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The role of chloroplast Ndh complex in resisting heat stress in tobacco strain

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Abstract. After exposure to high temperature (50℃), wild type of *Nicotiana tobacum* (WT) survived much longer than its *ndh*JK genes defective mutant (*ndh*JK mutant) did. Decrease in photochemical efficiency of PSII (Fv/Fm) under the condition was faster in *ndh*JK mutant. WT showed evident acceleration of re-reduction of $P700^+$ after turning off of far-red light and significant in post-illumination increase in Chl fluorecence under the condition, indicating that NDH-mediated cyclic electron transport around photosystem I (PS I) was heat-stimulated. Initial rise in non-photochemical quenching (qN) and its subsequent decline were faster in WT, suggesting that NDH is essential for enhancement of ΔpH across the thylakoid membranes and utilization of NADPH and ATP by carbon fixation under the stressed condition. Higher expression of Ndhk after heat treatment supports that Ndh is involved in the stimulated PS I-cyclic electron transport. A possible mechanism of the role of Ndh in resisting heat stress was discussed.

Introduction

NADH-ubiquinone-oxidoreductase (Ndh, or complex I) is a multi-subunit protein complex that passes electrons from NADH to ubiquinone coupling with formation of ATP. Genes with a high sequence homology to those encoding subunits of mitochondrial Ndh have been found in chloroplasts (Ohyama et al 1986, Shinozaki et al 1986). Cyanobacterial Ndh genes show a high sequence homology to those in chloroplast genes (Ellersiek and Steimmüller 1992). A series of work indicated that Ndh is involved in cyclic electron transport around PSI as well as respiratory electron flow in cyanobacteria (Mi et al. 1994, 1995) and in chloroplasts (Friedrich et al 1995，Shikanai et al 1998, Burrow et al, 1998, Kofer et al. 1998). The Ndh-mediated cyclic electron flow has been suggested to be essential for the adaptation of cyanobacteria to salt shock (Tanaka et al. 1997) and to function in protection against photoinhibition in tobacco (Endo et al. 1999). However, the physiological roles of NDH remain to be further clarified.

In this report, we investigated response of Ndh to a heat stressed condition between wild type and its *ndh*JK mutant of tobacco plants. Our results indicate that

the important physiological role of Ndh mediated electron transport in protecting plants against heat-damage.

Material and methods

1) Transformant plants of *Nicotiana tobacum c.v. Xanthi* in which the chloroplastic *ndh*JK genes were insertionally inactivated (*ndh*JK mutant) were cultivated along with wild-type (WT) in greenhouse (14h day at 25℃/10h night at 20 ℃, 200 μmol photon $m^{-2}s^{-1}$, 40% humidity). Young plants of 4-5 weeks old were used for experiment. For the heat treatment, the plants were transferred into a chamber at 50℃, 100 μmol photon $m^{-2}s^{-1}$ and 70 % humidity. Unless otherwise stated, all the experiments were done with the third fully expanded leaves.

2) Chl fluorescence and the redox state of P700 were measured with PAM Chl fluorometer (Walz, Effeltrich, Germany) with an emmiter-detector (ED-101 US) for Chl fluorescence and that (ED-P700) for P700 absorbance changes monitored by the absorbance at 810-830 nm. The fluorometer setup was done as described as by Schreiber et al (1986,1988) and Klughammer and Schreiber (1998).

3) Intact chloroplasts were isolated from freshly harvested leaves or the leaves after heat treatment with a method as described by Asada (1990), except HEPES buffer was replaced by STN buffer (0.4 M sucrose, 50mM Tris·HCl, 0.01M NaCl, pH 7.6). The chloroplasts were osmotically shocked with a buffer containing 50 mM Tris • HCl, 10mM NaCl supplementing with 2% Triton and incubated at 4˚C for 2 hours. After centrifugation at 10000×g for 5min, the supernatant was used for SDS-PAGE and Western blotting.

4) Denatured proteins were separated by SDS-PAGE in a 15% polyacryamide gel according to Laemmli (1970). Proteins in the gel were electrically transferred to a nitrocellulose membrane for Western blotting analysis by using an ECL immunoblotting kit (Amersham) according to its protocols. Protein concentration was determined by the method of Bradford (1976).

