# Kinetics of the Flash-Induced Electrochromic Absorbance Change in the Presence of Background Illumination. Turnover Rate of the Electron Transport. I. Isolated Intact Chloroplasts

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## Abstract

We investigated the flash-induced electrochromic absorbance change,[3] $\Delta A_{515}$, of isolated intact chloroplasts in continuous monochromatic background light of different intensities and wavelengths. From the variation of the amplitude of $\Delta A_{515}$ in background illumination the steady-state turnover time of electron transport was found to be around 100 msec and the slowest process could be assigned to a photosystem 1 driven cycle. The change of $\Delta pH$ induced by nigericin, ATP, or ADP did not modify substantially the turnover time.

In contrast to earlier observations the slow rise ($\sim 10$ msec) of $\Delta A_{515}$ in untreated chloroplasts persists also at high-intensity background illumination exciting both photosystems. The proportion of the slow rise of $\Delta A_{515}$ in nigericin-treated chloroplasts increases in the presence of background light. This result on the slow rise is discussed in terms of two different models existing in the literature.

**Key Words:** Intact chloroplasts; electrochromism; slow rise of $\Delta A_{515}$; turnover rate of electron transport.

## Introduction

The flash-induced electrochromic absorbance change of the pigments embedded in the thylakoid membrane is characteristic of the buildup of the

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[3] Abbreviations: $\Delta A_{515}$, flash-induced electrochromic absorbance change at 515 nm; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium methosulfate; PS, photosystem.

transmembrane electric field and the consecutive utilization or dissipation of the electric field energy (Witt, 1979).

The flash-induced $\Delta A_{515}$ in isolated intact chloroplasts exhibits three distinct kinetic components (Junge and Jackson, 1982). An initial rise ($\ll 1$ msec) is followed by a slow rise peaking around 10 msec, and then the signal decays monotonously with a half time of about 100–500 msec. The initial rise has been unambigously shown to originate from the primary charge separation in the reaction centers of the two photosystems. It is also commonly accepted that the decay of the transmembrane potential difference characterizes the ion permeability of the membrane and the activity of ATP-ase. In contrast, the interpretation of the slow rise of $\Delta A_{515}$ is still controversial. Models proposed to account for this kinetic component of $\Delta A_{515}$ are either based on an additional charge separation along a cycle (photosystem 1 cyclic electron transport or a Q-cycle) pumping extra protons into the lumen (Crowther and Hind, 1980; Velthuys, 1980; Bouges-Bocquet, 1981; Selak and Whitmarsh, 1982) or on changes in the configuration of the electric field (Vredenberg, 1981; Olsen and Barber, 1981; Zimányi and Garab, 1982).

The kinetic pattern of $\Delta A_{515}$ has been shown to vary greatly under different experimental conditions, e.g., excitation of both photosystems or photosystem 1 alone (Crowther *et al.*, 1979), addition of various inhibitors (Horváth *et al.*, 1979), adenine nucleotides (Morita *et al.*, 1982; Schreiber and Rienits, 1982; Girault and Galmiche, 1978), uncouplers (Crowther *et al.*, 1979; Horváth *et al.*, 1979), etc. The kinetics of $\Delta A_{515}$ are regulated by factors such as luminal pH of thylakoids (Bouges-Bocquet, 1981) or synthesis or hydrolysis of ATP (Morita *et al.*, 1982; Schreiber and Rienits, 1982; Peters *et al.*, 1983).

We investigated the variations in the kinetics of $\Delta A_{515}$ in the presence of continuous monochromatic background illumination. The measured dependence of the amplitude of $\Delta A_{515}$ on the intensity of background illumination was utilized to calculate the "overall turnover rate" of electron transport in isolated intact chloroplasts under different experimental conditions.

## Materials and Methods

### *Plant Material, Reaction Media*

Intact chloroplasts were isolated from two-week-old pea leaves grown in the greenhouse according to Nakatani and Barber (1977). Intactness of the chloroplasts was tested by measuring ferricyanide reduction (Walker, 1980) and routinely was about 90%. The dense chloroplast suspension was adapted

to dim white light ($5\,mW/m^2$) at 4°C for at least half an hour prior to measurements. The standard reaction medium contained 0.35 M sorbitol, 20 mM Tricine, and 5 mM $MgCO_3$ at pH 7.7. Other additions are indicated in the figure captions or in Table I. The chlorophyll content of the suspension was adjusted to about 50 $\mu$M. The absorbance of the samples along the direction of the background illumination was around 1.0 at 680 nm, and the estimated density of reaction centers, $n_0$, was $7.2 \times 10^{13}\,cm^{-3}$ using the assumption that one chlorophyll molecule out of 500 is part of either a PS1 or a PS2 reaction center.

The measurements were repeated on samples with much lower chlorophyll content ($\sim 15\,\mu$M) and yielded similar results.

