Modeling and simulation of the initial phases of chlorophyll fluorescence from Photosystem II
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A simple kinetic model structure for chlorophyll fluorescence (ChIF) from Photosystem II (PSII) offers practical usefulness in quantitative analysis and extraction of information from measured ChIF. In this work, the major PSII phototransduction kinetics was represented with only five state variables. Parameters were estimated through a least-squares algorithm. The developed model structure could produce the well-known OJIP pattern and fit measured ChIF. Influences of PQ pool size, active QB sites, and QA reduction rate on ChIF emission were simulated and discussed in light of the existing literature.

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1. Introduction

When a photon is captured by a plant leaf, the photon energy will ultimately be used for photochemical reactions or dissipated as heat or fluorescence (Goltsev et al., 2003; Taiz and Zeiger, 2006). The measurable chlorophyll fluorescence (ChIF) from Photosystem II (PSII) results from one of the three coupled energy pathways and therefore contains information about phototransduction and photochemical reaction kinetics (Lubitz et al., 2008). PSII ChIF is useful for different aspects of photosynthesis analysis (Zhu et al., 2005). It has been applied in detection of heavy metals, herbicides, and air pollution; sensing of light, temperature, and drought stresses; postharvest quality assessments of fruits and vegetables (DeEll and Toivonen, 2003; Rodriguez and Greenbaum, 2009; Zivcak et al., 2008). In these applications, certain stages of the ChIF induction curve are named and used as physiological indicators, such as F₀ (minimal ChIF), Fₘ (maximal ChIF), Fᵥ (variable ChIF, Fᵥ = Fₘ − F₀), and the O, J, I, and P steps (Kautsky and Hirsch, 1931; Stribet and Strasser, 1996; Rohacek and Bartak, 1999; Rohacek, 2002).

To enhance ChIF applications, it is important to relate measured ChIF with specific chemical reactions or reaction rates based on reaction kinetic analysis (Stribet et al., 1998). A PSII ChIF model that can fit experimental data is thus warranted. About a dozen ChIF models have been proposed in previous research and a review of them can be found in Zhu et al. (2005). The existing models have been used to simulate the processes, but few have been compared with experimental data (Chernev et al., 2006). Some previous models have a very complex structure. The model in Chernev et al. (2006) has 19 differential equations. Lazár and Jablonský (2009) compared eight PSII models for ChIF simulation. Some of these models include even more than 50 state variables. Lazár (2009) made a significant contribution for simplifying ChIF model structure; however, the reported model still contains more than 10 state variables. A model with many differential equations attempting to account for the photochemical reaction details may be theoretically complete but practically of little value because the model parameters cannot be easily determined from experimental data. It is not a surprise that a high-order model structure with a large number of parameters could fit ChIF measurements even if the model structure is not an accurate representation of the mechanisms for ChIF generation.

In this research, our goal was to determine a minimum model structure (form of model) that can describe the different phases of ChIF. A low-order, simple model structure would permit estimation of the model parameters from measured ChIF. After parameter estimation, the model was used to simulate the ChIF kinetics as affected by variations in plant conditions, and the results are discussed in comparison with what has been published in the literature.

2. Model structure for chlorophyll fluorescence from Photosystem II

A PSII antenna complex (A) can be excited by a photon (A*, excited antenna complex). The excited A* may relax to the ground state with the emission of heat and chlorophyll fluorescence. These
processes can be represented as:

$$A^+ + Q_A^+ → A + Q_A^-$$  \hspace{1cm} (1)

where $u$ is the excitation light intensity, $k_1$ is the light-capture efficiency of antennas, $k_2$ is the dissipation rate through heat and fluorescence.

$A^+$ may transfer the photon energy to P680 (PSII chlorophylls), which will then be excited (denoted as P680*$). The excited electron on P680* may be transferred through a pheophytin molecule to plastoquinone $Q_A$ (Goltsev and Yordanov, 1997; Blankenship, 2002), which can be several-magnitude faster than the electron transport after $Q_A$ (Zhu et al., 2005). To keep the model structure simple, we neglect the fast relaying steps and represent the process as if $Q_A$ was directly reduced by $A^*$. The reduced $Q_A$ may transfer the excitation energy back to the antenna through charge recombination (Goltsev and Yordanov, 1997; Guo and Tan, 2009). The photochemical reactions from the antenna to $Q_A$ can thus be simplified as:

$$A^* + Q_A^- → A + Q_A^-$$ \hspace{1cm} (2)

where $k_3$ is the rate at which $Q_A$ is reduced in the presence of $A^*$, $k_4$ is the overall rate of charge recombination, $Q_A^-$ is the reduced form of $Q_A$.

