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Shifts to $C_{675-670}$ and to $C_{696-684}$ in etiolated leaves illuminated with series of brief flashes

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Absorbance changes seen when etiolated primary bean leaves are given series of short, low-intensity flashes of light were recorded. Whatever its order in a series, each flash induced an initial, fast increase of the absorbance, producing an absorption band between 660 and 700 nm with a peak at 680 nm. This occurred in less than 30 msec and was followed within seconds by either a slow dark increase or a slow dark decrease of the leaf absorbance, depending on the order of the flash and the analytical wavelength. The dark absorbance changes were due to shifts of the initial absorption band produced by the flash, either towards 685 or 670 nm. The shift was always towards 670 nm after the first flash. It appeared to move progressively towards 685 nm from one flash to the next, this direction becoming predominant after a certain number (n_{inv}) of flashes. The (n_{inv}) depended on the light dose, but not on the flash frequency. Three proposals are made to account for the results.

The action of light on the protochlorophyll(ide) protein complexes $P_{645-630}$ and $P_{657-650}$ of fresh etiolated leaf yields two distinct chlorophyll(ide) proteins, $C_{675-670}$ and $C_{696-684}$. $C_{675-670}$ is normally found in much lower quantities than $C_{696-684}$. $C_{696-684}$ undergoes a dark shift, the Shibata shift, yielding the stable product $C_{680-672}$ in about 20 to 30 min; this very slow shift does not contribute to the events described in this paper which are completed within at most 4 min. According to Litvin et al. (*6*), $C_{675-670}$ is formed within a few seconds from a transient state of the pigment protein complex with a red absorption band at 676 nm, while $C_{696-684}$ arises within 30 sec from another transient state with a red absorption band at 678–680 nm (*3, 8*). Litvin et al. produced evidence showing that the 678–680-nm absorbing state is formed from the 676-nm absorbing state due to a particular light reaction (see **Discussion**). These transient states are ordinarliy trapped with liquid nitrogen immediately after a 1-msec flash (*8, 9*). They have been collectively called $P_{688-676}$ by Sironval and Kuiper (*10*).

In previous experiments, we were able to accumulate large amounts of $C_{675-670}$ by repetitively firing very low-intensity flashes. Under these conditions, either

Abbreviations: P_{m-n}, protochlorophyll(ide) protein complex absorbing in vivo at wavelength "n" and emitting at wavelength "m"; C_{m-n}, chlorophyll(ide) protein complex absorbing in vivo at wavelength "n" and emitting at wavelength "m". The wavelength is expressed in nanometers.

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$C_{675-670}$ or $C_{696-684}$ accumulated in the etiolated leaf depending on the frequency of the flashes (*7*).

To try to improve the description of these phenomena, we modified a technique of De Kouchkovsky and Fork (*2*) and looked at the effect of series of individual, low-intensity flashes on the leaf absorption. The results showed that $C_{675-670}$ and $C_{696-684}$ are formed in sequence, and that one single flash of a given intensity produces preferentially either the transient state which yields $C_{675-670}$, or the state which yields $C_{696-684}$ depending on the order of the flash in a series.

However, we were unable to see any change of the red absorption of the transients seen 30 msec after one flash when the flash order was increasing. Thus, 30 msec after Flash 1, the transient state evolving into $C_{675-670}$ has the same red absorption band as the transient state found 30 msec after flash 21, which evolves into $C_{696-684}$.

Material and methods

Culture conditions

Beans (*Phaseolus vulgaris*, variety Commodore) were grown in darkness at 23 $\pm 1°C$ in pots containing vermiculite moistened with tap water. The etiolated primary leaves were used between the 14th and 21st day after germination. All manipulations for cultivating the plants were done under dim, green safe light from an Osram 4543 lamp surrounded by a neutral filter. Leaf samples were prepared under dim, green light from a 4.5-V, 0.8-W tungsten lamp passed through two sheets of colored plexiglas, each 2 mm thick (ROHM and HAAS, W. Germany no. 303 and no. 701); this combination gave a transmission spectrum centered around 540 mm with a half-band width of 35 nm.

