Modulation of Chlorophyll b Biosynthesis and Photosynthesis by Overexpression of Chlorophyllide a Oxygenase (CAO) in Tobacco[1]

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[1] *A full length article will be published elsewhere*

Abstract: Chlorophyll (Chl) b is synthesized by oxidation of a methyl group on the B ring of the porphyrin molecule to a formyl group by chlorophyllide (Chlide) a oxygenase (CAO). The overexpression of *Arabidopsis thaliana* full length *CAO* (*AtCAO*) in tobacco (*Nicotiana tabacum*) resulted in an increased Chl synthesis and a decreased Chl a/b ratio in low-light-grown (LL) as well as in high-light-grown (HL) tobacco plants, where the effect was more pronounced. In HL-plants, increased [Chl b] resulted in efficient capture of solar energy and enhanced (40%–80%) electron transport rates at both limiting and saturating light intensities.

Keywords: Chlophyll b biosynthesis; Chlorophyllide a Oxygenase; *Nocotiana tabacum*; Photosynthesis

Introduction

Chlorophyll (Chl) b is a closed Mg-tetrapyrrole found in plants, green algae and some prochlorophytes. The main function of Chl b is to gather light energy and transfer it with 100% efficiency to Chl a. Chl b is synthesized from Chl a by oxidation of methyl group on the B ring to a formyl group at that position. The genes encoding chlorophyllide a oxygenase (CAO), responsible for Chl b synthesis, have been isolated from several different species (Tanaka *et al*., 1998; Espineda *et al*., 1999; Nagata *et al*., 2004). The CAO enzyme is localized in chloroplast envelope and thylakoid membranes and contains domains for a [2Fe-2S] Rieske center and for a mononuclear nonheme iron-binding site (Eggink *et al*., 2004). The conserved Rieske center and non-heme-iron binding motifs of CAO are likely to be involved in the electron transport from ferredoxin to moleclular oxygen. The recombinant CAO protein catalyzes the oxidation of Chlide *a* to Chlide *b* (Oster *et al*., 2000). We have previously reported that overexpression of full length *AtCAO* results in increased Chl b synthesis and decreased Chl a/b ratio in low-light, but more so in high-light-grown tobacco plants (Pattanayak *et al*., 2005). In the present study, we show that the overexpression of full length *AtCAO* modulates the flux of Chl biosynthesis pathway leading to increased Chl b synthesis both in low-light- and high-light-grown transgenic tobacco plants. We further show that increased Chl b biosynthesis in full-length CAO-overexpressing (CAOx) plants results in efficient capture of solar energy and increased electron transport mostly at limiting light intensities.

Materials and Methods

Plant Materials and Growth Conditions

Wild-type (WT) and CAO overexpressing (CAO_X) tobacco (*Nicotiana tabacum cv. Petit*

Havana) plants (Pattanayak *et al*., 2005) were grown in greenhouse in natural photoperiod for 25–30 days under light intensity of 200 μmol photons m^{-2} s^{-1} at 25 ± 2 °C. In our studies, these plants were transferred either to low light (LL) (70–80 μmol photons m^{-2} s^{-1}) or to high light (HL) (700–800 μmol photons m^{-2} s^{-1}) for additional 18–20 days in a greenhouse.

Chlorophyll a Fluorescence Measurements

Chl a fluorescence was measured with a PAM-2001 Chl fluorometer (Walz, Germany) at room temperature, from the front surface of leaves (see *e.g*., Dutta *et al*., 2009). Before each measurement, the leaf was dark-adapted for 20 min.

