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Kinetics of the Flash-Induced Electrochromic Absorbance Change in the Presence of Background Illumination. Turnover Rate of the Electron Transport. II. Higher Plant Leaves

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Abstract

The flash-induced electrochromic absorbance change[3] (ΔA_{515}) was measured in leaves of higher plants in the absence and presence of continuous monochromatic background illumination of different intensities and wavelengths. The variation of the amplitude of ΔA_{515} in background light was used to estimate the steady-state turnover time of the electron transport. In red light we obtained about 5 msec which was accounted for by the turnover of the linear electron transport. With far red background illumination or in the presence of the photosystem 2 inhibitor, DCMU, the steady-state turnover time tentatively assigned to photosystem 1 cyclic electron transport was much larger ($\geq$ 100 msec).

Increasing the intensity of background illumination with far red light gradually diminished the slow rise of ΔA_{515} in parallel with suppression of the initial rise generated by photosystem 1. At high intensities of the red light, however, while ΔA_{515} was attenuated, the slow rise was not eliminated and its proportion relative to the initial rise did not vary appreciably.

Key Words: Leaf; electrochromism; slow rise of ΔA_{515}; turnover rate of electron transport.

Introduction

In the preceding paper (Barabás *et al.*, 1985) we estimated the steady-state turnover rate of the overall electron transport in isolated intact chloroplasts

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[3] Abbreviations: ΔA_{515}, flash-induced electrochromic absorbance change at 515 nm; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS: photosystem.

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and revealed some factors which regulate the buildup of the transmembrane electric field in thylakoids. In this work we extended our investigations to leaves of higher plants, by using essentially the same method, measuring flash-induced absorbance transients in the absence and presence of background illumination of different intensities and wavelengths.

Materials and Methods

Plant Material

Freshly harvested leaves of two- to three-week-old seedlings of barley were treated and measured as previously described (Garab *et al.*, 1983). The leaves were infiltrated for several minutes in water until they became optically transparent. They were flushed with CO_2 gas and incubated for 2–5 min in CO_2-enriched atmosphere. The absorption at 680 nm varied between 0.9–1.1 in five independent measurements.

Measurement of Absorbance Transients

The flash-induced absorbance changes between 470 and 590 nm were measured in a setup described previously (Horváth *et al.*, 1979). The leaves set diagonally into a 1 × 1 cm spectroscopic cell were excited by Xe flashes (> 630 nm, 3 μsec duration at half peak emission). The other side of the leaves could be illuminated with monochromatic ($600 < \lambda < 750$ nm) background light (cf. Barabás *et al.*, 1985 for details). The measuring beam was perpendicular both to the direction of flash excitation and the background light beam. The kinetic traces of the flash-induced absorbance transients were collected in a multichannel analyzer (ICA 70, KFKI). When the dependence of the amplitude and kinetics of ΔA_{515} on the wavelength and intensity of the background illumination was determined, 20 scans were averaged at a repetition rate of 0.25 sec^{-1} started 2 min after the onset of the background light. When measuring the time course of the background light-induced changes in the amplitude of ΔA_{515} (or that of its relaxation) we used a series of flashes 200 msec, 2 sec, 10 sec, and 50 sec after the onset (or turnoff) of the continuous background light. Ten kinetic traces were averaged, and a 2-min break in the dark was allowed between flash series. The total amplitude was determined by curve fitting with a linear combination of two exponentials (Farineau *et al.*, 1980).

Determination of Rate-Limiting Turnover Time, τ, of Electron Transport

For the estimation of the rate-limiting turnover time of the electron transport, the same model was used as with isolated intact chloroplasts.

