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Defect in chloroplastic NAD(P)H dehydrogenase complex resulted in stromal over-reduction after exposure to strong light

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Introduction

The cyclic electron transport around PSI by NAD(P)H dehydrogenase complex (NDH) in tobacco chloroplast has been demonstrated in several laboratories, utilizing chloroplastic transformants in which *ndh* genes were insertionally inactivated (for review, see Shikanai and Endo 2000). Although these transformants grew normally in non-stressed conditions, they were more susceptible to photo-oxidative stress (Endo et al. 1999) or water stress (Horvath et al. 2000) than wild type plants. From these findings we hypothesized that energy dissipation was impaired in NDH-defective leaves which may result in stromal over-reduction. To date, we have obtained two independent experimental results which support the idea of post-illumination over-reduction. Chlrophyll fluorescence measurement showed reduction of the plastoquinone pool after exposure to strong light in NDH-defective tobacco (ΔndhB), which might reflect stromal over-reduction (Takabayashi et al. in preparation). P700 measurements showed lowered level of photo-oxidizable P700 after illumination of strong light in ΔndhB. Since the lowered $P700^+$ signal was recovered by the dark incubation with methyl viologen, it was ascribed to rapid P700 charge re combination within PSI complex due to acceptor limitation (Endo et al. 1999). Thus, this P700 data also is indicative of post-illumination stromal reduction. In this study we further characterized the post-illumination reduction of stromal components in ΔndhB in view of physiological relevance of this phenonenon.

Materials and methods

Plant materials
Chlroplastic transformants of Nicotiana tabacum cv. Xanthi, in which ndhB gene was insertionally inactivated (ΔndhB) (Shikanai et al. 1998) were cultivated on soil along with 4Y26 vector control in a growth chamber (50-100 μmol quanta $m^{-2}s^{-1}$, 25° C).
Chlorphyll fluorescence and P700 measurements
Chlorophyll fluorescence was measured with a PAM-2000 portable fluorometer (Walz). P700 redox was measured by a PAM fluorometer with an emitter-detector unit ED800T.

Results

Effects of developmental stage on the level of photo-oxidizable P700

In a previous study (Endo et al. 1999) stromal over-reduction was estimated by charge recombination of P700 after severe light stress (3,000 µmol $m^{-2}s^{-1}$ for 20 min) in ΔndhB. In this study, we examined whether such over-reduction occurred by mild and physiological light stress (800 µmol quanta m-2s-1 for 4 min). We measured photo-oxidizable P700 (ΔA_{MAX}) induced by multiple turnover flash (MT, 50 ms) which represents acceptor limitation at P700 (Fig. 1A), and far-red light oxidizable P700 (ΔA_{FR}) which represents electron donation from plastoquinone to P700, before and after the light stress (Fig. 1B). We also measured steady state yield of chlorophyll fluorescence (Fs) under low light which represents reduction level of plastoquinone (Fig. 1C) and Δ F/Fm' under low light which represents quantum yield of PS II (Fig. 1D).