### Measurement of Absorbance Transients

The absorbance changes measured between 470 and 590 nm were induced by saturating flashes from a xenon lamp ($> 630$ nm, 3 $\mu$sec duration at half peak emission). The measurements were carried out at room temperature in a single-beam kinetic spectrophotometer (Horváth *et al.*, 1979) equipped with a side illumination attachment. The monochromatic background beam was perpendicular to the measuring beam and parallel to the direction of the excitation by the Xe flash. The optical pathlength of the cell containing the chloroplast suspension was 1 cm along the measuring beam and 0.5 cm along the direction of the background illumination. The wavelength of the background light was selected from the beam of a 100-W Xe arc lamp by a high-intensity Bausch and Lomb grating monochromator with a spectral slit width of 9.6 nm. In experiments where higher light intensities were required, interference filters were used between 680 and 650 nm and at 725 nm (Oriel 680 nm with appropriate angles between the plane of the filter and the light beam, half bandwidth 10 nm; and Zeiss 725 nm filter with a half bandwidth of 11 nm). A fiber optic system was used to guide the monochromatic background light from a shutter (time resolution < 10 msec, A. W. Vincent Assoc. Inc., type L 2175) to the sample.

When the dependence of the amplitude and kinetics of $\Delta A_{515}$ on the wavelength and intensity of the background light was determined, a series of flashes at a frequency of $0.13\,sec^{-1}$ was started 1–2 min after the onset of the background illumination. This time was always sufficient to reach the steady state. Ten to twenty scans were collected in a multichannel analyzer (ICA 70, KFKI). When measuring the time course of the background light-induced changes in the amplitude of $\Delta A_{515}$ (or 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. The chloroplast suspension in these experiments was changed after each series; 10 kinetic traces were averaged this way.

The total amplitude of $\Delta A_{515}$ and the amplitude of the slow rise were 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

The amplitude of $\Delta A_{515}$ generated by a saturating flash is proportional to the density of open reaction centers. "Open" means the capability of performing a complete turnover of the electron transport (either linear or cyclic) accompanied by the appearance of the total electrochromic signal. After the onset of the background illumination, the density of open reaction centers eventually reaches its steady-state value. Evidently, both the steady-state level and the time course of approaching this level depend on the finite turnover rate of the electron transport. Specifically they are determined by the slowest process involved in the recovery of the field-generating capability of the reaction centers. The detailed calculation of the turnover time is given in the Appendix.

## Results

### Effect of Background Illumination on $\Delta A_{515}$ in Untreated Chloroplasts

When the chloroplasts were illuminated with continuous monochromatic background light, the amplitude of $\Delta A_{515}$ induced by saturating flashes decreased. (Similar changes were observed at other wavelengths between 470 and 540 nm.)

*Time Course.* Figure 1 shows that the steady-state value of $\Delta A_{515}$ is reached rapidly after the onset of the 650 and 725 nm background light (Fig.
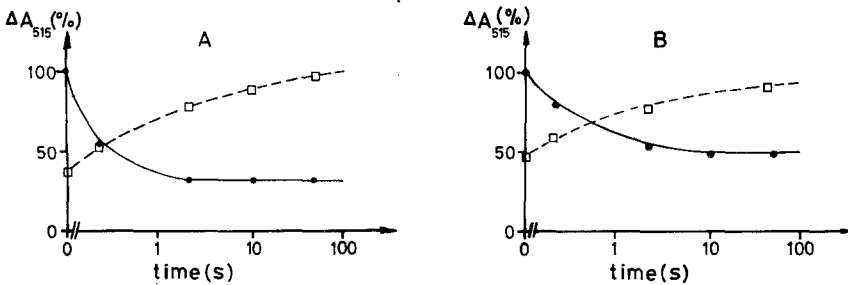


**Fig. 1.** Total amplitude of the flash-induced $\Delta A_{515}$ as a function of time after the onset (●) of the monochromatic continuous background light and restoration (□) of the signal following the offset of the background light after 2 min preillumination. A and B: 650 and 725 nm background light with an incident photon flux of $10^{15}$ and $1.7 \times 10^{15}$ cm$^{-2}$ sec$^{-1}$, respectively.

1A and B, respectively). On turning off the background light the amplitude of $\Delta A_{515}$ increased again and reached the original value within 1–2 min. Reversibility of the light-induced decrease of $\Delta A_{515}$ was retained after prolonged (5–8 min) illumination as well.

*Intensity Dependence.* The dependence of $\Delta A_{515}$ on the intensity of the background light of 650 nm and 725 nm is shown in Fig. 2A and B, respectively. When the experimental data are compared with the theoretical curves, one can see that the turnover rate, $\tau$, of the electron transport in the absence of externally added electron acceptors is very slow and does not depend significantly on the intensity of background illumination. It can also be seen that with high-intensity 650 nm background light the $\Delta A_{515}$ measured in the steady state could be almost completely abolished. However, the field-sensitive detector molecules are not appreciably affected by the background illumination since PMS can restore the decreased amplitude of $\Delta A_{515}$ in background light (see below).