Measuring device

The measuring apparatus was an adaptation of that described by De Kouchkovsky and Fork (*2*; block diagram in Fig. 1). The analytical light was provided by a 250-W, 21-V projector lamp (S) (Sylvania DKM, powered by a highly regulated, DC power supply), whose emission was dispersed by a grating monochromator (M) (Bauch and Lomb 500 mm, 1200 grooves per mm). The monochromatic output light was passed through a fiber optics pipe (LPa) and a mixing glass rod (LM); it was directed by this rod through the leaf in C and through a Corning CS 2-64 high-pass filter (F_2) on the photocathode of an EMI 9558B photomultiplier (PM). In order to catch as much light as possible, the distance between the lower face of the leaf and the photocathode was reduced to a minimum (about 5 mm). The voltage appearing at the load resistor of the photomultiplier was partly compensated by an adjustable biasing circuit (BC). The uncompensated part of the voltage was measured simultaneously with a Tektronix storage oscilloscope (OSC) and a Brush MK 220 oscillographic recorder (OR).

The actinic light was provided by a gas discharge tube (F.L.) (General Electric, Type FT 230). Flashes were obtained by discharging either a 0.1-μF or 10.1-μF capacity charged at a voltage between 1200 and 2000 V; the flash duration at half-peak was 88 μsec. The light of the flash was collected by a fiber optics pipe (LP_{fl}) connected to the mixing glass rod (LM) mentioned above; it was filtered though a Corning CS 4-96 filter (F_1). The analytical as well as the actinic light was passed

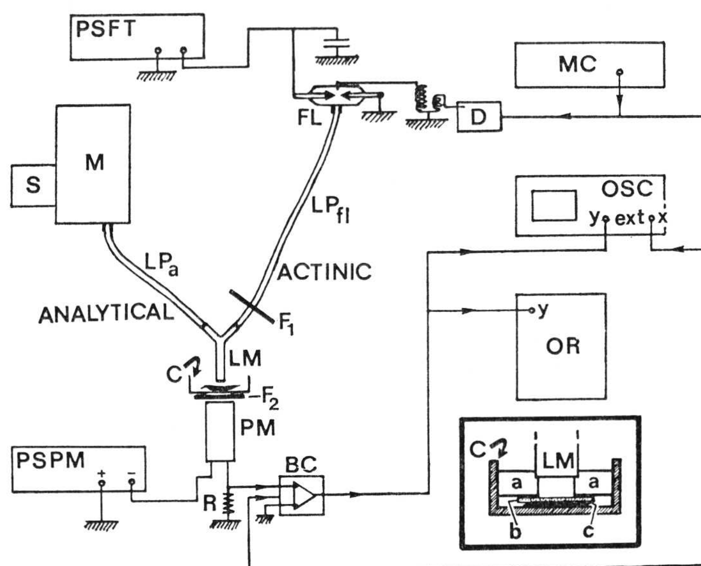

Fig. 1. *Block diagram of the measuring device.* PSFT, flash tube power supply; FL, flash tube; S, analytical light source; M, monochromator; LPa and LPfl, light pipes; LM, light mixing rod; C, cuvette; F1 and F2, optical glass filters; PM, photomultiplier; PSPM, photomultiplier power supply; R, photomultiplier load resistor; BC, signal biasing circuit; MC, master pulse generator; D, flash trigger pulse delaying circuit; OSC, oscilloscope; OR, oscillographic recorder.
The insert shows details of leaf positioning in the cuvette: a, centering plexiglass disc; b, glass slide; c, leaf.

through the mixing rod, shining perpendicularly on the leaf sample in the cuvette (C).

A master pulse generator (MC) commanded the time-base of the oscilloscope (OSC) and a delaying monostable multivibrator (D) whose output triggered the flash. This arrangement enabled recording of the base line on the oscilloscope screen from 10 to 200 msec before a flash was fired. A special circuit included in (BC) blocked the photomultiplier during the flash. This was done by polarizing the photocathode for 3 msec positively with respect to the first dynode; it took about 20 msec for the photomultiplier to recover its steady-state. Thus, the absorbance changes were estimated with a confidence of 30 msec after each flash.

Normalization of the data

When several leaves from the same culture were used, especially for measuring difference spectra, the data was normalized by adjusting to the same value the effect of either Flash 1 or 2 (depending on the experiment) as measured at 675 nm. In general, the ΔA_i defined in the first paragraph of the **Results**, were chosen to calibrate the intensity of the flashes when necessary.

