Analysis of electron flow around photosystem II in atrazine-resistant plants applying the three-state energy trapping model on the OJIP fluorescence induction curve

JJS van Rensen, GC Rodrigues, WJ Vredenberg

Laboratory of Plant Physiology, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands. Fax +31 317 484740. E-mail: Jack.VanRensen@PPH.dpw.wau.nl

Keywords: chlorophyll a fluorescence induction, electron flow rates, photosystem II, triazine resistance

Introduction

One of the first phenomena to be observed on a plant under stress is a decreased activity of photosystem II (PS II). This impaired activity can be easily demonstrated measuring chlorophyll a fluorescence. Chlorophyll fluorescence has become an important technique to estimate effects of stress on a plant. Most often the potential quantum yield of PS II is measured: the Fv/Fm value (variable over maximal fluorescence). However, the information of the Fv/Fm value is limited. Much more information becomes available from the full data of the fluorescence induction curve measured with the Hansatech Plant Efficiency Analyser (PEA), especially when the time is plotted on a log scale: the OJIP curve. This analysis was worked out mainly by the group of Reto Strasser (see, e.g. Stirbet et al. 1998).

Vredenberg (2000) has developed a three-state model for energy trapping and chorophyll fluorescence in PS II which takes into account that two turnovers are required for stationary closure of a PS II reaction center. An open reaction center is transferred with high efficiency into its semiclosed (-open) state. Full closure of a semiclosed (-open) center requires a second turnover. Based on this model we have developed a program to simulate the fluorescence induction curve (Vredenberg et al., 2001; these proceedings). This program yields information on the kinetics of the electron flow reactions on the acceptor as well as the donor side of PS II.

Triazine-resistant Chenopodium album plants have been characterized by the group of Van Rensen (see, e.g. Curwiel and Van Rensen, 1996). One of the properties of these plants is an impaired activity of PS II. Because such a lower activity is one of the early effects of stress on a plant, these plants have been used by this group as a tool to study stress effects in plants (Van Rensen and Curwiel, 2000). It has been reported earlier that the impaired activity of PS II in atrazine-resistant plants is related to a decreased rate of electron flow from the primary to the secondary quinone electron acceptor (Jansen and Pfister, 1990). The application of the simulation program of Vredenberg et al., 2001; these proceedings) on triazine-resistant Chenopodium album and Solanum nigrum plants revealed indeed that there are only minor differences between R and S plants with respect to kinetics of electron flow reactions at the donor side of PS II, but that rates of electron flow at the acceptor side are indeed much smaller.
Materials and methods

The origin of the *Solanum nigrum* was described by Kremer and Lotz (1998); that of the *Chenopodium album* by Jansen et al. (1986). Both type of plants were grown as reported earlier (Curwiel and Van Rensen, 1996). The plants were adapted to a light intensity of about 250 µmol m\(^{-2}\) s\(^{-1}\) and measurements were taken at about 30 days after planting from the abaxial leaf surface. Chlorophyll a fluorescence induction of dark-adapted intact leaves was measured with a Plant Efficiency Analyser (PEA), Hansatech Instruments Ltd, Norfolk, England.

![Graphs showing chlorophyll fluorescence transients of triazine-resistant (R) and wild-type (S) intact leaves of *Chenopodium album* and *Solanum nigrum*.](image)

**Fig. 1.** Chlorophyll fluorescence transients of triazine-resistant (R) and wild-type (S) intact leaves of *Chenopodium album* and *Solanum nigrum*. Upper panels, actual curves; lower panels, normalised curves.

Results

When the fluorescence transient is plotted on a log timescale, a so-called OJIP curve is observed. Such curves of *Chenopodium album*, and *Solanum nigrum*, R and S biotypes respectively, are presented in Fig. 1. While the Fo level is usual taken as the fluorescence at 50 µs, we have taken the extrapolated signal at 10 µs after the onset of the illumination as the Fo level. This was done by extrapolation of the log plot of the first derivative of the response in the 50-150 µs time domain. In general, this is a linear extrapolation due to an apparent exponential increase of the initial fluorescence (Vredenberg, 2000). The J-level is usually
Table 1. Parameters of triazine-resistant (R) and wild-type (S) PS II reactions of intact leaves of *Chenopodium album* and *Solanum nigrum*