Analysis of the kinetics of $\Delta A_{515}$ with 650 nm background illumination showed that the ratio of the amplitudes of the initial and slow rises, in contrast to the expectations (cf. Cramer and Crofts, 1982), remained about the same with or without background illumination of different intensities. (Hence the turnover rates could be calculated either from the total amplitude or the amplitude of the initial and slow rises.)

Upon illumination with 725 nm light both the initial and slow rises of $\Delta A_{515}$ decreased, but the relative decrease of the slow rise, in accordance with its close association with PS 1 (Junge and Jackson, 1982), was larger than that of the initial rise. With 725 nm background light $\Delta A_{515}$ could not be fully eliminated. Thus the theoretical curves were calculated using the assumption that 725 nm light can abolish only 50% of the total amplitude of $\Delta A_{515}$. If this value is inaccurate (e.g., if the stoichiometric ratio PS 2 : PS 1 is not 1 : 1), an error is introduced into $\tau$, which however, would not affect the main observation that the calculated turnover time is significantly higher in the case where PS1 is excited alone, than when both photosystems are excited by the background light.

*Wavelength Dependence.* Experiments in which the relative change of $\Delta A_{515}$ was determined as a function of the wavelength of background illumination showed that above 700 nm $\tau$ values were higher than below this wavelength (Fig. 3). (Values of $\tau$ above 700 nm are underestimated, since in the evaluation of the data in Fig. 3 we did not take into account the wavelength dependence of the fractional absorbance of PS 2.) The lowest values of $\tau$ were regularly obtained around 680 nm, which suggests that the optimal turnover rate of energization can be achieved by a balanced excitation of the two photosystems. Values of $\tau$ in different preparations varied in relatively broad intervals, e.g., for 650 and 680 nm they were obtained
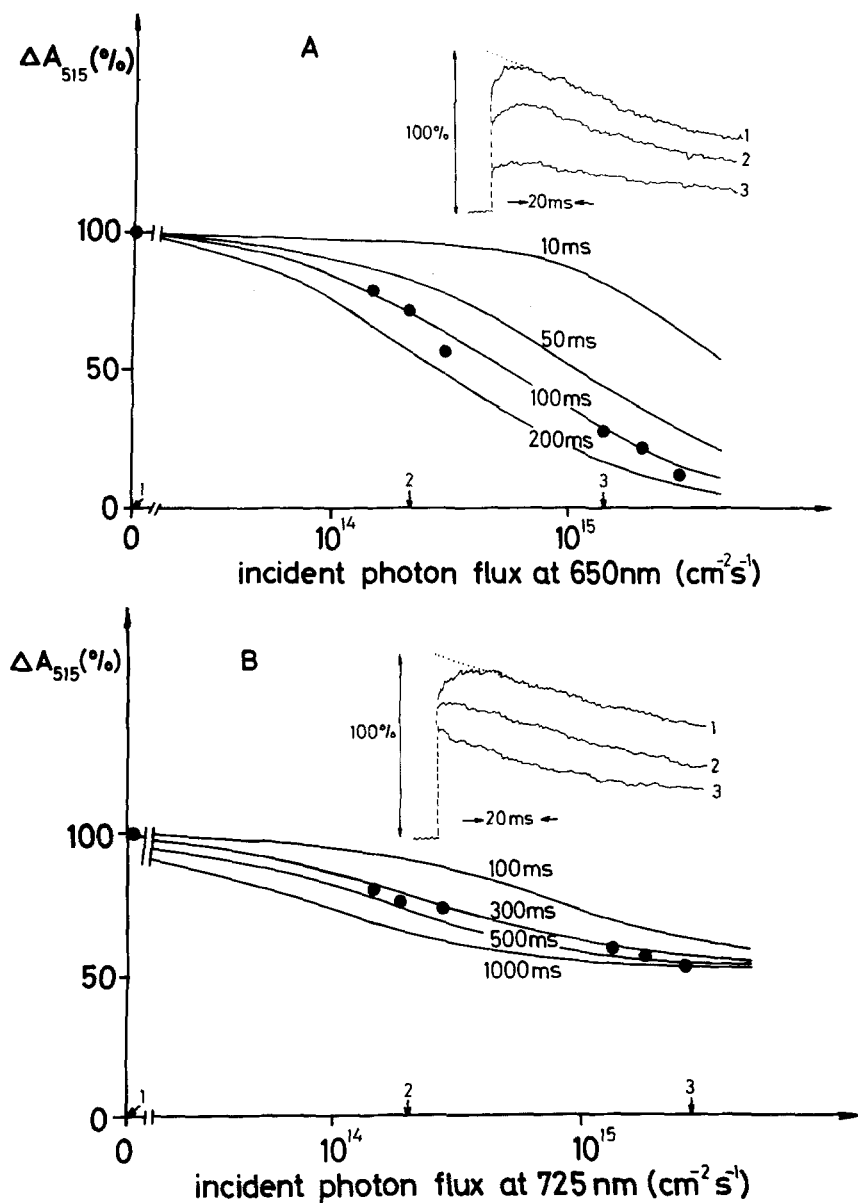
**Fig. 2.** Dependence of the steady-state total amplitude of $\Delta A_{515}$ on the intensity of the background light. Typical kinetics are shown in inset at different light intensities marked by 1, 2, and 3 on the abscissa. A and B: 650 and 725 nm background illumination, respectively. Individual points show the measured values; continuous curves are the theoretical intensity dependence plots calculated from Eq. (7). It was assumed that 100% of the total amplitude of $\Delta A_{515}$ could be eliminated with 650 nm and 50% with 725 nm background light.
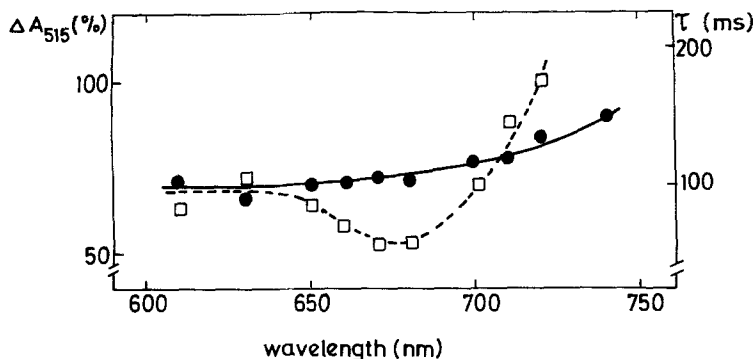
**Fig. 3.** Dependence of the total amplitude of $\Delta A_{515}$ on the wavelength of the background illumination (●) and the corresponding turnover time values ($\tau$) (□), calculated from Eq. (8). In the calculation of $\tau$ it was assumed that 100% of the total amplitude of $\Delta A_{515}$ could be abolished in the whole wavelength range. The incident photon flux was between 2.5–3.2 × $10^{15}$ cm$^{-2}$ sec$^{-1}$.

