side of the sun leaf is very similar to that of the upper side of the shade leaf. This behaviour favors the following hypothesis: The major differences in fluorescence induction in the upper and lower leaf side are caused by the same differentiation of the photosynthetic apparatus found in plants adapted to different light regimes.

Effect of Leaf Inversion: For the above hypothesis to be true, reversal of the light regimes for the two leaf sides should result in reversed fluorescence responses. As shown in Figure 4 for a bean leaf this is indeed the case. What was originally the lower side of the inverted leaf now shows fluorescence kinetics similar to that found in the upper side of noninverted leaves, and vice versa. The effect of leaf inversion increases with light intensity. At 0.1 mW cm^{-2} no effect was observed (not shown in

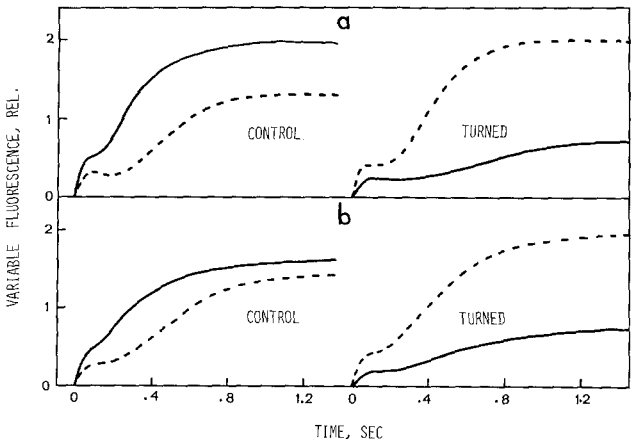

Fig. 5a and b. Effect of inversion on leaves grown in full sunlight. **a** ash; **b** blackberry. Solid lines: lower leaf sides; broken lines: upper leaf sides. Leaves were kept for 48 h in inverted position; responses were measured after 4 h darkness. $I=1$

the figures). With leaves grown in full sunlight the effect of inversion was even more dramatic than that shown in Figure 4. This is shown in Figure 5 for leaves of ash and blackberry.

3. Changes of Photosynthetic Parameters with Adaptation to Different Light Regimes

Photosynthetic adaptation to different light regimes involve the integrated changes of a number of features such as pigment composition, pigment concentration, enzyme activities, electron-carrier pool sizes, photosynthetic-unit sizes, grana stacking, and stomatal conductance (Anderson et al., 1973; Björkman et al., 1973; Boardman et al., 1974). Any of these features may also be involved in the photosynthetic adaptation of the two leaf sides, provided our hypothesis is correct that upper and lower leaf sides are characterized by "sun" and "shade" chloroplasts. Unfortunately most of the techniques applied for comparing sun and shade species cannot be used for the study of the two leaf sides, because of the difficulty in separating chloroplasts from them. In this respect the fluorescence method is unique, as it measures the surface of either side with high specificity.

Although there are still some doubts about the interpretation of some of the transients, we attempt in the following sections to characterize the changes in photosynthetic parameters with adaptation to different light regimes on the basis of fluorescence data alone. Such an approach appears justified as the general interpretation of fluorescence-induction kinetics is based on comparative studies with a number of other methods of photosynthesis analysis.

Photosynthetic-unit Size for Photosystem II Estimated from DCMU-treated Leaves

The photosynthetic unit size can be defined as the number of light-harvesting pigment molecules that serve one reaction center. Boardman et al. (1974) reported that there was not much difference in photosynthetic-unit size between sun and shade plants. This is surprising as Schmid and Gaffron (1971) found great variability in the unit size, which was influenced by light pretreatment. We investigated this problem by treating a spinach leaf with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and comparing the light-induced, rapid fluorescence rises in the upper and lower leaf sides. The DCMU fluorescence-rise reflects the reduction of the primary System II acceptor by quanta absorbed by pigment system II. The larger the light-harvesting unit connected to a System II reaction center the steeper the rise should be. As shown in Figure 6 the DCMU fluorescence-rise is substantially steeper for the lower leaf side. Comparison of the initial slopes indicates a difference in photo-synthetic-unit size by a factor of 1.5. In addition, similar measurements comparing sun and shade plants generally showed steeper DCMU fluorescence-rises in the shade plants (data not shown).

