

Saturation Pulse method for assessment of energy conversion in PS I

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Introduction

Light-induced charge separation at PS I and PS II reaction centers drives photosynthetic electron flow and transmembrane proton translocation, resulting in the formation of NADPH and ATP, a major part of which is used for CO₂ assimilation. Depending on the physiological state of the plant and the environmental conditions, alternative pathways of electron flow (e.g. O₂ reduction or cyclic electron flow around PS I) are possible, providing considerable regulatory flexibility in terms of NADPH/ATP ratios. Effective regulation is essential for survival under light-limited conditions as well as for protection against damage by excess radiation. While maximal energy conversion efficiency is required when light is limiting, down regulation is required when light is excessive. Hence, assessment of energy conversion efficiency of a plant under defined conditions can provide valuable physiological information.

During the past 20 years PAM fluorometry and the Saturation Pulse method have been applied in countless studies for measurements of the PS II quantum yield, resulting in important new insights into the mechanisms regulating plant performance and protection. In principle, by

using a modified emitter-detector unit the same instrumentation also allows to measure the redox state of P700, the reaction center chlorophyll of PS I, via its absorbance change in the near-infrared (peaking at 810-840 nm).

In first approximation, the fraction of reduced P700 may serve as a measure of active PS I. This does not hold, however, if the efficiency of charge separation is limited by acceptor availability. Klughammer and Schreiber (*Planta* 192: 261-268, 1994) introduced a special Saturation Pulse method that allows to distinguish between the two fractions of PS I centers with reduced P700 that are either photochemically active or inactive. As reliable application of this method puts considerable demands on sensitivity, time resolution and data acquisition software, so far its use has been quite limited. Recently, with the introduction of the *Dual-PAM-100 P700 & Chl Fluorescence Measuring System* a computer-controlled device has become generally available, which allows to apply the Saturation Pulse method with the same ease and reliability for assessment of energy conversion efficiencies both in PS I and PS II.