In the case of leaves it is reasonable to eliminate the thickness l from the expressions (7) and (8) in Barabás *et al.* (1985):

$$P = \frac{v_{ss}}{v_0} = \frac{1}{E'} \ln \frac{\tau S_0 E'/v_0 + e^{E'}}{\tau S_0 E'/v_0 + 1} \quad (1)$$

$$\tau = \frac{v_0}{2.3 A S_0} \frac{10^A - 10^{AP}}{10^{AP} - 1} \quad (2)$$

where $v(t) = n(t)l$, $v_0 = n_0 l$, $v_{ss} = n_{ss} l$ (cm^{-2}) are the area densities of open reaction centers, (i.e., those capable of initiating a complete turnover–either linear or cyclic–of the electron transport accompanied by a total electrochromic signal), S_0 is the incident photon flux ($cm^{-2}\ sec^{-1}$), $E' = El$ is the extinction of the leaf ($A = 2.3E'$ is the absorbance of the leaf), and P is determined as the proportion of the amplitude of ΔA_{515} with background light in the steady state and in the dark.

Results

Effect of Monochromatic Background Illumination on ΔA_{515}

Time Course. Upon onset of the monochromatic background light the amplitude of ΔA_{515} decreased and in about 10–100 sec reached steady state. The time course of the change of the amplitude of ΔA_{515} was markedly different in red ($\leqslant 680$ nm) and in far red ($\geqslant 700$ nm) light. Upon onset of the high intensity red background light (650 nm in Fig. 1A), ΔA_{515} decreased considerably. Prolonged illumination, however, reversed the tendency and led to a gradual increase of ΔA_{515}. The calculated turnover time of the overall electron transport (cf. Materials and Methods) is plotted in Fig. 1A (dashed line) and shows that the turnover was considerably accelerated by the red background illumination. This induction-like acceleration of the turnover is clearly linked to the activity of PS 2, because in 725 nm background light a gradual deceleration of the overall electron transport was observed (Fig. 1B). The amplitude of ΔA_{515} with far red background illumination underwent a monotonic decrease until it reached the steady-state value, usually in about 10–100 sec. Figure 1B also shows that when the background light was switched off, the amplitude of ΔA_{515} reversed rapidly to the dark value. The reversibility of changes was always retained in our experiments both with high-intensity red and far red light.

Intensity Dependence. An increase in the intensity of the background light resulted in a decrease in the amplitude of ΔA_{515}. (Hereafter, if not indicated otherwise, the flash-induced absorbance transients were measured after the steady state was reached.) In 650 nm light an appreciable decrease

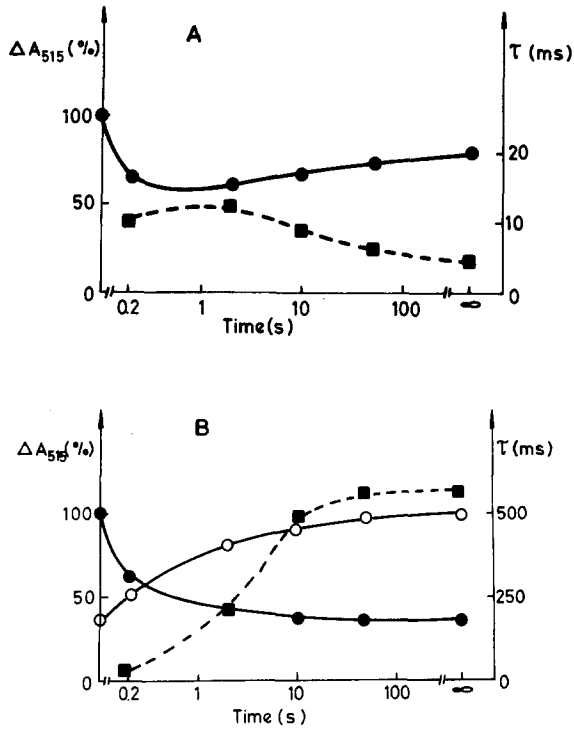

Fig. 1. Total amplitude of ΔA_{515} as a function of time after the onset (●) of the continuous monochromatic background light and the corresponding turnover time values (τ, msec) (■). A: 650 nm background light. Incident photon flux (S_0): 3.7×10^{15} cm^{-2} sec^{-1}; estimated area density of reaction centers (ν_0): 4×10^{13} cm^{-2}; absorbance (A): 0.4. In the calculation of τ it was assumed that 100% of the total amplitude of ΔA_{515} could be suppressed by sufficiently high intensity background light. B: 725 nm background light. Incident photon flux (S_0): 1.5×10^{16} cm^{-2} sec^{-1}; estimated area density of PS 1 reaction centers (ν_0): 2×10^{13} cm^{-2}; absorbance (A): 0.04. (○) recovery of ΔA_{515} after 2 min preillumination. In the calculation of τ it was assumed that a maximum of 65% of the total amplitude could be eliminated by the far red background illumination.