The electron on $Q_A^-$ may be transferred to another plastoquinone ($Q_B$), which may receive two electrons. These electron transport processes can be represented by:

$$Q_A^- + Q_B^0 → Q_A^- + Q_B^-$$ \hspace{1cm} (3)

$$Q_A^- + Q_B^- → Q_A^- + Q_B^-$$ \hspace{1cm} (4)

where $k_5$ through $k_8$ are the forward or backward reaction rates, $Q_B^0$ and $Q_B^-$ are, respectively, the single- and double-reduced forms of $Q_B$.

While $Q_A$ is tightly bound in the thylakoid membrane, $Q_B$ is loosely bound. After receiving two electrons, $Q_B^-$ will combine with two protons from the stroma to become plastoquinol (OH$_2$). OH$_2$ will diffuse from the $Q_B$ site to the thylakoid lumen. A plastoquinone (PQ) molecule from the PQ pool will then move to the $Q_B$ site and become $Q_B$. The protonation of $Q_B^-$ is assumed instantaneous (Zhu et al., 2005). The process of $Q_B^-$ exchanging with $Q_B$ can be represented by (Goltsev and Yordanov, 1997; Zhu et al., 2005):

$$Q_B^- + PQ → Q_B^- + PQ$$ \hspace{1cm} (5)

where $k_9$ is the overall rate at which $Q_B^-$ is protonated and then replaced by a new $Q_B$ from the PQ pool.

$Q_B^-$ will be oxidized by the cytochrome b6f complex and the resulting PQ will return to the PQ pool (Goltsev and Yordanov, 1997). According to Stribet et al. (1998) and Zhu et al. (2005), the reactions beyond Cyt b6f do not affect the PSI ChlF induction curve significantly, and inclusion of reactions beyond $Q_B^-$ oxidation does not improve curve fitting (Bailey and Schloder, 1992; Zhu et al., 2005). As a result, only the reactions up to $Q_B$ are included in the model structure. The oxidation of $Q_B$ could be represented by:

$$Q_B^- → Q_B^0$$ \hspace{1cm} (6)

where $k_{10}$ is the net rate of $Q_B$ oxidation.

There is one $Q_A$ site and one $Q_B$ site in each PSI reaction center. The probability for a $Q_A$ site to be in the state of $Q_A$ or $Q_A^-$ is unity. Because of chemical effects and existence of nonreducing $Q_B$ sites, some $Q_B$ sites may not accept electrons from $Q_A$. The ChlF from PSII Q$_B$ sites has different dynamics with that from PSII Q$_B$ reducing. Scientifically, any PSII is either Q$_B$-reducing or Q$_B$-nonreducing. If we try to differentiate these two types of ChlF sources, several more parameters or state variables should be used. In order to use as few parameters as possible, we consider the average effect of nonreducing and reducing $Q_B$ sites and treat a PSI in the nonreducing and reducing status with different probabilities and the total probability is 1. If the percentage of $Q_B$ sites that are active (accepting electrons from $Q_A$) in a sample is $r_2$ (0 ≤ $r_2$ ≤ 1), then the probability for a $Q_A$ site to be in the active state with $Q_B$ or $Q_B^0$ site is $r_2$. For the probability of (1 − $r_2$) part, the PSI produces ChlF as a $Q_B$-nonreducing PSI since electrons will not be passed from $Q_A$ to $Q_B$. One PSI unit contains about 290 chlorophyll molecules (Zhu et al., 2005), which was used as the antenna pool size $A_0$. Simulations indicate that $A_0$ around 290 does not affect the shape of responses significantly except for scaling the values.