Results

1 **Chl fluorescence analysis**

There was no clear difference in the photochemical efficiency of PS II, as judged by ratio of Fv (maximum yield of Chl fluorescence at closed PS II centers minus minimum fluorescence yield at open PS II centers) to Fm (maximum yield of fluorescence at close PSII center) between WT and *ndh*JK before treatment (Fig.1). However, after exposure to the

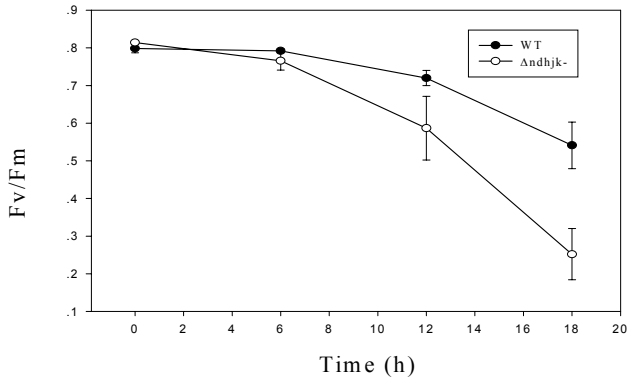

Fig.1 Changes in Fv/Fm during heat treatment at 50℃ in wild type (WT) and its *ndh*JK defective mutant (Δndhjk). Fo and Fm were measured at 28℃ after 5 min dark adaptation. Data points represent the mean ±S.E. of three independent measurements.

high temperature condition for 6 h, the Fv/Fm in *ndh*JK mutant decreased much sharper than that of WT. After 18 hours exposure, the Fv/Fm in WT still kept higher, but that in *ndh*JK mutant was almost undetectable. Actually, evident wilt was found in *ndh*JK mutant but almost not in WT after heat treatment for 12 h (data not shown).

2 Fig. 2 shows the initial rise and its subsequent decline in qN were almost the same in WT and *ndh*JK mutant before treatment. But after exposure to the high temperature for 10 h, both the initial increase and subsequent decline of qN in WT were faster than that in *ndh*JK mutant.

Before treatment, a transient increase in Chl fluorescence was observed in WT, but almost not in *ndh*JK mutant (Fig. 3). After exposure to the high temperature, the increase became significant in WT, but only a slight in *ndh*JK mutant (Fig. 3).

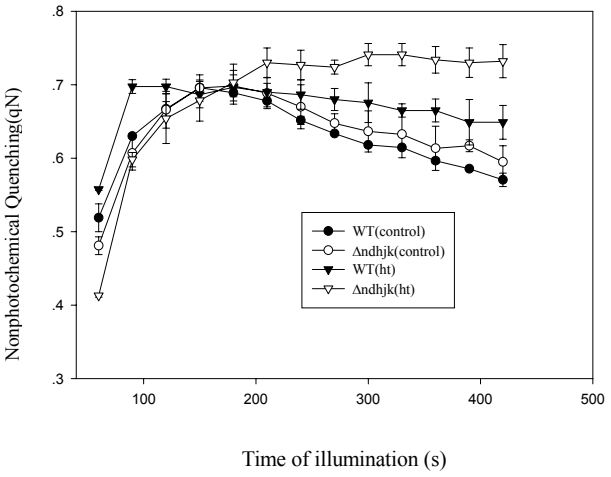

Fig. 2. Effect of temperature on qN of attached leaves in tobacco strains at 28℃ after heat treatment for 10 (ht) and 0 h (control) followed by 5 min dark adaptation, data points represent the mean ± SE of three replications.

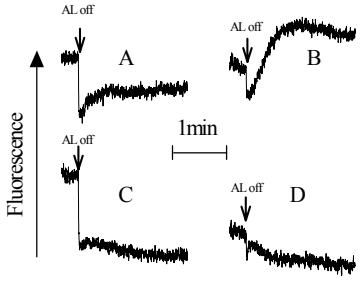

Fig. 3. Transient increase in Chl fluorescence after termination of actinic light (AL: 1000 μmol photon m^{-2} s^{-1}) for 1 min in tobacco plants. A, WT control; B, WT after heat treatment; C, *ndh*JK mutant control; D, *ndh*JK mutant after heat treatment. A and C were measured at 28℃. B and D were measured at 50℃ after heat treatment for 6h.

2. Reduction of $P700^+$ Table 1 shows a kinetic analysis of the dark reduction of $P700^+$ after turning off of far red light. Similar to the previous report (Bukhov 1999), natural logarithm plots of the dark reduction of $P700^+$ after far-red light illumination revealed bi-phase kinetics, fast phase and slow phase. Before heat treatment, there were no obvious difference in the halftimes ($t_{f1/2}$ and $t_{s1/2}$) and in the percentage of each phase (m_f(%) and m_s(%)) between WT and *ndh*JK mutant. After heat treatment, the halftimes of fast phase and of slow phase shortened to about 1/4 and 1/5, respectively, in WT and about 1/2, respectively, in *ndh*JK mutant. The percentages of fast phase decreased about 12.67%, accordingly the percentage of slow phase increased 12.67% in WT. However, there was not much change in *ndh*JK mutant.

