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Research article

Comparative analysis of mutant plants impaired in the main regulatory mechanisms of photosynthetic light reactions - From biophysical measurements to molecular mechanisms

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ABSTRACT

Chlorophyll (chl) fluorescence emission by photosystem II (PSII) and light absorption by P700 reaction center chl a of photosystem I (PSI) provide easy means to probe the function of the photosynthetic machinery. The exact relationship between the measured optical variables and the molecular processes have, however, remained elusive. Today, the availability of mutants with distinct molecular characterization of photosynthesis regulatory processes should make it possible to gain further insights into this relationship, yet a systematic comparative analysis of such regulatory mutants has been missing. Here we have systematically compared the behavior of Dual-PAM fluorescence and P700 variables from well-characterized photosynthesis regulation mutants. The analysis revealed a very convincing relationship between the given molecular deficiency in the photosynthetic apparatus and the original fluorescence and P700 signals obtained by using varying intensities of actinic light and by applying a saturating pulse. Importantly, the specific information on the underlying molecular mechanism, present in these authentic signals of a given photosynthesis mutant, was largely nullified when using the commonly accepted parameters that are based on further treatment of the original signals. Understanding the unique relationship between the investigated molecular process of photosynthesis and the measured variable is an absolute prerequisite for comprehensive interpretation of fluorescence and P700 measurements. The data presented here elucidates the relationships between the main regulatory mechanisms controlling the photosynthetic light reactions and the variables obtained by fluorescence and P700 measurements. It is discussed how the full potential of optical photosynthesis measurements can be utilized in investigation of a given molecular mechanism.

1. Introduction

1.1. From measurement of optical signals to regulatory mechanisms of photosynthetic light reactions

In nature, light conditions constantly fluctuate in many different time scales, both in respect to the quantity and, to a smaller extent, the quality of light. This sets specific demands on regulation of the photosynthetic light reactions, which require five large protein complexes to work in concert in order to safely convert light energy into chemical energy in the form of NADPH and ATP. Photosynthetic pigment–protein complexes, responsible for these reactions, not only convert light energy into chemical form but are also sources of optical signals reflecting the state of the energy conversion reactions. Our understanding on the photosynthetic light reactions is largely based on measurements of these fluorescence emission and light absorption variables. Pulse-amplitude modulation (PAM) spectroscopy is the most used method to probe the function of photosynthetic light reactions. A Dual-PAM (Walz, Germany) measurement records a few PSII fluorescence and PSI absorption variables from which a large number of different parameters are calculated and used to describe the function of the photosynthetic machinery. The actual molecular mechanisms affecting the

Abbreviations: CL, constant growth light; FL, fluctuating growth light; LL, low constant growth light; ML, moderate constant growth light; HL, high constant growth light; GL, growth light; MB, measuring beam; AL, actinic light; SP, saturating pulse.

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measured variables are, however, poorly understood. Despite the fact that the mechanisms affecting the PAM variables are elusive, the PAM parameters calculated from these variables are commonly used as given facts when interpreting the experimental data. During the last decade, targeted manipulation of genes encoding the photosynthetic proteins has largely improved our understanding on the structure and function of the photosynthetic machinery. Regardless the progress and availability of genetic tools and a number of new mutants, the factors affecting PAM variables and the validity of PAM parameters have not been tested by utilizing these novel resources. Here, we used comparative mutant analysis to demonstrate the correlation between the molecular mechanisms regulating the function of photosynthetic light reactions and the Dual-PAM PSII fluorescence and PSI light absorption signals. We also show that the information specific for given molecular mechanisms and present in the measured PAM variables easily becomes nullified when further treating of the authentic data.

1.2. Regulation mechanisms of photosynthetic light reactions and the corresponding mutants

Plants can survive under fluctuating light conditions only by strictly and dynamically regulating the energy utilization in the thylakoid membrane and by maintaining the functional balance between the different sub-reactions of the photosynthetic energy conversion machinery. The relationship between these molecular mechanisms and the Dual-PAM optical signals have, however, remained elusive. During the last decade, the availability of mutant plants with distinct deficiencies in the molecular mechanisms, traditionally investigated by the optical methods, have opened new possibilities to expand our understanding on the origin of the optical signals. The best-characterized regulatory mechanism of photosynthetic light reactions with clear optical indicators, is dependent on the development of trans-thylakoid proton gradient (ΔpH) and specific redox changes in the photosynthetic electron transfer chain (ETC). When the light energy is in excess as compared to the capacity of cellular metabolism, ΔpH increases via the PGR5 protein-dependent mechanism (Munekage et al., 2002). The strength of ΔpH, in turn, controls both the rate of electron transfer via Cytochrome b_6f (Cyt b_6f) and the light-harvesting efficiency of the thylakoid antenna system. The main mechanism to dissipate excess excitation energy as heat is also dependent on ΔpH via the function of the pH-sensitive PSBS protein (de Bianchi et al., 2010; Niyogi and Truong, 2013; Lambrev et al., 2012; Tikkanen and Aro, 2014; Tiwari et al., 2016). On the contrary, when the availability of light energy limits metabolism and when the ΔpH-dependent control of Cyt b_6f and PSBS-dependent thermal dissipation is relaxed, the light-harvesting is maximized optimally for both photosystems. This is attained with redox-regulated and reversible phosphorylation of the light-harvesting complex II (LHCII) phosphoproteins by the STN7 kinase and the TAP38/PPH1 phosphatase (Pesaresi et al., 2010; Rochaix et al., 2012).