Boardman et al. (1974) reported somewhat smaller differences in unit sizes in sun and shade plants after determining the ratio of light-harvesting pigment molecules to System II reaction centers in isolated chloroplasts. The assumption made was that following preillumination at $-5°C$ the fluorescence rise after 3 min dark time reflects reduction of Q, the primary acceptor of Photosystem II, alone. However, they also observed ca. 10 times faster rise in the presence of DCMU. Thus in the $-5°C$ preilluminated sample not only Q but also a substantial part of the secondary pool is reoxidized during 3 min dark time, and both are reduced during the following illumination.

Dynamic Changes of Photosynthetic-unit Size with Energy-distribution Changes: As shown in Figures 4 and 5 the photosynthetic apparatus adapts readily to changes in light intensity and the difference in slope of the DCMU fluorescence-rise indicates that one of the parameters changing is the photosynthetic-unit size. There is growing consensus that System I and System II are linked by a common light-harvesting complex (Thornber, 1975). The recent model of Butler and Kitajima (1975), which is based on low-temperature fluorescence studies, implies that energy distribution between the two photosystems is under the control of ionic and membrane conformational factors. We have recently presented data which indicate en-

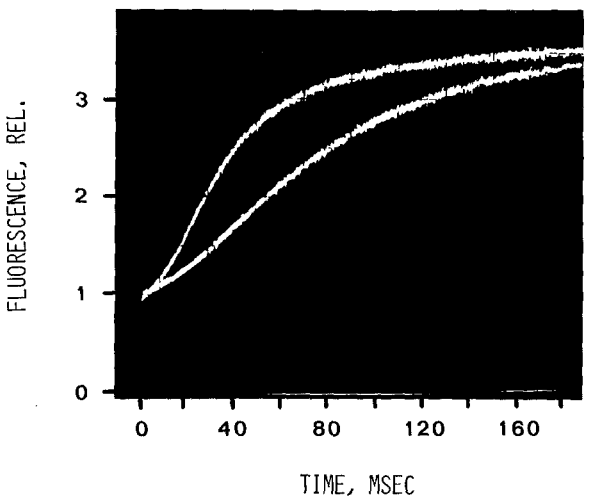

Fig. 6. Fluorescence-rise curves of spinach leaves in the presence of DCMU. Top curve: lower leaf side; bottom curve: upper leaf side. Sensitivity of oscilloscope adjusted to match initial fluorescence levels. Leaf vacuum-infiltrated for 15 min in 10^{-5} M DCMU solution. $I=1$

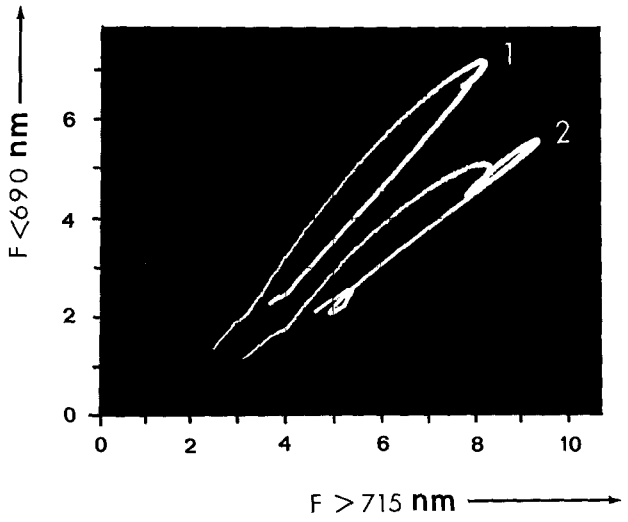

Fig. 7. x-y plot of short-wavelength ($F<690$ nm) versus long-wavelength ($F>715$ nm) fluorescence during the induction period in bean leaves. Curve 1, measured from lower leaf side; curve 2, from upper leaf side. A decrease in the $F<690/F>715$ ratio is considered to reflect a change to more favorable energy distribution towards System I. The lowest points on the curves correspond to the initial fluorescence yields. The part of the curves which reflect the slowest fluroescence changes may be distorted by slow changes in chloroplast conformation and concomitant changes in fluorescence reabsorption. $I=0.75$