between 80 and 200 msec and 50 to 150 msec, respectively. Around 725 nm the calculated values of $\tau$ were usually higher than 200 msec. The above results suggest a slow process associated with PS 1 in isolated intact chloroplasts. To explore this possibility we introduced various agents in chloroplast suspensions.

### Effect of Background Illumination on $\Delta A_{515}$ in the Presence of Different Agents

*DCMU + Na-dithionite.* After 20 preilluminating flashes following the addition of a saturating amount of DCMU ($> 10\,\mu$M), the amplitude of $\Delta A_{515}$ decreased to about 10–25% of that in the absence of the inhibitor (cf. Fig. 3 in Farineau *et al.*, 1984). This residual signal originated from PS 1 rather than from PS 2. This was judged from the fact that the DCMU-insensitive signal could amost be eliminated with relatively weak 725 nm background light (data not shown) or by the addition of 0.1 mM methylviologen (Farineau *et al.*, 1984).

The initial rise generated by PS 1 as well as the slow rise could be restored in DCMU-treated samples by addition of Na-dithionite in millimolar concentrations (Crowther *et al.*, 1979). When we tested intact chloroplasts both with 650 and 725 nm background light in the presence of $20\,\mu$M DCMU and 10 mM dithionite, we found that the initial and the slow rises decreased proportionally. The ratio of the initial and the slow rise remained approximately 1, while the amplitude of $\Delta A_{515}$ decreased in a similar manner

as in Fig. 2A and yielded $\tau$ values usually higher than 100 msec (data not shown). These experiments show that the slow turnover rate of the thylakoid energization can be obtained also in samples where PS 2 is not in operation.

*PMS.* PMS (in either oxidized or reduced form) maintains an artificial PS 1 cyclic electron transport bypassing the ferredoxin-NADP reductase (Hauska, 1977) and feeds electrogenic charge separation in PS 1 (Roux and Faludi-Dániel, 1977). PS 2 and PS 1 when combined in a linear electron transport (in the presence of PMS) yield a turnover time value not more than 20 msec (Table I). The above experiments strongly suggest that the slow process ($\geqslant 100$ msec) is part of the cyclic electron transport around PS 1. This possibility was further explored in experiments with antimycin-A.

