Energy storage in the photosynthetic electron-transport chain. An analogy with Michaelis-Menten kinetics

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Abstract: Simultaneous measurements of fluorescence and thermal emission have been performed by applying combined fluorescence and photoacoustic techniques on isolated thylakoids pretreated by prolonged illumination with saturating light. The traces were used to create Lineweaver-Burk type plots, proving clearly at least a formal analogy between the kinetics of the mechanisms governing fluorescence and thermal emission from isolated thylakoids and Michaelis-Menten kinetics of enzymatic reactions. Two characteristic parameters were calculated from them (energy storage and half-saturation light intensity) in order to obtain a basic, initial response of the photosynthetic apparatus functioning under photoinhibition stress.

Keywords: photosynthetic electron-transport, reaction centers, energy storage, fluorescence, thermal emission.

INTRODUCTION

As the most fundamental life process on earth, photosynthesis is the focus of a vast body of research, spanning studies of femtosecond reactions at the molecular level through field studies requiring a whole season of observation. Photosynthesis takes place in chloroplasts thylakoids membranes. In all oxygen-evolving organisms, photosynthesis involves the co-operation of two pigment-protein complexes, known as photosystems I and II (PSI and PSII). They function in series in the so-called non-cyclic electron transport chain (ETC) to oxidize water, reduce NADP⁺ and generate ATP. PSI can also function independently in a cyclic electron transport pathway to generate ATP.1 It is widely accepted that PSII and PSI function according to the “Z-scheme” by which electrons released from water pass through PSII and on to PSI, generating the strong reductant necessary for NADP⁺ reduction.2,3 The cross section of a thylakoid membrane (with two two-photosystems) showing the direction of photosynthetic electron transport (PET) is shown in Fig. 1.3 The two “energetic monettes” (NADPH and ATP), synthesized during the photosynthesis “light phase”, provide the necessary energetic input for the “dark phase”, consisting of cycles of biochemical reactions, and finishing with the final production of organic sugars.4
Isolated thylakoid membranes may be considered as photocatalysts for water decomposition in the presence of visible light. A manganese cluster plays a crucial role in the oxygen-evolving complex (OEC), in which a cycle-of-four oscillation (with the participation of a tyrosine residue) leads to the release of four protons, four electrons and the evolution of one O₂ molecule (at the lumenal side), all coming from two H₂O molecules – see Eq. (1).

The incident light absorbed by antennas of light-harvesting chlorophyll-protein (LHCP) complexes is used in two different ways: to drive photosynthesis upon charge separation in the reaction center complexes (RCs) of photosystems I and II, or it is dissipated in the form of fluorescence or thermal emission. The fluorescence trace is characterized by a non-variable component (\(F_\text{o}\)) that does not depend on photochemistry and a variable part (\(F_\text{v}\)) which is highly dependent on the photochemical activity of PSII. Thermal emission can be measured by photoacoustic spectroscopy (PAS), in which the released heat generates a pressure wave, detected by a sensitive microphone. The portion of incident light energy stored during the primary photochemical event (charge separation) in the electron-transport chain intermediates, defined as energy storage yield, can be estimated by comparing an (photochemically) active sample with an inactive one.

Energy storage has been detected by PAS techniques in various entities: isolated thylakoids, PSII particles, algae and leaves. Although it has not been clarified yet, the origin of the energy storage was mostly attributed to PSII activity. In the absence of artificial electron acceptors, photoreduction of the plastoquinone (PQ) pool is believed to be a predominant reaction for energy storage in PSII and in cyclic PSI. On the
other hand, variable fluorescence \( (F_V) \), and maximal fluorescence \( (F_M) \) indicate the partial and total closure of the PSII reaction centers, respectively, as a result of the reduction of quinones at the PSII acceptor side.

