A kinetic model structure for delayed fluorescence from plants

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In this research, we demonstrated that the plastoquinone-related electron-transport kinetics in photosynthesis could be sufficiently described with as few as three state variables, Q_A⁻, Q_B⁻, and Q_B²⁻. A third-order kinetic model structure was developed with delayed fluorescence as the measurable output. Delayed fluorescence emissions from drought-stressed, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] treated, or healthy plants were measured with a photon-counting system and used to verify the model structure through nonlinear least-squares optimization. While there were no visible differences between the healthy and the stressed plants, the model showed an obvious decrease of Q_A reduction rate in the drought-stressed samples and a clear decline of functional Q_AQ_B pairs in the DCMU-treated samples. The changes were consistent with the known mechanisms by which water and DCMU affect electron transport in photosynthetic plants. The results proved that the three-state formulation was a compact and practically useful model structure for describing delayed fluorescence from plants.

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1. Introduction

When light is absorbed by chlorophyll molecules for photosynthetic reactions in plants, part of the energy may be converted back into light in the form of prompt fluorescence (PF) and delayed fluorescence (DF) (Goltsev et al., 2003; Zaharieva and Goltsev, 2003; Govindjee, 2004). Both PF and DF from photosystem II (PSII) are emitted mainly from the antenna complex (Amesz and Van Gorkom, 1978), but there are fundamental differences between the two. A single molecule is sufficient for the generation of PF while the generation of DF depends on system interactions (Goltsev et al., 2003), which makes DF a potential indicator of photosynthesis efficiency and plant stresses (Bjorn and Forsberg, 1979; Jursinic, 1986; Wang et al., 2007). Goltsev and Yordanov (1997) suggested that the final stage of DF emission involves charge combination of P⁺ and I⁻. Detailed discussions on DF generation can be found in Jursinic (1986) and Goltsev et al. (2003).

DF was first discovered by Strehler and Arnold (1951) and subsequent investigations have yielded a significant amount of information. Much previous research focused on the correlations between DF or PF and photosynthesis-related factors such as drought stress and herbicide stress (Lu and Zhang, 1999; Christensen et al., 2003; Joly et al., 2005; Ilik et al., 2006). While these efforts contributed to the applications of DF, they were based on empirical observations and the results were not quantitative or generalizable. Mathematical modeling based on reaction kinetics is a complementary approach that has shown a great deal of potential (Goltsev and Yordanov, 1997; Stirbet et al., 1998; Lebedeva et al., 2000; Goltsev et al., 2003).

Lavorel (1975) used a sum of exponential functions to model DF. The model could represent measured DF after parameter fitting, but the model parameters could not be interpreted in terms of the underlying reaction processes. The hyperbolic model used by Scordino et al. (1996) suffers from the same limitation. Goltsev and Yordanov (1997) presented a seventh-order model for the reaction kinetics in PSII and used the model to analyze PF and DF. Although the model was complex and it was not compared with experimental data, the effort was a significant step forward in modeling DF based on the underlying photochemical reactions. Lebedeva et al. (2000) proposed a rather comprehensive and complex model for the catalytic cycles in PSII. Goltsev et al. (2003) developed a model to describe the relationship between DF and PF. Tyystjärvi et al. (2005) used a mathematical model and published parameter values to simulate the process of photo-inhibition in PSII.

The existing models or model structures vary in emphasis and complexity. Empirical models are relatively simple in structure but they lack physiologically meaningful interpretations. Reaction kinetics-based models have been developed to represent different aspects of the photochemical reactions in PSII. The published model structures, however, are complex and cannot be easily solved, analytically or numerically, for comparison with experimental results. In particular, there is a lack of parsimonious model structures that are convenient for plant stress analysis based on DF measure-
ments. Given the tremendous biological variability, it is unlikely that a model with fixed coefficient values can fit a wide range of species and environmental conditions. For a given purpose, however, a commonly useful model structure may be attainable because plants share a fundamentally similar phototransduction mechanism. This research was designed to develop a model structure for the DF-related reaction kinetics with a minimal set of variables and equations. The model structure developed involves only three state variables (QA−, Qb−, and Qb2−) and thus three first-order equations, significantly fewer than what has been reported in the literature. Comparison with measured DF from three different plants under normal and two stress conditions showed that the model could not only describe DF emissions closely but also indicate the stresses in a manner consistent with the known physiological mechanisms.