*Antimycin-A.* Antimycin-A, an inhibitor of the cyclic electron transport driven by PS 1 (Raven, 1976), inhibited part of the initial and the slow rise (Fig. 4 inset). In the presence of antimycin-A the background light caused considerably smaller variations in the flash-induced $\Delta A_{515}$ than in the absence of this inhibitor. (With antimycin-A concentrations smaller than 50 $\mu$M the background light-induced changes were reversible to about 90–100% of the signal in the dark.) When the effect of the background light on $\Delta A_{515}$ is plotted against the concentration of antimycin-A, it is seen that the inhibitor acts primarily on that part of $\Delta A_{515}$ which is very sensitive to the background illumination (Fig. 4). In our experiments antimycin-A had to be used at high concentrations probably because of its poor penetration into intact chloroplasts (Shahak *et al.*, 1981); the effectiveness of the inhibitor may also vary

**Table I.** Relative Amplitudes of the Kinetic Components of the Flash-Induced $\Delta A_{515}$ in the Dark and in 650 nm Background Light and the Corresponding Steady State Turnover Times $\tau$ of the Electron Transport in Isolated Intact Chloroplasts[a]

| Addition | Initial rise (%) | | $\tau$ (msec) | $\dfrac{\text{slow rise}}{\text{initial rise}}$ (%) | |
| | $\dfrac{\text{dark}}{\text{dark (control)}}$ | $\dfrac{\text{light}}{\text{dark}}$ | | Dark | Light |
|---|---|---|---|---|---|
| Control | 100 | 46 | 133 | 41 | 40 |
| 2 $\mu$M nigericin + 10 mM KCl | 95 | 49 | 117 | 44 | 93 |
| 330 $\mu$M ADP | 105 | 57 | 85 | 34 | 45 |
| 330 $\mu$M ATP | 89 | 52 | 104 | 22 | 18 |
| ATP + nigericin | 85 | 48 | 122 | 42 | 67 |
| 25 $\mu$M antimycin-A | 86 | 74 | 39 | 30 | 21 |
| 20 $\mu$M PMS | 115 | 86 | 18 | – | – |

[a] Mean values of the relative amplitudes were calculated from three to seven independent experiments; the incident photon flux at 650 nm was $4.5 \times 10^{15}$ cm$^{-2}$ sec$^{-1}$; turnover times were calculated according to Eq. (8) from the initial amplitudes. For other details see Materials and Methods.
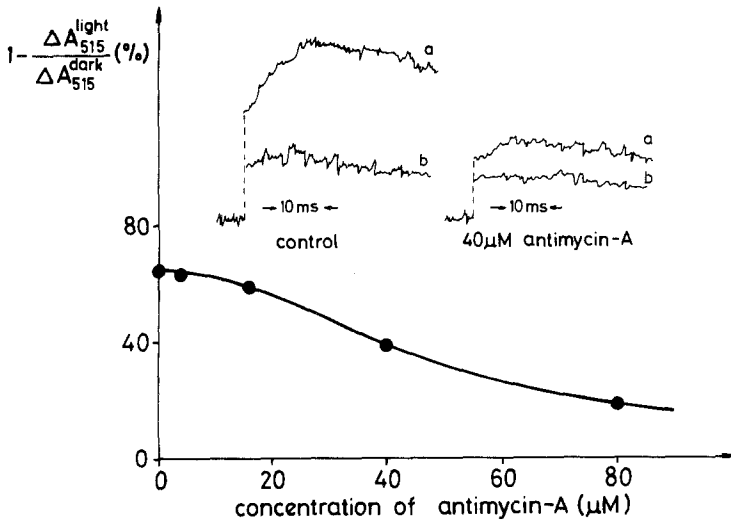
**Fig. 4.** The relative change of $\Delta A_{515}$ with background light in the steady state as compared to $\Delta A_{515}$ in the dark as a function of the antimycin-A concentration. Typical kinetics are shown in the inset; a and b: in the dark and in 650 nm background light with an incident photon flux of $10^{15}$ cm$^{-2}$ sec$^{-1}$.

over a very broad concentration range among different plant species (Woo *et al.*, 1983).

   *ATP.* Upon addition of ATP to the sample both the initial and slow rises decreased (Fig. 5). The relative decrease of the slow rise was larger than that of the initial rise (Table I). The slow decay was accelerated by ATP from 260 msec to 180 msec (mean values of half decay times from seven independent experiments). In background light both with and without ATP the decay was accelerated. The above data obtained in intact chloroplasts are in fair agreement with the results reported on the ATP effect on $\Delta A_{515}$ of chloroplasts in hypotonic medium (Schreiber and Rienits, 1982). We must emphasize that in our samples the concentration of the adenine nucleotides was probably much lower inside the chloroplasts than outside. Nevertheless, at external concentrations higher than $100\,\mu$M internal effects of ATP and ADP could clearly be recognized. Whereas the kinetic pattern of $\Delta A_{515}$ in the presence of externally added ATP changed considerably, no appreciable difference was seen between the calculated turnover rates of the electron transport with and without ATP (Table I; cf. also Fig. 5).