The relationship between Chl fluorescence and PAS signals has been studied in radish seedlings and in spinach leaves\(^{23-25} \) as well as in isolated thylakoids\(^{26} \) and PSII particles.\(^{27} \) It was found that energy storage was strongly correlated with the \( F_V \)\(^{28} \) expressed via photochemical fluorescence quenching, \( qPF = (F_M - F_V)/F_M \), and via its thermal emission counterpart, photochemical heat quenching \( qPH = (H_M - H_V)/H_M \). The evidence lies in the very similar dependence of \( qPF\&qPH \) on the intensity \( (I) \) of the photoacoustic modulated measuring (PAS) beam; a couple of parameters with significant photosynthetic relevance can be extracted from it.\(^{26,27} \) Such parameters can then be used to study the influence of some external factors with environmental aspects, such as temperature and photoinhibition. The latter, occurring “when plants are exposed to irradiation higher than it can convert or dissipate without harm”,\(^{29} \) is partly touched upon in this report.

**EXPERIMENTAL**

*Thylakoids isolation*

Thylakoids membranes were isolated from spinach leaves by grinding them in a Waring blender in the following buffer: 330 mM sorbitol, 20 mM N-tris(hydroxymethyl)methylglycine (Tricine) (pH 7.8), 10 mM NaCl, cooled to 0 °C. The homogenate was then filtered through 2 layers of Miracloth and centrifuged at 3000 × g for 2 min at 4 °C. The pellet was washed once with 50 mM Tricine (pH 7.8), 10 mM NaCl, and 5 mM MgCl\(_2\), and then resuspended in 20 mM 2-(N-morpholino)ethanesulfonic acid (Mes)-NaOH (pH 6.0), 330 mM sorbitol, 2 mM MgCl\(_2\), 1 mM NaCl, and 1 mM NH\(_4\)Cl at a chlorophyll concentration of 2 mg/cm\(^2\), which was adjusted spectrophotometrically.

*Fluorescence and photoacoustic measurements*

For the simultaneous fluorescence and photoacoustic measurements (FL&PAS), thylakoid membranes were diluted to 250 µg/ml in their respective resuspension buffer and 1 cm\(^2\) of the preparation was aspirated onto a nitrocellulose filter (Millipore, 0.4 µm pore size) using a gentle vacuum. The filter was cut to the proper dimensions for introduction into the photoacoustic cell. The measurements were made with a laboratory-constructed instrument using a MTEC photoacoustic cell in combination with a PAM-101 chlorophyll fluorometer (Walz, Effeltrich, FRG). The experiments were performed with 4 pulses over a 1278 s time scale with the following working parameters: first \( F_o = 5 \text{ s} \); 1st pulse delay = 5 s; pulse width = 2 s; 2nd pulse delay = 120 s; time between the pulses = 540 s; last photoacoustic time = 20 s; last \( F_o = 40 \text{ s} \) (see Fig. 2). The light intensity \( (I) \) range of the modulated photoacoustic measuring (PAS) beam were: 1.24 (1st one), 1.36, 1.76, 2.4, 3.04 and 3.84 W/m\(^2\) (the last one). Two excitation wavelengths of the PAS beam \( (\lambda_{PAS}) \) were employed: 680 and 700 nm.

The PAS beam (35 Hz) produces variable fluorescence \( (F_V) \) and thermal emission \( (H_V) \). A strong non-modulated background illumination from a quartz-halogen source (more than 150 W/m\(^2\)) was used to transiently close the PSII reaction centers, providing maximal fluorescence and thermal emission \( (F_M \text{ and } H_M, \text{ respectively}) \). The fluorescence initial level \( (F_o) \) was excited by using a 1.6 kHz fluorimeter modulated beam.

