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**On the approaches applied in formulation of a kinetic model of photosystem II:
Different approaches lead to different simulations of the chlorophyll *a* fluorescence
transients.**

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Abstract

Chlorophyll *a* fluorescence rise (O-J-I-P transient) was in literature simulated using models describing reactions occurring solely in photosystem II (PSII) and plastoquinone (PQ) pool as well as using complex models which described, in addition to the above, also subsequent electron transport occurring beyond the PQ pool. However, there is no consistency in general approach how to formulate a kinetic model and how to describe particular reactions occurring even in PSII only. In this work, simple kinetic PSII models are considered always with the same electron carriers and same type of reactions but some reactions are approached in different ways: oxygen evolving complex is considered bound to PSII or “virtually” separated from PSII; exchange of doubly reduced secondary quinone PSII electron acceptor, Q_B , with PQ molecule from the PQ pool is described by one second order reaction or by two subsequent reactions; and all possible reactions or only those which follow in logical order are considered. By combining all these approaches, eight PSII models are formulated which are used for simulations of the chlorophyll *a* fluorescence transients. It is shown that the different

approaches can lead to qualitatively different results. The approaches are compared with other models found elsewhere in the literature and therefore this work can help the readers to better understand the other models and their results.

Keywords

fluorescence induction; O-J-I-P; photosynthesis; theory

Abbreviations

F_0 , F_M – minimal and maximal fluorescence levels

FLR – fluorescence rise

O, J, I, P – particular steps of chlorophyll *a* fluorescence rise

OEC – oxygen evolving complex

P680 – electron donor in photosystem II

PQ – plastoquinone

PSII – photosystem II

Q_A , Q_B – primary and secondary quinone electron acceptors in photosystem II

RCII – reaction centre of photosystem II

S_i -states – particular redox states of oxygen evolving complex ($i = 0, 1, 2, 3$)

Introduction

When a dark-adapted photosynthetic sample is illuminated by high intensity of excitation light (about 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and more), usually two steps appear in chlorophyll *a* fluorescence rise (FLR) between minimal (F_0 ; denoted as O) and maximal (F_M ; denoted as P or M) levels: the first step at 2 – 3 ms and the second step at 30 – 40 ms. The steps are frequently denoted as J and I, respectively (Strasser and Govindjee, 1991, 1992) and the FLR

is called as O-J-I-P curve or transient (Strasser et al., 1995; see Fig. 1). It is commonly accepted that this variable fluorescence comes from photosystem II (PSII) whereas photosystem I emits in time only a constant fluorescence (for reviews see Dau, 1994; Govindjee, 1995; Lazár, 1999, 2006). Duysens and Sweers (1963) suggested that it is redox state of the primary quinone electron acceptor, Q_A , in PSII which determines the variable fluorescence: more reaction centers of PSII (RC IIs) with reduced Q_A accumulate, more fluorescence is emitted. In addition to the ' Q_A^- ' concept, many other hypotheses have been suggested so far to explain the O-J-I-P FLR: e.g., PSII donor side or redox state of the secondary quinone electron acceptor, Q_B , in PSII were suggested to play a role (for reviews see Lazár, 1999, 2006). As accumulations of electrons in PSII also depends on electron transport beyond PSII, also a role of these electron transport reactions on the O-J-I-P FLR was suggested (Schansker et al., 2003, 2005).

The O-J-I-P FLR was also studied by means of mathematical modeling. Most of these works simulated the FLR based on different models of PSII: simpler (e.g. Lazár et al., 1997; Tomek et al., 2001) or more complicated (e.g. Baake and Schlöder, 1992; Stirbet et al., 1998; Vredenberg et al., 2001; Lazár, 2003; Zhu et al., 2005; Belyaeva et al., 2006), but also complex models were considered describing, in addition to PSII, also subsequent electron transport reactions (e.g. Lebedeva et al., 2002; Kroon and Thoms, 2006; Laisk et al., 2006). However, inspection of PSII models used for simulations of the FLR reveals that there is no consistency in general approach how to describe particular reactions occurring in PSII.