   *ATP + Nigericin.* When nigericin was added together with or after ATP, the initial rise of $\Delta A_{515}$ decreased to about the same extent as with ATP in the absence of nigericin. In the presence of the uncoupler, however, upon addition of ATP the amplitude of the slow rise did not change markedly
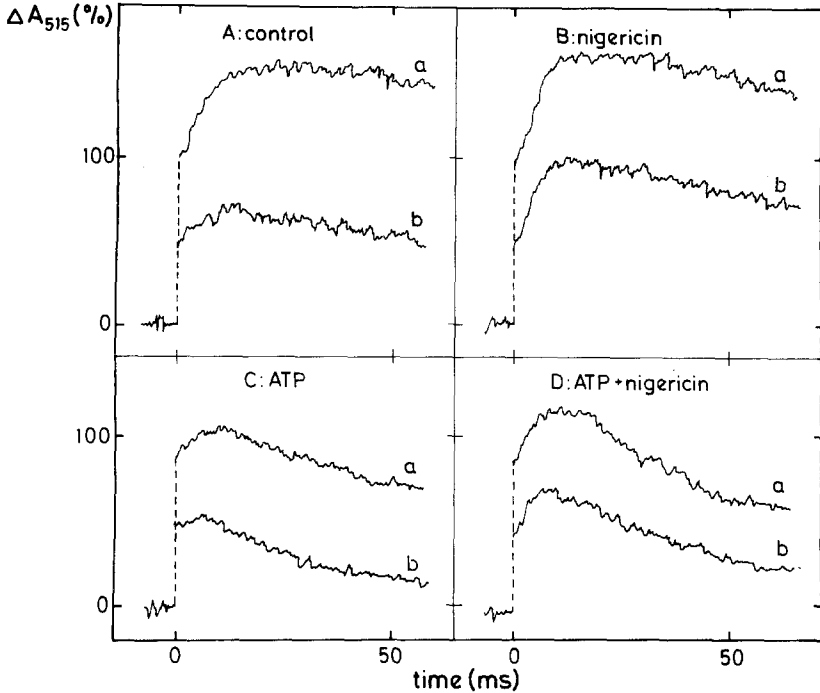
**Fig. 5.** Typical kinetics of $\Delta A_{515}$ in the dark (a), and in 650 nm background illumination with an incident photon flux of $4.5 \times 10^{14}$ cm$^{-2}$ sec$^{-1}$ (b). Additions were given in the following concentrations: nigericin, $2\,\mu$M plus 10 mM KCl; ATP, 330 $\mu$M.

(Fig. 5). Nigericin (both in the dark and light) prevents the inhibitory effect of ATP on the slow rise (Fig. 5). This result can be interpreted by taking into account the fact that $\Delta$pH caused by the hydrolysis of ATP plays an important role in determining the amplitude of this kinetic component (Bouges-Bocquet, 1981).

*Nigericin.* With 650 nm background illumination the initial rise of $\Delta A_{515}$ of chloroplasts in the presence of nigericin (40 nM–1 $\mu$M) decreased to about the same extent as in the control samples. However, the amplitude of the slow rise hardly decreased (Fig. 5, Table I). Thus, the proportion of the slow rise increased. Similar tendencies were observed in the presence of externally added ADP (Table I).

## Discussion

Under continuous excitation of chloroplasts the slowest process involved determines the recovery rate of the electrogenic activity of the reaction

centers. If the rate of two or more slow processes is about the same, or if the rate-limiting reaction is not a first-order process, a more sophisticated model than the one presented above should be applied. Nevertheless, the calculated $\tau$ values, as a first approximation, can be accepted as estimates of the steady-state turnover time of the overall electron transport.

The slow turnover rate of the electron transport could also be clearly recognized from the background illumination effect on the absorbance transients of cytochrome $f$. In background light the amplitude of the absorbance change $\Delta A_{554} - \Delta A_{540}$ in the presence of 1.5 $\mu$M valinomycin plus 10 mM KCl decreased in parallel with the amplitude of $\Delta A_{515}$ (data not shown).

The results indicate that the slow turnover rate is intimately linked to the cyclic electron transport around PS 1. The significance of the cyclic electron transport can also be shown by the large antimycin-A sensitivity of $\Delta A_{515}$ without background light (Fig. 4).

It is well known that in continuous light $\Delta$pH plays an important role in the energization of thylakoids. The results in the presence of ATP, ADP, or nigericin show that the slow turnover time is not affected markedly by $\Delta$pH.

PS 1 cyclic electron transport and the large slow rise in $\Delta A_{515}$ have been shown to be mutually associated under different experimental conditions in isolated intact chloroplasts (Crowther and Hind, 1980; Crowther et al., 1979; Crowther et al., 1983) and also in PS 1 enriched subchloroplast vesicles (Peters et al., 1984a). Our data show that the turnover rate of the PS 1 cycle is much slower than the rate of the slow rise of $\Delta A_{515}$. Furthermore, in our experiments no correlation was found between the actual values of $\tau$ and the amplitude or the rate of the slow rise. Thus direct correlation between this kinetic component of $\Delta A_{515}$ and the PS 1 cycle is not likely. We can assume that the slow rise does not originate from the PS 1 cycle but the cycle maintains (or helps to maintain) the proper redox conditions at the donor side of PS 1. Large slow rise of $\Delta A_{515}$ has been observed in isolated thylakoids in the presence of duroquinol and methylviologen (Selak and Whitmarsh, 1982), showing that the conditions necessary to induce the slow rise can also be independent of the PS 1 cycle.

