Rise Time of the Light Induced Electrical Field across the Function Membrane of Photosynthesis

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A repetitive laser giant pulse photometer is described which can resolve absorption changes of 0.1% in the time range of 10 ns. With this instrument the following results have been obtained.

The rise of the field indicating absorption change in photosynthesis has been measured at 515 nm. The upper limit of the half rise time is about 20 ns. This means that the field formation takes place within this time by a light induced transfer of electrons across the thylakoid membrane.

1. Nine of the ten different types of fast absorption changes in photosynthesis are caused a) by formation of metastable states, b) by primary light reactions, and c) by different electron transfer reactions. It has been shown that the tenth type is engaged in an intermediate reaction of phosphorylation. From the difference spectrum of these changes it was suggested in l. c. that this intermediate reaction is indicated by a chlorophyll-b.

2. It was assumed in l. c. that these absorption changes indicate a reaction of protons which are linked to phosphorylation.

3. In l. c. it has been shown that these absorption changes can be interpreted in a more general way, namely as being caused by the changes of a light induced electrical field across the thylakoid membrane.

The decay of the absorption changes is caused by a break-down of the field by movements of protons and other ions across the membrane.

4. Details on the electrical field phenomena are published in l. c. 4a–7.

5. In a primary note in l. c. 1–8 it was shown that the half rise time of the absorption changes which indicate the formation of the field is about \( \leq 2 \times 10^{-8} \) s.

6. Besides the absorption changes of chlorophyll-b absorption changes of the other bulk pigments indicate the field changes also.9

The value of the rise time is of great interest for the interpretation of these field phenomena. Therefore, we refined these measurements. Special precaution was taken to eliminate the extremely fast absorption change (type 1) which is caused by the formation of metastable states. In the preceding paper of this journal it is reported on new results on these metastable states. These results give suggestion how to arrange experiments in which any interference of the field indicating absorption changes with those of metastable states can be avoided.

Results

The measurements are performed by the repetitive laser giant pulse photometry (preliminary note

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5 W. Junge and H. T. Witt, Z. Naturforschg., 23 b, 244 [1968].
in l.c.\textsuperscript{12}, details see below). The kinetics were measured at 515 nm. At this wavelength the field indicating changes as well as those of metastable states take place\textsuperscript{10}. The decay time of the field indicating change is $\geq 10^{-3}$ s – depending on different conditions see l.c.\textsuperscript{7}. The decay time of type 1 is only $3 \cdot 10^{-6}$ s\textsuperscript{11}. The rise time of both is, however, extremely fast. In l.c.\textsuperscript{11} it has been shown that the magnitude of the changes of these two types in dependence of the flash energy are completely different. From fig.5 in l.c.\textsuperscript{11} it follows that at flash energies at which the field indicating changes are about 35\% of the saturation value, the contribution of the change of metastable states is negligible. Therefore the measurements in fig. 1 were performed under these conditions.

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Rise of the field indicating absorption change as function of time at 515 nm in a suspension of spinach chloroplasts.

Chlorophyll content: $4 \cdot 10^{-6}$ M, O$_2$ activity: $159 \cdot \frac{M(O_2)}{M(\text{Chl})}$ h

Buffer: Tris-HCl 5-10$^{-2}$ M, pH = 7.2. Electron acceptor: benzylviologen 10$^{-4}$ M, uncoupler of phosphorylation: NH$_4$Cl 2-10$^{-3}$ M. Temperature: 22°C. Optical path: 78 mm. Excitation: Q-switch laser pulses, $\lambda$ = 694.3 nm, half-width 25 ns, repetitive laser giant pulse technique, frequency 5 cps, 3750 pulses, pulse energy reduced so that the amplitude of the absorption change was only 35\% of that of the saturation amplitude. Monitoring light: interference filter 516 nm, half-width 13 nm, intensity 1 mW/cm$^2$. Rise time (10 – 90\%) of the whole apparatus including laser pulse: 48 ns.

For each measurements about 3000 laser giant pulses were used for excitation. The 3000 signals of absorption changes were averaged as described below. The activity of the suspension of spinach chloroplasts does not decrease during the set of $\sim$3000 shots (the magnitude of the absorption change was constant between the beginning and the end). Five different sets have been carried out (see table 1).

<table>
<thead>
<tr>
<th>Set No.</th>
<th>Number of fired laser pulses</th>
<th>Measured rise time $r_m$ of the averaged signal [ns]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2250</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>2250</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>3750</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>3750</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>3750</td>
<td>61</td>
</tr>
</tbody>
</table>

Table 1. Data of rise time measurements.

The time course for the rise of the field indicating absorption change at 515 nm measured under the conditions cited above is depicted in fig. 1. The figure shows the averaged signal produced by 3750 laser pulses. In table 1 the rise time $r_m$ (from 10 to 90\% of the amplitude) of each set is depicted. The mean value of $r_m$ and its standard deviation is $r_m = 60 \pm 10$ ns.

