Biochimica et Biophysica Acta, 1057 (1991) 337–345

ADONIS 0005272891001078

BBABIO 43374

Effect of light-induced changes in thylakoid voltage on chlorophyll fluorescence of *Aegopodium podagraria* leaves

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(Received 7 September 1990)

Key words: Charge separation; Chlorophyll fluorescence; Electrochromic absorption change; Thylakoid potential; Time constant

The millisecond kinetics of light-induced changes in thylakoid voltage as detected by absorbance changes at 520 nm were compared with the kinetics of the fluorescence yield on *Aegopodium podagraria* leaves. Frequency response measurements with red actinic light and the analysis of the response to far-red light flashes reveal that the light induced increase in thylakoid voltage is coupled to an increase in fluorescence yield. An increase in fluorescence yield of 5.4% per 10 mV increase in thylakoid voltage is found after calibration of the 520 nm absorbance changes by single-turnover flashes which are assumed to induce an increase in thylakoid voltage by 25 mV. The measured voltage sensitivity of the fluorescence yield compares well to calculations based on the assumption that the rate constant of PS II charge separation is sensitive to electric fields as proposed by Schatz et al. (Biophys. J. 54 (1988) 379–405). Based on the results of the frequency response measurements, it is estimated that about 7% of the O-I phase of the fluorescence kinetics and 10% of the fluorescence response to single-turnover flashes are due to the voltage effect. It is discussed whether transthylakoid voltage might be of relevance for the control of PS II electron flux.

Introduction

Upon illumination of chloroplasts, excitation energy collected by antenna pigments leads to a charge separation in the photosystems followed by vectorial electron transfer across the thylakoid membrane. One consequence of this process is an electric potential difference between the aqueous phases separated by the membrane with the more negative potential at the outer side. Therefore, under continuous illumination the light-driven reactions of the photosystems have to proceed in the presence of an electric field opposing the electron transfer across the thylakoid membrane. The question arises of whether and how the light-induced electric potential difference and the resulting electric field influence the electrogenic reactions of the photosystems.

The effect of electric fields on the reverse reactions, i.e., stimulation of reverse electron flow by the field, has been extensively studied by analysis of delayed fluorescence emission [1–5]. It is proposed that the rate of Photosystem II charge separation is controlled by the redox state of quinone acceptor, Q_a, by means of local electric fields. This hypothesis implies that also a delocalized field across the thylakoid membrane should diminish the rate of charge separation, resulting in an increase in the yield of chlorophyll fluorescence. Indeed, results of fluorescence studies on osmotically swollen chloroplasts subjected to electric field pulses [6] and on isolated giant chloroplasts of *Peperonia metallica* [7] using microelectrodes are in line with the idea that the thylakoid voltage influences the rate of charge separation.

Here, the influence of light-induced thylakoid voltages on the yield of PS II fluorescence of *Aegopodium podagraria* leaves are investigated. The results are of relevance as a test of the hypothesis that the rate of charge separation is highly sensitive to electric fields. Furthermore, a control of the rate of charge separation by light-induced thylakoid voltages might be of importance for the regulation of the photosynthetic light reactions in vivo.

By measuring electrochromic absorbance changes at

Abbreviations: A_{520}, absorption changes measured at 520 nm; F, fluorescence yield; F_m, fluorescence yield with reduced primary quinone acceptor; F_0, fluorescence yield with oxidized primary quinone acceptor oxidized; PS, Photosystem; P680, primary electron donor of PS II; Q_A, primary quinone acceptor of PS II.

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520 nm it is possible to detect changes of the thylakoid voltage in the range of milliseconds in chloroplasts [8] as well as in intact leaves [9–12]. Since chlorophyll fluorescence of higher plants originates almost exclusively from PS II at room temperature [13], the yield of PS II fluorescence can easily be monitored by measurements of modulated fluorescence. Changes in thylakoid voltage were induced by variations of the intensity of the actinic light. The changes of fluorescence yield occurring concomitantly to the changes of the thylakoid voltage were analyzed.

The problem of such an investigation is the complexity of the kinetics of chlorophyll fluorescence. However, the application of linearizing experimental conditions [14,15] enabled the detection of a common kinetic component in the changes of chlorophyll fluorescence and of absorption at 520 nm. From the related amplitude factors the magnitude of the voltage effect on fluorescence yield was determined and compared with a value calculated under the assumption that mainly the primary charge separation is sensitive to electric fields as proposed by Schatz et al. [4]. Possible implications for the interpretation of fluorescence induction curves and for the regulation of the photosynthetic light reactions are discussed.