ergy distribution changes even during the rapid part of the induction (Schreiber and Vidaver, 1976a, b). This leads to a more dynamic view of the photosynthetic-unit size than in the long time favored "separate package" model (for a review see Myers, 1971). In this dynamic view the number of light-harvesting pigment molecules per System-I and System-II reaction center is regulated by the thylakoid-membrane potential and conformation, both of which change in the light. With a dark-light transition energy distribution between the two photosystems undergoes very complex induction changes (Schreiber and Vidaver, 1976a, b) and the steady state is always characterised by favored distribution towards Photosystem I. As an example Figure 7 shows an x-y plot of short-wavelength fluorescence ($F<690$ nm) versus long-wavelength fluorescence ($F>715$ nm) for the upper and lower sides of a bean leaf. Generally speaking an increase in $(F>690\text{ nm})/(F>715\text{ nm})$ is believed to indicate an increase in energy distribution towards System II (a more detailed description of this technique is published elsewhere: Schreiber and Vidaver, 1976b). This should be equivalent to an increase in the effective size of the photosynthetic unit with respect to Photosystem II. We believe that similar energy distribution adjustments occur during adaptation of a plant to a certain light regime. The fact that the x-y plot for the lower leaf side in Figure 7 is shifted to smaller $F>715$ nm values compared to the upper side, appears to indicate that in the lower, shaded leaf side the overall energy distribution is more in favor of System II. This explains the steeper DCMU fluorescence-rise as an expression of higher System II activity in the lower leaf side (see Fig. 6). It also explains the difference in the responses from uninhibited samples, where curves from shade samples always show a steeper rise to the peak and a shorter intermediate step (see Figs. 1–5). The rise to the peak is a System II response, while the extent of the step is determined by the size of the secondary electron acceptor pool. This pool is continuously regenerated by System I activity, and thus will appear to increase with an increasing ratio of System I to System II activity.

Intersystem Electron-carrier Pool Size: An alternate explanation for the longer intermediate step in the fluorescence rise of the upper leaf side could be a higher content of intersystem electron carriers, in particular, plastoquinone and cytochrome *f*. Boardman et al. (1974) estimated electron-carrier pools in *Atriplex* plants grown in high light to be about twice the size of those grown in low light. An estimation of the pool size from the fluorescence rise (Malkin and Kok, 1966) is complicated in intact leaves by the fact that System I activity reoxidizes the pool during illumination. Therefore an extended intermediate step in the rise, as generally observed in upper-side

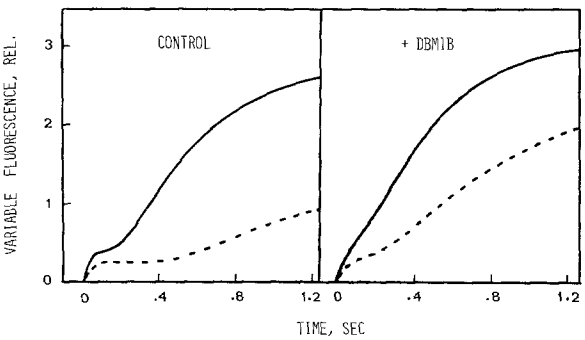

Fig. 8. Effect of DBMIB on fluorescence responses in wild-cucumber leaves. Solid lines: lower leaf sides; broken lines, upper leaf sides. Leaf vacuum-infiltrated with 5×10^{-5} DBMIB for 30 min

responses, may be as well explained by a favored energy distribution to System I as by a large secondary electron-acceptor pool. A way of distinguishing between these two possibilities is to add 2,5-dibromo-3-methyl-*iso*propyl-*p*-benzoquinone (DBMIB), which is known to inhibit electron transport between plastoquinone, constituting the main pool, and System I (Böhme et al., 1971). As shown in Figure 8, DBMIB removes most of the intermediate steps and brings the two leaf sides responses closer together. The same sample treated with DCMU gave approximately the same ratio in rise rates as with DBMIB (not shown in the figures, but see Fig. 6). Thus, if there were a substantial difference in pool sizes, it would have to be in Q. This appears unlikely since Q is generally considered to be in a 1:1 ratio with the reaction centers. We conclude that differences in energy distribution, along with differences in effective photosynthetic-unit sizes for the two photosystems, are of more importance in explaining the differences between the upper and lower leaf side responses, than differences in electron-carrier pool sizes.

