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Quantitative analysis of State 1–State 2 transitions in intact leaves using modulated fluorimetry – evidence for changes in the absorption cross-section of the two photosystems during state transitions

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Using a combination of modulated and non-modulated light with synchronized detection it has been possible to monitor State 1–State 2 transitions in intact leaves as changes in the yield of modulated chlorophyll fluorescence. In the presence of excess far-red non-modulated light (713 nm) absorbed mainly by Photosystem I (PS I), the modulated fluorescence intensity was taken to represent F_o – the emission yield which occurs when the reaction centres of Photosystem II (PS II) are all open. On the other hand, superimposing saturating non-modulated wide-band, blue-green light resulted in a transitory maximum yield of modulated chlorophyll fluorescence, F_m, due to the total closure of the PS II reaction centres. In the absence of these additional lights the fluorescence level assumed a steady-state value, F_s, between F_o and F_m. All these parameters changed as the leaf slowly adapted to light of a given spectral composition. It was found that both F_o and F_m increased reversibly (by about 15–20%) during the transition from State 2 to State 1 such that the ratio of F_m to F_o remained constant, indicative of changes in absorption cross-section of PS II and PS I rather than alterations in 'spillover' which would cause preferential changes in F_m. It was also possible to estimate the fractions of light, β and α, channelled to PS II and PS I, respectively, from the values of F_o, F_m and F_s. In one approach, β was estimated in State 1, using the assumption that $\alpha + \beta = 1$, and its variation during the subsequent state transition was assumed to follow proportional changes in F_o (or F_m). It was found that in State 2 there is a small loss (about 4%) of the total utilization of light in both photosystems. However, if such loss is neglected, assuming $\alpha + \beta$ is always unity, the calculated β was found to vary in the same direction and almost with the same magnitude as F_o (or F_m), indicating independently that a change in absorption cross-section in PS II (and PS I) had occurred. Consistent with these data were the light-saturation curves for the non-modulated far-red light-quenching effect in bringing the fluorescence from F_s to F_o in States 1 and 2. The ratio of the initial slopes of these curves indicates quantitatively both redistribution of light between PS I and PS II during the State 1–State 2 transitions and a partial loss of excitation energy in State 2.

Introduction

In photosynthetic organisms which evolve O_2, the distribution of different wavelengths of light to Photosystem I (PS I) and Photosystem II (PS II) is

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC-2, light-harvesting chlorophyll *a*/*b*-protein complex usually associated with PS II; PS, Photosystem; Q_A, primary electron acceptor of PS II; Q_B, secondary electron acceptor of PS II.

generally not even [1]. Certain ranges of the spectrum are absorbed preferentially by either one of the photosystems and may be called, correspondingly, Light 1 and Light 2. In green algae and higher plants, which contain chlorophyll *b* as a major accessory pigment, Light 1 is confined to the far-red edge of the spectrum in the region of 700 to 750 nm, while Light 2 stretches over the whole range of wavelengths below 700 nm, with maximum values corresponding to preferential absorption by chlorophyll *b* [2]. The precise absorption characteristics of PS II and PS I, however, are not fixed and can change depending on lighting conditions. Responses to changes in spectral quality are clearly seen at limiting light intensities and are termed 'state' transitions [3]. When the incident light is richer in Light 1 and PS I is preferentially excited, there is a tendency to a state where Light 2 is distributed maximally in favour of PS II, and is known as State 1. On the other hand, when the incident light is richer in Light 2 there is an adaptation to State 2, where Light 2 seems to be more evenly distributed between PS I and PS II. State 1-State 2 transitions have been conveniently detected by observing room temperature fluorescence emitted from the antenna chlorophyll of PS II [3,4]. This approach has been complemented by low-temperature (77 K) chlorophyll fluorescence measurements where the relative yields of PS I and PS II emission can be easily monitored at different wavelengths [4,5].

Two questions can be asked about the transition from State 1 to State 2.

(i) Is the decrease in the rate of excitation delivery to PS II, leading to a lowering of chlorophyll fluorescence, matched by a corresponding gain in the quantum yield of PS I? That is, does an efficient change of light distribution between PS II and PS I occur rather than a reversible deactivation of PS II?

(ii) If there are concomitant changes in PS II and PS I quantum yields, what is the mechanism which underlies the process? Does this mechanism involve changes in the light absorption cross-section of the two photosystems, or is the 'spillover' hypothesis of Myers [6] applicable which proposes that there is a change in energy transfer from PS II to PS I?

