Populations of photosystem 1 units rapidly and slowly reduced by stromal reductants represent photosystem 1α and photosystem 1β complexes: Evidence from irradiance-response curves of P700 photooxidation in intact barley leaves

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Abstract

Photon-induced absorbance changes at 830 nm (ΔA_{830}) related to redox transformations of P700, primary electron donor of photosystem 1 (PS1), were examined in barley leaves treated with diuron and methyl viologen. In such leaves, only soluble reductants localized in chloroplast stroma could serve as electron donors for $P700^+$. ΔA_{830} were induced by 1-min irradiation of leaves with "actinic light" (AL, 700±6 nm) of various irradiances. Two exponentially decaying components with half-times of 2.75 (fast component, relative magnitude of 62 % of ΔA_{830}) and 11.90 s (slow one, 38 % of ΔA_{830}) were distinguished in the kinetics of dark relaxation of ΔA_{830} after leaf irradiation with saturating AL. The components reflecting $P700^+$ dark reduction in two units of PS1 differed in the rate of electron input from stromal reductants. The decline in AL irradiance reduced steady state ΔA_{830} magnitude, which was also accompanied by a decrease in the contribution of fast component to the overall $P700^+$ dark reduction kinetics. The photon-response curves were obtained separately for rapidly and slowly decaying ΔA_{830}. The values of half-saturating irradiance were 0.106 and 0.035 μmol m^{-2} s^{-1} for rapidly and slowly reduced PS1 units, respectively. The ratio of rate constants of $P700^+$ dark reduction for rapidly and slowly reduced PS1 units was 1.4 times higher than the ratio of their half-saturating irradiances thus indicating higher relative antenna size in rapidly reduced PS1 units. The latter finding, taken together with higher relative amount of P700, favours the view that rapidly and slowly reduced PS1 units reflect $P700^+$ reduction by stromal reductants in spatially separated PS1α and PS1β complexes.

Additional key words: diuron; *Hordeum*; methyl viologen; photosystem 2.

Introduction

Heterogeneity of photosystems 1 and 2 (PS1 and PS2) constitutes a general property of electron transport system of a higher plant chloroplast. Two types of PS2 complexes, PS2α and PS2β, were distinguished on the basis of both their localization in different chloroplast membranes and antenna size (Melis 1991). PS2α is localized in appressed grana thylakoid membranes and its antenna size is 2–3 times larger than that of PS2β that is found in stroma lamellae (Irrgang 1999). Similarly to PS2, PS1 exhibits lateral heterogeneity. In membrane fractionation experiments, vesicles containing two different complexes of PS1, PS1α and PS1β, were separated (Albertsson 1995). Grana margin membrane fraction contained PS1α, whereas PS1β complexes were found in the vesicles originating from stroma lamellae (Svensson *et al*. 1991, Wollenberger *et al*. 1994).

PS1α complexes have the antenna size 30–40 % larger than those of PS1β (Albertsson 1995). They differ in redox properties: PS1α is responsible for the support of linear electron transport operating in series with PS2α, while physiological function of PS1β complexes is restricted to the support of cyclic photophosphorylation (Wollenberger *et al.* 1995).

Several different pathways contribute to PS1-driven

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Abbreviations: AL – "actinic light"; MV – methyl viologen; P700 – primary electron donor of photosystem 1; PS – photosystem, ΔA_{830} – absorbance changes at 830 nm.
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cyclic electron transport *in vivo* (Fork and Herbert 1993, Bukhov and Carpentier 2004). Ferredoxin:$NADP^+$ oxidoreductase (Shahak *et al.* 1981), ferredoxin:plastoquinone oxidoreductase (Bendall and Manasse 1995), and NAD(P)H dehydrogenase similar to mitochondrial complex I (Cuello *et al*. 1995, Sazanov *et al*. 1996) are involved in that process. Among these enzymes, NAD(P)H dehydrogenase was found only in stroma lamellae (Berger *et al*. 1993, Horvath *et al*. 2000, Quiles *et al*. 2000). This finding suggests probable difference between PS1α and PS1β in ability to use stromal reductants, NADH and/or NADPH, as a source of electrons.