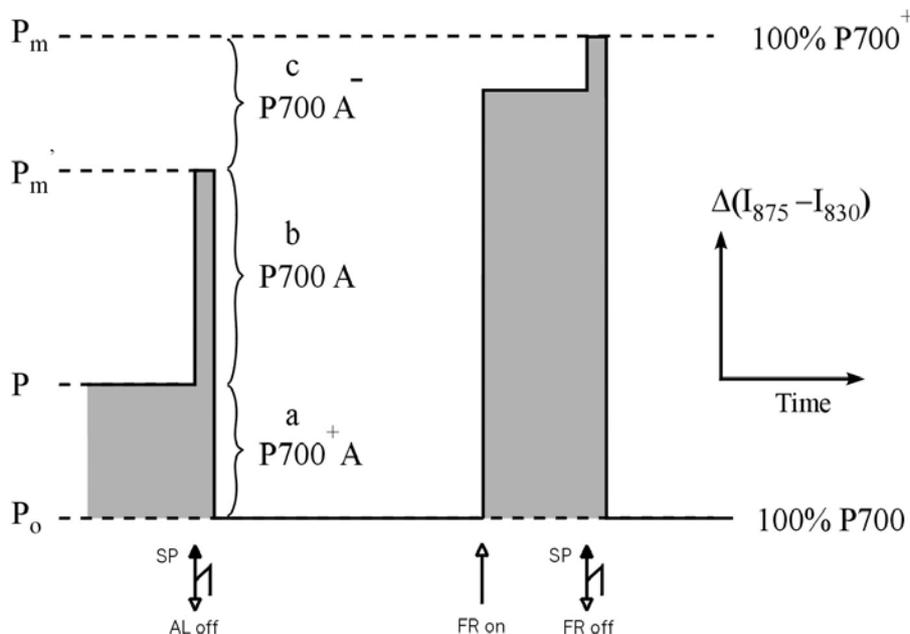


Fig. 1. Principle of Saturation Pulse method for determination of efficiency of energy conversion in PS I (modified from Klughammer and Schreiber, 1994). P700 is measured in the dual-wavelength mode (difference of intensities of 875 nm and 830 nm pulse-modulated measuring light reaching photodetector). P700 oxidation is characterized by a positive signal change. Complete P700 oxidation is induced by a Saturation Pulse (SP) in the presence of Far-Red (FR) light, with the maximal P700 signal denoted by P_m . Complete reduction is induced after the SP and cessation of FR-illumination, with the zero P700 signal denoted by P_0 . In the presence of Actinic Light (AL) a fraction a (donor-side limited closed centers P700+A) is oxidized by the AL resulting in an intermediate P700 signal denoted by P . In this state the SP-induced signal change corresponds to the oxidation of the active fraction b (open centers P700 A), with the maximal P700 signal being denoted by P_m' . The fraction c (acceptor-side limited closed centers P700 A-) that cannot be oxidized, corresponds to the difference between P_m and P_m' .

Basics of energy conversion at PS I reaction centers

Excitation energy captured by PS I antenna pigments is funneled to the reaction center chlorophyll, P700, where either charge separation takes place or the energy is dissipated in form of heat and fluorescence. In contrast to PS II, in PS I the fluorescence yield at room temperature is independent of the state of the reaction center and, hence, cannot serve as an indicator of photochemical efficiency. From its singlet excited state P700 donates an electron to the primary acceptor A₀ (pair of Chl a molecules) that is connected to a secondary electron transport chain, with the acceptors A₁ (phylloquinone), F_x, F_A, F_B (iron-sulphur centers), ferredoxin and eventually NADP or O₂. For the sake of simplicity, the effective ensemble of PS I acceptors here is denoted by A. Charge separation is partially reversible, as P700⁺ is long-lived and in the absence of oxidized secondary acceptors the radical pair P700⁺A₀⁻ recombines to the ground state.

The PS I quantum yield depends on the redox states of P700, and the PS I acceptors, A. In contrast to the situation in Photosystem II (PS II), there is no evidence regulated energy dissipation in PS I antenna pigments.

Therefore, it may be assumed that the photochemical quantum yield of open PS I reaction centers is unity. A center is open (quantum yield unity) only, when P700 is reduced and its acceptor A is oxidized. It is closed (quantum yield zero), when P700 is oxidized (non-photochemical energy dissipation to heat due to donor-side limitation) or its acceptor A is reduced (non-photochemical energy dissipation to heat due to acceptor side limitation). Consequently, for energy captured by PS

I three different, complementary quantum yields may be defined:

Y(I) quantum yield of photochemical energy conversion

Y(ND) quantum yield of non-photochemical energy dissipation due to donor side limitation

Y(NA) quantum yield of non-photochemical energy dissipation due to acceptor side limitation

$$Y(I) + Y(ND) + Y(NA) = 1$$

An individual PS I reaction center can occur in one of three different states, which during illumination are represented by the three fractions a, b and c:

a: P700⁺ A (closed) Y(I) = 0 Y(ND) = 1 Y(NA) = 0

b: P700 A (open) Y(I) = 1 Y(ND) = 0 Y(NA) = 0

c: P700 A⁻ (closed) Y(I) = 0 Y(ND) = 0 Y(NA) = 1

In a given state of illumination, the fractions **a**, **b** and **c** give detailed information on the photochemical quantum yield and its limitation by the PS I donor and acceptor sides. When fractions **a**, **b** and **c** are known, the three complementary quantum yields can be readily derived:

$$Y(I) = b/(a + b + c)$$

$$Y(ND) = a/(a + b + c)$$

$$Y(NA) = c/(a + b + c)$$

The Dual-PAM-100 allows to determine the fractions a, b and c using a special Saturation Pulse method.