Results

CAO Overexpressed (CAOx) Plants Grown in Low Light or High Light Regimes had Altered Chlorophyll a/b Ratio

The CAOx (CAOx1 and CAOx2) plants accumulated higher amounts of Chl in low light (LL; 70–80 μmol photons m^{-2} s^{-1}) as well as in high light (HL; 700–800 μmol photons m^{-2} s^{-1}) as compared to that in the wild-type (WT) plants grown under identical light regimes. As expected, WT-LL plants had higher Chl content and reduced Chl a/b ratio than the WT-HL plants (Fig. 1). The leaves of CAOx1-HL-plants were greener and accumulated 28% more Chl than WT-HL plants. The CAOx2-HL-plants accumulated 20% more Chl than the WT-HL plants (data not shown). Due to increased (70%) synthesis of Chl b in the CAOx plants grown in HL (CAOx1-HL), they showed an ~30% decline in Chl a/b ratio as compared to WT-HL plants. Similarly, CAOx2-HL-plants showed ~50% increase in Chl b synthesis as compared to WT-HL plants (data not shown). The CAOx plants grown in LL (CAOx1-LL) were greener than LL-grown WT plants (WT-LL) and had 15% lower Chl a/b ratio (Fig. 1). The Chl a/b ratio and Chl content of WT and transgenic plants showed some minor variations in different growth seasons. Since CAOx1 plants had high amount of Chl b and reduced Chl a/b ratio as compared to that of CAOx2, we characterized the CAOx1 plants in detail, henceforth referred simply as CAOx.

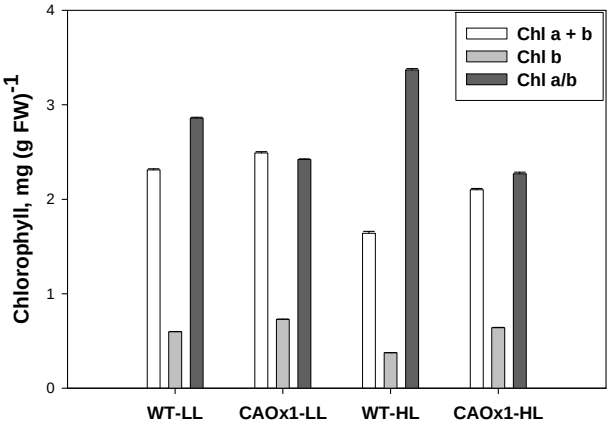

Fig. 1 Chlorophyll content and the leaf phenotype of wild-type (WT) and CAO overexpressing (CAOx1) tobacco plants grown under low-light (LL) and high-light (HL). Total chlorophyll (Chl a+b) content, chlorophyll b (Chl b) and chlorophyll a/b (Chl a/b) ratio of WT and CAOx1 plants grown in LL (WT-LL, CAOx1-LL) and HL (WT-HL, CAOx1-HL). Plants grown for up to 20–25 days under light intensity of 200 μmol photons m^{-2} s^{-1} were transferred to LL (70–80 μmol photons m^{-2} s^{-1}) and HL (700–800 μmol photons m^{-2} s^{-1}) for additional 18–20 days in the greenhouse. The 2nd leaf was harvested from each plant types for pigment analysis. Each data point is the average of five replicates.

Photosynthetic Responses of CAOx Plants Grown in Low Light and High Light Regimes

To ascertain if increased Chl b content had the expected effect on photosynthetic apparatus, Chl *a* fluorescence of leaves of both WT and CAOx tobacco plants grown in LL (70 μmol photons m^{-2} s^{-1}) or HL (700–800 μmol photons m^{-2} s^{-1}) was measured.

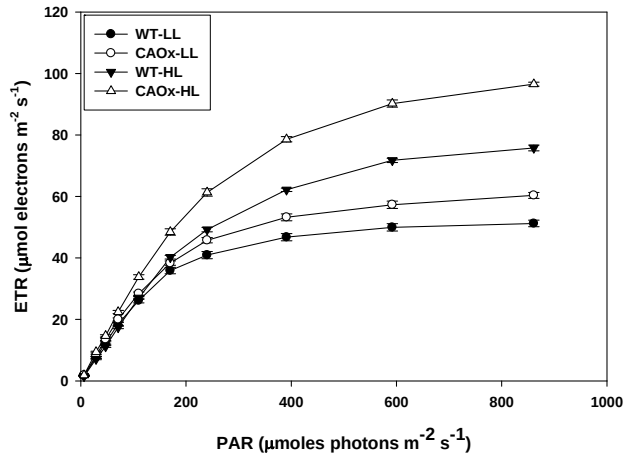

Fig. 2 Electron transport rate (ETR) of WT and CAOx plants. LL and HL-grown WT and CAOx plants were dark adapted for 20 min before readings were taken by PAM 2100 fluorometer. The ETR values were calculated from these fluorescence data (Table 1). The experiment was repeated thrice and each data point is the average of ten replicates.