Materials and Methods

Plants. Plants of *Aegopodium podagraria* were grown in pots on a window shelf (north east). During the experiments the leaf remained connected to the whole plant. It was positioned between the light conductors described below.

Light. The actinic light was provided by a red LED (660 nm, Stanley H-3000) and filtered by a long-pass filter (Schott RG 630). The intensity of the LED was modulated sinusoidally with frequencies between 1 Hz and 1000 Hz controlled by the LED current.

The measuring light for chlorophyll fluorescence (mean value 3.5 W m^{-2} at the surface of the leaf) was supplied by a red LED (660 nm, Stanley H-3000) and filtered by a shortpass filter (Balzers DT cyan special). The LED current was modulated by a sine wave of 40 kHz provided by a signal generator.

The measuring light of the A_{520} signal (520 nm, 1.5 W m^{-2}) was supplied by a halogen bulb (Osram Xenophot HLX 250W, filter 520 BP 10 from Omega Optical) and modulated by a chopper (Mastermodell 220, HMS-Elektronik) with a frequency of 10 kHz.

Non-modulated background light was used in some experiments. Broadband blue light (BG 18, Schott) was taken from a halogen bulb (Osram Xenophot HLX 250W).

The light guide comprising four arms for the light sources described above and 1 for the fluorescence detector was made from 70 Crofon fibers (Schölly) and had a terminal diameter of 10 mm at the leaf surface. The transmitted light was collected at the opposite side of the leaf by a second light guide which led the transmitted light to the A_{520} detector.

Detection of fluorescence yield and absorbance changes at 520 nm. The detectors were laboratory-made devices consisting of a photodiode (Hamamatsu S 1723-04) and an operational amplifier (OP37). The following optical filters were located in front of the photodiodes: chlorophyll fluorescence: 735 DF 30 (Omega Optical Inc.), A_{520}: 520 DF 10 (Omega Optical). The output signal of the fluorescence and the A_{520} detector passed fourth-order highpass filters with corner frequencies of 3.3 kHz and 12 kHz, respectively.

The output signals of the two highpass filters were correlated by laboratory-made lock-in amplifiers with the reference signals from the signal generator (40 kHz) and from the chopper device (10 kHz), respectively. The amplifiers were operated without any low-pass filter in the output stage. Instead, the amplifiers performed a true integration over one period of the measuring light resulting in a temporal resolution of 100 μs and 25 μs, respectively. The output signals of these two lock-in amplifiers give the fluorescence yield (F) and the transmittance of the leaf at 520 nm (A_{520} signal), respectively. The response of these two signals to the sinusoidal changes of the actinic light were studied by means of frequency response measurements as decribed below.

Identification of individual kinetic components by means of linearizing experimental conditions. Linearity of the responses induced by the actinic light is the premise for curve fitting by means of a sum of a priori known standard functions (Eqns. 1 and 2). Thus, linearity helps to overcome one basic problem of photosynthetic signals, namely, that normally the non-linearity of complex kinetics prevents curve-fitting based assignment of individual kinetic components to certain reactions [14–17]. Linearity could be achieved by measuring frequency responses as described below. It could be shown that the apparatus gave linear responses up to a modulation depth of 50%, as was also found in the low-frequency region [14,15].

Frequency response measurements. The actinic light was modulated with a sequence of sine waves with 15 to 20 different frequencies (f) ranging from 1 Hz to 1 kHz. The sinusoidal modulation of the actinic light causes sinusoidal changes of the fluorescence yield and of the A_{520} signal. For each frequency the amplitude and the phase angles of these changes relative to the sine-wave of the actinic light were determined as follows.

The output signals of the two lock-in amplifiers mentioned above were fed into four actinic correlators, where they were multiplied by the actinic signal itself (real component) and with an actinic signal delayed by 90° (imaginary component). After low-pass filtering

(time-constant of about 300 s) the two real components and the two imaginary components were plotted on a chart recorder. From these records amplitude and phase of the fluorescence and the A_{520} response to the sinusoidally modulated actinic light were calculated, and frequency responses were composed as shown in Fig. 1.