In this work, electron transport reactions occurring between S_i -states of the oxygen evolving complex (OEC; $i = 0, 1, 2, 3$), P680 (electron donor in PSII), Q_A , Q_B and plastoquinone (PQ) pool are considered. However, approaches to describe some reactions and consideration of some reactions themselves are different. It is shown that the different approaches can lead to qualitatively different simulations that consequently might affect

conclusions made from these simulations. Therefore this work can help the readers to orientate in the PS II models used to simulate FLRs found elsewhere in the literature.

Material and methods

Chlorophyll *a* fluorescence rise was measured with control dark-adapted mature pea leaf using fluorometer Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., Norfolk, UK). The fluorometer excites fluorescence by red light (emission maximum at 650 nm) and detects fluorescence above 700 nm. Used intensity of excitation light was 3000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. All theoretical simulations were performed using free software GEPASI version 3.21 (P. Mendes, The University of Wales, Aberystwyth, United Kingdom) that was designed for the simulation of chemical and biochemical kinetics (Mendes, 1993).

Defining explored problems and applied approaches

When any model to simulate experimental data is formulated, there is always the problem of balance between correctness of the model (with respect that it includes all “important” reactions) and requirement for simplicity of the model (because of easier calculations and better orientation in the model and its results). “Easy” models can be obtained by making simplifications to potentially complete reaction schemes. The final structure of a model depends on used approach, i.e. the way in which the description of the function of the modeled system was simplified. There are also procedures, the so-called model reduction, for the simplification of complicated models, which already exist (see e.g. Riznichenko et al., 1999; Nedbal et al., 2007).

In the case of a kinetic model of PSII in which the electron carriers are given (see below), the model simplification can be made by consideration of only some of the reactions and by simplified reaction kinetics (see below). But these model simplifications have also physiological consequences which are often overlooked and not discussed in the literature. The goal of this work is not to exactly study origin of particular steps of the O-J-I-P FLR by

means of its mathematical modeling but to show that different approaches (type of used kinetics and consideration or not of some reactions) applied in formulation of even a rather simple model can lead to very different simulations of the FLR.

It is assumed in all following descriptions and formulated models that variable fluorescence during the FLR originates in PSII only and therefore electron transport reactions occurring only in PSII and PQ pool are considered. The demand for simplicity of a model but also the fact that the FLR occurs in time range of tens of microseconds to hundreds of milliseconds (Fig. 1) restrained considered electron carriers and reactions between them. Therefore, the S_i -states, P680, Q_A , Q_B and PQ pool are considered as electron carriers in all considered models (see below).

In modeling of the FLR generally, a PSII model consists of its particular states (model forms also called state variables) which are transformed from one state to another state via particular reactions with given rate constants, describing thus formation of particular redox states of PSII. When the four PSII electron carriers (S_i -states, P680, Q_A , Q_B ,) are considered then there are four S_i -states (S_1 , S_2 , S_3 , S_0), two states of P680 (P680, P680⁺), two states of Q_A (Q_A , Q_A^-), and four states of Q_B (Q_B , Q_B^- , Q_B^{2-} , E, where E means empty Q_B -pocket). The combination of the particular redox states of the four electron carriers leads to $4 \times 2 \times 2 \times 4 = 64$ possible model forms (i.e. 64 differential equations) of PSII mutually connected by particular electron transport reactions.

However, to have a simpler model, some additional assumptions are often made. For example, the S_i -states of OEC are often considered as separated states (model forms) (e.g. Zhu et al., 2005) and redox states of PSII are described only by combinations of possible states of P680, Q_A and Q_B . This leads to $2 \times 2 \times 4 = 16$ model forms describing all possible redox states of PSII plus 4 model forms describing the four S_i -states, that is, 20 model forms (differential equations) describing OEC and PSII. The consideration of the S_i -states as

“virtually“ separated states leads to use of the second order kinetics for description of P680⁺ reduction by the S_i-state, that is, e.g., $S_1 + P680^+Q_A^-Q_B^{2-} \rightarrow S_2 + P680Q_A^-Q_B^{2-}$, instead of the first order kinetics, $S_1P680^+Q_A^-Q_B^{2-} \rightarrow S_2P680Q_A^-Q_B^{2-}$. But the second order kinetics is fully justified only for freely moving substrates in a medium which is not the case for S_i-states of OEC and particular redox states of PSII because it is well-known that OEC is bound to PSII (see Wydrzynski and Satoh 2005).

