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A mathematical model of non-photochemical quenching to study short-term light memory in plants

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ABSTRACT

Plants are permanently exposed to rapidly changing environments, therefore it is evident that they had to evolve mechanisms enabling them to dynamically adapt to such fluctuations. Here we study how plants can be trained to enhance their photoprotection and elaborate on the concept of the short-term illumination memory in *Arabidopsis thaliana*. By monitoring fluorescence emission dynamics we systematically observe the extent of non-photochemical quenching (NPQ) after previous light exposure to recognise and quantify the memory effect. We propose a simplified mathematical model of photosynthesis that includes the key components required for NPQ activation, which allows us to quantify the contribution to photoprotection by those components. Due to its reduced complexity, our model can be easily applied to study similar behavioural changes in other species, which we demonstrate by adapting it to the shadow-tolerant plant *Epipremnum aureum*. Our results indicate that a basic mechanism of short-term light memory is preserved. The slow component, accumulation of zeaxanthin, accounts for the amount of memory remaining after relaxation in darkness, while the fast one, antenna protonation, increases quenching efficiency. With our combined theoretical and experimental approach we provide a unifying framework describing common principles of key photoprotective mechanisms across species in general, mathematical terms.

1. Introduction

Plants require light for photosynthesis, but excessive light is dangerous, because it can inflict irreparable damage to the photosynthetic apparatus. As sessile organisms, plants therefore require adaptive mechanisms to dynamically react to changing light conditions. A common strategy that has evolved in eukaryotic phototrophs [1] is the dissipation of excess absorbed energy in the form of heat, through processes collectively termed as non-photochemical quenching (NPQ) [2], which can be experimentally assessed by monitoring chlorophyll (Chl) fluorescence dynamics [3]. Analyses of the dynamics of Chl fluorescence quenching identified different NPQ components, which have been assigned to different NPQ mechanisms [4–6]: (1) the pH-regulated mechanism, qE [7], (2) the state transition mechanism, qT [8], (3) the zeaxanthin dependent quenching, qZ [4,9], and (4) the photoinhibitory quenching, qI [10].

The discovery of the role of the xanthophyll cycle [11–13] in NPQ [14] and the identification of xanthophyll cycle mutants [15] provided a significant breakthrough in the understanding of NPQ. Since then, numerous studies supported a critical role of the xanthophyll zeaxanthin (Zx) in energy dissipation. Related to the qE mechanism, a synergistic action of Zx and the thylakoid lumen pH has been proposed [16], explaining why highest qE levels are inducible only in presence of Zx [4,17,18].

Based on titration studies under *in vitro* conditions, Horton and co-workers suggested that Zx shifts the pH-dependence of qE by about 1.5 pH units towards higher lumen pH [18,19]. Furthermore, Zx was shown to modulate the kinetics of NPQ induction (faster in presence of Zx) and relaxation (slower in presence of Zx) [4,20,21], and was proposed to accelerate the reorganisation/aggregation of the PSII antenna [22] that accompanies qE [16,23-26]. These characteristics led to the development of the 4 state model of qE [16,25,27], which consistently explains the modulation of qE by the lumen pH and Zx, irrespective of the underlying quenching mechanisms and quenching sites involved in qE [24,28].

Moreover, correlations of the Zx reconversion kinetics with the relaxation of the slower NPQ components qZ and qI indicate a function of Zx also in these processes [4,29-34], and Zx reconversion can be considerably down-regulated or inhibited under stress [35,36] and photoinhibiting [36,37] conditions. This gradual

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down-regulation of Zx epoxidation in response to different light stress conditions thus allows the light stress-specific adjustment of the time of Zx accumulation, and hence the modulation of the NPQ response in dependence of previous light stress, over the full time range from minutes to days or weeks [37]. It should be noted, however, that the accumulation of Zx in parallel with the activation of the different NPQ mechanisms (qE, qZ and qI) does not necessarily imply a direct role of Zx in quenching, but could simply reflect the modulation of the efficiency of quenching or a general photoprotective effect of Zx in the membrane [38]. Plants can thus store information about the illumination history to optimise their photosynthetic performance, simultaneously avoiding damaging effects of over-excitation, and Zx seems to play a crucial role for such a memory effect [28,39,40].

Motivated by this apparent connection between the xanthophyll cycle and NPQ induction [41], we used pulse amplitude modulated (PAM) chlorophyll fluorescence analysis to systematically investigate whether a memory of light exposure can be detected on the time-scale of minutes to hours (in contrast to other studies that investigated the illumination memory on a longer timescale [42]). We next constructed a mathematical model on the basis of our current knowledge, to provide a general description of NPQ dynamics and the associated short-term memory.