Calibration of A_{520} by single-turnover flashes. Single-turnover flashes were provided by a xenon flash-lamp (PRA model 610c, half-time 8 μs, 0.3 mJ cm^{-2}). The flash lamp was positioned in front of one arm of the light guide instead of the LED for the actinic light. The flash light was optically filtered by a OG 590 longpass and a Balzers DT cyan special 705 nm short-pass filter. The fluorescence yield (F) and absorption at 520 nm (A_{520}) were measured as described above with intensities of the measuring lights of 2 W m^{-2} and 1.5 W m^{-2}, respectively. The detectors were short-circuited during the flash by a CMOS-switch (CD 4066) for 50 μs. Even then, they were 'blind' for about 100 μs after the flash. Decreasing the intensity of the flash by 50% resulted in a reduction of the responses by less than 2%, indicating that saturation was reached. For signal averaging, the responses of F and A_{520} were taken from the output of the lock-in amplifiers and fed into a personal computer with a sampling rate of 0.1 ms. 50 to 200 runs with a flash frequency of 1 Hz were averaged.

Far-red actinic light pulses. In the experimental arrangement described above, an optical long-pass filter RG 715 (Schott) replaced the Balzers filter. Artefacts caused by the far-red actinic flashes distorted the signal from the fluorescence detector for 1.2 ms. For evaluation of the responses, the fluorescence data measured within 1.2 ms after the far-red flash were omitted.

Results

The occurrence of a common component in the kinetics of chlorophyll fluorescence and A_{520}

Fig. 1 shows typical frequency responses of F and A_{520}. As the tests mentioned above have shown that linearity holds, the measured frequency responses can be fitted by transfer functions $H(p)$ [15–17]

$$H(p) = \sum_{i=1}^{N} \frac{A_i}{(1 + p\tau_i)} \tag{1}$$

with

$$p = 2\pi f\sqrt{-1} \tag{2}$$

The parameters A_i (amplitude factors) and τ_i (time constants) are identical to those obtained from curve-fitting by means of Eqn. 3 of the response $h(t)$ to a short light pulse (e.g., a single-turnover flash) provided linearity is guaranteed (e.g., by the use of non-saturating light-pulses of low energy).

$$h(t) = \sum_{i=1}^{N} a_i \exp(-t/\tau_i) \tag{3}$$

with

$$a_i = A_i/\tau_i \tag{4}$$

The transition from Eqn. 1 (f = frequency) to Eqn. 3 (t = time) can be done by means of the Laplace transformation [18].

The frequency responses of chlorophyll fluorescence were fitted by six time-constants ($N = 6$ in Eqn. 1). Fig. 1 shows data points and calculated curves obtained from curve-fitting. The fastest time-constant of about 50 μs, which yields a better fit of the last two points in the high-frequency range, originates from the measuring device and is omitted in the following. In Table I, mean values of the parameters obtained from curve fitting are shown.

τ_2 was outside the range of applied frequencies. However, its existence is known from previous investigations [14,15], and its inclusion in Eqn. 1 resulted in a better fit of the last low-frequency point of Fig. 1. The other four time-constants had equal signs of the amplitude factor. This makes fitting difficult and results in the relatively high errors shown in Table I. However, omission of one of these time-constants causes significant deviations of the fitted curve from the data, especially in the phase plot. The dotted line in Fig. 1 shows the curve which is obtained if the time-constant at 5 ms is omitted in a fit with five time-constants. The neighboring time-constants move towards the omitted one, resulting in a more positive phase-shift in the region around 10 Hz and in a more negative shift around 100 Hz. Also the deviation at 1000 Hz is pronounced in fits with five time-constants. The error sum decreases by about 25% if six time-constants are used instead of five.

A full discussion of all components related to the time constants in Table I will be given in a subsequent paper, since here we concentrate on those components which enable conclusions to be reached about the effect of transthylakoid voltage on chlorophyll fluorescence. Nevertheless, some short statements are given now. τ_{1a}, τ_{1b}, and τ_2 of chlorophyll fluorescence are known from previous studies in the frequency range of 10^{-4} to 1 Hz [14–17].

τ_0 corresponds to the fast phase of the responses to single-turnover flashes [19–21]. Thus, $(\tau_0)^{-1}$ gives the apparent rate constant for the reoxidation of the primary quinone acceptor, Q_a.

