Kinetic Monte Carlo Simulation of the Initial Phases of Chlorophyll Fluorescence from Photosystem II

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Abstract
Kinetic Monte Carlo (KMC) simulation is employed to represent the photochemical reactions involved in the initial phases of chlorophyll fluorescence (ChlF) emission from photosystem II (PSII). Comparison with a differential equation representation reveals similarities and differences. Both KMC and differential equation models can describe the kinetic variations and show the main characteristics of ChlF emission. Differential equation models are simpler to implement but have limitations that warrant future improvements.

Keywords: Chlorophyll Fluorescence, Kinetic Monte Carlo, Reaction Kinetics, Photosynthesis

1. Introduction

In the plant photosynthetic process, absorbed light energy by chlorophyll molecules may be transferred forward and used for photochemical reactions, or dissipated as heat or fluorescence (Butler, 1978; Goltsev et al., 2003; Krause and Weis, 1991; Lavergene and Trissl, 1995; Stirbet et al., 1998; Vredenberg, 2004; Taiz and Zeiger, 2006). Because chlorophyll fluorescence (ChlF) competes for energy with photochemical reactions (Lubitz et al., 2008), the dynamics of ChlF is affected by photosynthetic activities (Kautsky and Hirsch, 1931; Stirbet and Strasser, 1996; Rohacek and Bartak, 1999;
This makes ChlF a useful indicator of plant physiology and environmental changes (DeEll and Toivonen, 2003; Guo and Tan, 2010; Rodriguez and Greenbaum, 2009; Zivcak et al., 2008).

Kinetic models are used to describe ChlF dynamics and to extract quantitative information from measured ChlF (Lazár, 2009; Lazár and Jablonský, 2009; Vredenberg, 2000; Vredenberg, 2008; Vredenberg and Prasil, 2009; Vredenberg, 2011). Differential equations have been commonly employed to represent the reaction kinetics (Chernev et al., 2006; Baake and Schloder, 1992; Goltsev and Yordanov, 1997; Guo and Tan, 2009 & 2011; Lazár and Schansker, 2009; Zhu et al., 2005). While differential equations are compact and convenient to use, they have limitations in representing certain aspects of the process. These limitations may or may not be significant for a given application but need to be analyzed and understood.

Each reaction center (RC) functions as an individual unit and each has one plastoquinone A (QA) site and one plastoquinone B (QB) site (Guo and Tan, 2009; Guo et al., 2010). An electron entering an RC is carried first by QA, then QB and later steps (Goltsev and Yordanov, 1997; Blankenship, 2002). The individuality of the RCs and the order of events within an RC can be represented by first-order differential equations with possible combinations of QA and QB states as state variables as was done in Guo et al. (2010). ChlF emission, however, involves numerous antennas and a pool of plastoquinones corresponding to each RC. There are then thousands of combinations of redox or excitation states, which makes it practically impossible to use first-order kinetics to represent the reactions. Tokarčík (2012) attempted to model ChlF from PSII by using pi-calculus though potential limitations of differential equations were not specifically
discussed. Using concentrations of individual chemical species as state variables result in a compact set of second-order differential equations as done in Guo and Tan (2011). The second-order differential equations, however, implicitly assume a well-mixed system. The effects of this assumption have not been demonstrated. Xin et al. (2013) simulated PSII ChlF through Monte Carlo method with explicit description of PSII activities; however, a comparison of output from Monte Carlo method and differential equations was not discussed.

In this work, kinetic Monte Carlo (KMC) simulation (Gillespie, 1976; Gillespie, 1977) is used to represent the discrete events involved in the initial phases of PSII ChlF emission. Since KMC simulation can represent a large number of individual RCs and other members of the electron transport chain without resorting to assumptions, this gives an opportunity to compare KMC and differential equation models.

