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A pronounced light-induced zeaxanthin formation accompanied by an unusually slight increase in non-photochemical quenching: A study with barley leaves treated with methyl viologen at moderate light

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Summary

Light-induced deepoxidation of violaxanthin to antheraxanthin and zeaxanthin in plants is associated with the induction of pronounced xanthophyll-dependent non-photochemical quenching (NPQ). To date, a misbalance between a high amount of zeaxanthin in thylakoid membranes and low NPQ has been explained by an absence of lumen acidification (e.g. when NPQ is measured in the dark after high light stress). In this study, we report that this misbalance can also be observed under moderate light. We found this result (deepoxidation state, DEPS, above 55% and NPQ~0.9) in barley leaves treated with 10 μM methyl viologen (MV) under white light (100 μmol photons $m^{-2} s^{-1}$, photosynthetically active radiation (PAR), growth irradiance). The addition of MV at this moderate light did not accelerate electron transport in thylakoid membranes, and induced only slight oxidative stress (no lipid peroxidation, almost unchanged maximum yield of photosystem II photochemistry, a

Abbreviations: A, antheraxanthin; APX, ascorbate peroxidase; DEPS, deepoxidation state of the xanthophyll cycle pigment pool; GR, glutathion reductase; LHC, light-harvesting complexes; MV, methyl viologen; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation (400–700 nm); PC, plastocyanin; PSI(II), photosystem I(II); P700 (P680), the primary donor of PSI(II); TBARPs, thiobarbituric acid reactive products; V, violaxanthin; VAZ, violaxanthin+antheraxanthin+zeaxanthin, total pool of xanthophyll cycle pigments; VDE, violaxanthin deepoxidase; Z, zeaxanthin.

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decrease in activity of ascorbate peroxidase, and an increase in that of glutathion reductase). We suggest that, in leaves treated under the conditions used here, the lumen acidification induced by light-limited electron transport in thylakoid membranes was high enough to activate violaxanthin deepoxidase, but not sufficiently high to form the expected number of zeaxanthin-dependent quenching centers in photosystem II antennae.

Introduction

The xanthophyll cycle plays an important role in the protection of chloroplasts against oxidative stress under high light. In thylakoid membranes, violaxanthin (V) released from chlorophyll-containing complexes is converted to zeaxanthin (Z), via intermediate antheraxanthin (A), by violaxanthin deepoxidase (VDE) activated by light-driven lumen acidification (for reviews, see Eskling et al., 1997; Lin et al., 2002; Latowski et al., 2004). Low lumenal pH also triggers several processes leading to the formation of quenching centers in light-harvesting complexes (LHC) (for reviews, see Holt et al., 2004; Horton and Ruban, 2005; Horton et al., 2005), where Z plays the role of direct quencher (Ma et al., 2003) or allosteric activator (Horton et al., 2005; Crouchman et al., 2006). Non-radiative dissipation of absorbed light (non-photochemical quenching, NPQ) by these centers, protecting reaction centers of photosystem II (PSII) against overexcitation, is called xanthophyll-dependent NPQ. For high NPQ levels, the xanthophyll-dependent NPQ is taken as a major component of the energy-dependent NPQ.

A correlation between high NPQ level and large amounts of Z in thylakoid membranes has been found in numerous *in vivo* studies (e.g. Demmig et al., 1987; Demmig-Adams, 1990). The pronounced decrease of NPQ in leaves after high light exposure indicates that the formation of Z-dependent quenching centers requires light-driven lumen acidification. Model experiments with chloroplasts have suggested that some threshold lumenal pH must be passed in order to observe the xanthophyll-dependent NPQ (Gilmore and Yamamoto, 1993). Recent *in vivo* studies by Kramer's group (e.g. Kramer et al., 2003), supported by previous *in vitro* results (e.g. Günther et al., 1994), propose that VDE is active at lumenal pH below 6.5, and thus even moderate light should be enough for activation of VDE. The limiting step of V deepoxidation appears to be the availability of free V for VDE in the lipid phase of thylakoid membranes (for review see Morosinotto et al., 2003) as at normal conditions, V is bound in chlorophyll-containing complexes (see Yamamoto and Bassi, 1996). As stress-induced (e.g. high light, heat stress) changes in the complexes cause a release of V into the lipid phase, VDE can mediate V deepoxidation.

Here, we present results showing that V deepoxidation *in vivo* also takes place at moderate light when the electron transport rate (ETR) in thylakoid membranes is limited by light. This was demonstrated with barley leaves treated with 10 μM methyl viologen (MV) under moderate light (100 $\mu mol\, m^{-2}\, s^{-1}$, photosynthetically active radiation (PAR), growth conditions). The huge accumulation of Z in MV-treated leaves was accompanied by an unusually slight induction of NPQ, implying that the lumen acidification in treated leaves was not sufficient for the formation of Z-dependent quenching centers. Our results support the view that VDE *in vivo* is active even at normal light conditions.

Material and methods

Plant material and treatments

Barley seedlings (*Hordeum vulgare* L. cv. Akcent) were cultivated in a perlit substrate supplied with Knop solution at $24 \pm 1\,^{\circ}C$ under a periodic 16/8-h day/night cycle (100 $\mu mol\, photons\, m^{-2}\, s^{-1}$, PAR). All experiments were performed with 8-d-old primary leaves (1.1 growth phase according to Feekes, 1941) over a day period. Leaf segments (5 cm) were cut out 1 cm below the leaf tip and infiltrated with 10 μM MV in Petri dishes by floating on the solution surface up to 5 h. Leaf segments floating on distilled water were used as control samples. The segments were initially incubated in solutions in the dark for 1 h and then exposed to white light (100 $\mu mol\, photons\, m^{-2}\, s^{-1}$, PAR; fluorescent tubes Tungsram 18 W, F33 cool white, Hungary) for up to 4 h. For an estimation of MV penetration into a leaf, the segments were incubated in MV solutions up to 5 h in the dark.

Absorption measurements

Measurements of absorption changes at 820 nm (ΔA_{820}) in dark-adapted leaf segments after the onset of light (white light, 100 $\mu mol\, photons\, m^{-2}\, s^{-1}$; PAR), reflecting