τ_v so far was not resolved in studies on fluorescence responses to single-turnover flashes. It is the aim of this study to assign this kinetic component to changes in the

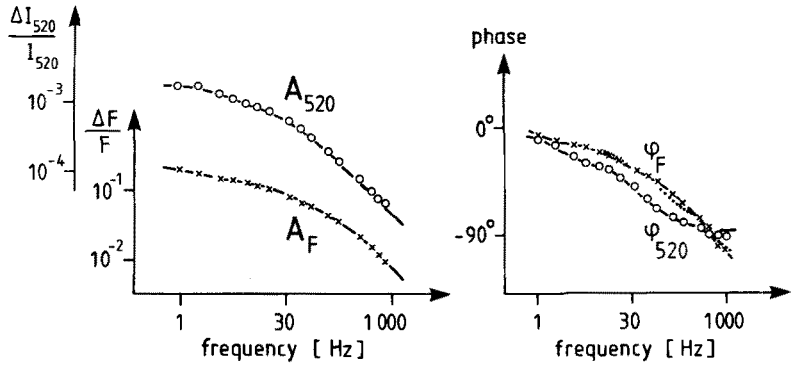

Fig. 1. Amplitude (A_F, A_{520}) and phase responses (ϕ_F, ϕ_{520}) of the fluorescence yield, F, and the A_{520} signal as induced by sinusoidal modulation of red actinic light between 5 and 15 W m^{-2} with frequencies given at the abscissa. The assignment of the scales to the curves is given by the indices. The amplitude of the actinic changes in light intensities was 5 W m^{-2}. The smooth curve results from curve-fitting according to Eqn. 1. Amplitude factors and time-constants as obtained by curve fitting are shown in Table I. The dotted line shows the deviations obtained if one time-constant less is used.

fluorescence yield which originate from light-induced changes in thylakoid voltage.

τ_{1a} corresponds the slow phase of the responses to single-turnover flashes. Possibly, the τ_{1a} component is related to the binding and unbinding of plastoquinone at the Q_b binding site [22].

τ_{1b} is of still unknown origin. Possibly this kinetic component originates from PS II heterogeneities and has to be assigned to the reduction of non-B type units.

τ_2 can be associated to the changes of the redox-state in that part of the ETC which comprises the plastoquinone pool and the pool X at the acceptor side of PS I [15–17].

In the A_{520} signal two kinetic components with positive amplitudes were found (Table I). A fit with more than two time-constants did not lead to a significant decrease of the error sum. In our experiments on leaves of *Aegopodium podagraria* we have never detected a kinetic component with a negative amplitude as sometimes observed in flash experiments [12,23,24].

Biphasic decay kinetics of the electrochromic absorbance changes in the range of wavelengths from 515 to 525 nm were also found in chloroplasts and in intact leaves in the case of excitation by single-turnover flashes [24,25]. The faster phase reflects the decay of the thylakoid voltage by ATP-hydrolase activity and passive ion transport systems. Their spectrum differs clearly from the spectra of apparent absorbance changes which originate from light-induced changes in the light-scattering properties [26]. In leaves and in chloroplasts changes in light-scattering occur on a much slower time-scale [9,11,26–31]. In dark-adapted plant material the apparent rate constant of the fast phase is smaller than observed here on light-adapted plants. The reason is that preillumination leads to a drastic acceleration of the fast decaying phase [10,27]. The origin of the slow decaying phase is less obvious. The amplitude of this phase seems to be correlated to the degree of activation of ATP-hydrolase. Schapendonk [25,26] assumes that this phase originates from conformational changes in the membrane core which lead to electrochromic absorbance changes.

TABLE I

Time-constants (τ_i) and amplitude factors (A_i) as obtained from curve-fitting according to Eqn. 1

The experimental conditions are given in the legend of Fig. 1. (Mean values of seven experiments are shown. The numbers in brackets denote the standard deviation. The amplitude factors give the amplitude of the changes in fluorescence yield and in A_{520} signal in %.

	Fluorescence yield					A_{520} signal	
	τ_0	τ_v	τ_{1a}	τ_{1b}	τ_2	τ_{v1}	τ_{v2}
τ_i (ms)	1.3	5.3	20	109	5600	6.1	42
	(0.4)	(1.3)	(4.2)	(20)	(500)	(2.4)	(17)
$A_i \times 10^{-2}$	4.2	2.3	14	12	−156	0.07	0.13
	(1.5)	(1.7)	(5.0)	(6.0)	(86)	(0.01)	(0.03)

In the context of this investigation the most remarkable feature of the kinetic parameters of the A_{520} signal shown in Table I is the numerical coincidence of the faster time-constant of about 5 ms (τ_{v1}) with τ_v of chlorophyll fluorescence.