2. KMC Simulation of ChlF

Light may excite a PSII antenna complex (A). An excited antenna complex (A*) may dissipate the absorbed energy as heat or ChlF, or it may transfer the absorbed energy forward for photochemical reactions. When A* transfers the absorbed energy to P680 (PSII chlorophylls), P680 becomes excited (P680*) and may pass the excited electron through a pheophytin molecule to plastoquinone QA, thus reducing QA (Goltsev and Yordanov, 1997; Blankenship, 2002). The electron carried by the reduced QA (QA -) may be transferred back to the antenna complex (Goltsev and Yordanov, 1997, Guo and Tan, 2009), or may be transferred to another plastoquinone (QB), which is capable of receiving two electrons. QA is tightly bound in the thylakoid membrane, but QB is loosely bound.
After receiving two electrons, $Q_B^{2-}$ will combine with two protons to become plastooquinol ($QH_2$) and $QH_2$ will diffuse from the $Q_B$ site to the thylakoid lumen. A plastooquinone (PQ) molecule from a PQ pool will then move to the vacated $Q_B$ site and become a new $Q_B$. $QH_2$ will be oxidized later and becomes a PQ. The resulting PQ will return to the PQ pool (Goltsev and Yordanov, 1997). These processes have been modeled at different levels of complexity in the literature and a comparison can be found in Lazár and Jablonský (2009). Some of the models reported have 50 or more state variables. While these models reach a high degree of completeness in representing the activities involved, they cannot be practically used for simulation and analysis because the complexity does not allow easy determination of model parameters from experimental data. To make model parameter estimation possible, Guo and Tan (2011) developed a minimized model for the initial phase of PSII ChlF based on the following 10 chemical reactions.

\[
A \xleftrightarrow{k_1u}{k_2} A^* \tag{1}
\]

\[
A^* + Q_A \xleftrightarrow{k_3}{k_4} A + Q_A^- \tag{2}
\]

\[
Q_A^- + Q_B \xleftrightarrow{k_5}{k_6} Q_A + Q_B^- \tag{3}
\]

\[
Q_A^- + Q_B^- \xleftrightarrow{k_7}{k_8} Q_A + Q_B^{2-} \tag{4}
\]

\[
Q_B^{2-} + PQ \xrightarrow{k_9} Q_B + QH_2 \tag{5}
\]
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, \( k_3 \) is the rate at which \( Q_A \) is reduced in the presence of \( A^* \), \( k_4 \) is the overall rate of charge recombination, \( k_5 \) through \( k_8 \) are the forward or backward rates of electron transfer between \( Q_A \) and \( Q_B \) sites, \( k_9 \) is the overall rate at which \( Q_B^{2-} \) is protonated and then replaced by a new \( Q_B \) from the PQ pool, \( k_{10} \) is the net rate of \( QH_2 \) oxidation.

By assuming continuous mixing and continuously-varying concentrations or probabilities of occurrence of \( A^* \), \( Q_A^- \), \( Q_B^- \), \( Q_B^{2-} \), and PQ, the reactions in Eqns. 1 through 6 were modeled with five continuous-time differential equations with the initial phases of ChlF being proportional to \( k_2 A^* \) in previous work (Guo and Tan, 2011). These assumptions have also been made in other differential equation models for ChlF in the literature (Goltsev and Yordanov, 1997; Zhu et al., 2005).

The chemical reactions in Eqns. 1-6 can be simulated by using the KMC method. Since it can represent the reactions as discrete stochastic events as they occur in individual reaction centers, KMC simulation should be a much closer presentation of reality than the differential equations. The general procedure for KMC modeling can be found in Gillespie (1976, 1977). KMC simulation of the 10 reactions is summarized below.

The reactions involve five independent chemical species (\( A^* \), \( Q_A^- \), \( Q_B^- \), \( Q_B^{2-} \), and PQ) and 10 reactions (represented by \( k_1 u \) through \( k_{10} \)). The amount of the \( i^{th} \) species at iteration \( p \) is denoted as \( x_{pi} \) \( (i=1, 2, \ldots, 5) \), and the reaction velocity for the \( j^{th} \) \( (j=1, 2, \ldots, \)
10) reaction at iteration $p$ is denoted as $r_{pj}$, where $p$ is an integer to denote Monte Carlo iterations. The simulation steps are:

**Step 1:** Set time $t=0$ and iteration index $p=0$.

**Step 2:** Initialize the species ($x_{0i}$, $i=1, 2, \ldots, 5$).

**Step 3:** Compute the 10 reaction velocities at iteration $p$ ($r_{pj}$) according to the current values of $x_{pi}$ ($i=1, 2, \ldots, 5$) as summarized in Table 1.