If $x_1$, $x_2$, $x_3$, $x_4$, and $x_5$ are denoted to the probabilities of $A^*$, $Q_A^-$, $Q_B^0$, $Q_B^-$, and PQ, respectively; the reactions in Eqs. ((1)–(6)) will depend on all the involved component probabilities. For example, the event of $Q_A$ passing an electron to $Q_B$ will be influenced by the product of $x_2$ and $x_3$. The reactions in Eqs. ((1)–(6)) may thus be represented by the following differential equations:

$$\frac{dx_1}{dt} = k_1 u(A_0 - x_1) - k_2 x_1 - k_3 x_3(1 - x_2) + k_4(A_0 - x_1)x_2$$ \hspace{1cm} (7)

$$\frac{dx_2}{dt} = k_2 x_1(1 - x_2) - k_4(A_0 - x_1)x_2 - k_5 x_2 x_3(2 - x_3 - x_4) + k_6(1 - x_2)x_4$$ \hspace{1cm} (8)

$$\frac{dx_3}{dt} = k_5 x_2(2 - x_3 - x_4) - k_6(1 - x_2)x_3 - k_7 x_2 x_3 + k_8(1 - x_2)x_4$$ \hspace{1cm} (9)

$$\frac{dx_4}{dt} = -k_9x_2x_5 + k_7 x_2 x_3 - k_8(1 - x_2)x_4$$ \hspace{1cm} (10)

$$\frac{dx_5}{dt} = -k_9 x_4 x_5 + k_6(A_0 - x_5)$$ \hspace{1cm} (11)

where PQ$_0$ is the initial PQ concentration or the size of the PQ pool per reaction center. If the reaction center concentration in a sample is $R$, then the concentrations of the chemical species ($A^*$, $Q_A$, $Q_A^-$, $Q_B^0$, and PQ) will be $R_k$ ($i = 1, 2, \ldots, 5$).

Zeaxanthin provides nonphotochemical quenching at a slower rate (Horton et al., 1996; Niyogi, 1999) and the influence of Zeaxanthin on the OJIP kinetics is ignored as done in Zhu et al. (2005). Both PSI and photosystem I (PSI) can produce ChlF. PSI ChlF is only about 10–25% of F$_0$ (Lavergne and Trissel, 1995; Stribet et al., 1998). PSI ChlF is neglected because it usually does not change with environmental stresses and does not contribute to F$_0$ (Butler, 1978; Zhu et al., 2005).

ChlF is mainly emitted from the antenna complexes (Krause and Weis, 1991) and is thus proportional to the concentration of excited $A^*$ (Zhu et al., 2005). The measured ChlF intensity can thereby be expressed as:

$$F = Gk_2x_1$$ \hspace{1cm} (12)

where $G$ is an overall gain factor accounting for sample size, reaction center concentration, and instrumentation gain.

3. Results

3.1. Model validation

The model parameters in Eqs.((7)–(12)) were estimated through a least-squares optimization algorithm. The Levenberg–Marquardt
method was used to adjust the model parameters to achieve optimal fit to measured ChlF (Levenberg, 1944; Marquardt, 1963; Constantinides and Mostoufi, 1999). The optimization algorithm was implemented in Matlab (The Mathworks, Natick, MA). Three sets of chlorophyll fluorescence data from the literature were used to verify the capability of the model structure in describing ChlF kinetics. Comparisons between model predictions and experimental data are shown in Fig. 1. The ChlF in Fig. 1(a) was measured from a fully mature pea leaf (Strasser et al., 1995; Zhu et al., 2005). The pea was 3–4 weeks old and grown in a greenhouse, with the temperature of 22°C/18°C (day/night) and natural sunlight. The ChlF in Fig. 1(b) was from a leaf of a 6-week-old Jatropha curcas L. seedling (Liang et al., 2007). The seedling was raised at 28°C with a 14-h light (150 μmol photons m⁻² s⁻¹)/10-h dark photoperiod. The ChlF in Fig. 1(c) was measured from a wheat (Triticum aestivum) leaf, which was grown in Knop solution and temperature around 20°C with 20/4 h light/dark cycle (Mehta et al., 2010). All the ChlF in Fig. 1(a)–(c) was measured by the instrument of plant efficiency analyzer (Hansatech Instruments Ltd., King’s Lynn, Norforlik, UK). The ChlF was induced by red light of about 3000 μmol photons m⁻² s⁻¹ with the peak at 650 nm. The model parameters used were listed in Table 1. Since the complex photosynthesis processes have been simplified and the model is reaction center based, all the estimated reaction rates would be reaction-center-based effective rate, which are useful for quantifying plant status for future research. The difference in the estimated parameter sets for Fig. 1(a)–(c) might be caused by the different plant species, different amount of captured excitation light, and different growing environment.