A real coincidence of τ_{v1} and τ_v would mean that the fluorescence yield increases concomitant with the formation of the thylakoid voltage, as measured by the A_{520} signal. However, the numerical coincidence indicated by the data given in Table I may be fortuitous. Therefore it was investigated whether the coincidence persists if the status of the photosynthetic system is varied by changing the conditions which define the actual steady state.

An useful means of changing steady state conditions is a change of the mean light intensity [32]. The results shown in Fig. 2 were obtained from experiments with mean light intensities ranging from 10 to 35 W m^{-2}

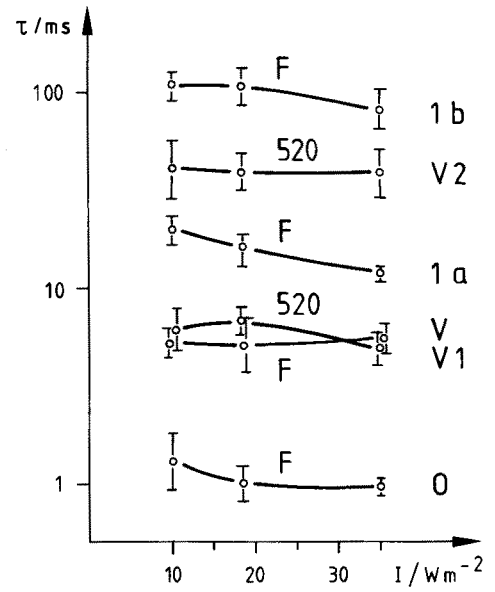

Fig. 2. Time-constants of the fluorescence yield (F) and the absorbance changes at 520 nm (520) measured at different mean light intensities. The time constants were obtained from frequency responses as shown in Fig. 1 by curve-fitting according to Eqn. 1. The indices are explained in the text. The bars indicate standard deviations.

consisting of the mean intensities of the measuring lights for A_{520} and for chlorophyll fluoresence, of the actinic light and, in the case of the experiments at 10 and 35 W m^{-2}, of an unmodulated background light. As indicated in Fig. 2 the coincidence of τ_{v1} and τ_v persists. Furthermore, the amplitude factors of τ_{v1} and of τ_v show a monotonous dependence (Fig. 3). Even though the scatter of the mean is smaller by a factor of about 3 due to averaging 7 to 13 experiments per data point, the scatter does not permit a final conclusion that this relationship is linear. The slope of the straight line gives the scaling factor $s_A = 26$, indicating that a change of 10^{-3} of the A_{520} signal corresponds to a change in fluorescence yield by 2.6%. (These data are used for quantitative estimations below.)

Far-red actinic light

The coincidence of τ_{1v} of the A_{520} signal and of τ_v of the fluorescence can be explained as follows. An increase in light intensity leads to an increase in thylakoid voltage with the time-constant τ_{1v}. This increase of the thylakoid voltage causes an increase of the fluorescence yield. In contrast, the other components of the fluorescence kinetics in the range of milliseconds originate from an increasing reduction of the quinone acceptors and the resulting trap closure as induced by higher light intensity absorbed by PS II.

In order to strengthen the interpretation given above, experiments with far-red actinic light were carried out. Now, pulses of a flash lamp served as actinic light instead of sinusoidal light modulation of a light-emitting diode. These far-red pulses are predominantly absorbed by PS I. Thus, the actinic flash leads to charge separation in the PS I units, resulting in a change of thylakoid voltage. Since the actinic effect of the far-red light on PS II is relatively small the amplitude factors of the kinetic components which originate from Q_a and Q_b reduction should be reduced as compared to the τ_v component which results from changes in the thylakoid voltage. The effect of thylakoid voltage on fluorescence yield as measured by the ratio of the amplitudes of the τ_v and τ_{v1} component should remain unchanged.

Adequate normalization of the amplitude factors in the last line of Table II enables the comparison with the amplitude factors of the response to red actinic light given in Table I. In the case of far-red actinic light the kinetics of both signals are dominated by the τ_v or τ_{v1} component, respectively. Kinetic components which originate from Q_a and Q_b reduction are damped by a factor of 20 as compared to the response to red actinic light.