*Photoinhibition treatment*

Defrosted thylakoids (in the bulk) were thermostated at 21 °C, prior to the photoinhibition experiment. The photoinhibition experiment was performed with defrosted, thermostated thylakoids in the bulk, before the preparation of the filtered samples (and so, before the FL&PAS combined experiment, as well). The source of photoinhibition was the strong, saturating white light from a “Fiber Lite” lamp, model 170 D.
saturation light from an optic fiber connected to the lamp was directed to the center of the cuvette containing the thylakoids bulk solution. The bulk solution was stirred with a magnetic stirrer, thus equalizing the average amount of light that the thylakoids absorb during light saturation. The bulk solutions were light-saturated for a few periods of time (tph): 15, 30, 45, 60 and 100 min. A fresh bulk solution was always employed for a new photoinhibition time. At the end of the photoinhibition experiment (for the given time period), the bulk solution was kept in the dark and on ice. When required, 1 cm³ aliquots of the bulk solution were taken, the filtered thylakoids were prepared in described manner, and then used for the combined FL&PAS experiment.

RESULTS

Typical simultaneous FL&PAS traces obtained from isolated thylakoids are shown in Fig. 2. The weak 1.6 kHz fluorometer excitation beam yielded the initial fluorescence (F₀), indicating that all reaction centers (RCs) were in the open state. The counterpart, H₀, was

![Fig. 2. Record from the simultaneous measurements of fluorescence (upper trace) and thermal emission (lower trace) in isolated and photoinhibited thylakoids. The numbers adjacent to arrows indicate: 1 - fluorescence probe beam; 2 - photoacoustic modulated measuring (PAS) beam (680 nm, 3.84 W/m², 35 Hz); 3 - saturated non-modulated background illumination. The other wavelength of the PAS beam (700 nm) as well as the other intensities produce very similar traces. The pulses width was 2 s, the distance between the 1st and 2nd pulse was 120 s, between the 2nd and 3rd and the 3rd and 4th 540 s. For some reason, the 2nd pulse on the thermal emission trace is hardly seen and is so unusable for calculations. The final buffer suspension with isolated thylakoids (the bulk solution) contained 250 μg/ml. F₀ – the initial fluorescence level, induced by the fluorescence probe beam; Fv, Hv – variable fluorescence and thermal emission level, respectively, induced by the PAS beam; Fm, Hm – maximal fluorescence and thermal emission level, induced by saturating, non-modulated light.](image-url)
not directly detectable.\textsuperscript{21,28} The modulated PAS beam caused the fluorescence emission to rise from \( F_0 \) to a variable \( F_v \) level. Simultaneously, a corresponding equivalent variable level of thermal emission (\( H_v \)) was achieved.

Subsequent addition of saturating pulses of non-modulated light induced maximal levels of fluorescence (\( F_m \)) and thermal emission (\( H_m \)). Unlike the \( F_v \) level, which reflects partial closure of the PSII reaction centers, \( F_m \) marks their total closure due to complete reduction of the plastoquinone pool and saturation of the electron transport.\textsuperscript{7}

A ratio \( F_v/F_m \) and \( qPF \), photochemical fluorescence quenching, defined as \( (F_m - F_v)/F_m \), may then serve as a measure of the RCs openness or closure\textsuperscript{30,31} (the nomenclature used in this paper does not follow strictly the van Cooten and Snel recommendation\textsuperscript{32} in a formal sense, but it has the same basic meaning). On the other hand, \( H_m \) is related to the state where no energy is stored. The energy storage yield, defined via photochemical heat quenching, \( qPH = (H_m - H_v)/H_m \), represents the amount of absorbed energy, stored in the ETC redox intermediates, and therefore not released as heat during the modulation period of the PAS beam.\textsuperscript{8}