Another example of the PSII model simplification is that the exchange of double reduced Q_B (after its implicitly assumed protonation) with PQ molecule from the PQ pool is instead of two subsequent reactions, e.g., $S_2P680Q_A^-Q_B^{2-} \leftrightarrow S_2P680Q_A^-E + PQH_2$ followed by $S_2P680Q_A^-E + PQ \leftrightarrow S_2P680Q_A^-Q_B$, described by only one second order reaction $S_2P680Q_A^-Q_B^{2-} + PQ \leftrightarrow S_2P680Q_A^-Q_B + PQH_2$ (e.g. Stirbet et al., 1998). The use of the second order kinetics in the latter case is partially justified with respect to the free movement of the substrates because PQ molecules freely move in the membrane. As the latter case ignores presence of the empty Q_B-pocket, there are only three states of Q_B and the PSII model simplified in this way has only $2 \times 2 \times 3 = 12$ (reflects redox states of P680, Q_A and Q_B, respectively) plus 4 (for assumed “virtually” separated S_i-states), that is, 16 model forms (differential equations) describing PSII and OEC.

Another way how to simplify the PSII model is to consider only those electron transport reactions which follow in “logical” order instead of consideration of all possible electron transport reactions. In other words, because it is usually assumed that there is only one initial (dark-adapted) state of PSII (represented e.g. by S₁P680Q_AQ_B model form), a model is constructed in a way that starting from the initial state particular reactions are added as they follow in “logical” order until a “final” state (model form) is reached (S₀P680Q_A⁻Q_B²⁻ in this case; before Q_B²⁻ is exchanged with PQ molecule from the PQ pool; see Scheme 5 in the Appendix). This approach was used e.g., by Vredenberg et al. (2001). As this approach

discards some reactions, formation of some model forms does not occur. An example is shown in Scheme 5 (part A) in the Appendix, where there are only 32 PSII model forms instead of 64 PSII model forms (for the case of PSII model in which the empty Q_B -pocket is considered and the S_i -states are not considered as separated states). Consideration of only those reactions which follow in logical order can be also applied in the case when the S_i -states are considered as separated states. In this case only those S_i - S_{i+1} -transitions are considered which follow in logical order with respect to initial distribution of the S_i -states.

In summary, a PSII model consisting of the S_i -states, P680, Q_A and Q_B electron carriers can be simplified by:

- i) Consideration of the S_i -states as separated states, i.e., OEC is assumed “virtually“ separated from PSII (i.e., the second order kinetics) instead of bound to PSII (i.e., the first order kinetics).
- ii) Description of exchange of doubly reduced Q_B with PQ molecule from PQ pool by one second order reaction instead of two subsequent reactions.
- iii) Consideration of only those reactions which follow in logical order instead of all possible reactions.

The three simplifications described above are explored in this work: by combination of the approaches to simplify the model, eight models are formulated and fluorescence transients are simulated on the basis of these models. For more details on the used models, see the Appendix. For easier orientation and cross-reference of discussed results, particular models are denoted by numbers in the text. A sketch summarizing approaches used in particular models is shown in Scheme 1.

Results

Models with OEC “virtually“ separated from PSII

Model 1: A model with OEC “virtually“ separated from PSII where all possible reactions are considered and where exchange of Q_B^{2-} with PQ molecule from the PQ pool is described by the second order reaction was chosen as a starting point. The reaction scheme of this model is shown in Scheme 2 in the Appendix. This model was chosen as the first one because it directly follows from a simplification of one of most complex models of PSII used for modeling of the O-J-I-P FLR so far (Lazár, 2003). Result of simulation of FLR based on Model 1 is presented in Fig. 2 (curve a). The simulation shows a step at 3 – 4 ms, which is at position of the J step in experimental FLR (Fig. 1), and the F_M level is reached at 70 ms which roughly corresponds to appearance of the I step in the experimental FLR (Fig. 1). Qualitatively very similar curve was also simulated on the basis of the complex model of PSII of Lazár (2003) (see the inset in Fig. 2). The qualitative agreement between both simulations shows that a reduction of the complex PSII model used in Lazár (2003) into Model 1 is justified.

