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ARTICLE

The effect of exogenous NO on PSI photochemistry in intact pea leaves

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ABSTRACT We investigated the effect of exogenous NO on PSI photochemistry by measuring P700 absorbance changes at 810-870 nm in intact pea leaves treated with the NO donor GSNO. Our results indicate that NO increases PSI quantum efficiency and the pool size of electrons in the intersystem chain.

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KEY WORDS

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P700 absorbance
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Nitric oxide (NO) becomes an increasingly recognized signaling molecule in the plant world as its influence over a growing number of physiological functions is revealed (Neill et al. 2003; Besson-Bard et al. 2008). Recently, *in vivo* target sites of NO in photosynthetic electron transport have been studied by non-invasive chlorophyll fluorescence measurements on intact pea leaves, which indicate that NO slows down electron transfer between Q_A and Q_B, binding sites, and inhibits charge recombination reactions of Q_A^- with the S_2 state of the water-oxidizing complex in photosystem II (PSII) (Wodala et al. 2005). Consistent with these results, NO was also shown to inhibit steady-state photochemical and non-photochemical quencing processes and also appears to modulate reaction-center-associated non-photochemical quenching (Wodala et al. 2008).

Reflecting changes in fluorescence yield of PSII, chlorophyll fluorescence results center around PSII photochemistry, thus provide limited information on the intersystem electron transport chain and on photosystem I (PSI). Monitoring the oxidation state of the reaction-center chlorophyll in PSI (P700) via the absorbance of near infra red light by the $P700^+$ radical provides valuable information on PSI photochemistry (Klughammer and Schreiber 1994). P700 absorbance changes also allow the assessment of the linear electron flow between PSII and PSI, including the pool size of electrons in the intersystem chain (Asada et al. 1992), and may contribute to the elucidation of cyclic electron flow around PSI (Asada et al. 1993). Chlorophyll fluorescence and P700 absorbance measurements thus provide complementary information on the electron transport processes in PSII and PSI. Having studied *in vivo* target sites of NO in PSII the aim of this study was to investigate the potential effects of exogenous NO on PSI photochemistry.

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Materials and Methods

Plant material and experimental solutions

Sterilized seeds of pea (*Pisum sativum* L. cv Petit Provençal) were germinated for 3 d at 24°C, and the seedlings were grown in a semi-controlled growth chamber for 2 weeks under a 12-h-light (150 µmol m^{-2} s^{-1}) /12-h-dark cycle and temperature of 22°C. Leaf disks of the youngest fully expanded leaves were prepared by a 15 mm diameter leaf punch and used for each measurement. Prior to measurements, leaf disks were individually incubated for 70 min under 150 µmol m^{-2} s^{-1} white light in covered but not sealed Petri dishes (with a volume of approximately 10 cm^3) in the presence of 3 ml distilled water as control, or 3 ml of the NO donor S-nitrosoglutathione (GSNO) and the NO scavenger hemoglobin (Hb) in aqueous solution used in a concentration of 1 mM or 4 mg mL^{-1}, respectively. To increase its stability, thus prevent early and unwanted NO release, GSNO stock solution was prepared daily and kept in dark on ice until the start of experiments (Feelisch 1998). GSNO was light inactivated by placing 1

Table 1. Φ_{PSI} and electron pool size values calculated from P700 oxidation curves measured in pea leaf disks that were incubated for 70 min under 150 µmol m^{-2} s^{-1} white light in distilled water (Control) or solutions of GSNO and Hb, as indicated. GSNO and Hb were used in concentrations of 1 mM and 4 mg mL^{-1}, respectively. Values represent means ± SD (n = 8).

	Φ_{PSI}	Pool size of electrons in the intersystem chain	Pool size of electrons from stromal donors
Control	0.41 ± 0.01	13.16 ± 2.75	28.10 ± 9.39
GSNO	0.43 ± 0.02	18.30 ± 6.29	42.17 ± 18.09
GSNO + Hb	0.33 ± 0.09	12.48 ± 1.66	26.04 ± 6.48
GSNO (light inactivated)	0.33 ± 0.03	9.48 ± 2.42	13.69 ± 3.08