All calculations were based on the 4 pulses in the case of \( qPF \) data (Fig. 2, upper trace), and on the 3 pulses in the case of \( qPH \) data (Fig. 2, lower trace). The 2\textsuperscript{nd} pulse was almost absent in the thermal emission trace, or was of very irregular shape, unsuitable for calculation. Generally, the thermal emission trace was always weaker than its fluorescence counterpart. The values of \( qPF \) and \( qPH \) were calculated for the same numbered pulse; then, they were averaged since the particular \( qPF\&qPH \) vs. \( I \) plots almost overlapped, on the same time scale. A clear correlation between \( qPF \) and \( qPH \) was seen from the shapes of the corresponding \( qPF\&qPH \) vs. \( I \) plots, for both \( \lambda_{\text{PAS}} \) values (680 and 700 nm), and for different photoinhibition times (as in the absence of any photoinhibition - see previous reports\textsuperscript{26,27}). At least in the case of fluorescence, the decline of \( qPF \) with increasing PAS beam intensities (\( I \)) was expected from the progressive closure of the RCs, as both photosystems are exposed to higher light intensities.

A mathematical model describing the kinetic behaviour of the photosynthetic electron transport chain, originally derived by Howel and Vieth,\textsuperscript{5} was based on a few very defined assumptions:

(a) A thylakoid membrane can be considered as a microheterogeneous photocatalyst (permitting the decomposition of water under visible light), consisting of a collection of identical PET chains.

(b) The PET chains function independently of one another.

(c) There are two reaction centers (RCs) per one PET chain, one belonging to PSII (excited by \( \lambda = 680 \) nm), and the other belonging to PSI (excited by \( \lambda = 700 \) nm).

(d) The absorbed photons are equally distributed between the two reaction centers; the net excitation rates of the two reaction centers are equal and can be represented by a single photophysical process \( (P + h\nu \rightarrow P^*) \)

(e) The electron-transport rate is independent of the concentration of the terminal electron acceptor.
Bearing these assumptions in mind, water photocatalysis by thylakoid membranes may be presented by the following scheme:5

\[
2 \text{H}_2\text{O} + P^* \xrightarrow{k_D} P + \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \\
k_d \downarrow k_c I \\
P + \text{hv}
\]

where \(P\) and \(P^*\) represent the concentrations of the RCs in the ground state and excited state, respectively; \(k_c\) is the rate constant for the excitation of RCs, and \(k_d\) is the rate constant for \(P^*\) deactivation, \(I\) represents the excitation intensity, and \(k_D\) is the overall photochemical rate constant, i.e., the overall rate constant for all PET-related processes and reactions, permitted by the absorbed sunlight and leading to \(\text{O}_2\) evolution.*

The net rate of \(P^*\) formation is:

\[
dP^*/dt = k_c IP - k_d P^* - k_D P^*
\]

Within a very short time after illumination, a steady-state concentration of the excited reaction centers (\(P^*\)) is established. Setting the net rate equal to zero (\(dP^*/dt = 0\)) and solving for \(P^*\):

\[
P^* = \frac{k_c IP}{(k_d + k_D)}
\]

Introducing the following stoichiometric invariance:

\[
P_o = P + P^*
\]

where \(P_o\) is the total concentration of the RCs in the system (both PSII and PSI), and combining Eq. (4) with Eq.(3) to eliminate \(P^*\) and \(P\) from the formulation, the following equation is obtained:

\[
P^* = \frac{IP_o}{[(k_d + k_D)/k_c + I]}
\]

Since the rate of photosynthetic electron transport is defined as:

\[
R_e = k_D P^*
\]

then \(R_e\) becomes:

\[
R_e = \frac{k_D IP_o}{[(k_d + k_D)/k_c + I]}
\]

Combining the individual rate constants gives:

\[
K_1 = (k_d + k_D)/k_c
\]

which yields upon substitution:

\[
R_e = \frac{k_D IP_o}{(K_1 + I)}
\]

* This is why the whole photosynthesis light phase is sometimes considered as an entire photochemical reaction, leading to the formation of the photochemical products, ATP and NADPH.
By introducing the maximum rate of PET:

\[ R_m = k_D P_o \]  \hspace{1cm} (10)

in Eq. (9), one obtains

\[ R_e = \frac{R_m I}{(K_I + I)} \]  \hspace{1cm} (11)

Equation (11) expresses the functional relationship between the PET rate, \( R_e \), and the light intensity, \( I \). At low light intensity (\( K_I \gg I \)), the PET rate is nearly proportional to the incident light, \( I \) (“light-limited region”). However, at higher \( I \) values (\( K_I \ll I \)), the rate asymptotically approaches a maximum value, \( R_m \), and is essentially zero-order with respect to the light intensity (“light-saturated system”).