Model 2: Possible imperfection of Model 1 is that it considers that any model form with $P680^+$ can be reduced by any S_i -state independent of logical order of the S_i - S_{i+1} -state transitions in case when initial distribution of the S_i -states is given. For example, because it is assumed here that there is 100% of OEC in the S_1 -state initially (dark-adapted state; see the Appendix), then, $P680^+$ after the second charge separation (formation of the $P680^+Q_A^-Q_B^-$ model form; see Scheme 2) can be reduced only by the S_2 -state (not considering the misses and the double hits) but not by any other S_i -state (e.g. S_1 -state which can be accumulated to some extent from previous “imperfect” S_1 - S_2 -transition) as used in Model 1. When this restraint on logical order of the S_i - S_{i+1} -transitions (see Scheme 2 in the Appendix) is applied, a drastically changed fluorescence transient is simulated as shown in Fig. 2 (curve b). This curve shows a maximum at 3 ms which is followed by a decrease of fluorescence signal which is completely in disagreement with experimental FLR (Fig. 1). The reason for such

fluorescence transient is that when the restraint on logical order of the S_i - S_{i+1} -transitions is applied, no S_0 - S_1 -transition is realized (see Scheme 2 in the Appendix) and as a consequence the S_0 -state starts to accumulate at 2 – 3 ms and it amounts to about 95% of all S_i -states at 1 second (data not shown). The accumulation of only the S_0 -state in turn leads to predominant accumulation of non-fluorescent model forms (model forms with Q_A and not with Q_A^- ; see Scheme 2) that causes the decrease of fluorescence signal after its peak value at 3 ms.

Model 3: Models 1 and 2 assumed that exchange of Q_B^{2-} with PQ molecule from the PQ pool was described by second order reaction (see Scheme 2 in the Appendix). However, as described above, the Q_B^{2-} /PQ exchange occurs, in fact, via two subsequent reactions. The model with OEC “virtually” separated from PSII where the Q_B^{2-} /PQ exchange is described by two subsequent reactions is shown in Scheme 3 (c.f. Scheme 2) in the Appendix. Result of simulation of a FLR based on Model 3, where all possible reactions are considered (related to the S_i - S_{i+1} -transitions; c.f. Model 1), is shown in Fig. 2 (curve c). The FLR is characterized by a higher and shallow step at about 10 ms and F_M -level reached later at about 120 ms, when compared with curve a in Fig. 2, simulated using the “same” model but with the Q_B^{2-} /PQ exchange described by the second order reaction (see Model 1). The reason for the difference in the step appearing between 3 – 4 ms (curve a) and at about 10 ms (curve c) is different time courses of $P680Q_A^-Q_B^{2-}$ model forms (data not shown) in the two models which result from different time courses of oxidized PQ pool (data not shown) as a consequence of different approaches used for descriptions of the Q_B^{2-} /PQ exchange. The different time courses of $P680Q_A^-Q_B^{2-}$ model forms are also reasons (data not shown) why the F_M -levels of curves a and c (Fig. 2) are reached at different times.

Model 4: Model 3 considered all possible S_i - S_{i+1} -transitions. Therefore, in analogy with Model 2, only the S_i - S_{i+1} -transitions which follow in logical order are considered in Model 4. A scheme of the model is shown in Scheme 3 in the Appendix and the simulation of a

fluorescence transient based on Model 4 is presented as curve d in Fig. 2. This curve is similar in shape to curve b in Fig. 2 but a maximum is reached at about 7 ms which corresponds to a shift of the step in curve a (Fig. 2) appearing at about 3 – 4 ms to the step in curve c (Fig. 2) appearing at about 10 ms. The shift of the step to about 7 ms (curve d) is caused by a delayed and increased accumulation of the $P680Q_A^-Q_B^{2-}$ model form when compared with curve b in Fig. 2 (data not shown).

Models with OEC bound to PSII

All the four models described in the previous section considered OEC “virtually” separated from PSII. In this case, there are only 12 (when the Q_B^{2-}/PQ exchange is described by the second order reaction) or 16 (when the Q_B^{2-}/PQ exchange is described by two subsequent reactions) possible model forms (redox states) of PSII. But it is well known that OEC is bound to PSII (see Wydrzynski and Satoh, 2005). In this case, there are four (it results from four S_i -states) times more (i.e. 48 or 64, respectively) possible model forms (redox states) of PSII. Note that the term “bound” in the following text is understood in a way and results in the fact that the S_i -states of OEC do not form separated model forms but are included into model forms which now consist of S_i -states, P680, Q_A and Q_B . The different amounts of PSII model forms (redox states) in both approaches to the incorporation of the S_i -states to a model indicate that assumption of OEC being “virtually” separated from PSII (Models 1 – 4) or bound to PSII (Models 5 – 8) might affect simulations of the FLR. Therefore, FLRs simulated on the basis of PSII models with OEC bound to PSII are explored using Models 5 – 8.