Our present-day understanding of the thylakoid membrane organization is that PS II and PS I are laterally segregated such that PS I is restricted to the non-appressed lamellae and the majority of PS II is located in the appressed regions [7]. Thus, the former mechanism proposed to explain changes in quantum yield of PS I and PS II could involve the lateral movement of antenna chlorophyll-protein complexes in the plane of the membrane, while the latter would require changes in the distance between PS II and PS I reaction centres which would also involve lateral movement of complexes [7].

A previous paper [8] presented analysis of photoacoustic signals measured with intact tobacco leaves which reflected changes in the quantum yield of O_2 evolution in response to different lighting conditions. It was concluded from this work that there are changes in the distribution of absorbed Light 2 between PS II and PS I on going from State 1 to State 2, typical values for 480 or 640 nm light being 64 : 36 in State 1 and 46 : 43 in State 2, respectively. Such a change is beneficial, since it optimizes the quantum efficiency of whole chain electron transport. A careful analysis of the data further suggested that the mechanism bringing about the state transitions in mature tobacco leaves involved changes in the absorption cross-section indicative of redistribution of light-harvesting antenna complexes, presumably light-harvesting chlorophyll *a/b* complexes (LHC-2) [8].

In the present study we have measured chlorophyll fluorescence changes which accompany the State 1-State 2 transitions in intact leaves. A quantitative analysis of the signals has been possible for the first time by scaling the fluorescence changes in terms of the parameters, F_o and F_m, as was also shown in Ref. [9]. Theoretically, F_o and F_m are defined as the relative emission yields when PS II reaction centres are fully open and closed, respectively. In this way we are able to give support to the concept that the State 1 to State 2 transition in mature leaves involves redistribution of energy by changes in absorption cross-section and not by the 'spillover' mechanism.

Materials and Methods

Chlorophyll fluorescence from freshly picked intact leaves (usually taken from young pea plants grown in a greenhouse as previously described or

from sources as described in the text) was measured using modulated (chopped) light and detected with the aid of a lock-in-amplifier which was selectively tuned to the chopping frequency in the range 10–50 Hz. The modulated measuring light (Light 2) was transmitted by a 480 nm Balzer interference filter and brought to the leaf surface using one arm of a three-branched optical fibre system (see Ref. 4). Its intensity at the leaf surface was about 7 W/m^2. The resulting fluorescence was transmitted via a second arm to an E.M.I. photomultiplier shielded by a 685 nm Balzer interference filter. Superimposing non-modulated light using the third branch of the optical fibre system had no effect on the modulated signal when the sample was either a filter paper impregnated with chlorophyll solution or a heat-inactivated leaf. However, with a freshly picked leaf the effect of the non-modulated light was to perturb the quantum yield of the modulated fluorescence. The extent and nature of this perturbation was dependent on the intensity and spectral quality of the non-modulated 'actinic' light, employing two types: saturating blue-green Light 2 (using a 4-96 Corning filter or alternatively a 3 mm BG-18 Schott filter) or a far-red light, as Light 1 (using a Balzer 713 nm interference filter with a 12 nm half waveband width). The light intensities were varied using dielectric coated neutral density filters and values are given in the figure legends. The time resolution of the recording system was altered depending on the type of measurement being made, with a minimum value of 0.2 to 0.3 s and a maximum of 1 to 3 s.

All measurements were carried out with detached leaves and no special attempt was made to control temperature, gas composition or humidity. Signals were recorded within 2 h of picking the leaves and were totally reversible.

Results

The assumption in the experiments to be presented is that adjustments in the electron transport rate and the redox levels of electron carrier intermediates, brought about by any sudden change in the light intensity or spectral distribution, are momentary on the time-scale of other adaptive changes, which usually take several minutes. It can be estimated on the basis of pool sizes and kinetic constants that light of medium intensity, as used here, will bring about rapid perturbations within a few seconds or less. Becuase of this, it is possible to use chlorophyll fluorescence yield as an assay of the momentary redox state of the PS II reaction centre [10,11] and, more specifically, the resulting macroscopic photochemical yield [12]. Thus, a fluorescence level, F_o, is defined here as the minimum yield corresponding to all PS II reaction centres being 'open' (i.e., a functional state with the primary quinone acceptor, Q_A, fully oxidized) and a maximum fluorescence level, F_m, when all PS II centres are closed (i.e., non-functional with Q_A fully reduced). Slow adaptive changes, such as 'state transitions', will alter these fluorescence yield parameters as well as the steady-state intermediate fluorescence values, corresponding to the light-induced poising of the PS II reaction centre in various redox states.