For a given set of experimental data, the relative fitting error was calculated as \[\sqrt{\frac{\sum_{i=1}^{N}(y_{i}^* - y_i)^2}{\sum_{i=1}^{N}(y_{i}^*)^2}},\] where \(y_{i}^*\) is the \(i\)th experimental data and \(y_i\) is the \(i\)th simulated data, \(N\) is the number of total data points. For Fig. 1(a)–(c), the average relative fitting error is 0.84 ± 0.47%. In Fig. 1, there is a visible difference between experimental data and model prediction especially at the initial phase (0 to J). It is caused by the logarithmic scale, which pulls the time axis at small scale and compresses it at the big one. It makes the fitting looks not as good as indicated by the relative fitting error although very few data points do not exactly match experimental data at the small time scale. The three sets of data represent different experimental conditions and thus varied ChlF kinetics. The model structure can successfully capture the complex OJIP kinetics with small relative errors, indicating the capability of the model structure.

Although the overall fittings are very good, careful examination of Fig. 1(a)–(c) reveals that model prediction looks smoother than experimental data. It might be caused by the simplification of

### Table 1
Model parameters for the curve fitting in Fig. 1(a)–(c).

<table>
<thead>
<tr>
<th></th>
<th>Fig. 1(a)</th>
<th>Fig. 1(b)</th>
<th>Fig. 1(c)</th>
<th>Fig. 1(a)</th>
<th>Fig. 1(b)</th>
<th>Fig. 1(c)</th>
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<tr>
<td>(k_1)</td>
<td>0.68</td>
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<td>0.18</td>
<td>6134.28</td>
<td>23700.40</td>
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<td>(k_2)</td>
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<td>1.25</td>
<td>3013.28</td>
<td>1398.06</td>
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<tr>
<td>(k_3)</td>
<td>3622.68</td>
<td>10418.40</td>
<td>4046.55</td>
<td>30.96</td>
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<td>1287.39</td>
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<tr>
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<td>80.00</td>
<td>642.34</td>
<td>9.21</td>
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<tr>
<td>(k_5)</td>
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<td>31435.70</td>
<td>1.00</td>
<td>0.99</td>
<td>0.77</td>
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<tr>
<td>(k_6)</td>
<td>1123.98</td>
<td>304.70</td>
<td>889.07</td>
<td>9.04</td>
<td>26.07</td>
<td>2.12</td>
</tr>
</tbody>
</table>
electron transferring from water to QA, the representation of QA-reduction and QB-nonreducing PSII, and neglecting the chlorophyll fluorescence from PSI or other sources. All these approximations make the order of the model structure not as high as the real system. It is thus the model prediction looks smoother than experimental data.

3.2. OJIP steps and species concentrations

Fig. 2 shows the simulation results of chlorophyll fluorescence emission (F) and the component concentrations of QA, QB, QH2, and PQ based on parameters estimated from experimental data shown in Fig. 1(a). r2 was set as unity for simplicity. Obviously, the model has the ability to produce the OJIP curve pattern. Different from previous models that use the combinations of QA and QB redox states as variables, the current model directly demonstrates the relationship between OJIP steps and species concentrations. As Fig. 2 indicates, the J step corresponds to the moment when QB begins to change quickly to QB. The I step corresponds to the maximum of QB, the first plateau of QA, and the time when PQ moves to the QA site. The P step corresponds to the steady state of the developed model structure. To compare these results with previous research, the joint probabilities of QA and QB redox states were checked. The new results are similar to previous reports that the J step corresponds most closely to the peaks of QA, QB, and the P step corresponds to the first shoulder of QA− and QB−. The QB sites may be rendered inactive by herbicides or other mechanisms. The influence of active QB sites on ChlF emission was simulated at two light intensities. The results are shown in Fig. 4(a) and (b). Fewer active QB sites make chlorophyll fluorescence stronger. This makes sense because with fewer active QB sites, less absorbed light energy will be carried forward for photosynthesis and ChlF will thus become stronger. The simulations also indicated that QB-nonreducing PSII may have more influences on the phases before the J at high light condition. This might be explained by more percentage of the low light is used for forward photosynthetic reactions and the low light cannot affect the later involved state variables as significant as the high light, which will not trigger the ChlF difference between QB-reducing and QB-nonreducing PSII earlier.