Model 5: A scheme of PSII model with OEC bound to PSII where the Q_B^{2-}/PQ exchange is described by the second order reaction and where only the reactions which follow in logical order are considered is shown in Scheme 4 in the Appendix. The term logical order of reaction is now related to subsequent order of all possible reactions in the model when initial (dark-adapted) state is given and not only to reduction of $P680^+$ (in particular PSII model

forms) by only those S_i -states which follow from logical order of the S_i - S_{i+1} -transitions, as it was done in Models 2 and 4. A view to Scheme 4 reveals that OEC is able to go through all its S_i -states in a circle that might predict that no such unusual FLR curves resulted from logical order of reactions when OEC was “virtually” separated from PSII (Models 2 and 4; curves b and d in Fig. 2) will be simulated based on Model 5. This is demonstrated by curve a in Fig. 3 which shows FLR simulated on the basis of Model 5 (Scheme 4). Moreover, a comparison of this curve with any curve from Fig. 2 clearly demonstrates that assumption of binding of OEC to PSII leads to a different FLR. There is a plateau between 2 – 3 ms followed by a fluorescence rise to a shallow step at 10 ms and finally the fluorescence signal rises to the F_M -level which is reached at about 80 ms (curve a in Fig. 3). Positions of the plateau and of the shallow step and the time at which the F_M -level is reached roughly agree with experimental FLR (Fig. 1). Curve a in Fig. 3, and also other simulations based on models where OEC is bound to PSII (Models 5 – 8), demonstrates that it is the assumption of OEC being bound to PSII which causes an appearance of two steps, instead of one step (Models 1 and 3), between the F_0 - and F_M -levels in the FLR. Origin of the shape of the FLR curve is evident from panel A of Fig. 4 where time courses of the redox states with reduced Q_A , which mostly accumulate during the FLR, are presented: the plateau of curve a in Fig. 3 is caused mainly by a transient accumulations of $S_2P680Q_A^-Q_B^{2-}$ redox state (see also Scheme 4 in the Appendix). On the other hand, the shallow step at 10 ms of curve a in Fig. 3 is mainly caused by accumulation of $S_2P680Q_A^-Q_B^{2-}$ and $S_0P680Q_A^-Q_B^{2-}$ redox states (panel A of Fig. 4). The two redox states of Q_B -reducing PSII, together with the $S_2P680Q_A^-N$ redox state of the Q_B -nonreducing PSII (panel A of Fig. 4; see the Appendix) mainly contribute to the F_M -level. The redox state of the Q_B -nonreducing PSII also contributes to formation of the two steps in the FLR curve but because the time course of this PSII model form is the same in all models

explored in this section, this model form is not responsible for changes in the shape of simulated FLR using Model 7 (curve b in Fig. 3).

Model 6: Model 5 considered only the reactions which follow in logical order. But this logical order causes that some model forms resulted from all possible combinations of redox states of the S_i -states, P680, Q_A and Q_B are not formed at all in the reaction scheme: there are 48 possible model forms (redox states) of (Q_B -reducing) PSII when the Q_B^{2-}/PQ exchange is described by the second order reaction but there are only 24 model forms (redox states) of (Q_B -reducing) PSII in Scheme 4. A rationale why to consider all reactions and all possible model forms is that even if initial amounts of the “additional” model forms is zero, these model forms could be non-zero during the FLR thanks to reversibility of considered reactions, that is, there could be an additional accumulation of Q_A^- (i.e., additional fluorescence) caused by accumulation of these “additional” model forms. Therefore, it was further explored if consideration of all reactions leading to formation of all possible model forms will affect simulation of the FLR. A scheme of Model 6 is not presented because it is not possible to make a transparent and simple scheme. However, it was found that the model with OEC bound to PSII where all possible reactions are considered and where the Q_B^{2-}/PQ exchange is described by the second order reaction gives absolutely the same FLR (i.e., the difference between both FLRs is zero for whole interval of the simulation) as the model where only the reactions which follow in logical order are considered (curve a in Fig. 3; Model 5). It means that the reversibility of reactions mentioned above does not lead to any additional accumulation of Q_A^- and therefore it is enough to consider only the reactions which follow in logical order (Scheme 4 in the Appendix) as described by Model 5.

