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Involvement of nitric oxide in light-mediated greening of barley seedlings

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Summary

When seedlings are grown in the dark, proplastids of the developing leaf differentiate into etioplasts. Greening of etiolated plastids is stimulated by light, which is sensed by various types of photoreceptors. Nitric oxide (NO) has been shown to be a bioactive molecule that could take part in this light-mediated process in plants. In this paper, we show that emission of NO in barley seedlings increased concomitantly with increasing activities of nitric oxide synthase (NOS) during the greening. Treatment with sodium nitroprusside (SNP), a NO donor, increased the accumulation of chlorophyll contents, enhanced the accumulation of thylakoid membrane proteins, such as light harvesting complex of photosystem II (LHCII) and PSIA/B, and then improved the effective quantum yield of photosystem II (PSII) (Φ_{PSII}) in the light. Instead, treatment with either NO scavenger 2-phenyl-4,4,5,5-tetramentylimidazoline-1-oxyl-3-xide (PTIO) or NOS inhibitor N^{ω}-nitro-L-arginine (L-NNA) retarded the greening of etiolated-seedlings. Moreover, sodium ferrocyanide, an analog of SNP, nitrite and nitrate, two NO-decomposition products did not have any effect on the greening process. These results indicated that NO, as an endogenous signaling molecule, participates in light-mediated greening of barley seedlings, and exogenous NO accelerates this process.

Introduction

When seedlings are grown in the dark, proplastids of the developing leaf differentiate into etioplasts (Robertson and Laetsch, 1974). During this developmental stage, a number of changes take place, such as an increase of plastid number/cell, volume/plastid, and an increase of expression of most nuclear and plastid genes encoding plastid

Abbreviations: F_0, initial chlorophyll fluorescence; F_m, maximum chlorophyll fluorescence; F_v, variable chlorophyll fluorescence; F_v/F_m, maximum photochemical efficiency of photosystem II; LHCII, light harvesting complex of photosystem II; L-NNA, N^{ω}-nitro-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PSII, photosystem II; PTIO, 2-phenyl-4, 4, 5, 5-tetramentylimidazoline-1-oxyl-3-xide; SNP, sodium nitroprusside; Φ_{PSII}, photochemical efficiency of photosystem II

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polypeptides (Klein and Mullet, 1987; Baumgartner et al., 1989). However, nuclear-encoded chlorophyll *a*/*b*-binding antennae proteins and plastid-encoded chlorophyll *a*-binding proteins fail to accumulate because etioplasts do not synthesize chlorophyll. Only under illumination does chlorophyll accumulate concomitantly with the accumulation of the plastid-encoded chlorophyll *a*-binding apoproteins and the assembly of the photosynthetic apparatus (Klein and Mullet, 1986).

Previous studies have showed that newly synthesized D1 and CP43 apoproteins were stabilized in the presence of chlorophyll and accumulated in the light (Mullet et al., 1990). In leaves left in the light in the presence of chlorophyll synthesis inhibitors (e.g. levulinic acid), the pre-accumulated light harvesting complex of photosystem II (LHCII) was degraded (Anastassiou and Argyoudi-Akoyunoglou, 1995). Thus, chloroplast-encoded reaction center proteins and LHCII apoproteins may compete for the limited amount of chlorophylls, which are required to anchor the proteins in the thylakoid membrane, rescuing them from degradation.

Greening of etiolated plants is stimulated by light, which is sensed by various types of photoreceptors. Recently, nitric oxide (NO) has been suggested to act as a signal molecule mediating responses to biotic and abiotic stresses in plant kingdom. It could induce germination instead of red light (Beligni and Lamattina, 2000), affect growth and development of plant tissue (Durner and Klessig, 1999) and enhance plant cell senescence (Leshem et al., 1998). Also, NO was suggested to be involved in the responses to drought stress, salt stress, heat stress, disease resistance and apoptosis (Delledonne et al., 1998; Durner and Klessig, 1999; Mata and Lamattina, 2001; Zhao et al., 2004). In animals, nitric oxide synthase (NOS) generating NO from L-Arg was identified (Bredt et al., 1991), and NOS-like activity has been detected widely in plants, and inhibitors of mammalian NOS were shown to inhibit NO generation (Cueto et al., 1996; Durner and Klessig, 1999; Foissner et al., 2000). Recently, a hormone-activated NOS was identified in plants (Guo et al., 2003). Nevertheless, the production of NO in plants is not restricted to NOS-like activity, and it can also be generated from nitrate reductase (Yamasaki and Sakihama, 2000). Recent reports indicated that NO stimulated de-etiolation and an increase in chlorophyll in potato, lettuce and *Arabidopsis* (Beligni and Lamattina, 2000). NO also increased chlorophyll contents in pea leaves, particularly in guard cells, and retarded chlorophyll loss in *Phytophthora infestans*-infected potato leaves (Laxalt et al., 1997).

Here, we investigated the function of NO in the accumulation of chlorophylls, assembly of chlorophyll protein complexes and activation of photosynthetic electron transport. In this report, we showed that NO stimulates chlorophyll biosynthesis, thylakoid membrane polypeptides accumulation and activates photosynthetic function during greening of barley leaves.

Materials and methods

Plant materials

Seeds of barley (*Hordeum vulgare L. var Morex*) were surface-sterilized with 0.5% hypochlorite for 20 min and soaked in water overnight. The etiolated seedlings were germinated in vermiculite inside a light-proof incubator at 23 °C for 5 d. Five-day-old dark-grown seedlings were cut 1 cm above the seed under distilled water, and then floated in Petri dishes containing distilled water or 100 μM sodium nitroprusside (SNP; Sigma, USA), an NO donor. 2-Phenyl-4,4,5,5-tetramentylimidazoline-1-oxyl-3-xide (PTIO; Sigma, USA) and N^{ω}-nitro-L-arginine (L-NNA; Sigma, USA) were used. One hundred micromoles of sodium ferrocyanide, and a mixture containing 100 μM $NaNO_2$ and 100 μM $NaNO_3$ were used as additional controls. To ensure proper uptake of the chemicals, the Petri dishes containing the seedlings were subjected to vacuum infiltration for 60 min. After vacuum infiltration in the dark, the samples were illuminated at a light intensity of 120 $\mu mol\, m^{-2}\, s^{-1}$ for 0, 6, 12, 24, and 36 h. All the treatments were performed when possible in complete darkness. When required, a dim green safelight was provided which was unable to photoconvert protochlorophyllide to chlorophyll (Klein and Mullet, 1986).

Nitric oxide content determination

NO content was determined as described by Murphy and Noack (1994) with some modifications. Barley seedling sections were incubated with 100 U of catalase and 100 U of superoxide dismutase for 5 min to remove endogenous ROS before addition of 10 mL oxyhemoglobin (5 mM). After 2 min incubation, NO was measured spectrophotometrically by measuring the conversion of oxyhemoglobin to methemoglobin.

Determination of NOS activity

NOS activity assay was performed according to Murphy and Noack's (1994) method with some