According to Bukhov *et al.* (2001, 2002), two types of PS1 unit differing in the rate of $P700^+$, oxidized primary electron donor of PS1, and reduction from stromal reductants were found in intact leaves using the kinetics and irradiance-saturation pattern of absorbance changes at 830 nm (ΔA_{830}). In particular, dark decay of ΔA_{830} consisting of two first order kinetic components with highly different half-times but rather similar magnitudes confirm that type of PS1 heterogeneity. In both types of PS1 units, the rate of electron input was determined by the amount of stromal reductants available for $P700^+$ reduction (Bukhov *et al.* 2002). It was unclear, however, whether rapidly and slowly reduced PS1 units were related to PS1α or PS1β type of heterogeneity.

The goal of this study was thus to establish whether the above PS1 units differ in the relative antenna size. To determine this parameter, we examined ΔA_{830} induced by various irradiances of "actinic light" (AL) in barley leaves treated with diuron and methyl viologen to prevent both electron flow from PS2 and electron recycling around PS1 *via* ferredoxin-dependent pathway. Then, irradiance-response curves were obtained separately for rapidly and slowly reduced populations of $P700^+$, and the ratios of the rate constants of dark $P700^+$ reduction for the above components were compared with corresponding ratios of half-saturating AL-irradiances. The simple calculations revealed 1.4 times higher relative antenna size in rapidly reducing PS1 units, which accounted for more then 60 % of total P700 in a leaf. These findings favour the view that PS1 units exhibiting fast and slow reduction of $P700^+$ by stromal reductants represent PS1α and PS1βcomplexes.

Materials and methods

Plants: Barley (*Hordeum vulgare* L.) seedlings were cultivated in a phytotrone chamber in a mixture of top-soil, sand, and peat (5 : 1 : 1, m : m : m) at 22/20 °C day/night temperature under "white light" provided by a set of fluorescence tubes. The photoperiod was 16 h. The distance between seedlings was 3 cm to minimize the difference among plants owing to self-shading. Primary leaves of 8–9-d-old seedlings were studied. Leaf segments were taken at 2–4 cm from the top of a leaf blade. Treatment of leaves with diuron *plus* methyl viologen was done by floating the segments of dark-adapted leaves on a solution containing 200 μM diuron and 2 mM methyl viologen. The segments were kept in the above solution in complete darkness for 30 min.

The redox changes of P700 were monitored as the irradiance-induced changes in absorption at 830 nm with a *PAM* fluorometer (Schreiber *et al*. 1988). A ΔA_{830} signal was detected with pen recorder. AL of 700±6 nm for selective excitation of PS1 was obtained by passing AL from a 150 W incandescent lamp through a *KS18* red glass filter and an interference filter with a peak of transmittance at 700 nm and half bandwidth of 12 nm (*LOMO*, Russia). Irradiance was attenuated with different combinations of wire-nets, whereas the slits of monochromator were kept constant not to change the spectral distribution of AL. Calibration of transmittance was done for each set of wire-nets using a parallel beam of "white light" in order to reduce errors related to low measuring irradiance of 700-nm radiation provided by a detector of photons. Irradiance was measured with a *LI-250* light meter (*LI-COR*, USA). As the meter was adapted to the spectral range of photosynthetically active radiation, it could not give correctly absolute values of AL irradiance at 700 nm. However, as the transmittance of wire-net combinations was experimentally found to be insensitive to a spectral region of radiation (blue, green, or red), relative irradiances of AL at 700 nm were assumed to be correct.

The data are mean ± SE of 7 measurements, each done with new leaf.