The method for measuring F_o and F_m levels which employs the modulated fluorescence system, introduced to demonstrate State 1-State 2 transitions in intact leaves [4], was as follows. A leaf was illuminated with 'low'-intensity modulated Light 2 until all induction effects, characteristic of the transition from dark to light, ceased and the leaf adapted to the prevailing illumination with a certain steady level of the fluorescence, F_s (Fig. 1a). Then 'high' intensity non-modulated Light 1 or Light 2 were superimposed on the weak modulated Light 2 for a few seconds to induce F_o and F_m respectively. In the case of leaves adapted to State 2, the introduction of Light 1 allowed a continuous record of the F_o level during the subsequent transition to State 1. At any time during this transition, F_s could be determined by turning off Light 1 and F_m levels would be measured by the brief introduction of the high intensity non-modulated Light 2. The light-saturation curves for the determination of the F_m and F_o levels were measured and the minimum saturating intensities which still induced the maximum changes were chosen for routine measurements. The concept behind these measurements is as follows. Under light-limiting conditions, F_s is determined by the balance of excitations in PS II and PS I. For a balanced rate an automatic adjustment is made for initially unequal excitation rates by a (partial)

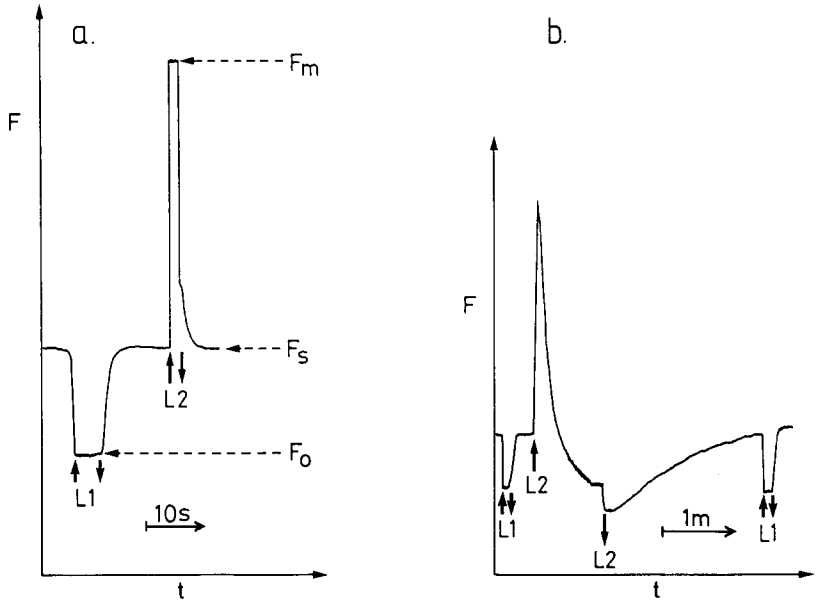

Fig. 1. (a) demonstration of the method for measuring maximum (F_m) and minimum (F_0) levels of modulated chlorophyll fluorescence in pea leaves. F_s is the steady-state level of fluorescence (after adaptation to State 1 in this experiment). F_o was induced by illumination with non-modulated 713 nm light (L1), 2 W/m^2 (small arrows, up = on, down = off) and F_m by 340 W/m^2 of broad band, blue-green light (L2) (large arrows). (b) Demonstration of the reversibility of the high intensity (L2)-induced fluorescence quenching of F_m in pea leaves. For other details see (a) above.

closure of the reaction centres of the photosystem which is more often excited. In our case this is reflected in F_s being always larger than F_o with the modulated Light 2 alone, indicating partial closure of PS II reaction centres. With the addition of Light 1, PS II tends to be in a better excitation balance with PS I, and F_s correspondingly decreases towards the minimum value, F_o. For light-saturating conditions obtained with the addition of bright Light 2, the rate of excitation is always much higher than the limiting rate of electron transfer, and reaction centres tend towards being completely closed, bringing the fluorescence to the maximum level, F_m (cf. also Ref. 9).

Adaptive changes in the fluorescence level induced by different illumination conditions were found to be fully reversible, with an example shown in Fig. 1b. Here the F_o level was determined by superimposing Light 1 after adaptation to the modulated Light 2 excitation (State 2). Addition of continuous high intensity Light 2 over a period of minutes was found to bring about a large decrease in fluorescence (after the initial rise to F_m), probably attributed to the pH gradient formed across the thylakoid membranes which quenches all fluorescence parameters [13]. On turning off light 2 and subsequently turning on continuous Light 1, both the F_s and F_o levels of this 'high light' adapted state could be determined. In the absence of continuous strong Light 2 and Light 1 a realtively slow adaptation (2–3 min) back to the initial 'low light' state took place. The quenched F_s fluorescence level increased to its original value and addition of Light 1 induced the same decrease to F_o seen before adaptation to 'high light'. This demonstrates the complete reversibility of adaptation of leaves to a change in lighting conditions. The 'high light' adaptation was not further studied in this work.