**Table 1: Reaction velocities**

<table>
<thead>
<tr>
<th>Reaction rates</th>
<th>Reaction velocities at the $p^{th}$ step</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1 u$</td>
<td>$r_{p1} = k_1 u(A_0 - A^*) = k_1 u(\Delta_0 - x_{p1})$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>$r_{p2} = k_2 A^* = k_2 x_{p1}$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$r_{p3} = k_3 A^* Q_A = k_3 x_{p1}(1-x_{p2})$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$r_{p4} = k_4 A Q_A = k_4 (A_0 - x_{p1}) x_{p2}$</td>
</tr>
<tr>
<td>$k_5$</td>
<td>$r_{p5} = k_5 A^* Q_B = k_5 x_{p2}(1-x_{p3}-x_{p4})$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$r_{p6} = k_6 A Q_B = k_6 (1-x_{p2}) x_{p3}$</td>
</tr>
<tr>
<td>$k_7$</td>
<td>$r_{p7} = k_7 A^* Q_B = k_7 x_{p2} x_{p3}$</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$r_{p8} = k_8 A Q_B = k_8 (1-x_{p2}) x_{p4}$</td>
</tr>
<tr>
<td>$k_9$</td>
<td>$r_{p9} = k_9 Q_B^2 = k_9 x_{p4} x_{p5}$</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>$r_{p10} = k_{10} Q_H = k_{10} (PQ_0 - x_{p5})$</td>
</tr>
</tbody>
</table>

where $A_0$ and $PQ_0$ are the number of antenna complexes and PQ pool size per reaction center, respectively.

**Step 4:** Calculate the cumulative reaction velocities $R_{pj} = \sum_{q=1}^{j} r_{pq}$, $j=1, 2, \ldots, 10$.

**Step 5:** Sample a uniformly-distributed random number $\mu \in (0, 1]$.

**Step 6:** Determine the event ($j$) to carry out for which $R_{pj(j-1)} < \mu R_{p0} \leq R_{pj}$ ($R_{p0}=0$). Note that the condition and the definition of $R_{pj}$ in Step 4 mean that events associated with greater reaction velocities are more likely to occur and events for later reactions (larger $j$ values) would not occur right after excitation light $u$ is applied to an initially dark-adapted leaf.

**Step 7:** Carry out event $j$ as summarized in Table 2.
Table 2: Events to carry out

<table>
<thead>
<tr>
<th>$j$</th>
<th>Events to carry out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$A^*$ increased by 1</td>
</tr>
<tr>
<td>2</td>
<td>$A^*$ decreased by 1</td>
</tr>
<tr>
<td>3</td>
<td>$A^<em>$ decreased by 1, $Q_{A^</em>}$ set to 1</td>
</tr>
<tr>
<td>4</td>
<td>$A^<em>$ increased by 1, $Q_{A^</em>}$ set to 0</td>
</tr>
<tr>
<td>5</td>
<td>$Q_{A^*}$ set to 0, $Q_B$ set to 1</td>
</tr>
<tr>
<td>6</td>
<td>$Q_{A^*}$ set to 1, $Q_B$ set to 0</td>
</tr>
<tr>
<td>7</td>
<td>$Q_{A^*}$ set to 0, $Q_B$ set to 0, $Q_{B^2}$ set to 1</td>
</tr>
<tr>
<td>8</td>
<td>$Q_{A^*}$ set to 1, $Q_B$ set to 1, $Q_{B^2}$ set to 0</td>
</tr>
<tr>
<td>9</td>
<td>$Q_{B^2}$ set to 0, $PQ$ decreased by 1</td>
</tr>
<tr>
<td>10</td>
<td>$PQ$ increased by 1</td>
</tr>
</tbody>
</table>

Step 8: Draw another uniformly-distributed random number $\mu' \in (0, 1]$.

Step 9: Update time with $t = t + \ln(1/\mu') / R_{p10}$. Note $\mu' = 1$ leads to no time increase.

Step 10: Increment $p$ and go to Step 3.

It is worth noting that in the KMC algorithm, many RCs can be simulated and each RC is simulated individually. This means that different RCs may have different model parameters. For example, when $k_5$ is set to zero for an RC, the $Q_B$ site in the RC is rendered inactive. This would be difficult to achieve in a differential equation representation.

