ACTIVATION OF THE REACTION II COMPONENT OF P515 IN CHLOROPLASTS BY PIGMENT SYSTEM 1

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1. Introduction

The kinetics of the P515 absorbance changes, induced by single turnover saturating light flashes, sofar have been interpreted to be a reflection of the rise and decay of a trans-membrane potential associated with the charge separation in photosystem 1 and photosystem 2 [1–3]. The multiphasic decay kinetics of the P515 response after energisation with multiple flashes, fired at high frequency, have been suggested to be caused by an altered H^+ permeability at the moment at which the trans-membrane potential exceeds a critical value [4–8]. Recently, however, it has been shown that the multiphasic rise and decay kinetics of the P515 response in intact chloroplasts, which show close correspondence with the P515 response in algae [9], are the composite result of at least two different processes: reaction I and reaction II [10]. The kinetics of reaction I, characterised by a rise time of <0.5 ms and a decay rate constant of 9–13 s^{-1}, have been shown to be similar to the kinetics of the trans-membrane potential as measured by means of microcapillary glass electrodes [10–12]. Reaction II, characterised by a rise time of 100–150 ms and a decay rate constant of 1.6–2.5 s^{-1}, has been suggested to be a reflection of slow intra-membranal changes induced by field-dependent charge displacements in the vicinity of the P515 pigment complex.

It has been reported by some authors that photosystem 1 and photosystem 2 participate to a similar extent in the generation of the ΔA_{515} [4,13]. Other workers have attributed the ΔA_{515} mainly to photosystem 1 activity [14,15]. A slow phase of the flash-induced A_{515} increase in the 0–100 ms time range after a flash, was first observed in *Chlorella* and was shown to be mainly activated by system 1 [16]. Recently, a similar A increase has been observed in intact chloroplasts [10,17–19]. This communication deals with results and analyses of the P515 ΔA in chloroplasts, under conditions at which one of the photosystems was activated. The slow component of the P515 ΔA (reaction II) is suggested to be induced by (changes in) a local electric field associated with the charge interaction between the acceptor of photosystem 1 and cytochrome *f* or plastocyanine.

2. Materials and methods

Intact chloroplasts were isolated from fresh grown spinach leaves by the method in [10]. The assay medium contained 330 mM sorbitol, 2 mM Hepes (pH 7.6), 0.5 mM K_2HPO_4, 10 mM $NaHCO_3$ and 10 mM $MgCl_2$. Broken chloroplasts were obtained by osmotic shocking in water, containing 10 mM KCl. The assay medium contained 330 mM sorbitol, 30 mM tricine (pH 7.7) and 17.5 mM KCl. Chloroplast concentration was equivalent to ~50 μg chl.ml^{-1}. Chloroplasts were kept in the dark for 1 h. Pre-incubation and measurements occurred at 2°C. $\Delta A_{460-540}$,

Abbreviations: ΔA, absorbance change(s); chl, chlorophyll; DCPIP, (2,6-dichlorophenol indophenol); DCMU, (3-(3,4-dichlorophenyl)-1,1-dimethylurea); PMS, (phenazine methosulphate)

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induced either by single flashes or series of 3 successive flashes with a half-life time of 8 μs and at wavelengths >665 nm or at 717 nm, were measured as in [10]. Inhibition of the electron transport between photosystem 2 and photosystem 1 was achieved by pre-incubation of the sample in the presence of KCN, by the method in [20], or by addition of DCMU.

3. Results

Figure 1a and 1b show representative examples of the ΔA_{515} in intact chloroplasts upon saturating single turnover light flashes, which excite both photosystems ((a) excitation light of wavelengths >665 nm), or preferentially photosystem 1 ((b) light of narrow wavelength band ~717 nm), respectively. According to analyses, illustrated and discussed in [10], the 515 nm response can be resolved into two different components. These have been interpreted to be due to rise and decay of the trans-membrane electric field (reaction I) and the rise and decay of a local electric field in the membrane core (reaction II), respectively. The result of these analyses with respect to the response shown in fig.1a,b are given by the solid curve (reaction I) and broken curve (reaction II). The response of reaction I, characterised by a single exponential decay, in this case was obtained by measuring the ΔA in flashes fired at a repetition rate of 4 s^{-1} after 3 pre-illuminating flashes which were necessary to