When \( K_I \) becomes numerically equal to \( I \), the PET rate achieves half of its potential maximum (\( K_I = I, R_e = R_m/2 \)). So, the constant \( K_I \) can be replaced by \( i_{50} \), the half-saturation light intensity, i.e., half of the intensity necessary to reach the maximal rate of electron-transport. So, Eq. (11) can be rewritten as:

\[ R_e = \frac{R_m I}{(i_{50} + I)} \]  \hspace{1cm} (12)

or, taken inversely:

\[ \frac{1}{R_e} = \frac{(i_{50} + I)}{R_m I} = \frac{1}{R_m} \left( \frac{i_{50}}{I} + 1 \right) \]  \hspace{1cm} (13)

Finally, \( R \) in Eq. (12) can be replaced by \( qPF \) (or \( qPH \)). The PET rate and fluorescence quenching are undoubtedly two proportional entities: an increase of the PET rate means a decrease of the variable fluorescence level, \( F_v \) and, consequently, an increase of \( qPF \). Analogously, \( R_m \) can be replaced by \( q^0PF \) (or \( q^0PH \)), meaning maximal potential conversion into ET intermediates, i.e., maximal energy storage of the absorbed light.

Hence, by substituting the change into Eq. (13), and using \( I/i_{50} \) relationship instead of \( i_{50}/I \) in the case of PAS data, the following equation results:

\[ \frac{1}{qPF} = \frac{1}{q^0PF} \left( \frac{I}{i_{50}} + 1 \right) \]
\[ \frac{1}{qPH} = \frac{1}{q^0PH} \left( \frac{I}{i_{50}} + 1 \right) \]  \hspace{1cm} (14)

The maximal energy storage yield corresponding to the state with all RCs in the open state, has been expressed here in the terms of fluorescence (as \( q^0PF \)), and thermal emission (\( q^0PH \)). Indeed, the linear relationship between the reciprocals of the two photochemical quenchings and \( I \) has been confirmed for a couple of photoinhibition times (\( t_{inh} \)), both for fluorescence and thermal emission (Fig. 3a–b). Introduction of the PAS beam of 700 nm did not change anything in the linearity of the plots (not shown). The calculated \( q^0PF \), \( q^0PH \) and \( i_{50} \) values for the both excitation wavelengths are presented in Table I.

**DISCUSSION**

There is a clear, at least formal, analogy between the mathematical model describing the kinetic behaviour of the PET chain, derived through Eqs. (2–14), and the Michaelis-Menten kinetic model describing a reaction between a substrate A, and a catalytic en-
zyme E, via a potentially reversible transient complex:

\[
A + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [EA] \underset{k_2}{\rightarrow} \text{Product} + E
\]  

(15)

The transient complex [EA] corresponds to the transiently excited reaction center, \(P^*\) (Eqs. (1–6)). It can undergo back reaction (\(\leftarrow \), \(k_{-1}\) rate constant) leading to the initial reactants, which corresponds to \(P^*\) deactivation to its ground state, \(P\) (deactivation rate constant, \(k_d\)). On the other hand, [EA] may decompose into final products (rate constant \(k_2\)), whereby the catalytical enzyme is restored. Just in a formal sense, it corresponds to the decomposition of water: RC does not react with water in a classical chemical reaction, but catalyzes its decomposition via the excited state \(P^*\). However, the same steady-state condition can be applied this time on the transient complex concentration (\(d[EA]/dt = 0\)) – like it was applied on the excited \(P^*\) species (\(d[P^*]/dt = 0\)) – which yields the same form of the final equation:

\[
\nu = k_2 E_{\text{tot}} A / (k_A + A)
\]

(16)
as Eq. (9). The reaction rate \(\nu\) corresponds to the PET rate \(R_e\); the total enzyme concentration, \(E_{\text{tot}}\), to the total RCs concentration \(P_o\); the constant \(K_A\) to the constant \(K_I\). The \(I\) and \(A\) analogy will be analyzed in the following text.