of ΔA_{515} could only be achieved by relatively high-intensity background illumination (Fig. 2A). This indicates that under steady-state conditions the density of the open reaction centers remains relatively high. A comparison with the theoretical curves shows that the turnover time in 650 nm light can be estimated to be between 5 and 10 msec. This turnover time can be assigned to that of the linear electron transport.

In 725 nm light the amplitude of ΔA_{515} could be suppressed relatively easily (Fig. 2B). (Note that the number of absorbed photons per unit time is much smaller at 725 nm than at 650 nm.) This indicates that in 725 nm light the steady-state turnover rate of the electron transport is considerably slower

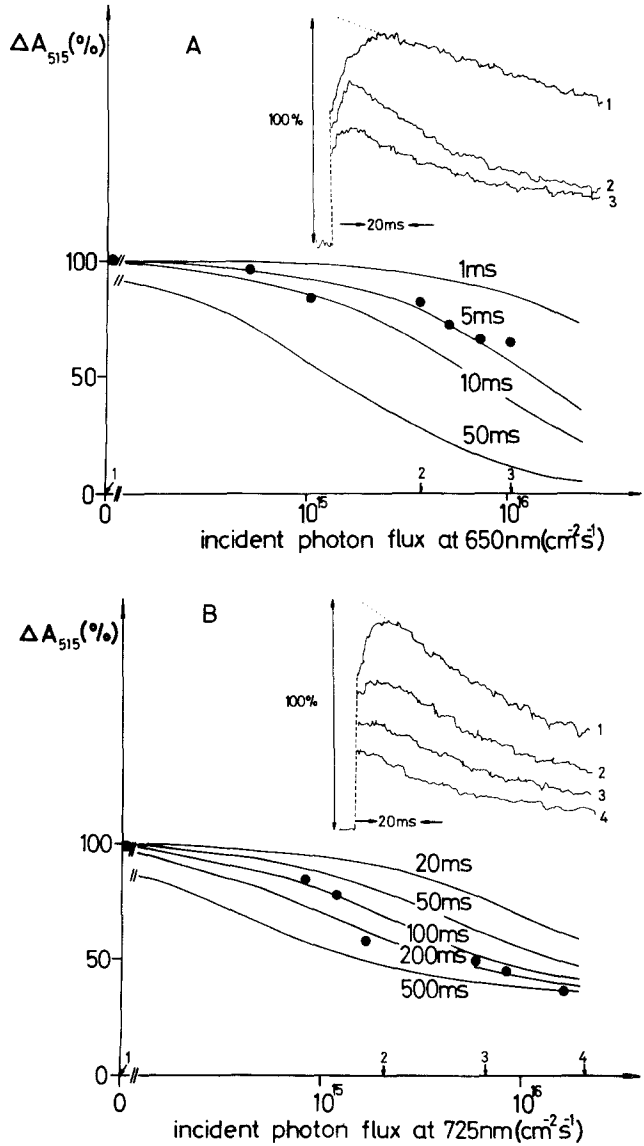

Fig. 2. Dependence of the total amplitude of ΔA_{515} on the intensity of the background light. Typical kinetics are shown in inset at different light intensities marked by 1, 2, and 3 on the abscissa. A and B: 650 and 725 nm background illumination, respectively. Individual points show the measured values, continuous curves are the theoretical intensity dependence calculated from Eq. (1). It was assumed that 100% of the total amplitude of ΔA_{515} could be suppressed with 650 nm and 65% with 725 nm background light. Estimated area density of reaction centers (v_0): 4×10^{13} cm^{-2} in the case of 650 nm and 2×10^{13} cm^{-2} in the case of 725 nm background light. Absorbance (A) of the leaf: 0.4 at 650 nm and 0.04 at 725 nm.

than that in red light. Since the 725 nm light excites predominantly PS 1, no considerable suppression of the PS 2 field generation can be expected.