The measured rise time $r_m$ is connected with the rise time $\tau$ of the reaction by the equation

$$\tau = \sqrt{(r_m)^2 - (\tau_i)^2 - (\tau_{app})^2}$$

(1)

$\tau_i$ = rise time of the laser pulse energy (33 ns), $\tau_{app}$ = rise time of the measuring apparatus (35 ns) (see below). According to (1) the rise time $\tau$ (from 10 to 90\% of the amplitude) of the reaction and its standard deviation is $\tau = 35 \pm 20$ ns.

and the “half rise time” $(1/2 \tau)$

$\tau_{1/2} = 18 \pm 10$ ns.

Because of only five sets of experiments we prefer to regard this value only as an upper limit. Measurements with shorter pulses and with higher numbers of sets are in preparation.

**Discussion**

The function unit of photosynthesis is the membrane of the thylakoid\textsuperscript{4}. This membrane is built up by a bilayer of lipids and proteins. It is assumed that the electrical field is formed across the poorly conducting lipid layer and caused by two light induced electron transfers (charge separations) with a component perpendicular to the membrane\textsuperscript{5}. The electrical field is coupled to a translocation of protons across the membrane into the inner phase of the thylakoids. The efflux of H$^+$ is coupled with the synthesis of ATP from ADP and P$_i$\textsuperscript{7}.

The thickness of the lipid layer is $\approx 30 \text{ Å}$. According to the results reported above this distance has to be overcome by the electrons in the order of 10 ns or faster. The mechanism of the electron transfer depends on the structure of the membrane. Although some details of its structure have been obtained no information is available to discuss how the electrons are pumped across the membrane in particular.

It may be that the electron translocation through the membrane is supported at special places by molecules with conjugated chains. Such compounds are e.g. the carotenoids which are attached to the lipid layer. The $\pi$-electrons of such molecules are freely movable along the molecules. Therefore they could serve as a "pipeline" for electrons between an electron donor on one side of the membrane and and electron acceptor on the other side.

The repetitive laser giant pulse photometry

For measurements of chemical reactions in the $10^{-8}$ s range Q-switched lasers can serve as the stimulation sources. The large bandwidth which is necessary for this time range causes such a high noise level that signals of absorption changes of $10^{-3}$ (photosynthesis) in the range of $10^{-8}$ s are totally masked by the noise level (signal-noise ratio $\ll 1$). Therefore by single excitation with a Q-switched laser the sensitivity is too low for the evaluation of any information in this time range. By the repetitive method, however, we succeeded in such an increase of the sensitivity that the signal-noise ratio is about 3 (see fig. 1). The difficulty for repetitive measurements with time sampling and averaging technique is to run the Q-switched ruby laser repetitively (e.g. 5 cps). As such a laser system has been specially developed for our purpose, we could realize a time resolution down to the order of 10 ns.

The measurements of the rapid signal, time sampling, averaging, integration and recording are performed in exactly the same way and with the same equipment as described in the ultra short flash photometry apparatus (see preceding paper in this journal) (see fig. 2).

The repetitive Q-switched ruby laser ($\lambda = 694.3$ nm) was developed by the Raytheon Com-

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pany. The maximum optical output power is 25 MW. The laser head consists of ruby-rod (R), Pockels-cell (Pc), polarizer (Pol), and TIR-prism (Pr) (see fig. 2). The maximum repetition rate is 5 cps. The heat dissipated by flashlamp and ruby rod is about 5 kW so that an effective cooling system is necessary. This was realized by a circular water flow system with a flow of 12 liters/min. The water is thermostated by a 9 kW cooling machine (Escher Wyss) at a temperature of 5 to 10 °C.

Because of electrical interference by the laser on the very sensitive measuring system both have been completely separated and supplied by different power lines with the measuring equipment installed 12 m apart from the laser.

The time course of the laser pulse intensity as monitored by a fast photodiode (Korad, KD 1) is depicted in fig. 2 with a half width of 25 ns.

From this time course the emitted pulse energy as function of time can be found by integration. It results for the rise time \( \tau^l \) of the laser pulse energy:

\[ \tau^l = 33 \text{ ns } \pm 2 \text{ ns}. \]

The output power varies ± 20 – 30% within 20 pulses. These variations have been controlled during each measurement (2250 – 3750 pulses) and found to run statistically.

The rise time of the measuring apparatus \( \tau^{app} \) (photomultiplier, amplifier, sampling oscilloscope, integrating network, and recorder) is

\[ \tau^{app} = 35 \text{ ns } \pm 2 \text{ ns}. \]

This was tested under measuring conditions with a short flash of only 1 ns duration.¹⁴

With the equipment in fig. 2 “half rise times” down to

\[ \tau_{1/2} \approx 10 \text{ ns} \]

can be resolved.

With 3000 laser pulses absorption changes \( \Delta I/I \) in the range of 10 ns are measurable down to

\[ \Delta I/I = 0,1\% \]

(see fig. 1).

Because of the low stability due to the high electrical input energy of the above described laser we have introduced recently a much less energized but therefore more stable lasers system for excitation (YAG-laser, Laser Associates Inc.).

Materials

The absorption changes were measured on spinach chloroplasts in an aqueous suspension. The spinach chloroplasts were prepared by fragmentation of spinach leaves by an Omnimixer at 10,000 cpm for 10 s in a solution after WINGET et al.¹⁵ with addition of \( 10^{-2} \text{ M ascorbate} \). From the sap of this preparation chloroplasts were isolated.