The same stands for the reciprocal plots:

\[
1/\nu = 1/\nu_{\text{max}} (K_A / A + 1)
\]

(17)
corresponds to Eq. (13), and its direct derivation, Eq. (14). Hence, the same linear plot, already well known for the \(1/\nu\) vs. \(1/A\) dependence (permitting the calculation of \(\nu_{\text{max}}\) and the Michaelis constant, \(K_A\)) should be expected for the fluorescence and photoacoustic data. Fig. 3 (a–b) clearly proves its validity. The plots obtained for different photoinhibition times all permit the calculation of the maximal energy storage (expressed as \(q^oPF\) for the fluorescence data and as \(q^oPH\) for the photoacoustic data) from the intercepts of the plots and of the half-saturation light intensity \(i_{50}\) from the intercepts and the slopes. The calculated values are presented in Table I for both the \(qPF\) and \(qPH\) data, for the used excitation wavelengths (680 and 700 nm), and for different photoinhibition times.

Attempts could be made to reveal more of the formal analogy shown above. A long time ago, photosynthesis was divided into two phases, the “light phase”, beginning with light absorption and finishing with NADPH and ATP formation (preserving the absorbed light in their chemical bonds), and the “dark phase” consisting of a few cycles of biochemical reactions, but having the NADPH and ATP as the necessary precursors. The division comes not only from the mechanistic point of view (the light initiated phase, and the thermal phase) but also from the kinetic point of view. However, if one looks at the kinetic dimension of the light phase itself (shown together with the redox-position of every participant in the ETC – Fig. 434), it is possible to distinguish ultrafast photophysical processes (light absorption and redistribution among the pigment molecules in the LHC complexes, occurring in the femtosecond time scale; primary separation occurring in reaction traps of
the RCs of the two photosystems and subsequent transfer of the released electrons to the
primary acceptors of PSII and PSI, occurring in the picosecond time scale) and the slower
processes, such as decomposition of water, or diffusion controlled reduction of the plasto-
quinone pool (see Fig. 1), which occur in the millisecond time scale. Hence, it could be said 
that, whereas the ultrafast events are pure biophysical processes, the other ones, with the 
indirect or direct participation of proteins (PSII and PSI, as the entities; the ETC components: 
plastocyanin, cytochromes are metal-protein complexes) are more biochemical in nature. 
Concretely speaking, the redox state of the primary PSII electron-acceptor QA, which di-
rectly controls the $F_v$ fluorescent level and so the $qPF$ value, is defined by the precedent 
electron-transfer from pheophytine, and subsequent reduction of QB (secondary PSII elec-
tron-acceptor), the first one occurring in less than 400 ps, and the latter in 100–600 μs (Fig. 
434). Both electron-transfers take place inside the PSII RCs complex, which is clearly pro-
tein-embedded (see Fig. 1), and so controlled by the protein composition and geometry. 
This is a possible connection between Eqs. (14) and (17).