Results

Absorbance changes after Flash 1

Two classes of absorbance changes occurred: The first class included changes

in the range of a few msec whose kinetics was not recorded by the experimental set-up. Only the resulting end state of these changes was seen. Each change of this class was characterized by the difference between the position of the oscilloscope trace just before the flash and its position 30 msec later. We call this difference the "initial" absorbance change ΔA_i (Fig. 2). From 660 to 700 nm, ΔA_i was positive (absorbance increase) and from 630 to 655 nm, it was negative (absorbance decrease). The second class included kinetics, in the second time range, of the difference between the absorbance before the flash and the absorbance at increasing times during 10 to 20 sec after the flash ($=\Delta A$). These kinetics were at least 1000 times slower than those of the first class. They depended on the wavelength of the analytical light and on the order of the flash (see Fig. 2 and 3).

Note that before as well as after Flash 1, the analytical light from 680 to 700 nm was found to be inactinic by itself. Thus, it did not interfere with the kinetics. However, the analytical light from 660 to 675 nm induced a slow, linear increase of the absorbance. This increase was most evident at 665 nm with a magnitude of the order of 10^{-4} per second. It was deduced when establishing the kinetics of ΔA.

a) *ΔA_i after Flash 1*

Fig. 3A (full line) shows the ΔA_i's measured after Flash 1 from 660 to 700 nm; it is a difference spectrum: [absorbance 30 msec after Flash 1—absorbance before the flash]. The product found 30 msec after Flash 1 had a red absorption band centered around 680 nm.

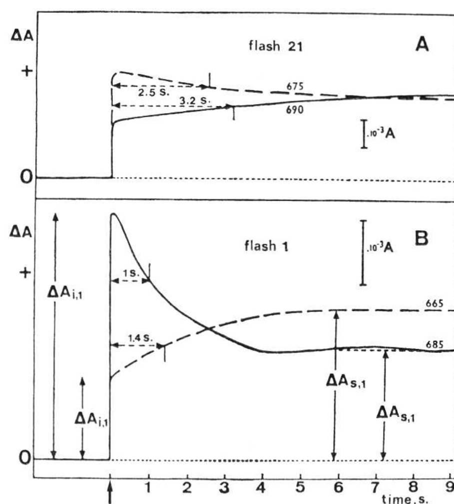

Fig. 2.

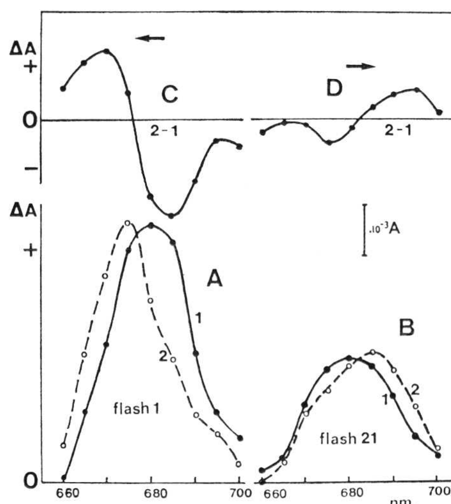

Fig. 3.

Fig. 2. *Kinetics of absorbance changes after Flash 1 (B) and 21 (A) observed respectively at 665 and 685 nm and at 675 and 690 nm.* ΔA_i and ΔA_s are defined in the text.

Fig. 3. *Difference spectra: [absorbance 30 msec after the flash minus absorbances before the flash] (Curve 1) and [absorbance 10 sec after the flash minus absorbances before the flash] (Curve 2) for Flash 1 (A) and 21 (B).* Curves C and D are the differences: [absorbances 10 sec after the flash minus absorbances 30 msec after the flash] for Flash 1 and 21, respectively.

b) *ΔA kinetics after Flash 1*

At all wavelengths including and between 680 to 700 nm, ΔA decreased during the seconds following Flash 1. Fig. 2B, curve (685), shows the kinetics of this decrease at 685 nm. When the analytical light was set at 675 nm, the absorbance decreased slightly during the first second after the flash, but this decrease was changed into a slight increase during the next few seconds. When observed at 670, 665 and 660 nm, the ΔA kinetics showed an increase of the absorbance. Curve (665), Fig. 2B, shows this increase at 665 nm.