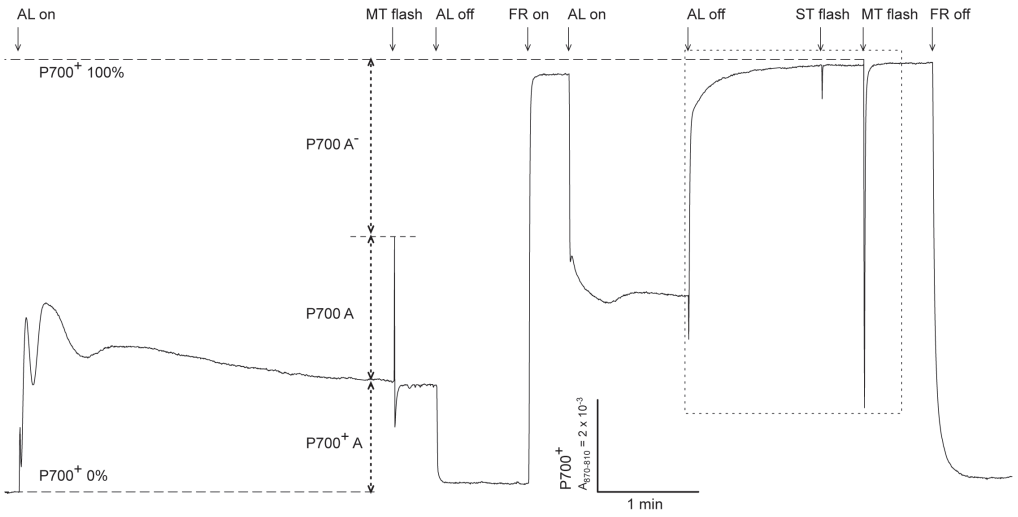

Figure 1. A typical P700 oxidation curve obtained from a pea leaf disk incubated for 70 min under 150 µmol m^{-2} s^{-1} white light in distilled water, following the cycle of illumination indicated by arrows and light sources above the trace.

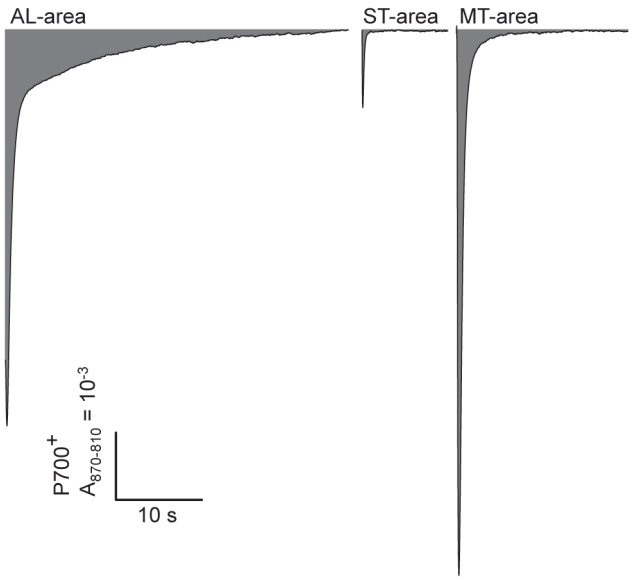

Figure 2. A section of the P700 oxidation curve from Fig. 1 (indicated by the dotted rectangle in Fig. 1) showing AL-, ST- and MT-areas.

mM GSNO solution under 1900 µmol m^{-2} s^{-1} white light for 10 h. All chemicals were purchased from Sigma-Aldrich.

P700⁺ absorbance measurements

The redox changes of P700 were monitored as the light induced changes in absorption of the $P700^+$ radical measured with a PAM fluorometer (PAM-101, Walz, Effeltrich, Germany) equipped with a dual-wavelength emitter-detector unit (ED-P700DW, Walz). The difference signal is obtained from absorbance of measuring lights peaking at 810 and 870 nm. This ensures high selectivity for P700 absorbance by minimizing all non-specific signal changes due to plastocyanin and light scattering. The signal was recorded with a computer connected to the PAM Data Acquisition System (PDA-100, Walz) and the sampling rate was set to 10 ms/point.