It is generally agreed that the slow rise of $\Delta A_{515}$ is linked to the operation of the Fe–S–cytochrome $b/f$ complex and reflects the electrogenic nature of an inward pump of protons at the donor side of PS 1.

Interpretation of the relative enhancement of the slow rise in background light in the presence of ADP or nigericin (Table I) can be different in the different models explaining the origin of the slow electrochromic rise.

(i) It has been suggested by several investigators (Velthuys, 1980; Bouges-Bocquet, 1981; Selak and Whitmarsh, 1982; Jones et al., 1984) that

the slow rise of $\Delta A_{515}$ originates from an additional charge separation occurring in the dark after flash excitation along a Q-cycle pumping extra protons into the lumen. An electrogenic Q-cycle has been proposed to function between the two photosystems (Velthuys, 1978; Bouges-Bocquet, 1977; Rich, 1984) or in close association with the cyclic electron transport driven by PS 1 (Crowther and Hind, 1980). In both cases the extra charge separation is thought to occur via the involvement of cytochrome $b6$. However, there is evidence suggesting that the slow rise and the cytochrome $b6$ reaction are not directly correlated (van Kooten *et al.*, 1983; Girvin and Cramer, 1984; Peters *et al.*, 1984$b$). To explain the effect of nigericin in the frame of this model, we must suppose that nigericin treatment in the presence of background illumination considerably increases the $H^+/e^-$ ratio relative to both the "dark" value as well as the control with 650 nm background illumination. Taking into account the close correlation between PS 1 charge separation and the slow rise (Junge and Jackson, 1982; Peters *et al.*, 1984$a$), the increased $H^+/e^-$ ratio can be accounted for either by multiple turnover of the Q-cycle upon each PS 1 photoreaction or by a decreased PS 2/PS 1 ratio of the field-generating reaction centers.

(ii) Other investigators suggested that the slow rise of $\Delta A_{515}$ can be explained in terms of changes in the configuration of the electric field (Vredenberg, 1981; Olsen and Barber, 1981; Zimányi and Garab, 1982). It has been shown that when charges are translocated from the dielectric membrane into the conductive phases of the thylakoid the intensity of the uniform transmembrane field detected by the field-sensitive pigments may increase (Zimányi and Garab, 1982). A particular charge translocation which was suggested to be responsible for a slow rise was the inward release of protons at the donor side of PS 1. To interpret the effect of nigericin on background light-illuminated chloroplasts we can assume again that the ratio of active reaction centers, PS 2/PS 1, decreases. Alternatively, if the ratio of the active reaction centers does not significantly vary, we must assume that the reaction pathway is changed so that charge translocation effects on $\Delta A_{515}$ become more discernible. A possible change may occur if the positive hole is buried more into the dielectric membrane and the consecutive proton release gives rise to a large increment of the transmembrane uniform field (Zimányi and Garab, 1982).

The present data do not allow us to distinguish between the above two (not necessarily conflicting) hypotheses for the origin of the slow rise.

## Appendix

Let us consider a sample of thickness $l$ (cm); let $n(x, t)$ (cm$^{-3}$) be the density of open reaction centers at time $t$ and distance $x$ measured in the

sample along the background light beam. Let us define $\sigma$ ($cm^2$) as the average nominal absorbance cross section per reaction center. The background light intensity at a distance $x$ in the sample is $S(x)$ (number of photons $cm^{-2}\,sec^{-1}$), with $S_0 = S(x = 0)$ being the incident light intensity. The initial value of the density of open reaction centers, $n(x, t = 0)$, equals $n_0$ everywhere in the sample; $t = 0$ corresponds to the onset of background illumination.

The change of the density of open reaction centers at a distance $x$ is described by the differential equation

$$\frac{d}{dt}\,n(x,\ t)\ =\ -S(x)\ \sigma n(x,\ t)\ +\ \frac{1}{\tau}[n_0 - n(x,\ t)] \tag{1}$$

where $\tau$ is the rate-limiting turnover time of the electron transport, or more precisely the lifetime of the slowest process involved in the recovery of the field-producing ability of a previously excited reaction center.

In general, $\tau$ itself may depend on time, but if its change is slow enough in the time domain determined by the characteristic rate $k(x)$ (see below), Eq. (1) is a good approximation. The slow variation of $\tau$ can be taken into account in this case as if the system went through a set of steady states obtained from the solution of Eq. (1) with different fixed values of $\tau$. Hence in most cases Eq. (8) (see below) may be used also when the time course of $\tau$ upon the onset of background illumination is to be determined.