TABLE II

Time-constants (τ_i) and amplitude factors (a_i) as obtained by curve-fitting according to Eqn. 3 of the responses to far-red light pulses shown in Fig. 4

The amplitude factors a_i give the related changes in fluorescence yield and in A_{520} signal in% of the mean value. Last line: for the comparison with the data in Table I, $A_i = a_i \tau_i$ were divided by '15' in order to give equal relative values for the A_{520} amplitude factors.

	Fluorescence yield			A_{520} signal	
	τ_0	τ_v	τ_{1a}	τ_{v1}	τ_{v2}
τ_i (ms)	1.3	8.1	26	7.9	41
$a_i \times 10^{-2}$	2.45	3.8	0.54	0.14	0.055
$a_i \times \tau_i/15$	0.21	2.1	0.8	0.07	0.15

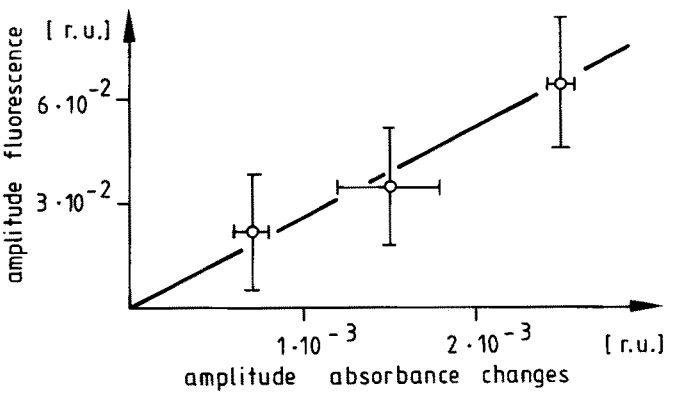

Fig. 3. Amplitude factor of the τ_v component of fluorescence versus the amplitude factor of the τ_{v1} component of the A_{520} signal. The three data points are mean values which were obtained by curve-fitting of frequency response measurements with a mean light intensity of 10, 18 and 35 W m^{-2}, respectively. The bars indicate the standard deviation. The mean values of the related time-constants are shown in Fig. 2. The amplitude of the sinusoidally modulated actinic light was always 50% of the mean light intensity.

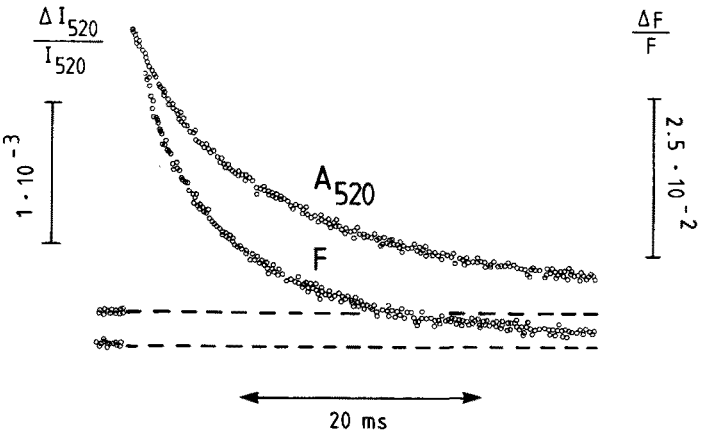

Fig. 4. Time-courses of fluorescence yield and the A_{520} signal as induced by a flash of far-red actinic light.

From the far-red experiments we obtain a value of $s_A = a_v/a_{v1} = 27 \pm 5$ (seven measurements) which compares well to the s_A determined from the data in Fig. 3.

Calibration of the A_{520} signal

Calibration of the A_{520} signal in terms of the involved changes in voltage is done by means of single-turnover flashes as described in Materials and Methods.

The relative change in the A_{520} signal as induced by a single-turn over flash was obtained from backward extrapolation of curves like those in Fig. 4. Single-turnover flashes resulted in an absorbance change of (mean value of ten measurements): $A_{520o}/A_{520ss} = (5.2 \pm 0.9) \cdot 10^{-3}$.

By means of a careful comparison with voltage changes as induced by salt jumps, Schapendonk and Vredenberg [26,33] found that the voltage changes as induced by single-turnover flashes are 12 to 37 mV with a mean value of 25 mV. Using this value we obtain a calibration factor of c = change in voltage/$(A_{520o}/A_{520ss}) = 4.8$ mV/10^{-3}.