Having established the technique for measuring F_m and F_o in leaves adapted to any particular light intensity or spectral quality, we then investigated the mechanism involved in state changes. A reduction in the absorption cross-section of PS II should bring about an equal fractional decrease in F_m and F_o, whereas increased 'spillover' to PS I should

preferentially decrease variable fluorescence ($F_m - F_o$) in relation to F_o [14–16]. Both mechanisms have been considered to explain State 1-State 2 transitions [3,6,8,16,17].

The F_m and F_o levels in pea leaves during the time-course of the transitions between the two states were measured as follows. During the transition from State 2 to State 1, F_o gradually increased (see Fig. 1b). At intervals, Light 1 was turned off to induce F_s and then non-modulated saturating Light 2 was turned on to induce F_m. The F_o level was then restored by turning Light 2 off and Light 1 on again. Care was taken to measure F_s and F_m for only a few seconds in order not to interrupt the transition to State 1. A similar method was employed to measure the time-course of the reverse transition (State 1 to State 2), which occurred by illuminating the leaf with the modulated Light 2 alone. F_o and F_m were monitored at intervals, by switching on and off the far-red and blue-green lights, respectively.

Fig. 2 shows the time-course of the decrease in F_m and F_o that occurred during the transition from State 1 to State 2. There was approximately a 14–15% change in both the level of F_m and F_o, which followed essentially the same time-course. The $t_{1/2}$ of 3 min is similar to that shown previously for the F_s in pea leaves [4]. The time-course of the F_m and F_o changes during the reverse State 2 to State 1 transition is shown in Fig. 3 for a different sample. These changes are slower ($t_{1/2} = 9$ min), but still F_m and F_o change relatively equally

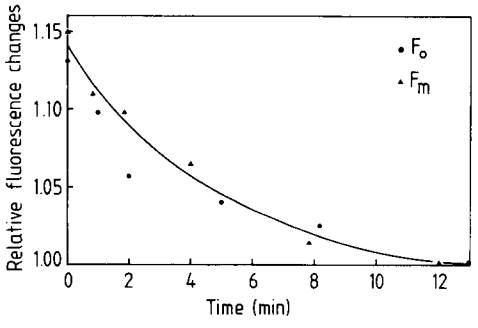

Fig. 2. Time-course of the relative change in F_m and F_o in a pea leaf during the transition from State 1 to State 2 induced by illumination with modulated Light 2. Both F_m and F_o values at various times have been normalized to the fluorescence levels determined in State 2. Other conditions as Fig. 1a.

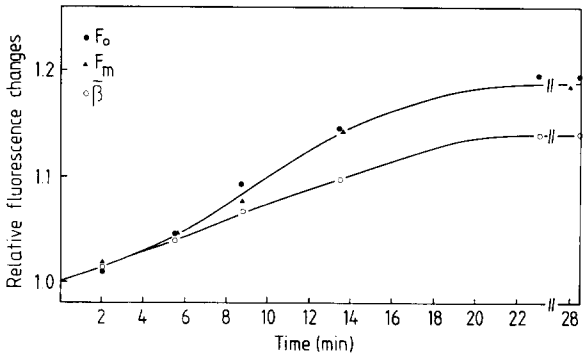

Fig. 3. Time-course of the relative change in F_m, F_o (see Fig. 2 legend) and $\bar{\beta}$ in a pea leaf during the transition from State 2 to State 1 induced by illumination with non-modulated 713 nm light, 2 W/m^2. $\bar{\beta}$ is calculated from the data of F_o, F_m and F_s according to Eqn. 2, assuming $\alpha + \beta = 1$.

throughout the time-course. These data show that changes in fluorescence yield associated with adaptation to lights of different spectral qualities can be fully accounted for by changes in the absorption cross-section of PS II.

The F_s level of fluorescence was found to follow approximately the same time-course as F_m and F_o during the transitions between State 1 and State 2 (data not shown) although the extent of the change was greater. A quantitative estimation of the relative balance of excitation distribution can be made based on F_s values relative to F_o and F_m. In the extreme case, when F_s equals the F_o level, the rate of excitation of PS I must be equal to or greater than excitation of PS II and any increase of the F_s level above that of F_o level is a measure of the extent to which PS II is excited more than PS I. The macroscopic quantum yield of photochemistry in PS II, ϕ_p, is related directly to the variable fluorescence function, f (Ref. 12), as follows:

$$\phi_p = f = \frac{F_m - F_s}{F_m - F_o} \qquad (1)$$

assuming a microscopic quantum yield of 1 for a photon absorbed in an 'open' reaction centre.