However, if one tries to find a closer connection between the plots represented by Eqs. 
(14) and (17), i.e., to reveal more of the biochemical background of the plots shown in Fig. 
3 (a–b), then one arrives at the question: what is the reactant A (Michaelis-Menten sub-
strate) in these plots (since $I$ appears there instead of $A$). The answer comes from the fact 
that the fluorescence level $F_v$ is directly controlled by the redox state of the quinones (Q) at 
the acceptor side of the very fluorescent PSII.35 The quinones, primary acceptors (QA) at 
the PSII acceptor side, are fluorescence quenchers in the oxidized form, while the reduced 
form (QA–) contributes to the increased fluorescence level.36 Hence, at lower excitation in-
tensities (“light-limited region”), the oxidized QA forms prevail, the PET rate $R_e$ increases 
and the fluorescence $F_v$ level is very low. However, at higher excitation intensities 
(“light-saturated regime”), the reduced QA– form dominates causing lower PET rates and 
leading to the increase of the $F_v$ level. Consequently, $qPF$ and $qPH$ decrease (Figs. 3a–b). 
In conclusion, the formal counterpart of the reactant A in Eq. (17) is the primary elec-
tron-acceptor QA (not appearing in Eq. (14)) – which is why it is the “formal” counterpart – 
in the oxidized form at lower, and in the reduced form at higher excitation intensities.

The PSII excitation, $\lambda_{PAS} = 680$ nm. The $q^PF$ and $q^PH$ (“maximal energy stor-
age”), which marks the total openness of the reaction centers, generally decrease with in-
creasing length of the photoinhibition time ($t_{ph}$). However, in the $t_{ph}$ period of 15–60 min, 
$q^PF$ and $q^PH$ slowly decrease (from 42.7 % to 33.3 % in the case of the $qPF$ data, and 
from 39.4 % to 34.7 % in the case of the $qPH$ data), and then there is a drop up to $t_{ph} = 100$ 
minutes (19.9 % and 18.9 %, for the $qPF$ and $qPH$ data, respectively). So, it seems that in 
t_{ph} = 15–60$ min period, a slow, but continuous inhibition takes place and then there is a se-
rious “hit” occurring in the $t_{ph} = 100$ min period. Such a behaviour is partly confirmed by a 
continuous decrease in $i_50$ with increasing $t_{ph}$ time (with the exception of $t_{ph} = 45$ min). 
Both these facts seem reasonable: it does have a logic that the maximal openness of the re-
action centers (expressed either through $q^PF$ or $q^PH$), as well as the half intensity (of 
photoacoustic measuring, the PAS beam) needed to saturate the reaction centers, decrease 
with increasing $t_{ph}$ time. Since the FL and PAS experiments were done immediately after 
photoinhibition, it is maybe premature to assume that the inhibition effects are permanent.
On the other hand, \( q^{PF} \) and \( q^{PH} \) are very close, sometimes almost equal (for \( t_{ph} = 30 \) min \( q^{PF} = 36.8 \% \), and \( q^{PH} = 36.4 \% \)). Mutual comparison of \( i_{50} \) offers a similar conclusion, with the same exception of \( t_{ph} = 45 \) min, the \( i_{50} \) from the \( q^{PF} \) data is 2.8, and from the \( q^{PH} \) data 2.0 W/m².

**PSI excitation, \( \lambda_{PAS} = 700 \) nm.** The \( q^{PF} \) and \( q^{PH} \), as well as the \( i_{50} \) behaviour, are contradictory compared to the \( \lambda_{PAS} = 680 \) nm data. It is difficult to see a persistent behaviour. For example, for \( t_{ph} = 30 \) min, \( q^{PF} = 2.5 \% \) and \( q^{PH} = 53.2 \% \), and \( i_{50} \) from the \( q^{PF} \) data is 0.4, while from the \( q^{PH} \) data 3.1 W/m². On the other hand, for \( t_{ph} = 45 \) min, \( q^{PF} = q^{PH} = 50 \% \), while \( i_{50} \) from the \( q^{PF} \) data is more than double compared to its \( q^{PH} \) counterpart (7.1 vs. 3.3 W/m²). This is an inherent impersistent behaviour, when one is aware that these differences do not come from one or two FL and PAS combined experiments. It was necessary to perform six successful combined FL and PAS experiments after each photoinhibition time (corresponding to six PAS beam intensities values), to create 4 plots (corresponding to the 4 pulses), from which \( q^{PF} \) and \( q^{PH} \) and \( i_{50} \) were calculated. In addition, for \( t_{ph} = 60 \) and 100 min, the \( q^{PF} \) and \( q^{PH} \) values are very close (53.5 \% vs.