We previously argued that a major challenge of theoretical biology is to provide simple, yet robust mathematical models that are not specially tailored for one model organism but describe a variety of species [43], because only such generic cross-species models will allow discovery of common generalised principles while understanding the species-specific features. Mathematical models range in complexity from very simplified and abstract models to extremely detailed ones aiming at including all known interactions. Here, our decision on the level of the model complexity depended strongly on the specific research question that the model is designed to address.

Our aim is to find a compromise between an accurate reproduction of experimental observation and a highly reduced complexity. For this, we simplified a number of processes, which are not directly involved in the NPQ mechanism. One particular objective to derive this model is to provide a general framework, that is not specific to one organism, but can be easily adapted to different species and is specifically designed to be convenient to implement and easy to use. By providing the full documented source code, we envisage that it will be further developed by the scientific community.

The model was initially calibrated for the model organism *Arabidopsis thaliana*, a sun-tolerant higher plant. Its flexibility is demonstrated by adapting it to the non-model organism *Epipremnum aureum*, a shadow-tolerant, ornamental plant, for which measured kinetic parameters are sparse. Our model is able to realistically reproduce experimentally obtained fluorescence traces and simulate all main features of chlorophyll induction, including transient activation of NPQ [44], the dynamics of fluorescence relaxation in darkness and qualitative differences in the quenching response to different light intensities. Thus, the model serves as a tool in which the role of the main quenching components can be computationally assessed and quantified, allowing simultaneously to test existing hypotheses on short-term light memory.

2. Experimental approach

Using PAM chlorophyll fluorescence analysis we quantitatively investigated the effect of short-term light memory on NPQ by comparing the fluorescence patterns of the first and second light periods. In the present study (see Fig. 1A), we examined two factors affecting the light memory in plants: intensity of incident light (varying from 100 to 900 $\mu E m^{-2} s^{-1}$) and the relaxation time between first and second light exposure (15, 30 or 60 min).

We first analysed whether the quenching patterns differ between the two phases of light. We directly analysed the originally measured maximal fluorescence ($F'_M(t)$) data instead of derived NPQ values (visualised in Fig. S3), to avoid mathematical distortion of the kinetics and provide more reliable information on the mechanism [45]. Fluorescence measurements are a non-invasive method for monitoring photosynthetic dynamics, providing information on the photosynthetic efficiency, protection and energy dissipation. However, each measurement can only be relative [46], and therefore at first data is normalised to the maximal fluorescence (measured after the first saturating pulse of light applied to a dark adapted plant, F_M) and then averages and standard deviations of the three replicates are calculated. In Fig. 1B we visualise the maximal fluorescence kinetics in the first (training) and second (memory) light phase (shifted to time 0). To highlight the key features which we aimed to explain with the mathematical model, only the last two measurements taken in dark phase (marked by grey background) and the first five measurements taken in consecutive light phases are displayed (for full traces of all 22 points taken during the experiment see either the NPQ trace in Fig. S3 in the Supporting Information (*SI Text*) or extract the data from the database).

It can be observed that for all light intensities the last F'_M in the relaxation phase (denoted $F_M^\star$) is consistently lower than in the training phase (F_M). Likewise, the first measurement in light at 61 s shows lower fluorescence than the corresponding point in the training phase (see Table S1 for statistical significance). This timed response to previously experienced illumination clearly demonstrates a short-term memory. The extent of the incomplete relaxation is influenced both by light intensity and the time spent in darkness (see Fig. S4).

Based on our current understanding, these observations can be attributed to the dynamical changes in the pigment composition, especially the slow epoxidation of zeaxanthin to violaxanthin in darkness. To quantify the zeaxanthin contribution to the memory effect we measured the pigment composition at the end of each phase of the experiment (full analysis summarised on Fig. S5). Fig. 2 shows that after exposing the samples for 15 min to high light intensities, Zx levels significantly increased up to 50% of all xanthophyll cycle pigments (sum of violaxanthin, antheraxanthin (Ax) and zeaxanthin). Simultaneously, 1 h in dark was sufficient to reduce this by half, explaining lower quenching effects in samples kept for longer periods in dark. This decrease was not as pronounced under illumination with the lowest light intensity. Moreover, zeaxanthin concentrations alone cannot explain that under 100 $\mu Em^{-2}\ s^{-1}$ the fluorescence signal for the later time points is higher in the memory phase compared to the training phase. This indicates that also the relaxation of the transient NPQ depends on the light memory, a conclusion consistent with the previous findings by Johnson et al. [20].

3. Mathematical model

Based on our experimental results and our current understanding of NPQ, we developed a small kinetic model to verify our hypothesis on the induction of light memory and to quantify the contribution of its molecular effectors. A general schematic of the model of the electron transport chain is shown in Fig. 3. A mathematical description of the processes, as well as the source code to solve the system numerically, can be found in the *SI Text*. Since the variable chlorophyll fluorescence originates from the antennae associated with PSII [47,48], we limit our model to the photosynthetic reactions around photosystem II, reducing the system to only six differential