Table 1. Effect of heat treatment on parameters of re-reduction of $P700^+$ after terminating off FR-illumination in wild type (WT) and its *ndh*JK defective mutant (ΔndhJK). $t_{f1/2,}$ the half time of fast phase; $t_{s1/2}$, the half time of slow phase; m_f(%), the relative magnitude of fast phase(% of total); m_s(%), the relative magnitude of slow phase(% of total)

	Before treatment				After treatment			
	$t_{f1/2}$(s)	$t_{s1/2}$(s)	m_f(%)	m_s(%)	$t_{f1/2}$(s)	$t_{s1/2}$(s)	m_f(%)	$m_{s(\%)}$
WT	1.31±0.07	7.33±0.91	71.95±2.13	28.05±2.13	0.32±0.01	1.42±0.07	59.28±2.04	40.72±2.04
Δndhjk	1.21±0.09	7.92±0.76	68.00±1.14	32.0±1.14	0.54±0.02	3.32±0.41	72.58±2.05	27.42±2.05

3**Expression of NdhK** To check changes in expression of NdhK under the high temperature condition, Westen blotting was carried out. As showed in Fig. 4, expression of NdhK increased after exposure to the high temperature for 6 h.

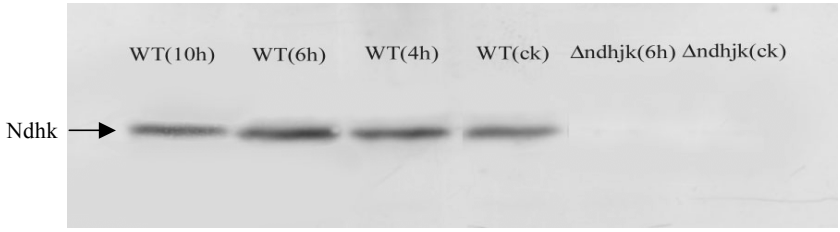

Fig. 4. Western blotting of expression of Ndhk in wild type (WT) and its *ndh*JK defective mutant (Δ*ndh*jk) of tobacco plants after SDS-PAGE. The plants were transferred to high temperature (50℃) for indicated time, ck, 0 h before sampling. 30μg protein/lane.

Discussion

It has been proposed that cyclic electron flow around PS I functions in adaptation to environmental stresses in eukaryotes or in cyanobacteria (Canaani 1990, Herbert et al. 1992, Heber and Walker 1992). The electron flow around PS I was stimulated by reduction of electron carriers in the intersystem chain by stromal components (Havaux 1996). Activation of cyclic electron flow around PS I is suggested to be capable of energizing thylakoid membranes in illuminated heat-stressed leaves even when PSII reactions suffer inactivation (Bukhov et al. 1999). The biphasic kinetics of re-reduction of $P700^+$ after turning off of far-red light revealed two different electron donor systems: the fast one probably a reduced ferredoxin (Bukhov et al. 1999), whereas the slow one a reduced pyridine nucleotide in the chloroplast stroma (Havaux 1996, Endo et al. 1997). Base on the data in Fig. 3 and

Table 1, we conclude that in addition to ferredoxin (Bukhov et al. 1999), NdhJK is also involved in the heat-stimulated cyclic electron flow in tobacco, as monitored by a transient increase in Chl fluorescence after termination of actinic light (Asada et al. 1993) and re-reduction of $P700^+$ (Mi et al. 1992). The expression of Ndhk was stimulated under the high temperature condition, suggesting that Ndh is involved in the heat-stimulated cyclic electron transport around PS I. Whether Ndh complex is activated under the high temperature condition or not, further investigation is on progressing. Lack of a PS I-cyclic electron flow by insertionally inactivated *ndh*JK genes caused severe photoinhibition, as judged by Fv/Fm in *ndh*JK mutant (Fig. 1). As a result, WT survived much longer than NdhJK mutant did under the heat stressed condition (data not shown).

The acceleration of both initial rise in qN and its subsequent decline in WT (Fig.2) indicate that Ndh is essential for enhancement of ΔpH across the thylakoid membranes and utilization of NADPH and ATP by carbon fixation (Ivanov et al 2000) under the stressed condition. The heat-stimulated cyclic electron transport probably functions in the downregulation of PSII maybe through the extra Δ pH across the thylakoid membranes (Heber and Walker, 1992), or through the redox potential of a stromal component (Ott 1999).

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