Results

Fig. 1 shows original traces of ΔA_{830} initiated in barley leaves treated with diuron and methyl viologen by 1-min exposures to 700-nm AL of various irradiances. Photon induced fast increase in absorption to a steady-state level, which was followed by its much slower relaxation to initial absorbance in the dark. Light-induced ΔA_{830} were due to P700 oxidation, whereas relaxation of absorbance changes in the dark was attributed to the input of electrons to PS1 with $P700^+$ reduction (Schreiber *et al.* 1988). Decrease in AL irradiance was accumulated in oxidized state under steady-state conditions. As diuron blocked electron donation from PS2 and methyl viologen prevented recycling of electrons around PS1 *via* ferredoxin-dependent pathway, stromal reductants such as NADH or/and NADPH could serve as the sole source of electrons for $P700^+$ reduction.

The implication of ΔA_{830} dark decay kinetics for characterizing the complex pattern of $P700^+$ reduction by

stromal reductants has earlier been argued (Cornic *et al.* 2000, Bukhov *et al.* 2001, 2002). The complex pattern of this process is demonstrated in Fig. 2, which shows kinetic analysis of the dark decay of absorbance changes induced by AL of various irradiances. For better comparison, ΔA_{830} values were normalized to the magnitude observed at each of the AL irradiances. Semi-logarithmic plots of ΔA_{830} dark decay *versus* time revealed two components of recovery of reduced P700 after P700 had been photooxidized by relatively strong AL (Fig. 2, curve *1*). These components were characterized by half-times of 2.75±0.09 s (fast component) and 11.9±0.29 s (the slow one). First order rate constants and relative magnitudes of these components were 0.250 s^{-1} and 0.62±0.02 (fast component) and 0.058 s^{-1} and 0.38±0.03 (slow component). Importantly, the decrease in AL irradiance not only reduced the absolute magnitude of light-induced ΔA_{830}, but changed relative magnitudes of fast and slow components of dark ΔA_{830} relaxation as well. The contribution of fast component to the overall ΔA_{830} decay kinetics declined following the decrease in AL irradiance (Fig. 2, curves *2–4*). Half-times of both fast and slow components of $P700^+$ reduction in the dark did not depend, however, on the AL irradiance.

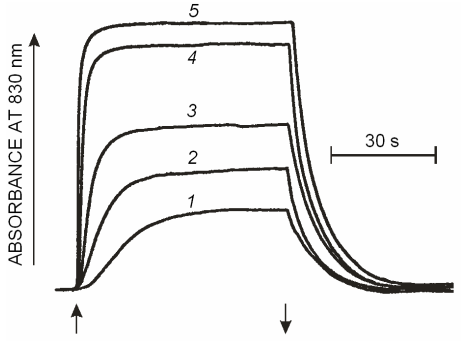

Fig. 1. Original traces of absorbance changes at 830 nm induced in barley leaf treated with diuron and methyl viologen by irradiance of 0.036 (*1*), 0.073 (*2*), 0.108 (*3*), 0.420 (*4*), or 1.050 (*5*) μmol m^{-2} s^{-1}. *Upward and downward arrows* indicate "actinic light" on and off, respectively.

Fig. 3*A* demonstrates the total P700 photo-oxidation (*1*) and the P700 photo-oxidation in rapidly (*2*) and slowly (*3*) reduced populations of PS1 complexes as a function of AL irradiance. The second and the third parameters were obtained at a given AL irradiance as the products of ΔA_{830} magnitude and contribution of a corresponding component to kinetics of ΔA_{830} dark relaxation. All magnitudes were normalized to ΔA_{830} magnitude at saturating AL irradiance (1.05 μmol m^{-2} s^{-1}). As expected, photo-oxidation of $P700^+$ was saturated more readily in slowly reduced population of PS1 than in rapidly reduced one (Figs. 3*A*, *2* and *3*).