The solution of Eq. (1) is as follows:

$$\frac{n(x,\ t)}{n_0}\ =\ \frac{1}{k(x)\tau}\ +\ \frac{k(x)\tau - 1}{k(x)\tau}\,e^{-k(x)t} \tag{2}$$

with $k(x) = \sigma S_0 e^{-Ex} + 1/\tau$, where $E = n_0\sigma$ is the extinction coefficient of the sample. The expression (2) must be averaged over the thickness $l$ of the sample:
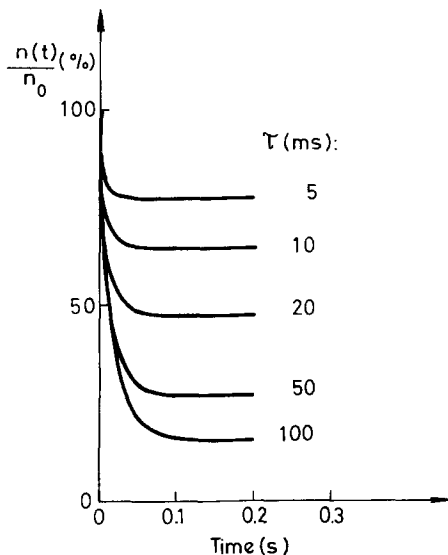
$$\frac{n(t)}{n_0}\ =\ \frac{1}{l}\int_0^l \frac{n(x,\ t)}{n_0}\,dx$$

In order to perform the integration, (2) is written in the form:

$$\frac{n(x,\ t)}{n_0}\ =\ \frac{e^{Ex}}{e^{Ex} + \alpha}\ +\ \frac{\alpha}{e^{Ex} + \alpha}\exp\left[-\frac{t}{\tau}(1 + \alpha e^{-Ex})\right] \tag{3}$$

with $\alpha = \sigma\tau S_0$. The average of the first term on the right-hand side of expression (3) then yields

$$\frac{1}{l}\int_0^l \frac{e^{Ex}}{e^{Ex} + \alpha}\,dx\ =\ \frac{1}{El}\ln\frac{\alpha + e^{El}}{\alpha + 1} \tag{4}$$

**Fig. 6.** Theoretical time course of the relative change of the density of open reaction centers $[n(t)/n_0]$ with different values of the rate-limiting turnover time ($\tau$, msec). Incident photon flux $(S_0)$: $3.7 \times 10^{15}$ cm$^{-2}$ sec$^{-1}$; density of reaction centers $(n_0)$: $8 \times 10^{13}$ cm$^{-3}$; extinction $(E)$: 0.8 cm$^{-1}$; pathlength $(l)$: 0.5 cm.

The average of the second term is

$$\frac{1}{l}\int_0^l \frac{\alpha\exp\left[-(t/\tau)(1+\alpha e^{-Ex})\right]}{e^{Ex}+\alpha}\,dx = -\frac{1}{El}\int_{(t/\tau)(1+\alpha)}^{(t/\tau)(1+\alpha e^{-El})}\frac{e^{-z}}{z}\,dz$$

$$= \frac{1}{El}\left[\ln z - z + \frac{z^2}{2\cdot 2!} - \frac{z^3}{3\cdot 3!} \pm \cdots\right]_{(t/\tau)(1+\alpha e^{-El})}^{(t/\tau)(1+\alpha)} \tag{5}$$

with the substitution $z = (t/\tau)(1 + \alpha e^{-Ex})$. Using expressions (4) and (5) we can get the average of $n(x, t)/n_0$ over $x$:

$$\frac{n(t)}{n_0} = 1 + \frac{1}{El}\left[-\frac{t}{\tau}(\beta - \gamma) + \left(\frac{t}{\tau}\right)^2\frac{(\beta^2 - \gamma^2)}{2\cdot 2!} - \left(\frac{t}{\tau}\right)^3\frac{(\beta^3 - \gamma^3)}{3\cdot 3!} \pm \cdots\right] \tag{6}$$

where $\beta = 1 + \alpha = 1 + \tau S_0 E/n_0$ and $\gamma = 1 + \alpha e^{-El} = 1 + (\tau S_0 E/n_0)\, e^{-El}$. Typical kinetics expected according to Eq. (6) are shown in Fig. 6.

The steady-state concentration of open reaction centers can be calculated by the substitution $t \to \infty$ in the expressions (4) and (5). In (5) the integrand converges to zero if $t \to \infty$; thus the whole integral equals zero when steady state (subscript ss) is reached. Therefore

$$\frac{n_{ss}}{n_0} = \frac{1}{El} \ln \frac{\alpha + e^{El}}{\alpha + 1} \tag{7}$$

By introducing $P = n_{ss}/n_0$ and $A = El/2.3$, the absorbance of the sample, $\tau$, can be expressed as

$$\tau = \frac{n_0 l}{2.3 A S_0} \frac{10^A - 10^{AP}}{10^{AP} - 1} \tag{8}$$

$P$ can be taken to be the proportion between the amplitude of $\Delta A_{515}$ with background light in the steady state and in the dark.

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