S20-026

Suppressive Effects of Nitric Oxide (NO) on Photosynthetic Electron Transport System

S Takahashi[1], H Yamasaki[2]

Laboratory of Cell & Functional Biology, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, JAPAN,

[1]takahashishunichi@hotmail.com, [2]yamasaki@sci.u-ryukyu.ac.jp

Keywords: alternative electron acceptor, electron sink, nitrate reductase, nitric oxide, photophosphorylation

Introduction

Nitric oxide (NO), a gaseous free radical, is a bioactive molecule that can act as a regulator for enzyme activities. Air pollution studies have shown that NO is a potent inhibitor for photosynthesis; NO rapidly and reversibly inhibits CO_2 uptake activity (Hill & Bennett 1970). However, inhibitory mechanism of NO has not yet been clarified. NO has been reported to be produced in leaves of higher plants (Wildt *et al.* 1997). Recent studies have suggested that plant cells have at least two types of enzyme that catalyze NO production, i.e. NO synthetase (NOS)-like protein (Durner & Klessig 1999) and nitrate reductase (NR) (Yamasaki *et al.* 1999). It has been suggested that NOS-like protein is involved in a various physiological responses such as hypersensitive response during interactions with pathogenic microorganisms. The physiological function of NR-dependent NO production is unknown. Here we show the effects of NO on photosynthetic activities in thylakoid membranes. Results demonstrate that NO inhibits photophosphorylation via suppressing the activity of photosynthetic electron transport.

Material and methods

Thylakoid membranes were prepared according to Yamasaki *et al.* (1991) with slight modifications. Spinach leaves were homogenized with a grinding buffer (pH 8.0) that contained 0.3 M sorbitol, 50 mM Tricine-KOH, 25 mM KCl, 5 mM $MgCl_2$ and 5 mM ascorbate. Thylakoids were suspended in a suspension buffer (pH 8.0) that contained 0.3

M sorbitol, 20 mM Tricine, 25 mM KCl and 5 mM $MgCl_2$, 4 mM EDTA and 1.5 mM K_2HPO_4. Photophosphorylation activity was measured by a bioluminescence assay using the luciferin/luciferase assay kit SZ101 (Yamato, Tokyo, Japan) in a reaction mixture (pH 8.0, 25 °C) containing 20 µmol Chl ml^{-1} thylakoids, 0.3 M sorbitol, 20 mM Tricine, 25 mM KCl, 5 mM $MgCl_2$, 4 mM EDTA, 1.5 mM K_2HPO_4, 50 µM methylviologen, 100 µM ADP and 30 mM ascorbate. ΔpH-dependent uptake of 9-aminoacridine (9-AA) was measured according to Gilmore & Yamasaki (1998). Measurements were carried out in a reaction mixture (pH 8.0, 25 °C) containing 20 µmol Chl ml^{-1} thylakoids, 0.1 M HEPES, 10 mM $NaCl_2$, 50 µM methylviologen, 50 µM ATP and 30 mM ascorbate. The activity of photosynthetic electron transport was determined from the rate of methylviologen-dependent O_2 uptake in a reaction mixture (pH 8.0, 25 °C) containing 20 µmol Chl ml^{-1} thylakoids, 0.1M sucrose, 10 mM $NaCl_2$, 0.1 M HEPES, 500 µM NH_4Cl, 50 µM methylviologen and 30 mM ascorbate. O_2 uptake was measured with a Rank Brothers O_2 electrode. NO concentration was measured electrochemically with a Clark-type NO electrode (ISO-NOP) in conjunction with an ISO-NO Mark II and Duo.18 data acquisition system.

Results

Inhibitory effects of NO on photosynthesis

Photosynthetic ATP synthesis (photophosphorylation), catalyzed by H^+-ATPase (CF_1-CF_0), is coupled with vectorial proton translocation across thylakoid membranes from lumenal to stromal sides. SNAP (*s*-nitroso-*n*-acetyl-DL-penicillamine), a chemical NO donor, was found to decrease photophosphorylation activity (Fig.1A). The extent of decrease was NO dependent and 50% inhibitory concentration (IC_{50}) was 0.7 µM NO.