Irradiance-response curves shown in Fig. 3*A* exhibited exponential dependence of photo-oxidized P700 on AL irradiance. This was especially clear when these curves were demonstrated in semi-logarithmic plots. Fig. 3*B* shows the values of $\ln[(\Delta A_i/\Delta A_{max})\times 100]$ as a function of AL irradiance, where ΔA_i and ΔA_{max} are the magnitudes of fast (curve *1*) or slow (curve *2*) relaxing component of ΔA_{830} induced by AL of i μmol m^{-2} s^{-1} and magnitude of

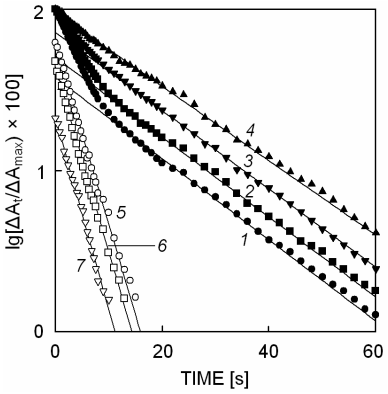

Fig. 2. Semi-logarithmic plots of the kinetics of dark decay of ΔA_{830} (*1–4*) and de-convoluted fast component of the decay (*5–7*) after 1-min irradiation of barley leaves treated with diuron and methyl viologen by "actinic light" (AL) of 1.050 (*1, 5*), 0.108 (*2, 6*), 0.073 (*3, 7*), and 0.036 μmol m^{-2} s^{-1} (*4*). Magnitudes of absorbance changes were normalized in the point when AL had been turned off (*zero point* on X-axis). *Straight lines* represent linear fits calculated for fast and slow components.

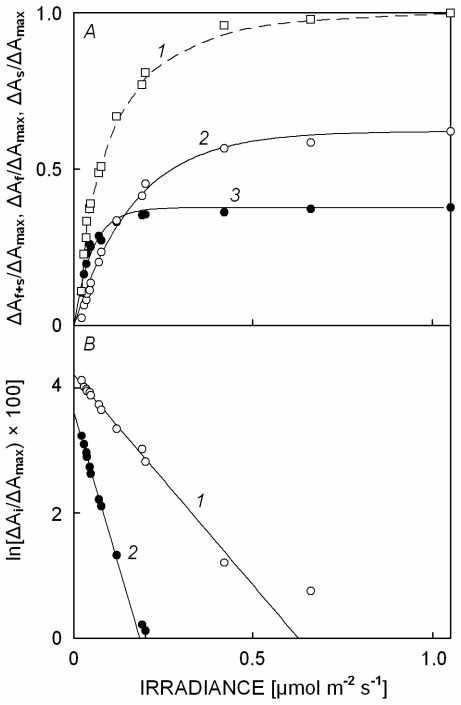

Fig. 3. *A*: Irradiance-response curves for total ΔA_{830} (*1*), and fast relaxing (*2*) or slow relaxing (*3*) component of ΔA_{830}. All magnitudes were normalized to maximum ΔA_{830} magnitude at saturating "actinic light". *B*: Semi-logarithmic plots of light-response curves for fast relaxing (*1*) or slow relaxing (*2*) component of ΔA_{830}.

ΔA_{830} induced by saturating irradiance, respectively. Such transformation gave straight lines with different slopes for irradiance-response curves of fast and slow relaxing components of ΔA_{830}. The values of AL, which oxidized P700 by one half, namely, $i_{1/2}$, were 0.106 and 0.035 μmol m^{-2} s^{-1} for rapidly and slowly reduced PS1 units, respectively. Solid lines on Fig. 3*A* demonstrate that initial irradiance-response curves were well fitted by expression

$$\Delta A_{j,i} = \Delta A_{j,max} (1 - e^{-k\, i}),$$

where $\Delta A_{j,i}$ and $\Delta A_{j,max}$ are magnitudes of kinetic component j (one of the two, fast or slow) of dark decay of ΔA_{830} induced by AL of i μmol m^{-2} s^{-1} and by saturating AL, and coefficient k is $\ln 2/i_{j1/2}$. The values of coefficient k were calculated to be 6.5 and 19.8 [(m^2 s) μmol^{-1}] for rapidly and slowly reducing PS1 units, respectively.