The decrease, as well as the increase of the absorbance, reached half-maximum amplitude within about 1 sec; both were completed within 5–6 sec. A steady-state was then reached, which persisted for at least 1 min. The steady-state value of the absorbance change (ΔA_s) was always positive.

Fig. 3A (dashed curve) shows the ΔA_s's measured from 660 to 700 nm; it is a difference spectrum: [absorbance 10 sec after Flash 1—absorbance before the flash]. The product seen 10 sec after Flash 1 had an asymmetrical red absorption with a maximum at 675 nm.

Fig. 3C shows the difference from 660 to 700 nm: $\Delta A_s - \Delta A_i$. It illustrates the shift of the spectrum towards shorter wavelength within the seconds following Flash 1.

Absorbance changes after Flash 21

Twenty-one flashes of the same duration and the same (low) intensity were fired at 10-sec intervals. The absorbance changes were measured after Flash 21. As after Flash 1, two kinds of changes were distinguished: a) the initial absorbance change measured by the ΔA_i seen 30 msec after Flash 21 and b) the change of the ΔA's during the next 10 sec up to a positive, steady-state value ΔA_s.

During the seconds which followed Flash 21, the analytical light from 680 to 700 nm had a negligible effect on the absorbance of the leaf. Below 680 nm, the effect of this light was much smaller after Flash 21 than after Flash 1. These effects were deduced, when necessary, from the measured absorbance changes.

a) *ΔA_i's after Flash 21*

The magnitude of the ΔA_i's was a little more than two times smaller after Flash 21 than after Flash 1. The difference spectrum [absorbance 30 msec after Flash 21—absorbance before the flash] is seen in Fig. 3B (full line). Its shape did not differ from that of the corresponding difference spectrum after Flash 1 (Fig. 3A, full line).

b) *ΔA kinetics after Flash 21*

In contrast to what was seen after Flash 1, the ΔA kinetics after Flash 21 was an increase of the absorbance at 685 nm and longer wavelengths, but was a decrease at 680 nm and shorter wavelengths. Fig. 2A shows the kinetics registered at 675 (ΔA decrease) and 690 nm (ΔA increase). The 675-nm ΔA decrease was practically over within 10 sec. The 690-nm ΔA increase tended asymptotically to a steady-state value of the leaf absorbance which was reached within 10 to 15 sec. In both cases, the half-maximum change was reached within some 3 sec.

The difference spectrum [absorbance 10 sec after Flash 21—absorbance before the flash] is shown in Fig. 3B, dashed line. Comparison with the ΔA_i spectrum

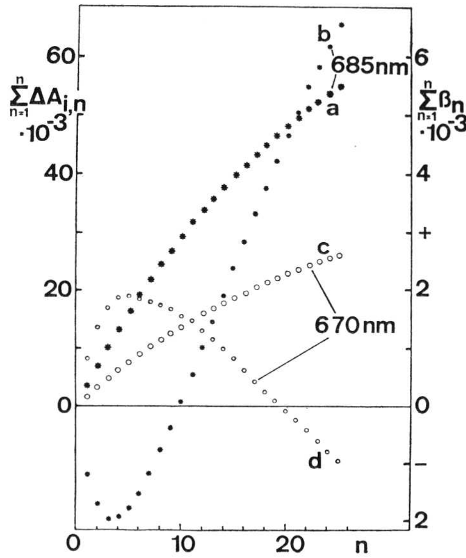

Fig. 4.

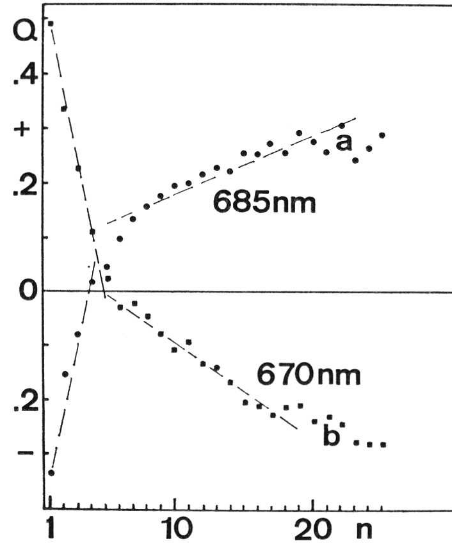

Fig. 5.