2. Model Structure Development

Under light excitation, P680 (PSII chlorophyll) absorbs photon energy or gets energy from around the antenna (Goltsev et al., 2003) and becomes excited (denoted as P680*). The excited electron may be transferred, via a pheophytin molecule, to the primary electron acceptor plastoquinone QA, which is tightly bound in the thylakoid membrane (Goltsev and Yordanov, 1997; Blankenship, 2002). The electron on QA− may be transferred to another loosely bound plastoquinone (Qb) or transferred back to recombine with P680+ (oxidized form of P680) and produce a re-excited P680* (Wong et al., 1978; Radenovic et al., 1994). The re-excited P680* may produce fluorescence either by emitting a photon directly or transferring the energy to around the antenna where the fluorescence will be given off (Goltsev et al., 2003). The re-excited P680* may also donate its gained electron to QB, which can carry two negative charges at the same time (Goltsev and Yordanov, 1997; Blankenship, 2002; Govindjee, 2004; Taiz and Zeiger, 2006). Then the next step of electron transport is either

\[ \text{QA}^- + \text{Qb} \xrightarrow{k_1} \text{QA} + \text{Qb}^- \]  
(3)

or

\[ \text{QA}^- + \text{Qb} \xrightarrow{k_6} \text{QA} + \text{Qb}^{2-} \]  
(4)

where \( k_1 \) through \( k_6 \) are reaction constants, and QA− and Qb− are respectively the single- and double-reduced forms of Qb.

After receiving two electrons, Qb2− will be reduced by two protons from the stroma to become plastoquinol (QH2), which will diffuse from the Qb site to the thylakoid lumen where it will be oxidized to become Qb (Goltsev and Yordanov, 1997; Blankenship, 2002; Govindjee, 2004; Taiz and Zeiger, 2006). For the purpose of DF modeling, this is a link in the electron transport chain that would allow minimization of the downstream effects on the plastoquinone-related kinetics under certain experimental conditions. The O–H bond in plastoquinol has finite bond energy, and oxidation of plastoquinol requires the involvement of the cytochrome b6f protein complex and occurs only after it diffuses to the lumen side. The oxidation of Qb2− is, therefore, practically irreversible. In terms of the plastoquinone activities, these reactions may be collectively represented as:

\[ \text{Qb}^{2-} \xrightarrow{k_7} \text{Qb} \]  
(5)

where \( k_7 \) is an overall reaction rate.

If the experiment sample is initially dark-adapted and the excitation is a short-pulse that is not sufficient to saturate the electron transport system, further simplifying assumptions may be made about the reactions represented in (5). Besides the Qb molecules recovered via plastoquinol oxidation, there is a plastoquinone (PQ) pool in each reaction center to refill the vacated Qb site. Kolber and Falkowski (1993) reported a pool size of up to 30 molecules and others estimated it to be about 9–10 (Malkin and Kok, 1966; Murata et al., 1966; Govindjee, 2004). In either case, the PQ pool provides a buffering capacity so that any time delays associated with the redox reactions and diffusions to replenish the PQ pool may be assumed not to limit the availability of a Qb molecule in a reaction center under dark-adapted and short-pulse conditions. Furthermore, both the stroma and the lumen have certain pH buffering capacities; therefore, the proton concentrations may be assumed steady for dark-adapted and short-pulse conditions and the overall Qb2− oxidation rate, \( k_7 \), may be considered as a constant.