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>C. album</em> S</th>
<th><em>C. album</em> R</th>
<th><em>S. nigrum</em> S</th>
<th><em>S. nigrum</em> R</th>
</tr>
</thead>
<tbody>
<tr>
<td>kL</td>
<td>15</td>
<td>13</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>k1</td>
<td>6.6</td>
<td>4.8</td>
<td>5.1</td>
<td>6.0</td>
</tr>
<tr>
<td>k2</td>
<td>2.6</td>
<td>1.9</td>
<td>2.95</td>
<td>1.35</td>
</tr>
<tr>
<td>k3</td>
<td>0.12</td>
<td>0.13</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>k4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>kAB1</td>
<td>1.8</td>
<td>0.68</td>
<td>1.8</td>
<td>0.30</td>
</tr>
<tr>
<td>kAB2</td>
<td>0.03</td>
<td>0.001</td>
<td>0.055</td>
<td>0.001</td>
</tr>
</tbody>
</table>

observed at 1-2 ms, the I-transient at about 30 ms, and between 0.5 and 1 s the maximal fluorescence P is reached.

Based on the three-state model of Vredenberg (2000) a simulation program was developed (Vredenberg et al., these proceedings) and applied on these four curves. In this simulation 7 parameters are used. The light excitation rate is kL. The rate constants of electron flow leading to the S2, S3, S4 (0), S1 states of the oxygen evolving complex are denoted as k1, k2, k3, and k4. The rate constant of reduction of Q_B by Q_A− is denoted as kAB1, and the rate constant of reduction of Q_B− by Q_A−, kAB2. In Fig. 2 simulated curves are presented.

![Fig. 2. Actual chlorophyll fluorescence induction curves (exp) of leaves of *Chenopodium album* and *Solanum nigrum* and simulated curves, using the numbers of Table 1. O-J-I- and P-levels are at 0.05, 1, 50 and 500 ms, respectively.](image-url)
using the numbers of Table 1. It appeared that differences in $k_4$ did not influence the simulation effect; the reason being that $k_4$ cannot be faster than the slowest one of $k_1$, $k_2$, or $k_3$. Therefore, $k_4$ is not determined. We have observed that in the presence of DCMU the fluorescence reaches the I-level, but not the P-peak. The I-level thus reflects the fluorescence yield at full closure of the RC’s. We suggest that the I to P transient is related with an electrogenic process in PS I (not yet published). For this reason the theoretical curves of Fig. 2 are simulated not further than the I-level.

**Discussion**

As can be observed in Fig. 1, the fluorescence induction curves of wild-type plants (S) of both *Chenopodium album* and *Solanum nigrum* have the typical OJIP transients: the J-level is at about 1-2 ms, the I-transient is at about 30 ms and the maximum P is reached at about 500 ms. Of both *C. album* and *S. nigrum* the resistant biotypes (R), however, have an increased J-level and the I-transient is much less pronounced. Comparable deviations from the “normal” OJIP curve in D1 mutants were reported earlier (Srivastava et al., 1995; Kohno et al., 2000).

It is known that the mutation in the D1 protein of triazine-resistant plants not only causes resistance against this type of herbicides, but that in addition, in the absence of herbicides, the electron flow rate at the acceptor side is slower (Jansen and Pfister, 1990). Our analysis, as illustrated in Table 1 and Fig. 2, confirms this phenomenon, but in addition, reveals more information. The rate constants of the oxygen evolving complex $k_1$, $k_2$, and $k_3$, are about the same in the resistant and the wild-type plants. The most important differences are on the acceptor side: the rate constants of the electron flow from $Q_A^-$ to $Q_B$ ($k_{AB1}$) are 3 to 6-fold slower in the R plants; the rate constants of electron flow from $Q_A^-$ to $Q_B^-$ ($k_{AB2}$) are 30 and 55 times slower in *C. album* and *S. nigrum*, respectively. There is incomplete correspondence between simulated and experimental curves in the 5-50 ms time domain (J-I phase), especially in the R biotypes (Fig. 2). This probably is caused by a simplification of the model as used here, in which 100% S-state homogeneity has been assumed with $S=S_1$ for a dark adapted system. Refinements which take into account an $S_1/S_2$ ratio below 1 give substantial improvement of the results (data not shown).

**References**

Vredenberg WJ, Rodrigues GC, Van Rensen JJS (2001) *These Proceedings*