In the steady state, the electron transport rates through PS II and PS I must be balanced so that if β and α are the light distribution coefficients of PS II and PS I, respectively, and $\beta < \alpha$, then it is

evident that:

$$\beta f = \alpha \text{ and hence } f = \frac{\alpha}{\beta} \quad (2)$$

If it is also assumed that $\alpha + \beta = 1$, one can solve Eqn. 2 using the value of f calculated from experimental data, and obtain the value of α and β:

$$\beta = \frac{1}{1+f} \quad (3)$$

$$\alpha = \frac{f}{1+f} \quad (4)$$

Table I shows a typical example of the calculation for a pea leaf adapted to State 1 and State 2. We assumed that in State 1 $\alpha + \beta = 1$ (see below for justification), which results in 60 : 40 distribution of light in favour of PS II, similar to that determined previously using the photoacoustic technique [8]. The transition to State 2 seems to involve a decrease in the relative absorption cross-section of Photosystem II, as indicated by the decrease in F_m and F_o, and therefore there is a change in β from 0.60 to 0.52 on transition to State 2 (see Table I). The α value in State 2 was then calculated from Eqn. 3 using the f value of State 2. As shown in Table I, the sum of α and β in State 2 is less than 1. This confirms the photoacoustic study of Canaani and malkin [8], who also showed that, on adaptation to State 2, the loss of excitation of PS II is not fully balanced by an increase in PS I activity.

That $\alpha + \beta$ is not constant is consistent with the repeated observation that the relative change in F_o or F_m during the state transitions is always larger than the relative change in β if calculated according to the assumption $\alpha + \beta = 1$ in State 1 (Eqn. 3). A verification of this statement is presented in the Appendix. In Fig. 3 we have plotted such 'virtual' values, denoted $\bar{\beta}$, together with F_o and F_m. $\bar{\beta}$ behaves only approximately as F_o or F_m by increasing with the same time-course from State 2 to State 1 but with a 25% smaller total increment. Parallelism of $\bar{\beta}$ and F_o (F_m) is, at the first approximation, consistent with changes in light absorption cross-sections and would be fully consistent if $\bar{\beta}$ could be corrected by considering the possibility that $\alpha + \beta \neq 1$. This behaviour of $\bar{\beta}$ and F_o (F_m) was always found, whether measured in the transition from State 2 to State 1 or vice-versa. It can be proved (cf. Appendix) that $\alpha + \beta$ in State 1 is always larger than $\alpha + \beta$ in State 2 and hence the justification to take arbitrarily the maximum possible value ($\alpha + \beta = 1$) in State 1, as was done in Table I. Using the same procedure as described in Table I, we calculated β from the fluorescence parameters and α from Eqn. 2 for intermediate situations during the transition from state 2 to state 1. The sum $\alpha + \beta$ is reproduced and plotted in Fig. 4.

The relative changes of β and α in the transition from State 2 to State 1 are significant and should show up also in the fluorescence quenching effect by Light 1. As the imbalance between PS II and PS I in Light 2 becomes greater, more far-red light will be needed to regain balance and saturate the fluorescence quenching from F_s to F_o. Such saturation curves will be sensitive to the difference $\beta - \alpha$ and hence should give an independent measure for the relative change in β and α. Fig. 5 shows the normalized saturation curves of the Light 1 induced decrease in fluorescence (from F_s to F_o) in leaves adapted to the two states. The

TABLE I

COMPARISON OF THE RELATIVE LIGHT DISTRIBUTION BETWEEN THE TWO PHOTOSYSTEMS IN A PEALEAF ADAPTED EITHER TO STATE 1 OR STATE 2

Values have been calculated as described in text.

	α	β	$\alpha+\beta$
State 1	0.40	0.60	1.00
State 2	0.44	0.52	0.96

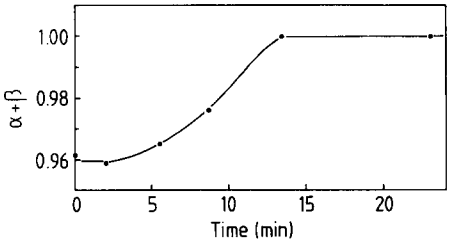

Fig. 4. Time-course of the change in the sum of α and β during the transition from State 2 (time zero) to State 1 (end of the experiment) using the experimental data of Fig. 3.