There is one QA site and one Qb site in an active PSII reaction center. The concentration or probability of presence for the total QA (including QA and QA−) or total Qb (including Qb, Qb−, and Qb2−) can be set to unity. The reactions represented by expressions (2), (3), (4) and (5) can then be described by the following three differential equations.

\[
\frac{dQ_A}{dt} = k_1 u (1 - x_1) - k_2 x_1 - k_3 x_1 (1 - x_2 - x_3) + k_4 (1 - x_1) x_2 - k_5 x_1 x_2 + k_6 (1 - x_1) x_3
\]  
(6)

\[
\frac{dQ_b}{dt} = k_3 x_1 (1 - x_2 - x_3) - k_4 (1 - x_1) x_2 - k_5 x_1 x_2 + k_6 (1 - x_1) x_3
\]  
(7)

\[
\frac{dx_1}{dt} = -k_7 x_3 + k_5 x_1 x_2 - k_6 (1 - x_1) x_3
\]  
(8)
where $x_1$, $x_2$ and $x_3$ respectively denote the average (over many reaction centers) concentrations (or the probabilities of presence) of $Q_A^-$, $Q_B^-$, and $Q_AQ_B$ in an active reaction center. DF generation is proportional to the rate at which $Q_A$ is generated from the reduced $Q_A^-$ state; i.e.,

$$y = Nk_0 k_2 x_1 = k_8 k_2 x_1$$

(9)

where $y$ is the DF intensity, $N$ is the total number of active reaction centers or $Q_AQ_B$ pairs under observation, $k_0$ is the instrumentation gain, and $k_8 = Nk_0$ is the overall gain factor.

3. Experimental Data Collection

Experimental data were collected to validate the model structure. Besides healthy plants, stresses were induced to test the effectiveness of the model under varied conditions. Three plant varieties: corn, soybean and green bean; and two stress conditions, drought and herbicide; were used in the work. The plant and stress types were chosen because they are commonly grown crops or frequently occurring conditions. More importantly, the mechanisms by which the chosen stress conditions affect electron transport in PSII are known, which makes it possible to determine if the modeling results make qualitative sense.

3.1. Plant Samples

Mature greenhouse-grown soybean plants were used for the drought stress experiments. Three healthy samples were used as control and three drought-stressed samples were measured. For the drought condition, the weight of the pots and soil was monitored. Measurements on the drought-stressed samples were taken when the soil moisture was between 10 and 20% of the dry soil weight, while the control measurements were taken when the moisture was between 60 and 70% of the dry soil weight.

Both corn and green bean samples were used for the herbicide stress experiments. Twelve- to fifteen-day-old yellow corn seedlings were used to measure DF emission with or without herbicide stress, and seven samples were used for each group. An atomizer was used to spray a $10^{-4}$-M DCMU [Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea] solution onto the corn sample leaves until dripping 15 min prior to DF measurements. Twelve-day-old green beans (Burpee Bush Bean Contender) seedlings were also used for DCMU stress tests. The sample bean leaves were soaked in tap water, $10^{-5}$-M or $10^{-7}$-M DCMU solution for 15 min before measurement.

All the samples were grown at room temperature in open air and under fluorescent lighting (Model F20T12/CW, Philips, New York, USA. Color temperature: 4100K. Wavelength range: 410–720 nm) controlled in 16:8-h on-off cycles. Few visible differences could be observed between healthy (control) and stressed plants. Whole leaves were excised and kept in the dark for at least 30 min before DF measurements were taken. Kato et al. (2002) showed that detached leaves could be used for photosynthesis evaluation within several hours. Our experiments indicated that for the excitation and plants used, the DF emissions did not change appreciably 2 h after detachment. All the experiments were conducted much within the 2-h timeframe.

3.2. Instrumentation and DF Measurement

The test samples were placed in a light-tight chamber. Illumination from three white LEDs (Model NSPW500BS, Nichia, Tokushima, Japan. Wavelength range: 400–750 nm) was delivered through an 8-mm liquid light guide (Model 77628, ORIEL, Irvine, CA). The illumination light guide was pointed at the sample from a 1-cm distance and at a 30° angle from the vertical direction. DF emission was collected via a second light guide positioned at the same distance and angle as the illumination light guide but on the opposite side of the vertical line. This emission light guide was fed into a photon-counting channel multiplier tube (CMT) (Model MH1372P, PerkinElmer, Waltham, MA) located outside the test chamber.