3. Results

3.1 Comparison of KMC with Differential Equations

Because the KMC simulation and the differential equations in Guo and Tan (2011) are based on the same set of chemical reactions (Eqns. 1 through 6), it would be meaningful to compare these two models when the same model parameters (reaction rates
Fig. 1 indicates that the initial sections (O and J) of the OJIP curve are almost the same for the two models, but the output of the KMC model from J to P is higher than that of the differential equation model. The similarity and difference are expected and can be explained. Upon excitation, ChlF starts with antenna complexes absorbing and immediately releasing light energy, and thus the initial phase of ChlF (O to J) mainly reflects this process. Each RC has approximately 290 antenna molecules (Zhu et al., 2005), which makes the excitation status change of antenna complexes close to a continuous and instantaneous phenomenon. The differential equation model describes the instantaneous bulk behavior and is thus in this phase very close to the KMC model.

Later in the process, QA, QB, and PQ are involved. There are only one QA site and one QB site, and a PQ pool of about 12 for each RC (Zhu et al., 2005). This makes the two models different. As noted in Step 6 of the KMC simulation process, the initial
reaction velocities are all zeros and later events in the transport chain would not occur till sufficient earlier events happen; in other words, reactions initially propagate down the transport chain rather than occur simultaneously throughout the chain. The differential equation model, however, assumes that all events happen simultaneously and continuously. As a result, time delays are not represented by the differential equation model and the forward photosynthetic activities would occur sooner than in the KMC model when the same reactions rates are used, which leads to lower ChlF emissions in the later phases (I to P) as shown in Fig. 1. The ten reaction rates used for the simulation of Fig. 1 are 0.68, 409.61, 3622.68, 1.40, 15983.00, 1123.98, 6134.28, 3013.28, 30.96, and 9.21, respectively. Many sets of model parameters are used and simulations revealed the same regularity. Due to simplification, the reaction rates in this research are effective rates. The simulation is single RC based and second-order kinetics is also involved, it is difficult to compare model parameters with those pseudo first-order reaction rates in literature. Because the focus of the manuscript is to compare the outputs from KMC and differential equations under the same set of model parameters, the exact model parameters used do not affect the comparison.

3.2 Influence of Number of RCs on Simulated ChlF

The differential equation model represents the average behavior of many RCs. The KMC model, however, represents the stochastic events in each RC separately. Many RCs should thus be represented in the KMC simulation; otherwise, one would expect strong noise in the simulation results. Fig. 2 shows the influence of the number of RCs on the simulated ChlF. It clearly indicates that the predicted ChlF is more stable when more RCs
are used. Another interesting phenomenon is that the noise level is not uniform and gradually increases from the O stage to the P stage. This is explainable. For an initially dark-adapted plant leaf, light excitation will progressively trigger a cascade of stochastic activities from the antenna complex, to $Q_A$, to $Q_B$, and to PQ. This leads to noise propagation and thus higher noise levels in the later parts of the OJIP curve. The stochastic cascade is not represented in differential equation models and thus the characteristic is not observed in differential equation simulations. This dependence of noise on number of RCs needs to be noted in ChlF simulations. In actual ChlF measurement, the number of RCs observed may not be so low that this noise is discernable, but one should pay attention to this phenomenon when a very small sample is used.

![Fig 2. Influence of the number RCs on ChlF emission](image)

### 3.3 OJIP Stages and Species Concentration

Fig. 3 shows variations of the average species concentrations relative to the OJIP stages of ChlF from KMC simulation. Both KMC and differential equation models show
similar patterns of variations and lead to the same observations. As shown in Fig. 3, the J stage emerges when $Q_A$ passes electrons to $Q_B$ and thus $Q_B^-$ starts to increase. The I stage corresponds to the $Q_B^-$ maximum, the first $Q_B^{2-}$ plateau, and the time when the available PQs begin to decrease. The P stage corresponds to the steady state.

![Fig 3. Relative ChlF intensity and species concentrations (arbitrary unit)](image)

4. Summary and Conclusions

The results indicate that KMC is a useful tool for simulating the reactions involved in ChlF emission. KMC simulation cannot only represent the kinetic variations and characteristics known from experimental measurements and differential equation simulations, but also show the influence of conditions such as number of RCs and percentage of active $Q_B$ sites, which is difficult to demonstrate with differential equations. Second-order differential equations give compact, practically useful representations of the reactions involved, but they underestimate ChlF in later stages of emission compared
with KMC simulation. The differential equation model is simple to implement, but refinements are warranted.

References