We note here that in 725 nm light no precise estimation of τ can be given; for example, the stoichiometry of PS 2/PS 1 reaction centers and the fractional light absorbance of PS 2 were not determined in our samples. Nevertheless, it is clear from Fig. 2B that in the case of 725 nm illumination the calculated τ values are much larger than when both photosystems are excited in red light.

In leaves "in the dark" the ratio of the amplitude of the slow rise over the initial amplitude varied between about 0.3 and 0.7. The mean value obtained from nine independent experiments was 0.56. Illumination of the leaves with red light did not cause appreciable change in the ratio of the amplitudes of the slow and initial rises, while in the presence of far red light a pronounced decrease of the slow rise as compared to the initial one can be seen, indicating its close association with the PS 1 photoreaction (cf. Junge and Jackson, 1982; Peters *et al.*, 1984).

Wavelength Dependence. The effect of the background illumination on ΔA_{515} was measured as a function of the wavelength of the background light (Fig. 3). It can be seen that while the calculated steady-state turnover rate of the overall electron transport is rapid in the case of red background light, it is quite slow in far red background light. The turnover time increases in parallel with the decrease of the fractional absorption of PS 2 (cf. Hoch and Martin, 1963).

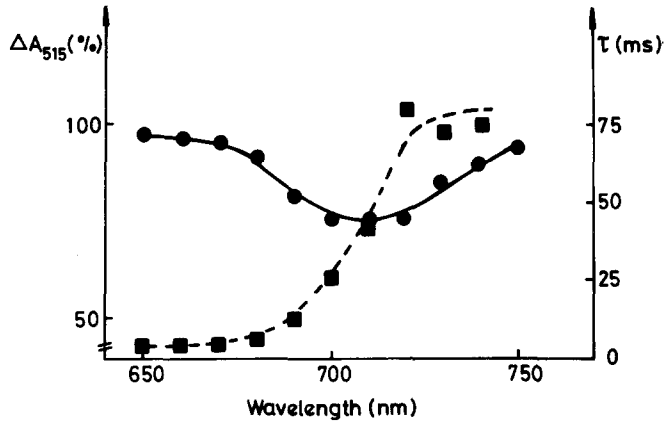

Fig. 3. Dependence of the total amplitude of ΔA_{515} on the wavelength of the background illumination (●) and the corresponding turnover time values (τ) (■) calculated from Eq. (2). In the calculations it was assumed that 100% of the total amplitude of ΔA_{515} could be abolished in the entire wavelength range. The incident photon flux (S_0) was between 1–1.8 $\times$ 10^{15} cm^{-2} sec^{-1}; estimated area density of reaction centers (ν_0): 4 $\times$ 10^{13} cm^{-2}; absorbance (A) at 680 nm: 0.92.

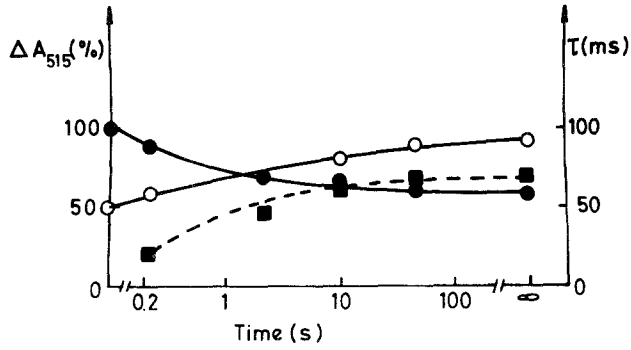

Fig. 4. Total amplitude of ΔA_{515} in DCMU-treated leaves as a function of time after the onset (●) of the 650 nm background illumination. (■) corresponding turnover time values (τ, msec). (○) the restoration of ΔA_{515} following the offset of background illumination after 2 min pre-illumination. Incident photon flux (S_0): 4×10^{14} cm^{-2} sec^{-1}; absorbance (A): 0.4; estimated area density of reaction centers (v_0): 2×10^{13} cm^{-2}. In the calculation of τ it was assumed that 100% of the DCMU-insensitive total amplitude of ΔA_{515} could be eliminated by the background light. (For other conditions see Fig. 1.)