The CMT was gated electronically. When a signal was given to turn the gating off, the high voltage for the CMT channel would be nullified to prevent photoelectron flux formation in the channel. The photoelectric output pulses from the CMT were recorded with a gated photon counter/multiscaler card (Model PMS-400, Becker & Hickl GmbH, Berlin) plugged in a host computer. The computer also controlled the LED illumination, CMT gating, and data acquisition timing. Dark-adapted samples were excited with a 0.5-s illumination pulse and DF emission following the pulse was recorded. DF photons were recorded with a 1-ms sampling interval for a 1-s period. The illumination intensity and duration were experimentally chosen so that the excitation was strong enough to give good signal-to-noise ratio and yet was not sufficient to cause observable saturation in the DF measurements.

4. Model Structure Validation

4.1. Parameter Estimation

Least-squares optimization (Constantinides and Mostouf, 1999) was used to estimate the model parameters in Eqs. (6)–(9). The optimization algorithm was implemented in Matlab (The Mathworks, Natick, MA). When a leaf is dark-adapted for tens of minutes, the plastoquinone acceptors of PSII ($Q_A$ and $Q_B$) become maximally oxidized (Baker, 1993). Rutherford et al. (1984), however, showed that there were about equal numbers of $Q_B$ and $Q_A$—after dark adaptation. As a result, the initial value for $x_2$ was set to 0.5 and those for $x_1$ and $x_3$ were set to 0. In each iteration of the parameter estimation process, the equations were numerically solved with the dark-adapted initial values for both the light-on and light-off phases of the experiment. Experimental data, however, were available for parameter correction only for the light-off phase.

4.2. Soybean and Drought-Stressed Samples

Fig. 1(a) and (b) shows the mean value and 95% confidence area of the DF emissions following a pulse excitation for the control group and the drought-stressed group, respectively. The DF intensity from the drought-stressed samples was typically a little weaker than that from the control samples, but few other quantitative specifics could be directly discerned about the changes in the electron transport process.

Fig. 2(a) and (b) is the model predictions of DF compared with measurements for all the control samples and all the drought-stressed samples, respectively. In the optimization process, all the model parameters were adjusted to give the optimal fit to the experimental data for all the controls samples. For the drought-stressed plants, however, the model parameters determined for the control samples were used and kept constant except for the one that was expected to differ. Under the experimental conditions, drought-related parameter changes should be primarily in $k_1$, the photoinduced reduction rate of $Q_A$ which depends on water as an electron supplier. As a result, only $k_1$ was re-determined for the drought-stressed group. It should be noted that drought changes $k_1$ but $k_1$ is not the only change drought induces. Drought leads to multiple changes in the longer term, including stomatal closure and decreased $CO_2$ assimilation. Stomatal closure helps the plant conserve water...
and thus $k_1$ should reflect this aspect of the effect. Decreased CO₂ assimilation influences the QH₂ oxidization rate (Baker, 1993). This longer term downstream effect was neglected for the short-pulse and dark-adapted conditions as discussed earlier.

From Fig. 2, it is obvious that the model predictions matched the experimental data well. Even when $k_2$ through $k_8$ were constrained to remain equal to the values of the control group, a $k_1$ value could be found to fit the measurements for the drought-stressed group. This indicates that the model structure sufficiently represents the kinetics governing delayed fluorescence emission for the samples. $k_1$ for the control samples in Fig. 2(a) was 398.10 and 271.60 for the drought-stressed samples in Fig. 2(b). This agrees with a known effect of drought. Water is oxidized to serve as the original electron donor. Since $k_1$ represents the overall efficiency for electron transfer from water to Qₐ by photo-activation of chlorophylls, a lack of water would impede the process and thus result in reduced $k_1$. For the two groups of samples tested, drought reduced the electron transport and thereby photosynthesis by about 32%.

4.3. Corn and DCMU-Treated Samples

The mean values and 95% confidence areas of DF the control and the DCMU-stressed corn samples are shown in Fig. 3. Similar to the drought stress experiments, the DF differed in intensity but the specific changes were not obvious without further analysis.

Fig. 4(a) and (b) is the model predictions of DF compared with measurements for all the control corn samples and all the DCMU-stressed samples, respectively. The parameter identification process was similar to that used for the drought stress experiments. The model parameters for the control samples were identified first and then used as constants for the DCMU-stressed samples except for $k_8$, which was expected to be influenced by DCMU stress. Only $k_8$ was re-determined to fit the DCMU-stressed sample group.