Fig. 4. *Cumulated initial absorbance changes, $\Delta A_{i,n}$ and cumulated subsequent dark shifts, β_n, for an increasing number, n, of actinic flashes measured at 685 and 670 nm.* Frequency of flashes: 0.1 Hz. See text for exact definition of the symbols.

Fig. 5. *Relative magnitude of the dark shift Q at each successive flash, n, measured at 685 nm (Curve a) and 670 nm (Curve b).* See text for an exact definition of Q. Frequency of flashes: 0.1 Hz.

(Fig. 3B, full line) shows that the spectrum found 30 msec after Flash 21 had shifted towards longer wavelengths within 10 sec. Fig. 3D gives the difference of ($\Delta A_s - \Delta A_i$).

Absorbance changes from Flash 1 to Flash 25

Curves (a) and (c) of Fig. 4 show the sums $\sum_{n=1}^{n} \Delta A_{i,n}$ of the initial ΔA_i's cumulated from Flash 1 to Flash n, the flashes being fired every 10 sec. The data are for measurements at 685 and 670 nm. The $\sum_{n=1}^{n} \Delta A_{i,n}$ was about two times larger at 685 nm than at 670 nm. The magnitude of each ΔA_i decreased regularly from flash to flash.

Curves (b) and (d) of Fig. 4 show the sum $\sum_{n=1}^{n} \beta_n$ for Flash 1 to 25 where $\beta_n = \Delta A_{5\,sec,n} - \Delta A_{i,n}$. Curve (b) shows the β_n's estimated at 685 and curve (d) those estimated at 670 nm. After Flashes 1, 2 and 3, the absorbance at 685 nm decreased in darkness ($\beta_n < 0$), but increased at 670 nm ($\beta_n > 0$); starting from Flash 4, the absorbance increased at 685 nm but decreased at 670 nm.

Fig. 5 shows the values of the ratio:

$$Q_n = \frac{\Delta A_{5\,sec,n} - \Delta A_{i,n}}{\Delta A_{i,n}} = \frac{\beta_n}{\Delta A_{i,n}}$$

the analytical light being again set, either at 685 nm (curve a) or 670 nm (curve b). Q_n is the absorbance change during the 5 sec which follow Flash n relative to $\Delta A_{i,n}$,

the initial absorbance change seen 30 msec after Flash n. Q_n varied monotonically at 685 nm as well as at 670 nm. At a given flash, the absolute value of Q_n was almost equal at the two wavelengths 685 and 670 nm, as can be seen from the symmetry of curves (a) and (b) in Fig. 5. Fig. 5 also shows that there was no break in the behavior of Q_n when, between Flash 3 and 5, the sign of ΔA_n changed. However the variation rate of Q_n from Flash n to Flash n+1 was larger before Flash 4 than after Flash 6, suggesting two distinct, successive phases.

Effects of flash frequency and intensity on the absorbance changes

Flash frequencies were varied from 0.05 to 0.33 Hz. Table 1 shows that, given a constant value for the light dose in one flash, the facts in paragraphs 1, 2 and 3 held true in the whole frequency range investigated.

The effect of varying the light dose (d) in one flash at a constant frequency (0.1 Hz) is illustrated in Fig. 6 for absorbance changes at 685 nm. Qualitatively, the same conclusions were drawn at all analytical wavelengths. The light doses were estimated by measuring the mean $\Delta A_{i,1}$ for 10 leaves after Flash 1 at 685 nm. The

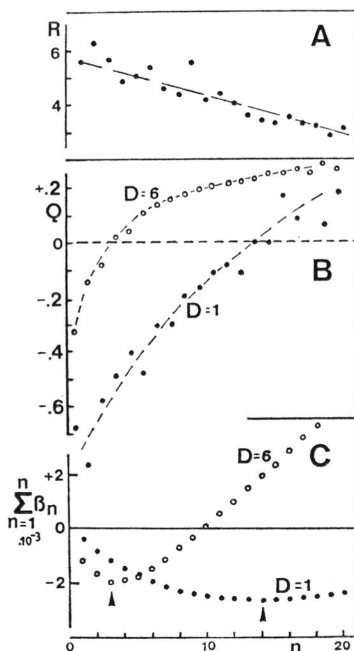

Fig. 6.