Calvin-cycle Activity: Carboxydismutase (ribulose-1,5-disphosphate carboxylase) appears to be a key rate-limiting enzyme of the Calvin cycle, and therefore of overall photosynthesis (Walker, 1972). It is the delay in NADPH reoxidation by Calvin-cycle activity which is believed to cause the transient reduction of the electron-transport chain and the concomitant rise of fluorescence to the peak level (Munday and Govindjee, 1968).

Activation of the Calvin cycle and reoxidation of the electron carriers could conceivably be reflected in the fluorescence decay from the peak. This may be true despite the existence of slow fluorescence changes independent of the redox state of the primary System-II acceptor, which overlap those caused by redox changes (for a recent review see Papageorgiou, 1975). In all examples of Figures 1 and 3–5 the amplitudes of peak fluorescence are consistently lower and the overall rates of the decay higher for the high-light samples. This indicates higher Calvin-cycle and presumably carboxydismutase activity in the high-light samples. Björkman (1968) has shown that the light intensity under which a given plant is grown, regulates the level of carboxydismutase in the chloroplast. In addition to an adaptation yielding changes in enzyme concentration there appears to be an adaptation involving the catalytic effect of Mg^{2+} ions, as indicated by Walker (1972). As far as fluorescence data are concerned this is reflected in the induction kinetics after different dark times following an extended illumination. As shown in Figure 9 the peak level is lower and the decay faster the shorter the dark recovery time. The short adaptation times involved exclude vast changes in the enzyme pool size.

A Proposed Role of Mg^{2+}: Hind et al. (1973) found a large, reversible, light-driven Mg^{2+} efflux from the thylakoid. During exposure to light this process results in a drastic depletion of Mg^{2+} inside the thylakoid and a corresponding rise in the stroma. In the dark the flux will be reversed. This has important consequences not only for the slow fluorescence transients, as outlined above, but also for the fast ones. Murata (1969) showed that Mg^{2+} inhibits "spillover" of energy from System II to System I, and recent data of Butler and Kitajima (1975) indicate an increase of energy distribution towards System

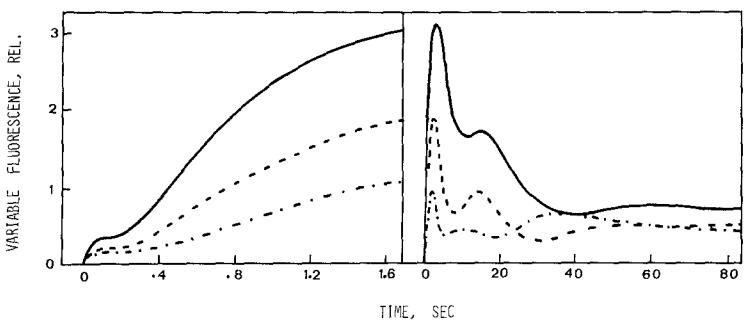

Fig. 9. Upper-leaf-side fluorescence-response dependence on dark time after an extended period of illumination of bean leaves. —— 4 h dark; --- 1 h dark; ·—·—· 10 min dark. $I=1$