In spite of the constraint of shared parameter values for the two sample groups, the model predictions were close to the measured DF for both the control and DCMU-stressed samples as shown in Fig. 4(a) and (b). This again shows the capability of the model structure. DCMU works by binding to the Qₐ sites (Rutherford et al.,...
When a DCMU molecule occupies the Q₈ site in a reaction center, electron transfer from QA to Q₈ is blocked and the reaction center becomes inactive for electron transport. k₈ is a coefficient proportional to the total number of active reaction centers under observation and should be affected by the application of DCMU. For the DCMU-treated group shown in Fig. 4(b), the k₈ value was reduced to 62.22% of the value for the control group in Fig. 4(a). This is consistent with the mechanism of DCMU and indicates that about 38% of the reaction centers were blocked 15 min after application of the herbicide.

4.4. Green Bean and DCMU-Treated Samples

Fig. 5(a) and (b) shows all the experimental measurements and model predictions of DF for healthy and DCMU-treated green bean samples, respectively. The model parameters were estimated by the procedure used for the corn samples, in which only k₈ was re-determined for the DCMU-treated group after optimization for the control group. The model predictions could closely match the experimental measurements. As with the corn samples, DCMU treatment reduced the k₈ value. For example, in one set of experiments, the 10⁻⁵-M DCMU treatment reduced k₈ to 52.83% of the value for the control group; and the 10⁻⁷-M DCMU treatment reduced it to 92.94% of the value of control group. The model again correctly indicated the DCMU influence.

5. Discussion

The sensitivity of the model to parameter changes was analyzed. Fig. 6 is a typical chart of the sum of squared prediction errors comparing the optimized minimum with those when ±10% change was made to each of the optimized parameters. The total fitting error was very sensitive to k₂, k₇ and k₈. The strong dependence of DF on k₂, k₇ and k₈ make sense because k₂ is the oxidation rate of QA⁻ to generate DF, k₇ determines how many electrons are transferred forward for photosynthesis and k₈ is the overall gain. Variation of k₁ or k₈ alone allowed close fit of the model to both the control group and stressed group. This, however, was not possible when one of the other parameters (k₂ through k₇) was allowed to change. This indicated that k₁ and k₈ respectively accounted for the most changes induced by drought and DCMU stresses as expected.

For a given system, there may be different choices of state variables and consequently multiple possible model structures. Also, depending on the purpose of modeling, certain variations may be included or neglected. Attempts to account for many effects in a comprehensive model are often not successful or meaningful as the resulting model is difficult to verify and may not be practically useful. In this work, the individual redox states of the plastoquinones were used as state variables and the longer term...
changes beyond O$_2$ diffusion were assumed insignificant under short-pulse and dark-adapted conditions. This allowed modeling of the plastoquinone kinetics with only three state variables and a third-order system. This model structure neglects many details and is not as theoretically comprehensive as some previously published models (e.g., Goltsev and Yordanov, 1997; Lebedeva et al., 2000). Experimental verifications, nonetheless, showed that the parsimonious model structure rather accurately described the DF measurements. Moreover, the model parameters correctly indicated the expected changes resulting from drought stress and DCMU stress. This shows that the model is a compact but sufficient structure for delay fluorescence from plants under short-pulse and dark-adapted conditions.

It should be noted that what was developed was a model structure for the DF-related reactions rather than a model with generally applicable coefficient values. For a specific application, the model coefficients must be determined. A universal set of coefficient values is unlikely to exist. The model structure shows the nature of the kinetics involved and the coefficient changes can reflect the physiological changes in the plant samples.

6. Conclusion

Electron transport in the early stage of PSII can be effectively described with three state variables and thus three kinetic equations. Experimental validation showed that the three-state model could effectively describe delayed fluorescence emission from three different plants under normal or two stress conditions. The estimated model parameter values correctly reflected the expected changes induced by drought and herbicide stresses. While further validation is needed, the model structure seems to provide an effective representation of the photosynthetic electron transport process involving plastoquinones.

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