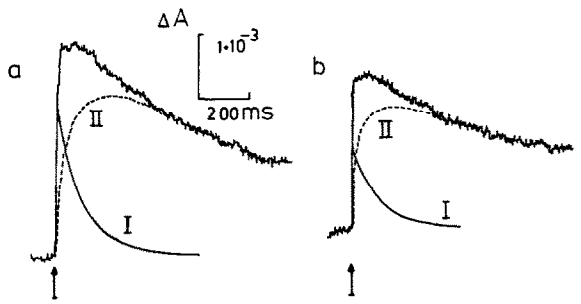

Fig.1. Time courses of the ΔA_{515} in intact chloroplasts upon illumination with single turnover light flashes at wavelengths >665 nm (a) and at a narrow region ~717 nm (b). Flash frequency was 0.05 Hz (noisy curves) or 3 Hz (solid curves). In case of the latter 3 pre-illuminating flashes were fired (see text). The dotted lines represent the resultant kinetic pattern (reaction II) after subtraction of the solid curves (reaction I) from the overall response.

complete and saturate reaction II [10]. The response of reaction II subsequently was obtained by subtracting the one of reaction I from the overall response. Figure 1 shows that the magnitude of reaction I and II in 717 nm light (system 1) is ~50% and 75%, respectively, of the magnitude in light absorbed by both photosystems.

Figure 2a,b show the ΔA upon illumination with 3 successive light flashes of wavelengths >665 nm and of 717 nm, respectively, in intact chloroplasts. The time intervals between the flashes was 100 ms. The dotted curves represent the P515 absorbance responses upon a single flash. As the absorbance decay in the dark, 300 ms after a flash, is exclusively due to that of reaction II (cf. fig.1), it appears, according to fig.2a and conclusive with results obtained with double flash experiments [10], that reaction II is saturated by a single light flash of wavelengths >665 nm. This apparently is not the case when light of 717 nm wavelength is used. Repetitive excitation of photosystem 1 gives rise to a further increase of reaction II in the second and third flash (fig.2b). It should be mentioned that, with 717 nm light, the third and the second flash were not completely saturating. Therefore it might be that the saturation level of reaction II can be reached after two successive excitations with system 1 light.

Figure 3 shows the responses in broken chloroplasts, in the absence (a) and presence of electron transport inhibitors DCMU (b,c) or KCN (d,e). In the presence of DCMU (b), the extent of the P515 response is ~50% of the ΔA associated with reaction I in the absence of DCMU (a). The ΔA in the presence of DCMU unfortunately cannot be analysed into components with sufficient accuracy. In general, reaction I can only be measured accurately by fast repetitive flashes [10]. However, as would be expected, no signal is observed under this condition in the presence of DCMU (see also [13]). Addition of DCPIP/ascorbate creates a condition in which a total recovery of the P515 response is measured (fig.3c). A similar response has been measured after addition of PMS to DCMU-poisoned chloroplasts (not shown). The response measured after pre-incubation of the chloroplasts in the presence of 30 mM KCN is shown in fig.3d. The magnitude of the signal is ~40% of that of the reaction I response in the control experiment. The decay of the response in the presence of KCN appears to be faster than the decay of the signal in DCMU-poisoned