With the calibration factor c we obtain the sensitivity, s_V, of the fluorescence yield to changes of the thylakoid voltage from the slope, s_A, of the straight line in Fig. 3:

$$s_v = 5.4\%/10\,\text{mV} \qquad (5)$$

Also, the response of fluorescence to the single-turnover flashes was measured. We found a mean value of 2.5 ± 0.4 for the ratio of the fluorescence yield detected 130 μs after and immediately before the flash. In the calculations given in the Appendix it is assumed that this ratio gives approximately the ratio between the fluorescence yields F_M of closed (Q_a reduced) and F_0 of open (Q_a oxidized) reaction centers.

Discussion

The results of Figs. 3 and 4 show that light-induced changes of thylakoid voltage are coupled to changes of the yield of chlorophyll fluorescence. In intact leaves of *A. podagraria* adapted to low light intensities the calibration of the A_{520} described above leads to a 5.4% increase in fluorescence yield per 10 mV increase in thylakoid voltage.

We assume that the reason for the influence of thylakoid voltage on fluorescence yield is an effect of the resulting electrical field on the primary charge separation of PS II. Since the light-induced electrical field opposes the vectorial charge separation, it is expected to decrease the free energy difference between the excited chlorophylls of the antenna and the charge-separated state. This free-energy difference is relatively small (about 40 m eV [4,5]). A further decrease may very well exert an influence on the rate of charge separation which has become detectable as a change in the fluorescence yield as demonstrated by the calculations given in the Appendix. The effect of artificial electrical fields on the fluorescence of thylakoids was interpreted in this sense [2,7]. Furthermore, in bacterial reaction centers an electric field effect on primary charge separation was demonstrated [34].

Schatz et al. [4] proposed a kinetic model of primary charge separation and its relation to chlorophyll fluorescence. They assume that mainly the rate constant of charge separation (trapping) is sensitive to electrical fields. Adopting this model, the effect of light-induced electric fields on fluorescence yield was estimated as shown in the Appendix. The calculated sensitivity and the measured sensitivity of the fluorescence yield to changes in thylakoid voltage are in keeping. Thus, the measured voltage sensitivity of the fluorescence yield is in line with a voltage sensitivity of the trapping rate constant, k_1, of Schatz et al., so far confirming their assumptions.

However, the calculated value is prone to error for several reasons. Most important, the calibration of the A_{520} signal might be uncertain, and the dielectric distance between the oxidized P680 and reduced pheophytin is not precisely known. Furthermore, the influence

of PS II heterogeneity (α- und β-units, non-B-type units, for a review see Ref. 35) was neglected.

In mature, wild-type chloroplasts, β-units and non-B-type units are probably given by the same population of photosystems [36–38]. The contribution of non-B-type units to variable fluorescence was estimated by fluorescence measurements as described by Chylla and Whitmarsh [39]. We found that in the *A. podagraria* leaves the contribution of non-B type units to variable fluorescence was less than 20% (data not shown). Thus we assume that the measured voltage sensitivity of the fluorescence yield reflects mainly the voltage sensitivity of PS II_α units.

As the chlorophyll fluorescence of PS II is influenced by thylakoid voltage the question arises as to whether this finding has an impact on the interpretation of fluorescence measurements which are of widespread use as a tool in photosynthesis research. The τ_0, τ_v, τ_{1a} and τ_{1b} components are related to the so-called O-I increase of fluorescence. The amplitude factors shown in Table I are identical to those obtained by fitting of a response to a stepwise increase in light intensity by a sum of exponential functions (provided the response is linear [17]). According to these data, the effect of thylakoid voltage is responsible for 7% of the O-I increase of chlorophyll fluorescence.

The contribution of the voltage effect to the I-D-P-S-T kinetics can not be estimated from the data given in Table I. However, in the induction phase of photosynthesis membrane voltages of about 100 mV may occur [11,40]. Thus, a significant influence on the slower fluorescence kinetics appears to be likely. Other authors [41,42] already tried to explain certain features of the fluorescence induction kinetics by an effect of light induced changes in thylakoid voltage.

For estimating the influence on the kinetics as induced by short flashes, e.g., single-turnover flashes, the amplitudes given in Table I have to be divided by the corresponding time-constant (compare Eqn. 1 and Eqn. 3). Thus, about 10% of the changes in fluorescence as caused by short flashes are due to the influence of the electric field. The response of chlorophyll fluorescence to single-turnover flashes are usually analyzed in terms of bi-exponential kinetics [19,21]. In case of the bi-exponential analysis the voltage component probably merges into the slower kinetic component. Thus, the amplitude determined for the slower kinetic component will be seriously influenced by the effect of the decaying thylakoid voltage on fluorescence yield.