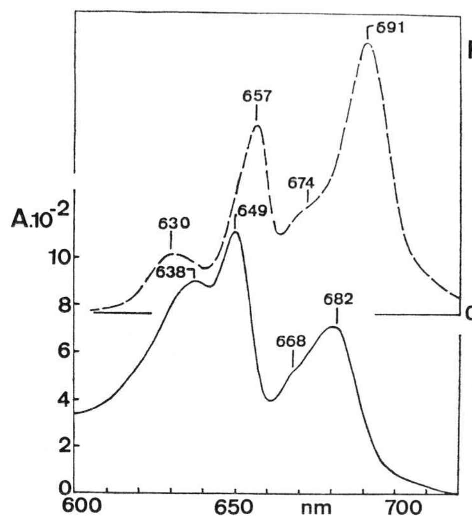

Fig. 7.

Fig. 6. *Effect of the light dose in one flash (d=1; d=6), as a function of the flash order, n, on the initial absorbance changes as expressed by the ratio R (defined in the text), the relative magnitude and sign of the dark shifts, Q (exact definition in text) and the cumulated dark shifts in absolute value β_n (exact definition in text).* Frequency of flashes: 0.1 Hz.

Fig. 7. *77°K Absorption (below) and fluorescence emission (above) spectra of an etiolated leaf which had received 20 low-intensity flashes at a frequency of 0.1 Hz.* The fluorescence spectrum was not corrected for the spectral sensitivity of the fluorimeter.

Table 1 *Effects of flash frequency on the absorbance changes at 685 nm*

Flash frequency (Hz)	0.33		0.29		0.25		0.22		0.20		0.13		0.10		0.07		0.05	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
$\Delta A_{i,1}$	4.4	100	4.8	100	3.2	100	3.3	100	2.7	100	3.6	100	3.5	100	3.4	100	2.7	100
β_1	−1.3	−30	−1.4	−29	−1.0	−31	−1.0	−30	−1.1	−41	−1.3	−36	−1.2	−34	−1.2	−35	−1.0	−37
$\sum_{n=1}^{n=3} \Delta A_{i,n}$	12.1	275	14.1	294	8.9	278	9.4	285	7.7	285	10.5	292	10.1	289	10.2	300	7.6	281
$\sum_{n=1}^{n=3} \beta_n$	−2.3	−52	−2.5	−52	−1.7	−53	−1.3	−39	−1.7	−63	−2.1	−58	−1.9	−54	−1.9	−56	−1.7	−63
$\Delta A_{i,21}$	1.6	36	2.0	42	1.6	50	1.5	45	1.3	48	1.6	44	1.5	43	1.5	44	1.0	37
β_{21}	+0.4	+9	+0.5	+10	+0.5	+16	+0.4	+12	+0.2	+7	+0.4	+11	+0.4	+11	+0.4	+12	+0.3	+11
$\sum_{n=1}^{n=21} \Delta A_{i,n}$	56.5	1284	65.0	1354	45.9	1434	45.7	1385	39.5	1463	51.0	1417	50.5	1443	51.9	1526	37.8	1400
$\sum_{n=1}^{n=21} \beta_n$	+4.8	+109	+6.0	+125	+4.8	+150	+5.7	+173	+4.1	+152	+5.0	+139	+4.9	+140	+4.2	+124	+4.7	+174

A=absolute values of the 685-nm absorbance changes; B=values in% of $\Delta A_{i,1}$. Each column refers to one experiment given as an example; variations seen from one column to the next thus include leaf variability. The absolute values are in $x \cdot 10^{-3}$ A; $\Delta A_{i,n}$ is defined in **Results**. For frequencies from 0.33 to 0.22 Hz, $\beta_n = \Delta A_{x\,sec,n} - \Delta A_{i,n}$ in which x is respectively 3, 3.5, 4 or 4.5; for other frequencies $\beta_n = \Delta A_{5\,sec,n} - \Delta A_{i,n}$. Intensity of the flash: d=6 as defined in the text. $n_{inv}=3$ all frequencies.

data in Fig. 6 are relative to a ratio of 6 between the doses $\Delta A_{i,1}=3.6\cdot10^{-3}$ (d=6) and $0.6\cdot10^{-3}$ (d=1).