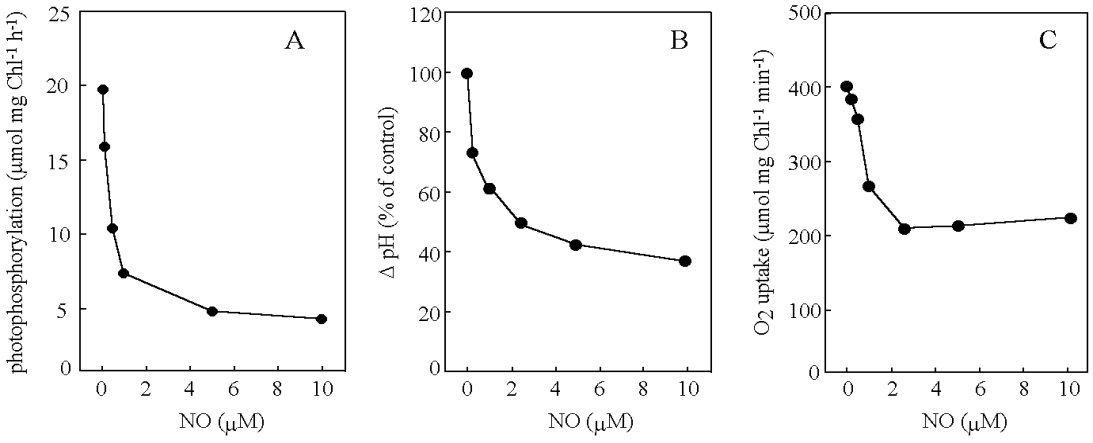

Figure 1. Inhibitory effects of NO on photophosphorylation rate (A), ΔpH formation (B) and methylviologen dependent O_2 uptake rate (C) in thylakoid membranes. Measurements were carried out under the illumination of 150 μmol m^{-2} s^{-1}.

Also, NO had a potential to inhibit ATPase (ATP hydrolysis) activity (IC_{50} = 50 μM NO) but the sensitivity of ATPase activity to NO was much lower than that of photophosphorylation activity. NO can be oxidized to NO_2^- along with trace amounts of NO_3^- in oxygen-containing aqueous solution (Ignarro *et al.* 1993). Thus, one may consider that the inhibitory effect of NO on ΔpH formation would be due to the uncoupling effects of these products. However, oxidized products of NO (NO_2^- and NO_3^-) showed little inhibition on ΔpH formation (data not shown). The linear electron transport from water to $NADP^+$ is a driving force for *in vivo* ΔpH formation. We measured the linear electron transport rate by methylviologen-dependent O_2 uptake. Consistent with inhibitory effects of NO on photophosphorylation and ΔpH formation, NO inhibited the O_2 uptake in a concentration-dependent manner (Fig. 1C). A similar inhibitory effect of NO on photosynthetic electron transport was observed by a PAM method (data not shown).

NO quenching by thylakoid membranes under illumination

We hypothesized that the inhibitory effects of NO on ΔpH forming electron transport would be due to electron leakage from PSII to NO. If NO functions as an electron acceptor, NO quenching would be observed as a result of NO reduction. We found that thylakoid membranes have a potential to quench NO under illuminating conditions (Fig. 2). The extent of NO quenching was light-intensity dependent. The NO quenching rate was 0.93 μmol NO min^{-1} μg Chl^{-1} under an illumination of 300 μmol m^{-2} s^{-1}. NO quenching was not affected by DCMU which inhibits electron transfer from Q_A to Q_B in photosystem II (data not shown).