The value of the thylakoid voltage under steady-state conditions is a matter of debate [8]. It was estimated that under continuous saturating illumination the thylakoid voltage sensed by intrinsic membrane proteins is in the order of 100 mV [43,44]. According to the calculations outlined in the Appendix, even a thylakoid voltage of 50 mV will decrease the rate constant of charge separation by 50%. Thus, we suggest that the thylakoid voltage is a factor controlling PS II electron flux in vivo in addition to the control by the redox state of the ETC and the high-energy state [45,46]. Furthermore, a high sensitivity of PS II charge separation to the thylakoid voltage might be the reason that in contrast to mitochondria [47,48] the protonmotive force in chloroplast is mainly established by the proton gradient. An electric potential gradient of 150 mV or more might almost completely disrupt charge separation.

Acknowledgement

These investigations were supported by the Deutsche Forschungsgemeinschaft (Ha 712/7-5). The flash-light was a generous loan of Professor D. Gradmann, Göttingen. We are grateful to Mrs. E. Götting for drawing the figures and to Mr. R. Döring for building the lock-in amplifiers.

Appendix

Calculation of the electric field effect on fluorescence yield

The sensitivity of the fluorescence yield to a change in the thylakoid voltage is estimated essentially following the suggestions of Schatz et al. [4] on the relationship between the rate constants of charge separation and of fluorescence yield. The model given by Schatz et al. is confirmed by recent data on the picosecond kinetics of fluorescence and absorbance changes [13,49–51]. However, there are also other approaches to the understanding of picosecond fluorescence data [52,53].

Here, we will estimate the changes in fluorescence yield as induced by an increase of thylakoid voltage of 10 mV. This leads to an increase of the potential difference between P680 and pheophytin by 3 mV if we assume that the dielectrically weighted distance between the electron donor P680 and the primary acceptor pheophytin spans about 30% of the thylakoid membrane [54], similar to the situation in bacterial reaction centers [55]. Thus, under the assumption of thermodynamically equilibrated states [4], the free-energy difference between the excited antenna chlorophylls and the charge separated state is increased by 3 meV. Therefore, the ratio of the charge separation rate constant, k_1 (trapping), to the rate constant k_{-1} of the backward reaction (detrapping) changes as follows:

$$\frac{k_1^*/k_{-1}^*}{k_1/k_{-1}} = \exp(-3\ \mathrm{meV}/kT) = 0.89 \qquad \text{(A1)}$$

with T being room temperature, and the rate-constants labeled by an asterisk being those changed by the additional field. Following Schatz et al. [4], we assume that it is mainly k_1 which is sensitive to electrical fields. Then, Eqn. A1 implies a decrease of the rate constant of

charge separation by 11% for an increase of the membrane voltage by 10 mV.

Calculation of the fluorescence yield following Schatz et al. [4] or Leibl et al. [5] reveals that we can approximate the F_0 to F_M ratio and the fluorescence yield F_0 by

$$\frac{F_0}{F_M} = \frac{k_d}{k_d + k_1}, \quad F_0 = \frac{k_f}{k_d + k_1} \qquad \text{(A2, A3)}$$

with k_f and k_d being functions of different molecular rate constants but not of k_1. As shown above for leaves of *A. podagraria*, the ratio of F_m to F_0 was 2.5, resulting in a ratio of 0.7 between k_1 and k_d according to Eqn. A2.

All measurements of this study were done on leaves adapted to light intensities below 40 W m^{-2}. In this intensity range, most PS II units are in an open state [45], i.e., the experimental conditions correspond to the F_0 state. Thus, Eqn. A3 is used for the calculation of the ratio of fluorescence yield with and without the additional membrane voltage of 10 mV

$$\frac{f^*}{f} = \frac{k_1 + k_d}{k_1^* + k_d} = \frac{1 + 0.7}{0.89 + 0.7} = 1.07 \qquad \text{(A4)}$$

The above calculations predict that fluorescence is increased by 7% due to an increase in thylakoid voltage of 10 mV. This value has to be compared with the experimentally determined value of 5.4% as given by Eqn. 5.

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