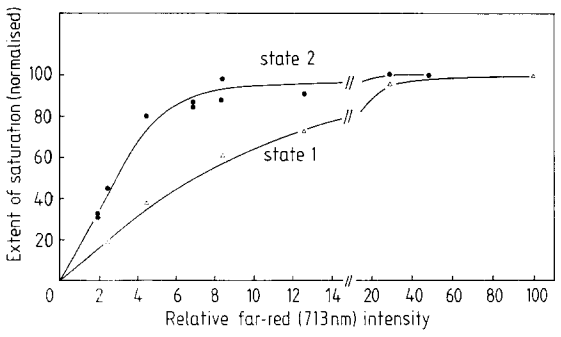

Fig. 5. Intensity dependence of the quenching of chlorophyll fluorescence by 713 nm light in a pea-leaf-adapted either to State 1 or State 2. The total fluorescence changes were normalized to the same extent (100%) for both states. 100% intensity was 6 W/m^2. Other details as in Materials and Methods.

figure clearly demonstrates the increase in effectiveness of PS I in State-2-adapted leaves to quench F_s to F_o due to the smaller $\beta - \alpha$. The ratio of the initial slopes for State 2 vs. State 1, respectively, is 2.3 ± 0.2. It can be shown (cf. Appendix) that this value is consistent with the values of α and β obtained in Table I, allowing for a reasonable partial distribution (about 20%) of the far-red light to PS II. In contrast, if the assumption that $\alpha + \beta$ remains constant at a value of 1 holds, then calculating β and α according to Eqns. 3 and 4 for both states leads to a ratio of slopes for the far-red light effect of 3.1, in disparity with the experimental results.

Discussion

In this paper we have demonstrated, using a modulated chlorophyll fluorescence technique, an equal proportional change in both the F_m and F_o values of pea leaves accompanying State 1-State 2 transitions. We conclude that in vivo regulation of excitation energy distribution can therefore be fully accounted for by changes in absorption cross-section of the photosystems and does not involve a significant alteration in the degree of 'spillover' from PS II to PS I. This confirms, independently, the conclusions of Canaani and Malkin [8] using a photoacoustic technique to measure oxygen yield in leaves. Similar conclusions were arrived at by Hodges and Barber [16] and Williams et al. [18], who examined chlorophyll fluorescence induction parameters in *Chlorella*. Moreover, we have also demonstrated that the effectiveness of PS I to quench fluorescence increases on transition from State 1 to State 2 reflecting a change in the balance of light distribution in favour of PS I.

It has been suggested that reversible phosphorylation of the surface of LHC-2, indirectly controlled by the redox state of the plastoquinone pool is responsible for the in vivo state transitions [7,19]. Such a conclusion has been implied by the finding that isolated thylakoids reversibly phosphorylate LHC-2 under the same spectral conditions which induce State 1-State 2 transitions in leaves and by studies of other related phenomena [20–22]. In addition, it was found that the transition to State 1 is inhibited in vivo by NaF, a specific inhibitor of phosphatases [23].

With isolated thylakoids the mechanism by which phosphorylation of LHC-2 controls the relative excitation of PS II and PS I is dependent on the background level of cations [15,24,25]. For example, at concentrations of Mg^{2+} below 5 mM the mechanism involves a change in 'spillover' as well as adjustments in the absorption cross-sections of PS I and PS II. With these low ionic conditions the ability to screen electrostatically the surface charges on the membrane, particularly the additional charge due to LHC-2 phosphorylation, is poor and there can be substantial unstacking of the membranes [25] and presumably considerable intermixing of PS II and PS I. At higher Mg^{2+} levels (above 5 mM), where electrostatic screening is more effective the phosphorylation of LHC-2 mainly causes absorption cross-sectional changes of PS II and PS I [24–27], and is therefore more like the in vivo situation. In this case, it has been proposed that a pool of LHC-2, when phosphorylated, moves laterally from the appressed to the non-appressed regions of the thylakoids [28]. This is thought to be due to the increased surface charge density after the addition of phosphoryl groups to the surface exposed portions of the complexes [7,29]. It is presumed that the phosphorylated LHC-2 complexes in the non-appressed regions are then able to interact and transfer their excitation energy directly to PS I. The conclusion that the in vivo state transitions involve only absorption cross-section changes in higher plants suggests that the level of Mg^{2+} in the stroma, the main screening cation within the intact

chloroplast [30,31], has an activity above 5 mM.