Leaf discs were illuminated by far-red light (FR, >720nm, 2 µmol m^{-2} s^{-1}, 102-FR, Walz), actinic white light (AL, 220 µmol m^{-2} s^{-1}, KL 1500 LCD, Schott, Mainz, Germany) and saturating (> 8000 µmol m^{-2} s^{-1}) single-turnover (ST, peak width at half-maximal intensity = 1.5 µs, XE-STL/XE-STC, Walz) and multiple turnover (MT, peak with = 50 ms, XF-103/XMT-103, Walz) flashes following the cycle of illumination in Figure 1. The quantum efficiency of PSI (Φ_{PSI}) was calculated according to Klughammer and Schreiber (1994). Indicated in Fig. 2, the pool size of electrons in the intersystem chain (MT-area/ST-area) and the number of electrons from stromal donors (MT-area/ST-area) x [(AL-area/MT-area) – 1] were determined according to Asada et al. (1992).

Results and Discussion

Figure 1. shows a typical P700 oxidation curve obtained by illuminating an untreated pea leaf disc with actinic and far-red light as well as saturating pulses according to the indicated cycle of illumination. This measurement provided data for calculating PSI quantum efficiency as well as pool sizes of electrons in the intersystem chain and from stromal donors. Under background FR illumination, ST and MT flashes cause a rapid and transient reduction of P700 followed by re-oxidation to the level preceding the flashes. ST- and

MT-areas represent complementary areas of the P700 oxidation curve between the onset and 10 s, or 20 s following the flash, respectively (Fig. 2). The ST flash only allows a single excitation event in PSII, while the 50 ms MT flash causes multiple excitation events, which results in total reduction of intersystem carriers. The ratio of the MT-area to the ST-area thus represents the functional pool size of electrons per reaction center in the intersystem chain (Asada et al. 1992). The AL-area was calculated as the complementary area of a 40 s section of the P700 oxidation curve following a 60 s AL illumination during FR background (Fig. 2). This area represents the pool of electrons accumulating during AL illumination that can be donated to P700. Using these area values, the number of electrons from stromal donors per P700 was also estimated according to Asada et al. (1992). Calculation of Φ_{PSI} values is based on the assumption that PSI reaction centers may occur in three different states: active state (P700 A), inactive state due to donor limitation ($P700^+$ A) and inactive state due to acceptor limitation (P700 A^-) as described by Klughammer and Schreiber (1994). These fractions can be identified by different P700 absorbance levels in a given illumination scenario and are indicated in Figure 1.

Treatment with GSNO caused a slight increase in Φ_{PSI} values of leaf disks, which indicates that NO does not significantly alter the optical cross-section of PSI centers (Table 1). GSNO, however, decreased the fraction of P700 A^- centers at the cost of increasing the ratio of $P700^+$ A centers (data not shown). This slight indication of donor side inhibition is in good agreement with previous chlorophyll fluorescence measurements indicating a slower rate of linear electron transport in the presence of GSNO, as GSNO was shown to slow down the rate of electron transfer in PSII, and reduce non-photochemical quenching (Wodala et al. 2008).

GSNO treatment modestly increased the number of electrons in the intersystem chain (Table 1), which is surprising given that NO is expected to slow down the rate of electrons entering the intersystem chain by reducing PSII optical-cross section, and speed up the rate of electrons leaving the chain by the modest increase of Φ_{PSI}. Still, measurements with light inactivated GSNO and in the presence of Hb restored the number of intersystem electrons to control values, which strongly indicates that NO is exclusively responsible for this increase. GSNO also increased the pool size of electrons from stromal donors (Table 1). Previous studies suggest reduced ferredoxin, NADPH and NADH as sources of stromal electrons (Asada et al. 1992) and these molecules roughly account for the number of electrons estimated in control leaf disks, but not the increased number caused by GSNO. Such increases have been reported in C_3 plants under anaerobiosis, when carbon dioxide fixation is suppressed, though it is unlikely that GSNO would bring about such conditions. Experiments in the presence of Hb and using light inactivated GSNO both demonstrate that degradation products of NO production from GSNO – such as nitric oxides and oxidized glutathione – are not responsible for the increase. Further investigation is required to see whether the surplus of electrons in the intersystem chain and from stromal donors are provided directly by NO, or NO induced alternative sources.

In summary, our results indicate that NO may influence PSI photochemistry in vivo. Further experiments are required to confirm and clarify these data for a better understanding on the effect of NO on the electron transfer processes in PSI.

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