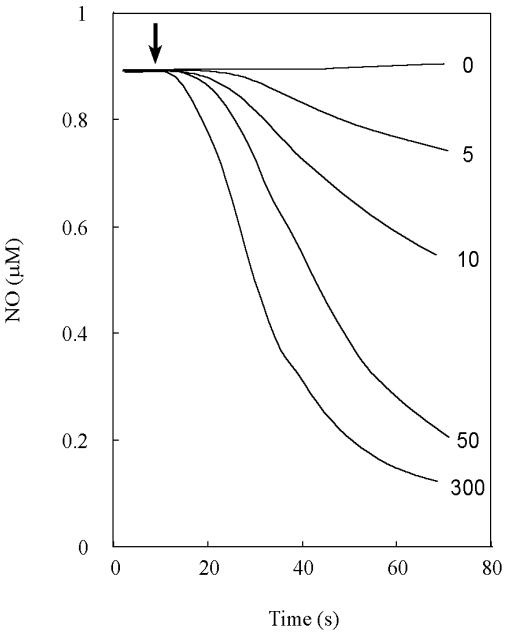

Figure 2. NO quenching by thylakoids during illumination. Thylakoids (20 μmol Chl ml^{-1}) were suspended in a reaction buffer (pH 8.0) contained 0.1 M HEPES, 10 mM NaCl2, 50 μM methylviolgen, 50 μM ATP and 30 mM ascorbate. Thylakoids were illuminated (300, 50, 10, 5 or 0 μmol m^{-2} s^{-1}) at the allow as indicated.

Discussion

NO inhibited the linear electron transport rate, ΔpH formation and photophosphorylation activity (Fig.1). Because electron transport coupled with ΔpH is a driving force for

photophosphorylation, the results suggest that NO primarily suppresses electron transport and subsequently inhibits ΔpH formation and eventually inhibits photophosphorylation. It has been reported that NO binds to the non-heme iron of PSII (Petrouleas & Diner 1990). If NO accepts electrons from PSII, NO quenching and inhibition of the ΔpH forming electron transport would simultaneously occur (Figs. 1C & 2). Further investigations are required to clarify the hypothesis that NO acts as an electron acceptor for PSII.

The result showing that NO suppresses photophosphorylation activity (Fig. 1) implies that NO could control the carbon assimilation activity. NO has been reported to be produced in leaves of higher plants (Wildt *et al.* 1997). We previously reported that nitrate reductase (NR) is capable of producing NO from nitrite (Yamasaki *et al.* 1999). NR-dependent NO production would occur when nitrite accumulates in cells under unfavorable conditions for nitrogen assimilation. The activities of nitrogen assimilation and carbon assimilation are clearly interdependent (Foyer *et al.* 1995). NO produced by NR may function as a regulator for the carbon assimilation system to tune a harmonic C/N assimilation balance.

Acknowledgments

We are grateful to Dr. Michael F. Cohen for critical reading of the manuscript. This work was supported by Grant-in-Aid for Scientific Research (B) and (C) from Japan Society for the Promotion on Science.

Reference

Durner J, Klessig DF (1999) *Current Opinion in Plant Biology* **2,** 369-374.

Foyer CH, Valadier MH, Ferrario S (1995) In *Environment and plant metabolism* (ed N Smirnoff), pp. 17-33. BIOS Scientific Publishers, Oxford, UK.

Gilmore AM, Yamasaki H (1998) *Photosynthesis Research* **57,** 159-174.

Hill AC, Bennett JH (1970) *Atmospheric Environment* **4,** 341-348.

Ignarro LJ, Fukuto JM, Griscavage JM, Rogers NE, Byrns RE (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8103-8107.

Petrouleas V, Diner BA (1990) *Biochimica et Biophysica Acta* **1015,** 131-140.

Wildt J, Kley D, Rockel A, Rockel P, Segschneider HJ (1997) *Journal of Geophysical Research-Atmospheres* **102,** 5919-5927.

Yamasaki H, Sakihama Y, Takahashi S (1999) *Trends in Plant Science* **4,** 128-129.

Yamasaki H, Furuya S, Kawamura A, Ito A, Okayama S, Nishimura M (1991) *Plant Cell Physiol.* **32,** 952-934.