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The xanthophyll cycle pool size controls the kinetics of non-photochemical quenching in *Arabidopsis thaliana*

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Abstract ***Arabidopsis*** **plants overexpressing β-carotene hydroxylase 1 accumulate over double the amount of zeaxanthin present in wild-type plants. The final amplitude of non-photochemical quenching (NPQ) was found to be the same in these plants, but the kinetics were different. The formation and relaxation of NPQ consistently correlated with the de-epoxidation state of the xanthophyll cycle pool and not the amount of zeaxanthin. These data indicate that zeaxanthin and violaxanthin antagonistically regulate the switch between the light harvesting and photoprotective modes of the light harvesting system and show that control of the xanthophyll cycle pool size is necessary to optimize the kinetics of NPQ.**

Keywords: Xanthophyll cycle; Thylakoid membrane; Non-photochemical quenching; Photoprotection

1. Introduction

The reversible enzymatic interconversion between the carotenoids violaxanthin and zeaxanthin (the xanthophyll cycle) regulates the induction of photoprotective non-photochemical quenching (NPQ) in the thylakoid membranes of plants, the main component of which is the ΔpH-dependent, rapidly-reversible qE [1]. Xanthophyll cycle carotenoids are bound to the LHCII proteins [2–5], mostly at the peripheral V1 site [6,7]. There are two theories to explain the mechanism of action of these carotenoids in qE. Firstly, it has been proposed that zeaxanthin, but not violaxanthin, is a direct quencher of chlorophyll excited states [8]. Secondly, these carotenoids were suggested to allosterically regulate a quenching process that is intrinsic to LHCII [9,10]. Although there is experimental evidence in support of both theories, it has not been possible to conclusively prove that either one explains the role of the xanthophyll cycle *in vivo*.

The first evidence used to support the allosteric model was the phenomenon of "light activation" of qE – pre-illumination of leaves to convert violaxanthin into zeaxanthin shifted the ΔpH requirement for qE but had little effect on the maximum qE capacity [11]. Light activation was also recognised in kinetic effects: the rate of qE formation was faster in the presence of zeaxanthin but the rate of dark relaxation was slower [12,13]. These kinetic effects on qE were consistent with the observation that the rate of quenching of isolated LHCII was accelerated by addition of zeaxanthin but slowed down by violaxanthin [14,15]. It was therefore suggested that violaxanthin and zeaxanthin work antagonistically and competitively, the former as a qE inhibitor and the latter as a qE promoter [9,10]. In order to further test this hypothesis it is necessary to determine whether these effects arise from changes in zeaxanthin concentration or from the change in ratio of zeaxanthin to violaxanthin, expressed as the de-epoxidation state (DES). Here we have used *Arabidopsis* plants in which the expression of the enzyme β-carotene hydroxylase 1 has been increased; these plants accumulate 2–3 times the level of violaxanthin with little perturbation of the content of other pigments [16,17]. Comparing these plants to wild-type plants we show that NPQ kinetics depend upon DES. Furthermore, the data point to a new explanation of why the size of the xanthophyll cycle pool is subject to fine control according to external environmental signals.

2. Materials and methods

Arabidopsis thaliana, cv C24 (*wt*) and β-carotene hydroxylase 1 overexpressing lines (*sChyB*) derived from it [16] were grown for 8–9 weeks in Conviron plant growth rooms with an 8-h photoperiod at a light intensity of 100 μmol photons m^{-2} s^{-1} and a day/night temperature of 22/18 °C. The composition of carotenoids was determined by HPLC for leaf disks rapidly frozen in liquid N_2 [17]. To completely inhibit violaxanthin de-epoxidation, leaves were vacuum infiltrated with a 5 mM dithiothreitol (DTT) solution. Chlorophyll fluorescence kinetic analyses of whole leaves was carried out using a Walz PAM-100 fluorimeter at an actinic light intensity of 1000 μmol photons m^{-2} s^{-1}, with light saturation pulses applied as indicated in the figures. NPQ data analysis used a SigmaPlot software curve-fitting procedure (SPSS, Chicago, IL).

3. Results

As observed previously [16,17], the leaves of dark-adapted *sChyB* plants have nearly three times the content of violaxanthin

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Abbreviations: DES, de-epoxidation state of the xanthophyll cycle pool; DTT, dithiotheitol; LHCII, the main light harvesting complex of photosystem II; NPQ, Non-photochemical quenching; ΔpH, pH difference across the thylakoid membrane; qE, the ΔpH-component of NPQ; *wt*, wild-type plants