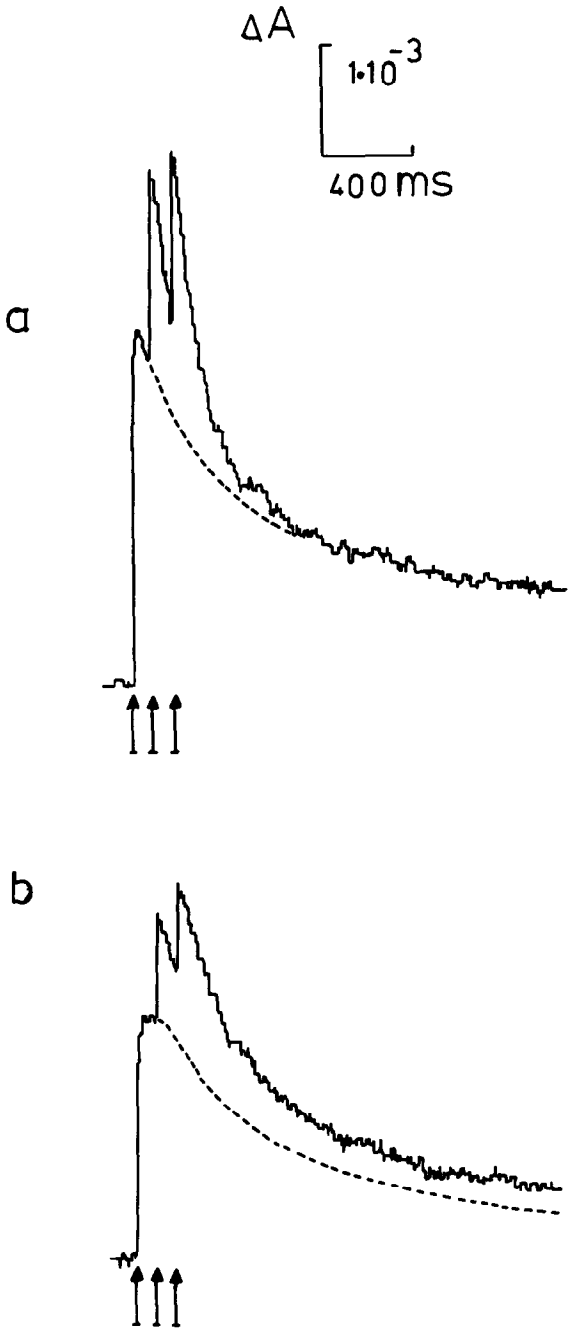

Fig.2. Time courses of the ΔA_{515} in intact chloroplasts upon illumination with triple flashes at wavelengths >665 nm (a) and at a narrow wavelength region ~717 nm (b). Time span between separate flashes was 100 ms. Series of 3 flashes were fired at a frequency of 0.05 Hz. The dotted lines represent the responses of single flashes fired at 0.05 Hz.

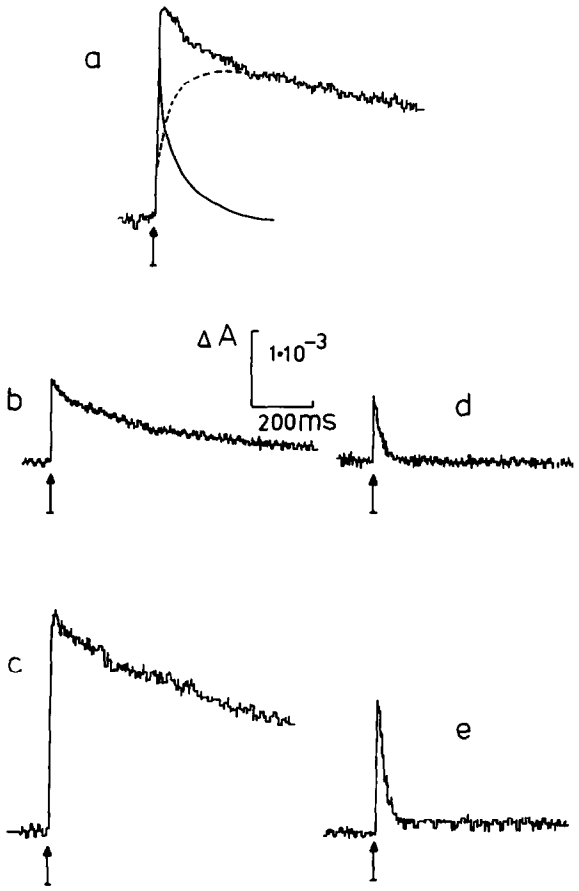

Fig.3. Time courses of the ΔA_{515} upon illumination with single turnover light flashes at wavelengths >665 nm fired at a frequency of 0.05 Hz in broken chloroplasts. Chloroplasts were suspended in 330 mM sorbitol, 30 mM tricine (pH 7.6) and either 17.5 mM KCl (a–c) or 17.5 mM KCN (d,e). (a) Control experiment; (b) in the presence of 2 μM DCMU after pre-illumination with 4 flashes; (c) in the presence of 2 μM DCMU, 3.3 mM ascorbate and 48 μM DCPIP; (d) after pre-incubation in 30 mM KCN; (e) after pre-incubation in KCN and subsequent addition of 3.3 mM ascorbate and 48 μM DCPIP.

chloroplasts and suggests the complete absence of reaction II and a somewhat faster decay rate of reaction I. Addition of DCPIP/ascorbate (fig.3e), in this case, creates a condition in which the flash-induced ΔA equals the reaction I response of the control, except for the somewhat faster decay. The dark kinetics suggest the absence of reaction II in KCN-inhibited chloroplasts in the presence of DCPIP/ascorbate.

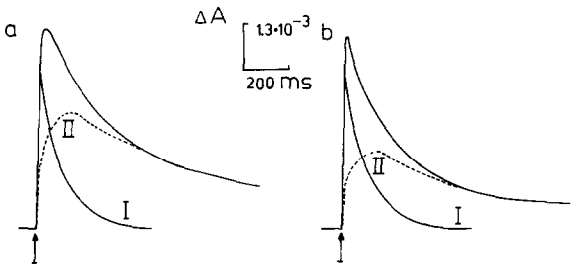

Fig.4. Resolution of the ΔA_{515} in intact chloroplasts upon illumination with single turnover light flashes at wavelengths >665 nm in the absence (a) and presence (b) of 200 nM valinomycin, into two components: reaction I and reaction II. Flash frequency was 0.05 Hz (overall responses) or 3 Hz (curves I). In case of the latter 3 pre-illuminating flashes were fired. The dotted lines represent the resultant kinetic patterns (reaction II) after subtraction of curves I from the overall responses.

Figure 4 shows the resolved responses of the reaction I and II component of the ΔA_{515} in the absence and presence of 200 nM valinomycin.