Discussion

The presented results support our earlier suggestion about functional heterogeneity of PS1 units with respect to their capacities to receive electrons from soluble stromal donors (Bukhov *et al*. 2001, 2002). Indeed, the half-times of $P700^+$ reduction after irradiation of leaves treated with diuron and methyl viologen were several orders larger than characteristic time of limiting step in linear electron transport, oxidation of plastoquinol by cytochrome b_6f complex (Witt 1971, Laisk and Oja 1994, Ott *et al*. 1999). As the linear electron flow and the alternative PS1-driven electron transport pathways share intersystem part of electron transport chain (Scherer 1990), the rate of $P700^+$ reduction was limited under our experimental conditions by the input of electrons from stromal donors to plastoquinone pool. If pathways of electron donation reflected by fast and slow components of $P700^+$ reduction compete within the same PS1 unit, the ratio of magnitudes of the above components has to be proportional to the ratio of their rate constants, which is the simple consequence of chemical kinetics rule. The ratio of rate constants of fast and slow components was found to be 4.3, whereas the ratio of magnitudes was only 1.6. This discrepancy evidences that slow and fast components reflect the processes of electron donation to $P700^+$ from stromal reductants proceeding in two different PS1 units.

For each type of PS1 units, simplified scheme of electron transfer from stromal donor, D, to methyl viologen, MV, can be presented as follows:

$$D \xrightarrow{k_1} P700 \xrightarrow{k_2} MV$$

where k_1 is a rate constant characterizing the limiting step in the sequence of electron transport reactions finally resulting in $P700^+$ reduction, and k_2 is a "light" rate constant characterizing P700 photooxidation. Under not extremely high irradiances (which means that no double hits occurred in PS1 antenna), the value of k_2 must be proportional to the number of quanta absorbed by a PS1 unit. This can be expressed as $k_2 = N\, i$, where N is the number of antenna chlorophyll molecules and, i, once again, is a fluence rate. Such simple approach has already been applied to characterize the electron transport through PS1 from stromal reductants in heat-treated barley leaves (Bukhov *et al.* 2000).

The k_1/k_2 ratio determines under steady state conditions the ratio $P700^0/P700^+$, where $P700^0$ and $P700^+$ are P700 species in reduced and oxidized states, respectively. For either rapidly or slowly reducing PS1 unit irradiated with half-saturating radiation, $P700^0 = P700^+$ and, correspondingly, $k_1 = k_2 = N\, i_{1/2}$. Two equations for rapidly and slowly reduced PS1 units can be re-written as follows:

$0.250 = N_{fast} \times 0.106$ (rapidly reduced PS1 units)
$0.058 = N_{slow} \times 0.035$ (slowly reduced PS1 units)

The N_{fast}/N_{slow} ratio obtained from the above equations is 1.42, which indicates higher antenna size in rapidly reduced PS1 units. As was noticed above, antenna size was reported to be 30–40 % larger in PS1α than in PS1β (Albertsson 1995). In addition, PS1α contains about 70 % of total amount of chlorophyll molecules binding to PS1 complexes (Albertsson 1995). Assuming that rapidly reduced PS1 units are PS1α, it is easy to calculate that the ratio of P700 belonging to rapidly and slowly reduced populations of PS1 must be (0.7/1.4) : (0.3) = 5 : 3, which corresponds to the experimenal data (see Fig. 2). Taken together, the data on relative antenna size and relative magnitudes favour the view that PS1 units, in which $P700^+$ is rapidly reduced by stromal reductants, represent PS1α, whereas PS1β can be reduced by stromal donors with much slower rate (slow component).

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