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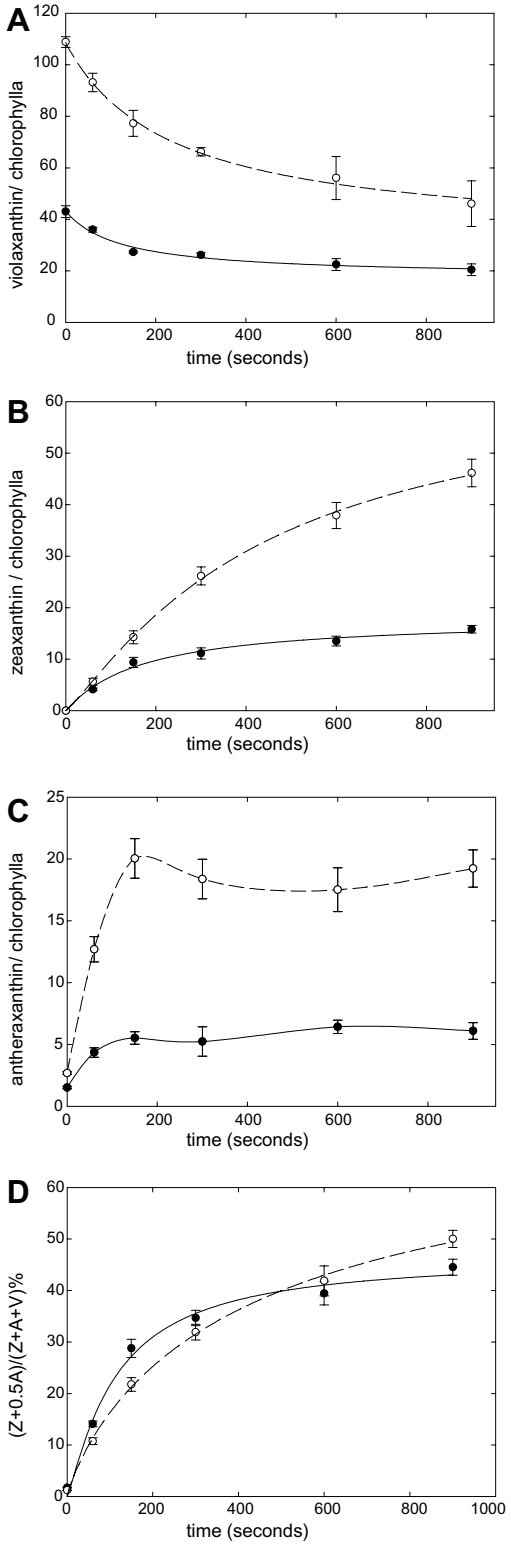

Fig. 1. Kinetics of de-epoxidation in *wt* (filled circles and line) and *sChyB*, (open circles and dashed line) leaves at 1000 μmol photons m^{-2} s^{-1}. (A) violaxanthin; (B) zeaxanthin; (C) antheraxanthin; (D) DES (Zx + 0.5Ax)/(Vx + Zx + Ax)%; A, B, and C values expressed as mmoles carotenoid per mole chlorophyll *a*. Data are means of three independent experiments ± S.E.M.

compared to the wild-type (*wt*) plants (Fig. 1A). Upon illumination, violaxanthin was de-epoxidised to antheraxanthin and zeaxanthin (Figs. 1A–C). During the first 300 s zeaxanthin accumulated rapidly in both *wt* and *sChyB*, after this point the *wt* zeaxanthin level saturated while in *sChyB* it continued to increase, slowing down only after about 15 min. The final levels of zeaxanthin and antheraxanthin were about three times larger in the *sChyB* plants compared to *wt*. Although the initial rate of zeaxanthin formation was apparently the same in *wt* and *sChyB*, the larger xanthophyll cycle pool size in the latter affected the rates of change in their DES (Fig. 1D). Thus, during the first 150 s of illumination the DES was significantly less in the *sChyB* plants compared to the wt (approx. 20% compared to 30%) (Fig. 1D), but after 15 min of illumination the DES was larger in the *sChyB* plants (approx. 50% compared to 40%). No further changes in DES were observed at longer illumination times (data not shown).

The kinetics of induction of NPQ in dark-adapted leaves of *sChyB* plants were different than in those of the *wt* (Figs. 2A and B). In both cases there was a similar initial fast phase of qE formation, reflecting the capacity for qE formation driven by ΔpH formation but without de-epoxidation. This is followed by a second slower phase of NPQ formation which is associated with zeaxanthin accumulation. This phase was much slower in the *sChyB* plants than in the *wt*: at around 100–200 s there was approx. 30% less NPQ in the *sChyB* plants compared to the *wt*. The final amplitude of NPQ obtained was not significantly different, even if the illumination period was extended (data not shown), as previously reported [16,17]. This

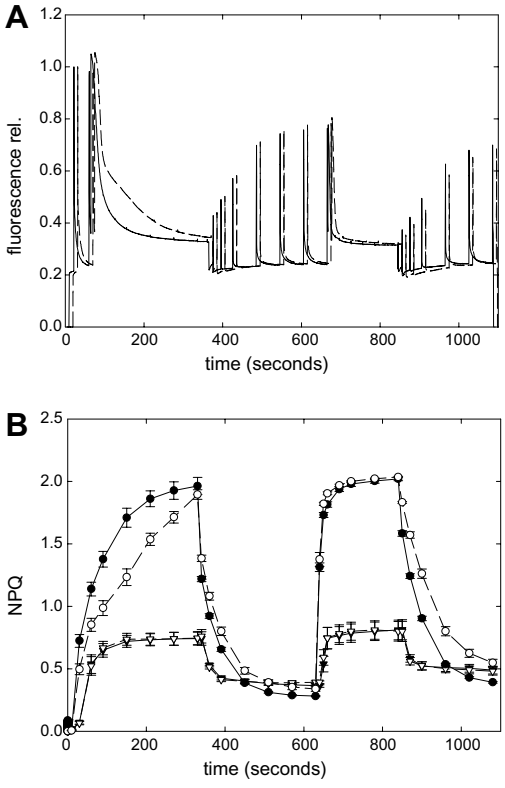

Fig. 2. Fluorescence induction curves (A) and NPQ (B) in *wt* (solid line and filled symbols) and *sChyB* (dashed line and open symbols) leaves at 1000 μmol photons m^{-2} s^{-1} actinic light. Control leaves (circles) leaves infiltrated with DTT (triangles). Arrows indicate actinic light on and off.