I with the efflux of Mg^{2+} from the thylakoid. Thus the Mg^{2+} level inside the thylakoid may actually control the relative photosynthetic-unit sizes of Systems I and II, which thus undergo dynamic changes during exposure to light and in the dark. This may in part explain the extended dark periods required for a "dark-adapted fluorescence response" (see Fig. 9). As far as rapid transients and the primary electron-transport reactions are concerned, complete dark adaptation, i.e. reoxidation of carrier pools, should be completed within seconds. The large intermediate step, and slow rise to the peak in a partially dark-adapted sample therefore appears to reflect a state of favored energy distribution towards System I. The proposed role of Mg^{2+}, and the implied dynamic changes of the photosynthetic unit sizes with illumination explains the complex changes of "quantum efficiency states" for the two photoreactions reported by Troughton and Fork (1974). These authors demonstrated a 60% stimulation of System I response with increased duration of illumination. The adaptive advantage of an increased effective size of the System I unit at higher light intensities may be that System I can make use of energy, which is in excess of that needed for non-cyclic electron transport, to drive cyclic photophosphorylation (for a review see Simonis and Urbach, 1973).

Fluorescence Responses in Isolated Chloroplasts from Differently Light-adapted Leaf Sides

Further characterization of the differences in photosynthetic parameters in the upper and lower leaf sides would be facilitated if isolated chloroplasts from the two leaf sides were obtained. We have not as yet succeeded in our attempts to achieve such a separation from any dicotyledonous leaf. Separation of a high-light adapted from a low-light adapted leaf side was however obtained, in *Typha*. For chloroplast isolation we used the method of Thorne and Boardman (1971) and Boardman et al. (1974). In Figure 10, the responses of the intact leaf sides are compared with those of the isolated chloroplasts. Obviously much of the difference between the chloroplasts was lost during chloroplast isolation. For reasons outlined above (see section on Aspects of Leaf Morphology) we do not think this indicates that the difference in fluorescence induction is caused by the different morphology of the two leaf sides. Chloroplast isolation drastically interferes with the ionic environment of the photosynthetic apparatus. Any particular Mg^{2+} gradient developed during adaptation to a particular light regime would be destroyed during isolation. The curves in Figure 10 were recorded immediately after chloroplast isolation (within 5–15 min). After 1 h at 5° the responses of the sun and shade chloroplasts were almost identical (not shown in the figures).

Conclusions: These observations support our earlier conclusions that the differences between sun-side and shade-side chloroplasts from the same leaf are in ionic factors, affecting membrane conformation, energy distribution and enzyme activity, rather than in structural factors. We believe that some of these considerations could also apply to differences in the photosynthetic apparatus between sun and shade plants. The fact that much of the previous work on this subject was done with isolated chloroplasts may have obscured the significance of ionic and conformational factors in the adaptation of chloroplasts to changing light regimes. Great care must be used in the interpretation of experimental results dealing with chloroplasts from whole leaves. Our data from inverted leaves indicate that mature chloroplasts maintain a considerable range of adaptive capability with respect to changes in light intensity and the characterization of this capability for different plant species may provide an interesting avenue of research in the field of ecophysiology.

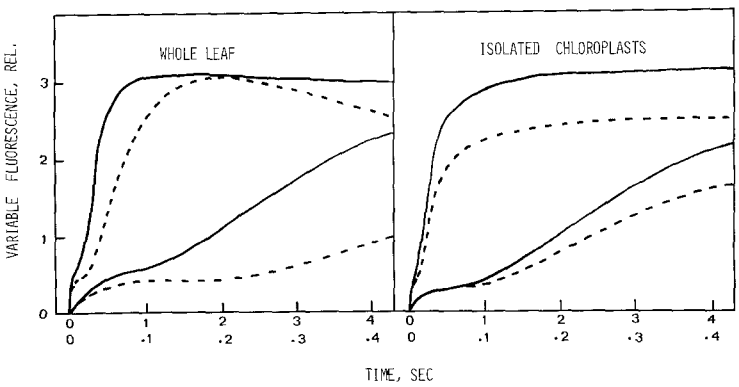

Fig. 10. Effect of chloroplast isolation on fluorescence responses from the sun and shade side of a *Typha* leaf. Leaf was split and chloroplasts isolated under dim light. Solid lines: shade samples; broken lines: sun samples, $I=2$. Top time scale for top set of curves; bottom scale for bottom set of curves. Experiments carried out ca. 6 h after collecting the sample from the natural habitat, in which one leaf side was shaded

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