Saturation Pulse method for assessment of PS I with the Dual-PAM-100

P700 is measured via its absorbance change in the near-infrared (transmission or remission difference signal 875 - 830 nm). This signal is minimal when P700 is completely reduced in darkness and increases upon light-driven oxidation. Hence, phenomenologically the P700 signal displays similar dark-light induction curves as chlorophyll fluorescence. However, while fluorescence can vary by more than a factor of 5 upon closure of PS II reaction centers, upon transition from the fully reduced to the fully oxidized state P700 signal changes are in the order of 0.1 to 1 % only. Nevertheless it is possible to assess Saturation Pulse (SP) induced changes within this small signal span with high time resolution. In conjunction with every SP, the zero P700 signal level **P₀** for fully reduced P700 is determined, so that any signal change is evaluated with respect to this reference level. In this way, the actual "measurements", i.e. assessment of P700 redox state and of the complementary quantum yields, are not affected by signal drift or any offset due to imbalance between the two measuring beams. The processed P700 signals are always referenced against the P₀ signal. The maximal P700 signal observed upon full oxidation is de-

noted by **P_m**. Hence, all P700 signal changes occur between P₀ and P_m, just like all changes of fluorescence yield occur between F₀ and F_m.

An illuminated sample displays a P700 signal (denoted by **P**) somewhere between P₀ and P_m, which reflects the **fraction a** (P700⁺A):

$$P - P_0 = a$$

The difference between minimal and maximal signals corresponds to the total P700, i.e. the sum of the fractions a + b + c:

$$P_m - P_0 = a+b+c$$

While complete P700 reduction is readily obtained in the dark, complete P700 oxidation can be problematic. Far-red light causes P700 oxidation by preferential excitation of PS I, which due to cyclic PS I electron flow, however, does not necessarily result in full P700 oxidation. Complete oxidation after far-red preillumination, however, can be achieved at onset of strong illumination, if the intensity is sufficiently high to cause maximal P700 oxidation before cyclic electrons or electrons from PS II can

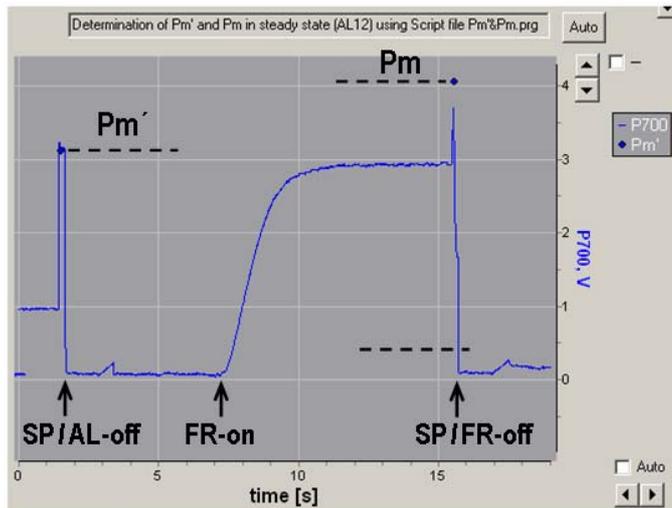


Fig. 2. Example for determination of energy conversion efficiency at the end of actinic illumination using 200 ms Saturation Pulses and a special extrapolation routine for assessment of the Pm' and Pm levels. Slow Kinetics recording with Dual-PAM-100. Detached rose leaf in transmission mode; PAR of AL, 200 μmol quanta/(m²·s).

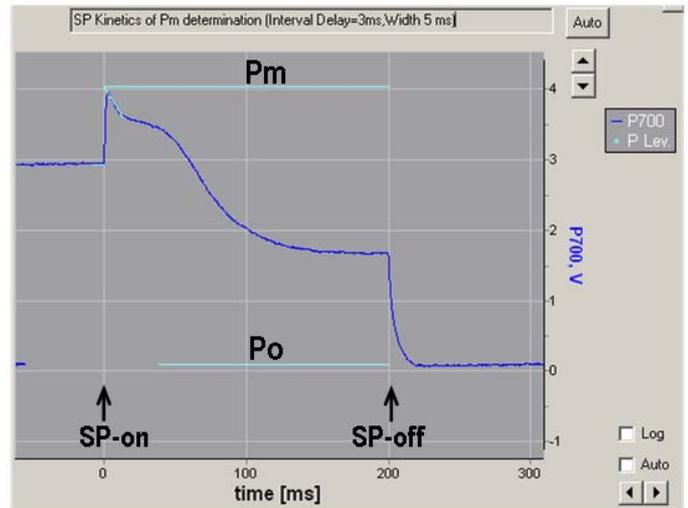


Fig. 3. SP Kinetics recorded in conjunction with Pm determination shown in Fig. 2. Note: Maximal SP-induced oxidation already reached within a few milliseconds is followed by biphasic re-reduction during the 200 ms SP. Full reduction (baseline) is reached briefly after SP termination.