Knocking out the regulatory proteins described above (PGR5, PSBS, STN7, TAP38/PPH1) can be utilized to create targeted functional imbalances between the five large protein complexes functioning in concert to convert light energy into chemical form, thus making it possible to follow not only the environmental condition-dependent but also the regulation-specific changes in the optical signals. To be able to comprehensively interpret the optical data, it is important to note that in constant growth light (CL), whether it is low, moderate or high, plants can compensate any imbalance in energy and electron distribution by changing the stoichiometry between photosynthetic protein complexes (Lunde et al., 2003; Tikkanen et al., 2006; Grieco et al., 2012). Such compensatory mechanisms are, however, largely inhibited in fluctuating light (FL) conditions (Grieco et al., 2012; Suorsa et al., 2012), and impose secondary consequences crucial to be taken into consideration in thorough interpretation of the optical data. Table 1 describes the molecular consequences and phenotypes resulting from specific depletions of the major regulatory proteins.

1.3. Description of Dual-PAM variables

The function of photosynthesis regulation mechanisms is traditionally investigated by recording the PSII-specific chl a fluorescence signals and the PSI-specific light absorption signals (P700). Simple chl a fluorescence and P700 measurement can be used to measure both the redox state of the ETC and the efficiency of excess energy dissipation (Fig. 1A). Importantly, the chl a fluorescence is critically dependent on both the growth history and the pre-acclimation of the leaves: (i) the leaves acclimated to darkness prior to measurement or leaves taken directly from light show completely different behavior of PAM variables and (ii) the behavior of PAM variables always depends on the relative difference between the previous growth light intensity and the actinic light (AL) applied in the experiment. Taking these facts into consideration and conducting concomitantly both the PSII (chl a fluorescence) and PSI (P700) measurements, a plethora of new information is expected to be gained about the functionality of the two photosystems, their interactions and thus eventually about the entire ETC.

A typical chl a fluorescence curve from the wild type (WT) *Arabidopsis thaliana* (from now on Arabidopsis) leaf is depicted in Fig. 1A. As fluorescence can be measured only after excitation of chl with light, the dark-acclimated leaves are first exposed to low measuring beam (MB) of the fluorimeter in order to detect the emitted fluorescence, assigned as F0, i.e. the initial fluorescence from dark-acclimated leaves. Subsequent application of a saturating light pulse (SP) closes all PSII reaction centers and the value for maximal fluorescence, Fm, is obtained. Exposing the dark-acclimated leaves to AL results in excess transfer of light energy to PSII in relation to the capacity of the entire ETC and leads to a strong increase in the initial fluorescence emission, which then relaxes and stabilizes in a few seconds due to the activation of the photosynthetic machinery. This fluorescence induced by the AL is called F′ and when it reaches a steady state after the induction phase, it is often called F's. After the induction phase, F′ remains very stable in WT plants independently of the intensity of AL. In sharp contrasts to WT, the mutant plants impaired in proper distribution of excitation energy to PSII and PSI or having problems in regulation of electron transfer between the two photosystems, show distinct, very dynamic and light intensity-dependent behavior of F' (Grieco et al., 2012; Tikkanen et al., 2011). Likewise, in such mutants, the maximal fluorescence in light-acclimated leaves is lower than Fm obtained from dark-acclimated leaves, and is called Fm'. Decrease in Fm' reflects the induction of thermal dissipation of excitation energy by processes dependent on the pH of the thylakoid lumen and by other still poorly understood yet minor mechanisms.

The lower curve in Fig. 1A, also recorded with the Dual-PAM fluorimeter, monitors the redox state of PSI. This P700 signal is determined as a difference in absorbance at 875 nm and 820 nm (Klughammer and Schreiber, 2008). Concomitant measurement of the P700 signal and fluorescence provide further information about the regulatory mechanisms of photosynthesis and has potential to disclose the so far unknown mechanisms. The Dual-PAM protocol is based on the determination of Pm (P700 maximally oxidized) and on the comparison of this value to the P700 signals induced by the AL (P′) and by SPs applied upon the measurement. As described in Fig. 1, Pm is obtained by first oxidizing the ETC by PSI-favoring far-