Increasing the light dose by a factor of 6 affected the initial absorbance changes $\Delta A_{i,n}$ depending on the order of the flash. This was reflected in the quasi-linear decrease of the ratio:

$$R=\frac{\Delta A_{i,n} \text{ for } d=6}{\Delta A_{i,n} \text{ for } d=1}$$

In the example of Fig. 6A, R varied from 6 to 3 from Flash 1 to 20.

Q_n (Fig. 6B; see definition in **Results**, third section) also depended on the light dose. At 685 nm, Q_n was negative for a higher number of flashes starting from Flash 1, and its absolute value was greater when the dose was smaller.

Finally, the smaller the dose, the more flashes were needed to inverse the sign of β_n. In Fig. 6C, three flashes were necessary for d=6, against 14 flashes for d=1.

At the point where the sign of β_n changed (inversion point), the value of $\Sigma_{n=1}^{n} \beta_n$ was independent of the light dose. When measured at 685 nm, this value was between -2.0 and $-2.5\cdot10^{-3}$. The same held true for the sum $\Sigma_{n=1}^{n} \Delta A_{i,n}$ of the initial absorbance changes cumulated from Flash 1 to the flash at the inversion point. When measured at 685 nm for the dose d=1, $\Sigma_{n=1}^{n} \Delta A_{i,n}$ amounted to $8\cdot10^{-3}$, while it was $10\cdot10^{-3}$ for d=6. It follows that at the inversion point, the ratio $\Sigma_{n=1}^{n} \beta_n / \Sigma_{n=1}^{n} \Delta A_{i,n}$ did not vary much, lying between 0.2 and 0.3 when estimated at 685 nm.

Discussion and conclusions

Spectra A and B (full lines) of Fig. 3, suggest that in our experiments each low-intensity flash produced the same complex in an amount measurable by the magnitude of $\Delta A_{i,n}$. However, when the flash order was below a certain value n_{inv} (for $n_{inversion}$), a dark shift resulted afterwards towards shorter wavelengths (→670 nm) with a half-life time of 1 sec; while for n greater than n_{inv}, the shift was towards longer wavelengths (→685 nm) with a half-life time of 3 to 4 sec (Fig. 2). Flash n_{inv} thus clearly appears as a singular point. It has the following properties: 1) it decreases when the intensity of the flash increases (Fig. 6C) and 2) it does not change with the flash frequency, at least for the intensity d=6, and within the limits of the selected frequencies (Table 1).

Two features distinguish the shift towards shorter wavelength (→670 nm) from the Shibata shift: 1) it is completed within a few seconds and 2) it starts from a chlorophyll(ide)-protein complex similar to $P_{688-676}$ (see below), while the Shibata shift starts from $P_{696-684}$, which is the complex produced from $P_{688-676}$ by the rapid shift described by Sironval and Michel, and Gassman et al. (*3, 8*). The (→670 nm) shift described here thus rather resembles the shift described by Litvin and Bielayeva (*5*), while the shift towards longer wavelengths (→685 nm) described here resembles that described by Sironval and Michel, and Gassman et al. (*3, 8*).

Both the quantity of the chlorophyll(ide) protein complex accumulated up to flash n_{inv} (as measured by $\Sigma_{n=1}^{n_{inv}} \Delta A_{i,n}$), and the quantity of that complex undergoing the short wavelength spectral shift (towards 670 nm, as measured by $\Sigma_{n=1}^{n_{inv}} \beta_n$)

appear more or less independent of flash intensity and frequency (Fig. 6 and Table 1). At flash n_{inv}, independently of flash intensity and frequency, the leaf absorbance due to that fraction of the complex whose absorption shifts to shorter wavelength is about $6 \cdot 10^{-3}$ (at 670 nm). This is estimated by adding the absorbance increase at 670 nm due to the shift towards 670 nm (about $1 \cdot 10^{-3}$) to the $\sum_{n=1}^{n} \Delta A_{i,n}$ at 670 nm (about $5 \cdot 10^{-3}$).