Inhibition of PS 2. When the leaves were infiltrated in water containing 50 μM DCMU, the amplitude of ΔA_{515} was about 60% of that in the absence of the PS 2 inhibitor. In the presence of DCMU the apparent amplitude of the slow rise was also considerably smaller than in the control (cf. Garab *et al.*, 1983 for details).

In DCMU-treated leaves red light of moderate intensities could significantly decrease the amplitude of ΔA_{515}, whereas in the control it could only be achieved with high-intensity background illumination. As a typical example, 650 nm background light of 4×10^{14} photons cm^{-2} sec^{-1} incident intensity caused about 5 and 50% decrease in the amplitude of ΔA_{515} in the control and in DCMU-treated leaves, respectively. It must be noted that in DCMU-treated leaves full reversibility of the background light-induced decrease in the amplitude of ΔA_{515} is not retained, but the signal is restored in the dark to about 80–90% of its original amplitude.

The time course of the 650 nm background light effect of ΔA_{515} in DCMU-treated leaves is depicted in Fig. 4. This time course resembles that measured in the control with 725 nm light. Similarly as in far red light the turnover is gradually decelerated after the onset of the background illumination and the calculated τ value under steady-state conditions is high relative to the control.

Discussion

Despite the very significant differences in the estimated turnover rate of the overall electron transport there is no significant difference between the

kinetic pattern of ΔA_{515} in leaves and isolated chloroplasts (cf. Barabás *et al.*, 1985).

The calculated τ values in leaves agree fairly well with those in algal cells. In algae a turnover time of 10–20 msec was found to be associated with the PS 1 electron transport (Joliot and Delosme, 1974), as determined from the dependence of the initial amplitude of ΔA_{515} on the time interval between two consecutive flashes.

In 650 nm light the amplitude ratio of the initial and slow rises was invariant to the intensity of the background light. This observation is at variance with earlier results. It was reported that in preilluminated algal cells the large slow rise diminished together with an acceleration of the decay (Witt and Moraw, 1959; Joliot and Delosme, 1974). In preilluminated samples the slow rise may be masked by the fast decay of ΔA_{515} also in our case. A very rapid decay of ΔA_{515} was observed in leaves deficient in CO_2 and was absent in leaves (or algal cells) supplied with CO_2 (Garab *et al.*, 1983).

Those reactions which are involved in the reaction route restoring the electrogenic capacity of the reaction centers are expected to be suppressed in background illumination. Indeed, we found a considerable suppression of the amplitude of cytochrome *f* absorption change both in red and far red light (data not shown).

The deceleration of the turnover rate with DCMU or in far red light can be explained by the gradual consumption of the reducing equivalents of the plastoquinone pool and of the fast PS 1 donors. The number of excitations per PS 1 reaction center during the halftime of the gradual decrease of ΔA_{515} upon the onset of the background light is estimated to be 10 and 7.5 in the case of 725 nm (Fig. 1B) and 650 nm (+ DCMU) illumination (Fig. 4). These numbers of the reducing equivalents at the donor side of PS 1 are within the reducing capacity range of the plastoquinone pool (Cox and Olsen, 1982; Graan and Ort, 1984).

The steady-state τ values in far red light illuminated or in DCMU-treated leaves are characteristic of the turnover rate of the PS 1 electron transport without the contribution of PS 2. It can be seen that the turnover rate of the PS 1 electron transport under these conditions is very slow. However, reductive poising of PS 1 does not cease even in the absence of PS 2 electrons, as indicated, for example, by the large ($\sim 60\%$) residual signal of ΔA_{515} in DCMU-treated leaves. As far as the continuous, though very slow, supply of electrons is concerned, we may assume that it originates from some metabolic poising of NADP or of PS 1 donors. These possibilities are inferred from the results obtained in bundle sheath chloroplasts of maize (Leegood *et al.*, 1983) and from the results on the interaction between the photosynthetic and respiratory electron transport chains (Bennoun, 1982; Vermeglio and Joliot, 1984).

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