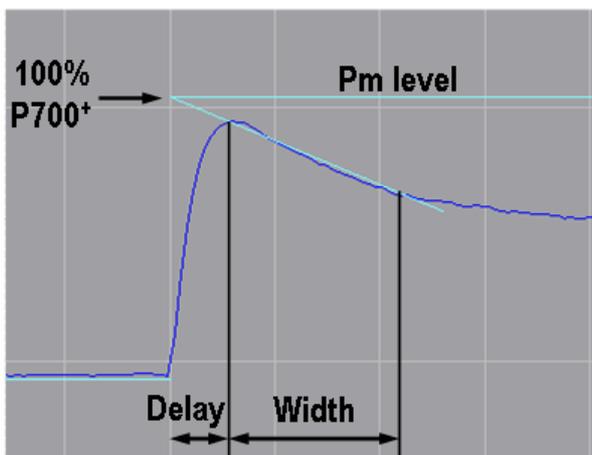


Fig. 4. Determination of 100 % P700⁺ signal level, Pm, by extrapolation routine (detail of SP Kinetics shown in Figure 3). The Interval Delay and Width parameters can be varied for optimal assessment of the Pm-level.

cause re-reduction. For this purpose, the Dual-PAM-100 employs Saturation Pulses of a powerful LED array with μs on-off characteristics that in open reaction centers (P700 A) enable full P700 oxidation within 3 ms. The fraction c (P700 A⁻) of closed centers, however, can not be oxidized even by the most powerful Saturation Pulses.

Hence, the P700 signal induced by a Saturation Pulse in a given state of illumination does not reach the Pm level. In analogy to maximal fluorescence yield Fm' of an illuminated sample, the maximal P700 signal induced by combined actinic illumination plus Saturation Pulse is denoted with Pm'. The difference between Pm and Pm' corresponds to the fraction c of closed centers (P700 A⁻):

$$Pm - Pm' = c$$

The fraction b of open centers (P700 A) corresponds to the difference between Pm' and P:

$$Pm' - P = b$$

The determination of the various P700 signal levels and

reaction center fractions by the Saturation Pulse method is illustrated schematically in Fig. 1. In Figs. 2 - 4 corresponding measurements with the Dual-PAM-100 are presented in form of original screenshots. In Fig. 5 a typical example of application of the Saturation Pulse method is given (Light Response Curve).

In contrast to the situation in PS II, Pm cannot be determined directly by application of a Saturation Pulse after dark-adaptation, as electron transfer at the PS I acceptor side requires light-activation and a substantial fraction c (P700 A⁻) is rapidly formed during a Saturation Pulse. Rapid accumulation of A⁻ can be avoided by far-red pre-illumination, which induces a "donor-side limitation". When in this donor-side limited state a Saturation Pulse is given, it takes about 4 ms until electrons from PS II reach P700, during which time maximal P700 oxidation can be obtained.

Based on the thus determined values of Po, P, Pm and Pm' the fractions a, b and c of PS I reaction centers as well as the complementary quantum yields Y(I), Y(ND) and Y(NA) are automatically calculated by DualPAM software and saved in a Report file. In the example of measurement depicted in Figs. 2 - 4 the steady state illumination state was characterized by:

$$Y(I) = 0.634$$

$$Y(ND) = 0.130$$

$$Y(NA) = 0.236$$

$$Y(I) + Y(ND) + Y(NA) = 1.000$$

Obviously, under the given experimental conditions the photochemical quantum yield of the investigated leaf, Y(I) was distinctly more limited by the acceptor side than by donor side. This may be due to the fact that the measurement was done using a detached leaf, the stomata of which presumably were closed.