4. Discussion

The results presented in this work indicate that the reaction I and reaction II components of the P515 ΔA upon single turnover saturating light flashes in dark-adapted chloroplasts are differently activated by photosystem 1 and 2. According to the data of fig.1,2 it appears that the magnitude of the fast rise of reaction I in system 1 (717 nm) light is ~50% of the magnitude measured in light absorbed by both photosystems. Moreover the magnitude of the P515 response measured in DCMU- or KCN-treated chloroplasts (fig.3b and d, respectively) upon flash light absorbed by both systems is ~50% of the reaction I response in the absence of the inhibitors (fig.3a). Illumination of DCMU-poisoned broken chloroplasts with 717 nm light flashes under these conditions did not result in an absorbance response in the absence of electron donors for system I. We therefore conclude, in agreement with the interpretation [13], that the response measured in the presence of these electron-transport inhibitors is brought about by charge separation in pigment system 2.

According to fig.3a,c,e, reaction I can be restored completely in DCMU- or KCN-poisoned chloroplasts by the addition of electron donors for system I. Thus it seems reasonable to conclude that reaction I is equally activated by photosystem 1 and photosystem 2. This conclusion is consistent with the suggestion that reaction I is the electrochromic response of the P515 complex upon generation and decay of the trans-membrane electric potential [10]. It has been shown that this potential, measured with micro-electrodes is equally activated by both pigment systems [11].

Reaction II appears to depend quite differently on system 1 and system 2 illumination. According to the data of fig.1,2, the magnitude of reaction II in a single flash of system 1 light is 75% and 65%, respectively, of the (saturated) magnitude of reaction II in light absorbed by both systems. We found for a variety of preparations that excitation of system 1 causes a 60–80% activation of reaction II. Figure 2 shows in addition that saturation of reaction II can be achieved after double or triple excitation of system 1. This would suggest that the trans-membrane electric field, which in case of system 1 excitation is only 50% of the field generated by both systems (see before), modifies the membrane conformation necessary for a maximal reaction II response. Thus we conclude that reaction II is mainly dependent on a process activated by system 1 excitation. This conclusion is substantiated by the observation (fig.3) that reaction II is relatively small, or even absent in DCMU- (fig.2b) and KCN-treated (fig.2c) chloroplasts, respectively, and can be completely restored in the presence of DCMU after addition of electron donors for system 1. The decay of the P515 response in the presence of DCMU (fig.2b) suggests a contribution of reaction II, which in this case is ~20% of the maximal response under conditions at which electron transfer through system 1 is possible (fig.2a,d). Whether this small contribution of reaction II has to be attributed to system 1 activity cannot be said with certainty. Although system 1 excitation under these conditions was found not to result in a P515 response, it is possible that, upon excitation of both systems, the reduced acceptor of system 2 is oxidized in the dark by a component which in its reduced form serves as a (weak) donor for oxidized carriers at the donor side of system 1. Thus, upon excitation, some electron transport through system 1 would be possible with the conse-

quent appearance of a small reaction II (and reaction I). The results in [19] indeed suggest that electron transfer in system 1 is partially restored in DCMU-treated chloroplasts in the presence of dithionite.

Our results do not permit definite conclusions about the process which is responsible for the appearance of reaction II. A slow A_{515} increase has been reported to occur in pre-illuminated chloroplasts [18], which has been suggested to be caused by a proton translocation at the oxidizing site of the plastoquinone pool. In our experiments however the resolved slow ΔA, associated with reaction II, occurs under conditions at which plastoquinone is in the oxidized form (system 1 illumination).

It has been suggested that the slow decay component of P515, which according to our analyses is attributed to that of reaction II, represents the decay of the transmembrane potential generated by cyclic electron transport in system 1 [19]. However, this suggestion is difficult to reconcile with the fact (fig.4) that the decay of reaction II is unaltered whereas its magnitude is suppressed in the presence of low concentrations of valinomycin. Additional evidence that the slow decay component of P515 (reaction II) is difficult to interpret in terms of a trans-membrane potential has been given in [10]. We have presumed that changes in the mutual orientation of fixed charges due to conformational changes in the membranal core, affect the interaction of the associated electric fields with the P515 pigment complex. The absence of reaction II in KCN-poisoned chloroplasts, in the presence of electron donors for system 1 (fig.3e) would suggest a functional role of plastocyanine or cytochrome *f* in this process. Thus it seems likely that changes in charge densities occurring at these sites during electron transport from system 2 or from artificial donors (in the absence of KCN), cause alterations in strength and orientation of inner-membrane electric fields. This interpretation is in agreement with the observation [21] that a distinct component of the P515 response is dependent on the redox condition of a component with a midpoint potential of +385 mV [21], which is about equal to the midpoint potential of cytochrome *f* and plastocyanin.

Acknowledgements

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