The calculations of α and β, the fraction of light absorbed by PS I and PS II, respectively, indicate that on conversion to State 2 there is an apparent loss of some excitation of PS II that is not accounted for by an increase in PS I excitation. This loss is also demonstrated by in the relative effectivity of PS I (as measured by the intensity of 713 nm light required to oxidize Q_A, see Fig. 5) in the two states. As shown in the Appendix, the observed ratio of the relative far-red effectiveness to quench F_m to F_o in State 1 and State 2 are consistent with α and β values having their sum decreased on the transition to State 2. This apparent loss of excitation could reflect the degree of inefficiency of phosphorylated LHC-2 to transfer its excitation energy to PS I. Since there is no evidence that the phosphorylated complex becomes physically associated with PS I, then the efficiency of transfer will be determined by statistical interactions at a spatial separation sufficiently close to facilitate energy transfer. It should be emphasized that our results and conclusions are probably only true for higher plants and green algae. In organisms which contain phycobilisomes it was reported that 'spillover' occurs during State 1-State 2 transitions [32].

Our conclusion that State transitions within mature leaves are a result of absorption cross-section changes is based on the determination of F_o and F_m values. These parameters, as defined here, are not a priori expected to have the same values for F_o and F_m as usually obtained from the fluorescence induction curves (F_o being then the initial fluorescence upon trnsition to light of a dark-adapted leaf and F_m the first peak value or the maximum value obtained by DCMU treatment – cf. Ref. 33). Adaptation to light could bring about several types of change, e.g., in the radiationless transition constant and in the interactions between the antennae pigments and the reaction centres, such as to modify F_o and F_m as well as their ratio. We have compared the F_m/F_o ratios determined from the modulated fluorescence technique in the steady state with those obtained by recording fast fluorescence induction curves with dark pretreated leaves. In general, we noted that under the latter conditions we obtained slightly higher F_m/F_o ratios, which may be attributed to a lower F_o level.

TABLE II

VARIOUS CHLOROPHYLL FLUORESCENCE PARAMETERS OF ATRAZINE-SENSITIVE AND -RESISTANT LEAVES OF *SENECIO VULGARIS*

		State 1	State 2
Sensitive	F_o	1.17	1
	F_m	5.57	4.8
	F_m/F_o	4.8	4.8
	β	0.56	0.48
	α	0.44	0.45
	$\alpha+\beta$	1	0.93
Resistant	F_o	1.20	1
	F_m	3.07	2.6
	F_m/F_o	2.6	2.6
	β	0.56	0.48
	α	0.44	0.46
	$\alpha+\beta$	1	0.94

This difference could suggest that the far-red light did not fully oxidize the primary acceptor Q_A as assumed for dark adaptation. This is consistent with the often observed initial fast phase in the fluorescence induction kinetics, reflecting a fraction of PS II reaction centres unattached to the plastoquinone pool and hence to the main stream of electron transport (see, for example, Ref. 34). Such centers will contribute to the fast phase in the fluorescence induction, but their fluorescence cannot be quenched by far-red light. This fact does not make any difference to the present analysis, which deals with those PS II and PS I centres constituting the majority which are able to normally communicate by electron transfer. This difference in the F_m/F_o ratios was even more marked when using atrazine-resistant *Senecio vulgaris* compared with the atrazine-sensitive biotype. However, when the α and β values were calculated for the resistant and sensitive plants in either State 1 or State 2 they were very comparable (see Table II). It is possible to conclude that the modulated fluorescence levels attributed to F_o and F_m are satisfactory for the analyses outlined in this paper.

Appendix

(1) Proof that $\alpha+\beta$ in State 1 is always larger than in State 2

The experimental result (Fig. 3) is that the

parameter $\bar{\beta}$ (i.e., the β calculated according to Eqn. 3 assuming $\alpha + \beta = 1$) changes in the State transition by a factor smaller than F_o or F_m; i.e.:

$$\frac{\bar{\beta}_1}{\bar{\beta}_2} < \frac{F_1}{F_2} = \frac{\beta_1}{\beta_2} \tag{A1}$$

where the index refers to the state and F stands for either F_o or F_m. From Eqn. 2 in the main text:

$$\alpha + \beta = \beta f + \beta \tag{A2}$$

From Eqn. 3 in the main text:

$$\bar{\beta} = \frac{1}{1+f} \text{ hence } f = \frac{1}{\bar{\beta}} - 1 \tag{A3}$$