Typical example of application

Light response curves provide valuable information on the efficiency with which photosynthetically active radiation is used by plants. Based on the special Saturation Pulse method outlined above, the Dual-PAM-100 not only allows to measure "Light Curves" of PS I quantum yield, $Y(I)$, and of the relative rate of electron transport via PS I, $ETR(I)$, but also of the complementary quantum yields of non-photochemical energy dissipation, $Y(ND)$ and $Y(NA)$.

In Fig. 5 a typical example of a Light Curve recording using a sunflower leaf is shown. The first measurement at $PAR = 0$ relates to the P_m measurement, where donor-side limitation is induced by far-red preillumination. Acceptor-side limitation is apparent at low PAR values, as $Y(NA) > Y(ND)$. Above $50 \mu\text{mol quanta}/(\text{m}^2 \cdot \text{s})$ there is a shift from acceptor-side to donor-side limitation. Above

$200 \mu\text{mol quanta}/(\text{m}^2 \cdot \text{s})$ the photochemical quantum yield, $Y(I)$, declines and the relative electron transport rate starts saturating, whereas $Y(NA)$ remains at a low level and $Y(ND)$ increases. These changes may be considered typical for a well-regulated, healthy leaf that is able to protect itself against damage by excess radiation by non-photochemical energy dissipation.

The thus obtained information on energy conversion efficiency in PS I is complemented by corresponding information on PS II obtained via analogous chlorophyll fluorescence measurements. The Dual-PAM-100 allows simultaneous measurements of chlorophyll fluorescence and P700. The same Saturation Pulses can be applied for assessment of energy conversion efficiency in PS I and PS II.

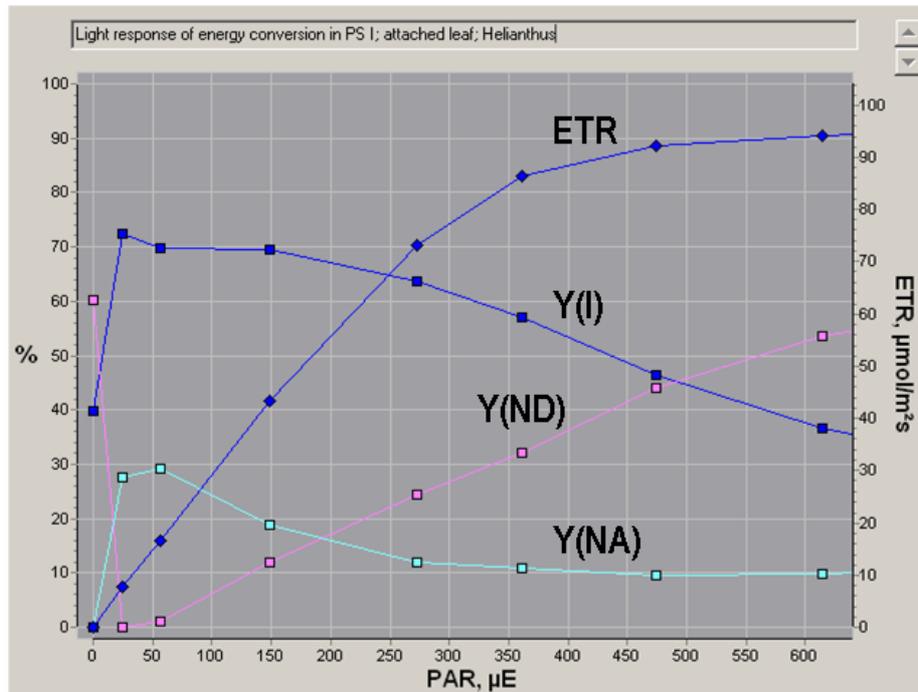


Fig. 5. Light response of the complementary PS I quantum yields $Y(I)$, $Y(ND)$, $Y(NA)$ and of the relative rate of electron transport, $ETR(I)$. Sunflower leaf at 27°C . Illumination steps, 3 min.