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**Fig. 4.** The Z-scheme of photosynthetic electron transport (PET), with the positions of the participants on the oxido-reduction scale. The time notations of ms, µs, and ps indicate the lifetimes of the respective ET steps. The abbreviations: the same as for Fig. 1. From: Kohen et al.: “Photosynthesis” in Photobiology, Academic Press, 1995, p. 185.
55.9% for \( t_{ph.} = 60\) min, and 37.0% vs. 35.5% for \( t_{ph.} = 100\) min), while the \( i_{50} \) values are very different (3.7 vs. 2.5 W/m² for \( t_{ph.} = 60\) min and 5.6 vs. 3.4 W/m² for \( t_{ph.} = 100\) min).

So, with increasing \( t_{ph.} \), neither of the two parameters show a clear behaviour. The \( i_{50} \) values oscillate and it is difficult to determine where the “maximum” is and where the “minimum” is. The \( q^{PF} \) and \( q^{PH} \) values both drop to 37.0 and 35.5% for \( t_{ph.} = 100\) min, after the two 50% values for the previous \( t_{ph.} \) periods (45 and 60 min).

Generally, \( \lambda_{PAS} = 700\) nm produced higher plot values for both the \( q^{PF} \) and \( q^{PH} \) data, then \( \lambda_{PAS} = 680\) nm (Table I). It is almost the same for \( i_{50} \) (Table I). Hence, PSI is ready to receive more photons, and generally needs more light to obtain saturation after the photoinhibition pretreatment. Clearly, PSI appears to be less affected than PSII.

**TABLE I.** The \( q^{PF} \) and \( q^{PH} \), maximal energy storage yield, corresponding to the maximal openness of the reaction centers, and the \( i_{50} \), half-saturation light intensity, calculated from the plots shown in Figs. 3a and b, for a couple of photoinhibition periods, \( \lambda_{PAS} = 680\) nm data (left), \( \lambda_{PAS} = 700\) nm data (right).

<table>
<thead>
<tr>
<th>Time of photoinhibition/min</th>
<th>( \lambda_{PAS}=680) nm</th>
<th>( \lambda_{PAS}=700) nm</th>
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<tbody>
<tr>
<td>( q^{PF} / % ) ( i_{50}/\text{Wm}^{-2} )</td>
<td>( q^{PH} / % ) ( i_{50}/\text{Wm}^{-2} )</td>
<td>( q^{PF} / % ) ( i_{50}/\text{Wm}^{-2} )</td>
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</tr>
<tr>
<td>60</td>
<td>33.3</td>
<td>2.5</td>
</tr>
<tr>
<td>100</td>
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**ИЗВОД**

СКЛАДИШЋЕЊЕ ЕНЕРГИЈЕ У ФОТОСИНТЕТСКОМ ЕЛЕКТРОН-ТРАНСПОРТНОМ ЛАНЦУ. АНАЛОГИЈА СА МИХАЕЛИС-МЕНТЕНОВОМ КИНЕТИКОМ

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Применом комбинованих флоресцентних и фотоакустичних техника у раду је извршено истовремено мерење флоресценције и термалне емизије из изолованих тилакоидних мембрана претходно изложеном продуженом дејству засићуће светлости. Из добијених сигнала конструисани су Лайневер-Баркови типови графика докazujući барем формалну аналогију између кинетике механизама флоресценције и термалне емизије из изолованих тилакоидних мембрана, а Михаелис-Ментенове кинетике за ензимске реакције. Из ових графика израчуната су два карактеристична параметра (складиштена енергија и полузасићући светлоски интензитет) у циљу добијања основног, почетног одговора о функционисању фотосинтетског апарате под условима фотоникиบรајућег стреса.

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REFERENCES