Chl *a* fluorescence is used as a nondestructive and noninvasive signature of photosynthesis, particularly of Photosystem II (for reviews, see Papageorgiou and Govindjee, 2005). The minimal fluorescence (F_0), the maximum fluorescence (Fm) and the maximum photochemical efficiency of PSII in dark-adapted leaves, measured as Fv/Fm (where $F_v = F_m - F_o$), were not substantially affected in LL- or HL- grown WT or CAOx plants (Table 1). The electron transport rate (ETR, μmol electrons m^{-2} s^{-1}) was estimated from fluorescence parameters. The light-response curves of ETR suggest that in limiting (up to 70 µmol photons m^{-2} s^{-1}) as well as saturating light intensities, the ETR was higher in CAOx plants than that in WT plants grown in LL- or HL-regimes suggesting better light absorption and utilization, especially in Photosystem II, in the transgenic plants (Fig. 2). In WT, at limiting light intensities, the rate of increase in ETR in LL-grown plants was essentially the same as that in HL-grown plants (Fig. 2) even though the latter have less Chl (Fig. 1). The ETR in LL-grown WT and CAOx plants saturated at lower light intensity, around 400 µmol photons m^{-2} s^{-1} than HL-grown plants that saturated at around 800 µmol photons m^{-2} s^{-1} (Fig. 2). Both LL and HL CAOx plants had higher ETR at higher light intensities than equivalent WT plants since they have higher [Chl] and higher Chl b: Chl a ratio, the former being more in Photosystem II, and thus, the ratio of PSII : PSI is likely to be higher. Further, at limiting light intensities (Fig. 2), CAOx – HL had higher ETR than that in CAOx –LL as well as both LL- and HL- WT plants.

Table 1 Chlorophyll a fluorescence parameter of WT and CAOx plants grown under different light intensities. WT and CAOx plants were exposed to LL and HL conditions as mentioned under methods. Leaves were kept in dark for 20 min before the measurement of their minimal fluorescence (Fo), maximum fluorescence (Fm) and photosynthetic efficiency (Fv/Fm) by PAM 2100 fluorometer. The experiment was repeated 3 times and the values are mean ± SD (n = 10).

Plant lines	Chl a fluorescence		
	Fo	Fm	Fv/Fm
WT-LL	0.245 (± .003)	1.150 (± .003)	0.786 (± .003)
CAOx-LL	0.246 (± .006)	1.239 (± .028)	0.801 (± .0029)
WT-HL	0.253 (± .003)	0.885 (± .022)	0.714 (± .003)
CAOx-HL	0.257 (± .005)	1.254 (± .012)	0.794 (± .0028)

Discussion

Studies from other research groups and that of ours have demonstrated that high light decreases total Chl and Chl b content. Overexpression of *AtCAO* in tobacco or *Arabidopsis* results in increased total Chl and Chl b content, decreased Chl a/b ratio both in LL- and HL-grown plants (Fig. 1) (Pattanayak *et al.*, 2005; Tanaka and Tanaka, 2005). As expected from WT plants, the ETR values, calculated from yield parameters of PAM fluorometry (Schreiber, 2004), were higher in HL-grown plants than that in LL-grown plants (Fig. 2). As compared to that of HL-grown plants, the ETR saturated at a relatively lower light intensity (400 µmol photons m^{-2} s^{-1}) in LL-grown plants (Fig. 2). Due to increase in the antenna size, the ETR values at limiting light intensities of LL-grown WT-plants were higher than that of HL-grown WT plants (Fig. 2). At higher light intensities, the HL-grown CAOx plants had elevated ETR than that of HL-grown WT plants.

Our studies reveal that regulated increase in Chl b biosynthesis by over-expression of full length *CAO* increases the light-harvesting potential. Our results further demonstrate that controlled upregulation of endogenous Chl b biosynthesis, by genetic manipulation of full length *CAO*, partially increases photosynthesis.

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