When an etiolated leaf is frozen in liquid nitrogen immediately after a 1-msec flash, a chlorophyll(ide) lipoprotein complex is trapped with an absorption band at 676 nm (emission at 684 nm) if no more than 10% of the reducible protochlorophyll (ide) had been reduced and with a red absorption band at 678–680 nm (emission at 688–690 nm), if more than 20% of this pigment has been reduced. The mixture of these chlorophyll(ide) protein complexes is called $P_{688-676}$ by Sironval and Kuiper (*10*). The absorption spectrum of $P_{688-676}$ is roughly similar to the difference spectrum of $\Delta A_{i,n}$ in Fig. 3. It belongs to some transitory species: in darkness, its red absorption is shifted within a few seconds, either to a band located at 670 nm (emitting at 675 nm) (*5*) when less than about 10% of the pigments is reduced, or to a band located at 685 (emitting at 696 nm) (*3,5,8,9*) for the rest of the leaf pigments. The resulting pigment-protein complexes have lifetimes extending over several minutes.

By applying a series of low-intensity flashes, we found that the direction of the dark shift does not depend on the quantity of protochlorophyll(ide) reduced in one flash but on the order of the flash. The first flashes, and only these, produce the complex which undergoes the short wavelength shift (→670 nm). We call it complex (A).

When a leaf is frozen in liquid nitrogen after some 20 low-intensity flashes, complex (A) appears as a shoulder at 668 nm in the absorption spectrum, which corresponds to a shoulder at 674 nm in the emission spectrum ($=C_{675-670}$, or better $P_{674-668}$, Fig. 7). It obviously amounts to a very limited quantity of the chlorophyll(ide) protein in a rather stable state.

The proportion of complex (A) in the chlorophyll(ide)produced by Flash 1, measured by the magnitude of Q (or in general by Q_n for flash n, $n < n_{inv}$; see **Results** and Fig. 6), increases when the light dose is lowered from $d=6$ to $d=1$ ($Q_1 = -0.3$ for $d=6$; -0.5 for $d=3$, and -0.7 for $d=1$). Thus, at least one product of Flash 1 does not take part in the short wavelength shift and the amount of this product increases with the light dose.

Moreover, the substitution around n_{inv} of the shift towards shorter wavelength by the shift towards longer wavelengths (=shift inversion) appears to be prepared before n_{inv} as shown by Fig. 4 and 5. Before n_{inv}, the amplitude of the 670-nm shift decreases progressively, while after n_{inv}, the amplitude of the 685 nm shift increases progressively. This suggests an overlap of distinct processes around n_{inv}.

Let us suppose that the product of Flash 1 has two components: complex (A) and complex (B). We cannot prove that (B) undergoes the long wavelength shift (→685 nm), but suggest that it does. Then, the ΔA kinetics at Flash 1 should result from the superposition of the kinetics of the shifts of complexes (A) and (B), and the inversion at n_{inv} should be due to an increase of the proportion of (B) in the product of the flashes.

Keeping in mind that each flash reduces a new and low quantity of protochloro-

phyll(ide), as proved by curves (a) and (c) in Fig. 4, we introduce in general terms at least three possible explanations for the shift inversion around n_{inv}. First, the etiolated leaf contains a small amount (a few percent) of a protochlorophyll(ide)-protein complex endowed with the intrinsic property of being transformed by light into a chlorophyll(ide) complex which shifts its red absorption from 680 to 670 nm within seconds following its formation. In this case, this small quantity must also be the first to be reduced due to a mechanism which provides it with the absorbed energy for some reducing power. This is conceivable since efficient energy transfers have been shown in etiolated leaves, (*1*, *4*). A second explanation is that the first pigment molecules to be reduced are reduced randomly, but in an environment peculiar to them, not because of some peculiar localization inside the prolamellar body membranes, but because they are the first to be reduced. This amounts to saying that some properties of the environment of the pigments change during illumination. These surounding changes are thought to impose the inversion of the shift direction. A third explanation rests on the assertion by Litvin et al. (*6*) that two photoreactions in series, called I and II, are necessary for the shift to 685 nm to occur. The first photoreaction I forms a complex $C_{684-676}$ which undergoes the short wavelength shift in darkness. Then photoreaction II transforms the product of I, $C_{684-676}$, into $C_{690-680}$ which undergoes the long wavelength shift. In this case, firing a series of low-intensity flashes causes Flash 1 to initiate photoreaction I giving $C_{684-676}$, and consequently, the dark shift towards 670 nm, and the shift inversion occurs as photoreaction II becomes more and more probable from flash to flash, espercially after n_{inv}.

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