Substituting back in Eqn. A2 it follows that:

$$\alpha + \beta = \frac{\beta}{\bar{\beta}} \tag{A4}$$

Hence, using Eqn. A1:

$$\frac{\alpha_1 + \beta_1}{\alpha_2 + \beta_2} = \frac{\beta_1}{\beta_2} : \frac{\bar{\beta}_1}{\bar{\beta}_2} = \frac{F_1}{F_2} : \frac{\bar{\beta}_1}{\bar{\beta}_2} > 1 \qquad \text{Q.E.D.}$$

(2) Analysis of the saturation curves of far-red induced quenching of chlorophyll fluorescence

From Eqn. 2 in the text:

$$f(\beta i + \beta' I) = \alpha i + \alpha' I$$

where i and I are the intensities of the modulated Light 2 and the additional non-modulated Light 1, respectively, α and α' are the fractions of Light 2 and 1 exciting PS I, respectively, β and β' are the fractions of Light 2 and 1 exciting PS II, respectively, and f is as defined by Eqn. 1 in text. Evidently this equation is correct so far as $f < 1$, i.e., when $\beta i + \beta' I > \alpha i + \alpha' I$, otherwise $f = 1$.

Therefore:

$$f = \frac{\alpha i + \alpha' I}{\beta i + \beta' I} \left(\text{for } I < \frac{\beta - \alpha}{\alpha' - \beta'} i \right)$$

and

$$f = 1 \left(\text{for } I > \frac{\beta - \alpha}{\alpha' - \beta'} i \right) \tag{A5}$$

f changes between $\frac{\alpha}{\beta} (I = 0)$ to 1 (excess far-red light).

The linear approximation of Eqn. A5 for the initial change of f vs. I when I has a low value is obtained by differentiation of Eqn. A5 at $I = 0$:

$$f = \frac{\alpha}{\beta} + \frac{\alpha'\beta - \beta'\alpha}{\beta^2 i} I \text{ (small } I) \tag{A6}$$

From Eqn. A6 it is possible to express the normalized change in f (from the initial value α/β obtained without Light 1) as a function of I. The change in f is $\Delta f = f - (\alpha/\beta)$ and the total span of Δf is $1 - (\alpha/\beta) = (\beta - \alpha)/\beta$; hence the normalized change in f, Δf^*, is obtained from Δf by division through the last factor.

$$\Delta f^* = \frac{(\alpha'\beta - \beta'\alpha)}{\beta^2 i} \cdot \frac{\beta}{(\beta - \alpha)} \cdot I \tag{A7}$$

It is assumined that Light 1 distribution has no loss, hence $\alpha' + \beta' = 1$ Substituting $\beta' = (1 - \alpha')$, Eqn. A7 becomes:

$$\Delta f^* = \frac{\beta - \beta'(\alpha + \beta)}{\beta(\beta - \alpha) i} I \tag{A8}$$

Therefore, the reciprocal slope of Δf^* against I is:

$$\frac{\beta(\beta - \alpha) i}{\beta - \beta' + \beta'\sigma}$$

where σ is a small correction factor for the difference between $\alpha + \beta$ and 1, i.e., $\sigma = 1 - (\beta + \alpha)$.

For all conditions treated here, σ is always very small compared to the other terms and can be ignored, so that:

$$\text{Reciprocal slope} = \frac{(\beta - \alpha) i}{1 - \frac{\beta'}{\beta}} \tag{A9}$$

Using Eqn. (A9) and the values of α and β given in Table I, the ratio of the reciprocal slopes can be calculated and compared with the measured ratio of the slopes in Fig. 5. It is assumed that $\beta' = 0.1$. State 2 parameters are $\beta = 0.52$ and $\alpha = 0.44$, and State 1 parameters are $\beta = 0.60$ and $\alpha = 0.40$. Therefore

$$\text{expected ratio} = \left(\frac{0.6 - 0.4}{0.52 - 0.44} \right) \cdot \left(\frac{1 - 0.1/0.52}{1 - 0.1/0.60} \right) = 2.4$$

If, however, the sum $\alpha + \beta$ equals 1, in State 2 as

well as State 1, the parameters for State 2 are now $\beta = 0.53$, $\alpha = 0.47$. The expected ratio would then be:

$$\left(\frac{0.6-0.4}{0.53-0.47}\right)\cdot\left(\frac{1-0.1/0.53}{1-0.1/0.60}\right) = 3.2$$

It should be noted that the above numerical computation is not very sensitive to β', so that a big change in the assumed value for β' over a wide range around $\beta' = 0.1$ will change the above ratios only slightly. This number reflects the quantum yield of electron transport in far-red light which is about 1/5 of the peak value [35] in the quantum yield spectrum, where one assumes an equal fraction (0.5) of light distributed to the two photosystems.

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