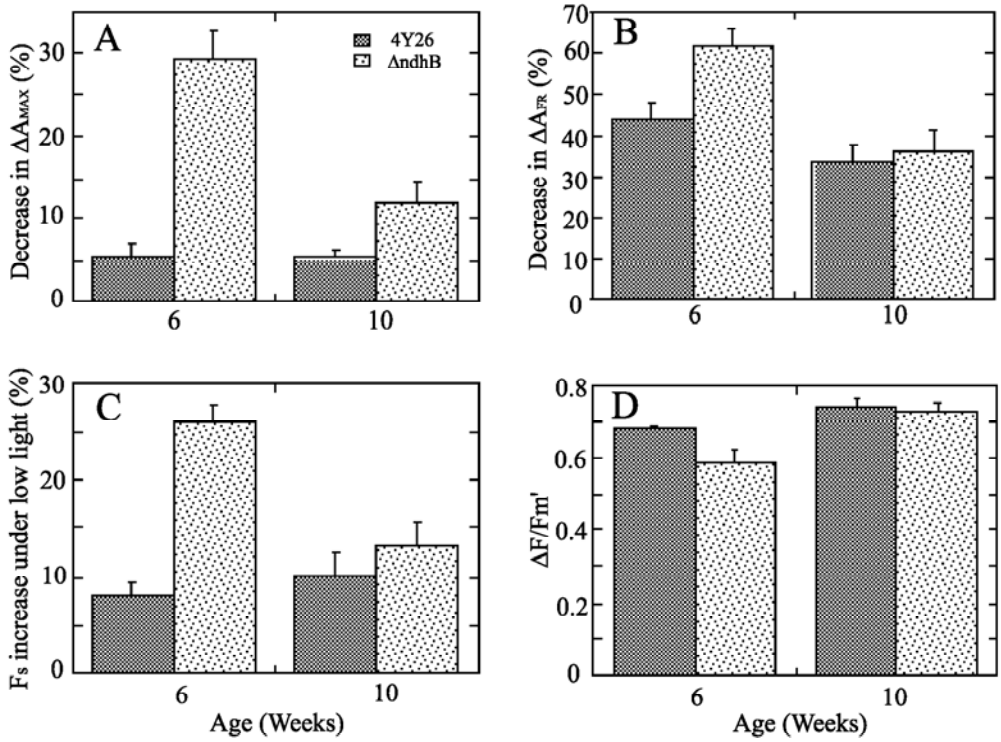

Fig. 1. Post-illumination reductions were compared in the 6- and 10-week old 4Y26 control and ΔndhB plants. All measurements were done 15 min after light (800 µmol $m^{-2}s^{-1}$ for 4 min) was turned off. (A) Stromal over-reduction, estimated by P700 charge recombination as judged from $P700^+$ level induced by white multiple-turnover flash, ΔA_{MAX}. Values are expressed relative to the control recorded before light stress treatment. (B) Electron donation from plastoquinone to $P700^+$, estimated from $P700^+$ level induced by far red light (>710 nm, 3 W m^{-2}), ΔA_{FR}. Values are expressed relative to the control recorded before light stress treatment. (C) Level of plastoquinone reduction, estimated from a increase in steady state fluorescence level Fs after light stress. (D) Quantum yield of PSII under low light after light stress expressed as chlorophyll fluorescence parameter, Δ F/Fm'.

After the light stress, ΔA_{MAX} decreased more significantly in ΔndhB than in the vector control (4Y26) (Fig. 1A). Interestingly, the difference between 4Y26 and ΔndhB is much greater in younger plants. This result suggested that stromal over-reduction occurred even by this mild light stress and that young leaves were more susceptible to stromal over-reduction. ΔA_{FR} (Fig. 1B) and Fs under low light (Fig. 1C), both were

indicators for redox level of plastoquinone, unanimously showed that ΔndhB had more reduced plastoquinone pool after light stress than vector control especially in young leaves. Apparently, the plastoquinone reduction found in ΔndhB was not via NDH but via unknown quinone reductase, that functions in a different manner as NDH, or via chemical equilibilium between the sromal and the plastoquinone pools. Quantum yield of PSII as judged by Δ F/Fm' after light stress was also decreased more in Δ ndhB than the vector control (Fig. 1D).

Suppression of electron transport at PSII after light stress

To examine the effects of stress-induced over-reduction on photosynthetic yields, relative electron flow at PSII was compared before and 1 hour after light stress. Relative electron flow was estimated from fluorescence parametrer Δ F/Fm' (Genty et al. 1989), which is intrinsically associated with reduction level of plastoquinone. Feild et al. (1998) proposed that the reduced plastoquinone after light stress could be oxidized in the light because PSI drained electrons out of the plastoquinone pool to the stroma. Indeed, mild over-reduction induced in vector control 4Y26 after light stress could keep electron transport suppressed only at low light intensity (Fig. 2A), and under high light condition, the rate of electron transport was indistinguishable from that of non-stressed control (Fig. 2B). However, in Δ ndhB, the reduction of plastoquinone seemed to be associated with stromal over-reduction as shown in Fig. 1. In such cases plastoquinone may be kept reduced even in the light, because of low PSI activity due to charge recombination and high electron donation from stroma to plastoquinone. As expected, the electron transport through PSII was consistently lower in light stressed ΔndhB leaves than non-stressed leaves even at sub-saturating light (Fig. 2B). Thus, over-reduction jammed electron transport and decreased quantum yields of both photosystems.