3.4. Influence of active QB sites on chlorophyll fluorescence

The QB sites may be rendered inactive by herbicides or other mechanisms. The influence of active QB sites on ChlF emission was simulated at two light intensities. The results are shown in Fig. 4(a) and (b). Fewer active QB sites make chlorophyll fluorescence stronger. This makes sense because with fewer active QB sites, less absorbed light energy will be carried forward for photosynthesis and ChlF will thus become stronger. The simulations also indicated that QB-nonreducing PSII may have more influences on the phases before the J at high light condition. This might be explained by more percentage of the low light is used for forward photosynthetic reactions and the low light cannot affect the later involved state variables as significant as the high light, which will not trigger the ChlF difference between QB-reducing and QB-nonreducing PSII earlier.

3.3. PQ size influence on chlorophyll fluorescence

The exact size of the PQ pool is not known (Papageorgiou and Govindjee, 2009). Four PQ pool sizes were used to simulate their effects on chlorophyll emission. Fig. 3 clearly indicates that chlorophyll fluorescence emissions begin to differ from the I step if the PQ pool size changes. This makes sense because PQ appears in the later reactions and should only affect the later part of chlorophyll fluorescence emission. A larger PQ pool will favor the forward transfer of photoelectrons and therefore reduce the later part of ChlF, as one would expect. Future experiments by using plants with genetically modified PQ pool size are needed to validate these observations.
Fig. 4. Active Q₅ site influence on chlorophyll fluorescence emission. The excitation light intensity in (b) is 10 times of that in (a). All the other model parameters are the same for (a) and (b).

Fig. 5. Chlorophyll fluorescence emissions for four levels of k₃.

of O to J because it is a rate relating to photochemical process, which dominates the energy pathway at the beginning. k₉ and k₁₀ mainly affect the P level. This is because they are parameters directly related to the efficiency of electrons transfer to PSI and appear at the later part of reaction kinetics. k₄ to k₈ mainly affect the transition from the J level to the P level.

4. Discussions and conclusions

In previous work (Stribet et al., 1998; Zhu et al., 2005), the combinations of species were used as state variables. For example, if combinations of the redox states of PSII (Z), Pheno, QA, and Q₂ with, respectively, 2, 2, 2, and 3 states are used, the total number of state variables will be 2⁴. When the species number increases, the number of state variables will increase drastically. By using second-order reaction kinetics, one state variable corresponds to one species. The developed model structure has only five state variables but produces the OJIP patterns similar to the 20-state-variable model of Zhu et al. (2005). Moreover, it could fit measured PSII fluorescence with less than 1% relative error.

The value of \( F_v/F_m \) or \( (F_m - F_0)/F_m \) has been defined as maximum quantum yield of PSII (Papageorgiou and Govindjee, 2009). Drought stress is known to affect photosynthetic plant growth, but it was found not to affect the quantity of \( F_v/F_m \) (Razavi et al., 2008; Živčák et al., 2008). This seems to be explained by the simulation results in Fig. 5. Drought stress or water availability may influence the k₃ value. \( F_m \) and \( F_0 \) will both increase or decrease when k₃ changes. Therefore, \( F_v/F_m \) may not be sensitive to drought stress.

The model structure developed in this work neglects many details, but it makes parameter estimation from experiments possible. There have been few reported attempts to fit a ChlF model to experimental data (Vredenberg, 2008; Vredenberg and Prasil, 2009; Vredenberg, 2011). Since each chemical reaction rate represents an aspect of plant physiology, the ability to estimate the model parameters is an important characteristic of the simple model structure proposed.

Simulations indicate that the J step corresponds to the moment when Q₅ begins to change quickly to Q₋; the I step corresponds to the maximum of Q₋, the first shoulder of Q₋² and the time when PQ becomes Q₅; the P step corresponds to the steady state.

Simulations show that the algorithm could converge to the optimum parameter sets listed in Table 1 even when parameter initializations are more than 100% difference from the optimum. However, sometimes the goodness of fitting is not sensitive to the coefficients of k₅ and k₆. It indicates that the model structure is locally identifiable. In the future, we will try to make the model structure globally identifiable. Further experiments are warranted to verify the model structure simulation results.

References