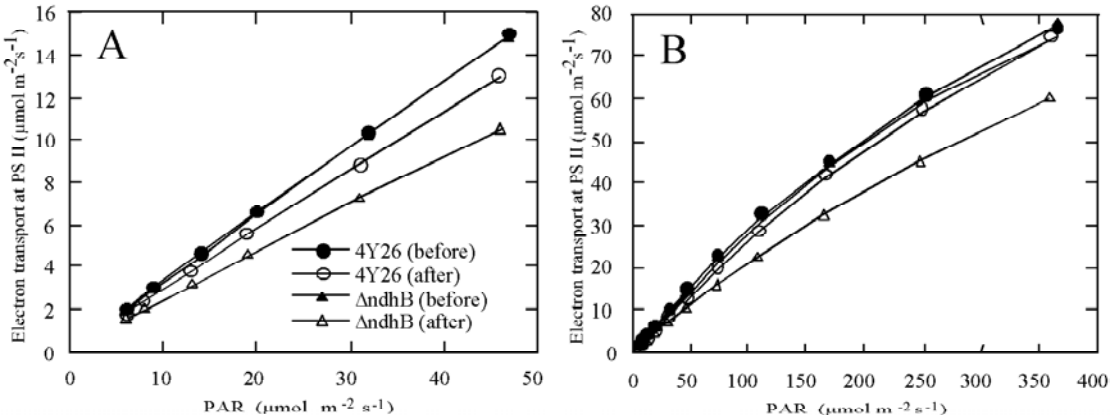

Fig. 2. Relative electron transport at PSII compared before and after the light stress (2,000 μmol m-2s-1, 4 min) in the 4Y26 control and ΔndhB, at low light (A) and sub-saturating ranges (B). PAR, photosynthetic active radiation. Seven- to eight-week old plants were used for the measurements. Values were calculated as Δ F/Fm'x PAR x 0.5 x 0.84 according to the operation manual of the fluorometer. Internsity of actinic red light was increased stepwise from about 6 to 370 μmol $m^{-2}s^{-1}$. Exposure to light at each intensity continued for 3 min, and at the end of each step, a saturating pulse (3,000 μmol $m^{-2}s^{-1}$ for 1 s) was applied for Fm' measurements. Values are the means of three independent measurements. Standard errors at 370 μmpl $m^{-2}s^{-1}$ are 1.6 to 6.3 $m^{-2}s^{-1}$.

Discussion

How can we link cyclic electron flow and over-reduction?
Heber and Walker (1992) proposed dual functions of cyclic electron transport, that is, supplemental ATP production to adjust ATP/NADPH ratio to drive the Calvin cycle and induction of ΔpH-dependent down-regulation of PSII. Both idea can explain post-illumination over-reduction in NDH-defective leaves, presented here; ATP shortage may result in surplus of NADPH in stroma, and dessensitization of PSII down-regulation may also result in accumulation of reducing equivalents in stroma. However we tentatively decline latter possibility because no significant difference was found in the formation of energy quenching of chlorophyll fluorescence even under strong light between 4Y26 and ΔndhB (data not shown).
Physiological relevance of post illumination over-reduction
After exposure to strong light, plastoquinone reduction as judged from an increase in apparent Fo' and stromal reduction as judged from a decrease in photo-oxidizable P700, in ΔndhB lasted for several hours under room light (data not shown), suggesting that effective safety valves such as terminal oxidase in thylakoids or envelope oxidase to discharge extra reducing power either do not function in low light or are not present. Therefore, mechanisms to avoid over-reduction are important for plant growth. In this sense, chloroplastic NDH may play a role in photoprotection. However, it should be noted that even wild type plants are susceptible to over-reduction and consequent photoinhibition under severe light stress. Thus, stromal over-reduction seems to be a quite general phenomenon that takes place under light stress, and the relevance of NDH in photoprotection may be restrited in its capacity. At present, little information is available regarding whether cyclic electron flow mediated by NDH is physiologically significant in the field. Most likely situation in which NDH-mediated cyclic flow is crucial for plant growth is when young plants are exposed to sudden and frequent changes in light condition such as sun flecks.

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