Model 7: The same way as in Models 1 and 2, Models 5 and 6 assumed that the Q_B^{2-}/PQ exchange was described by the second order reaction (see Scheme 4 in the Appendix). In analogy with previous text (Models 3 and 4), an effect of consideration of two subsequent

reactions for description of the Q_B^{2-}/PQ exchange on the FLR was further studied. Model 7, with OEC bound to PSII where only the reactions which follow in logical order are considered and where the Q_B^{2-}/PQ exchange is described by two subsequent reactions, is shown in Scheme 5 (c.f. Scheme 4) in the Appendix. A FLR simulated on the basis of Model 7 is presented as curve b in Fig. 3. This FLR is characterized by a shallow step at 2 – 3 ms followed by a higher and more pronounced step (when compared with curve a in Fig. 3) at about 15 ms and by reaching the F_M -level at about 100 ms. Positions of the steps and shape of the simulated FLR agree rather well with experimental data (Fig. 1). Comparison of curves a and b in Fig. 3 indicates that description of the Q_B^{2-}/PQ exchange by two subsequent reactions (curve b) leads to not so pronounced first step (at 2 – 3 ms) in the FLR, to much higher and more pronounced second step (at 15 ms) in the FLR, and to reaching the F_M -level at longer time, when compared with the case where the Q_B^{2-}/PQ exchange was described by the second order reaction (curve a). Panel B of Fig. 4 shows time courses of the model forms (redox states) with reduced Q_A , which mostly accumulate during the FLR shown as curve b in Fig. 3. Comparison of the time courses in panels A and B of Fig. 4 shows that changes in time courses of $S_2P680Q_A^-Q_B^{2-}$ and $S_0P680Q_A^-Q_B^{2-}$ model forms (redox states) are the reasons for different shapes of the FLRs presented as curves a and b in Fig. 3.

Curve b in Fig. 3 demonstrates that description of the Q_B^{2-}/PQ exchange by two subsequent reactions, instead of a second order reaction (curve a in Fig. 3), results in a better agreement between the simulated and experimental FLRs. But the difference in the description of the Q_B^{2-}/PQ exchange reactions themselves is not the reason why two steps appear between the F_0 - and F_M -levels in the simulated FLR because a change in the considered description of the Q_B^{2-}/PQ exchange did not lead to an appearance of a new step in simulated FLR in the case when OEC was assumed “virtually” separated from PSII (c.f. curve a and curve c in Fig. 2). Therefore, as mentioned above, it is the assumption of OEC being

bound to PSII which causes an appearance of two steps between the F_0 - and F_M -levels in the simulated FLR (curves a and b in Fig. 3), and description of the Q_B^{2-}/PQ exchange by two subsequent reactions only makes positions and heights of the two steps more realistic.

Model 8: A last model to be described and discussed is a PSII model where OEC is bound to PSII, all possible reactions are considered and where the Q_B^{2-}/PQ exchange is described by two subsequent reactions. Because of the same reasons as mentioned for Model 6, a scheme of this model is not shown. Similarly as for Models 5 and 6, also Model 8 gives absolutely the same FLR curve as curve b of Fig. 3 based on the PSII model where only the reactions which follow in logical order were considered (Model 7). Therefore, same as in the case of Models 5 and 6, also the simulation based on Model 8 indicates that it is enough to consider only the reactions which follow in logical order (Scheme 5 in the Appendix) as described by Model 7.

Discussion

The above results show that it is very important to care about approaches applied in formulation of PSII models to be further used for simulations of the chlorophyll *a* FLR. A simple visual comparison of model schemes (Schemes 2 – 5 in the Appendix) reveals that even if always the same electron carriers and same types of reactions occurring among them were considered, but treated by different approaches in formulation of particular models, it can lead to reaction schemes of different complexity. It was demonstrated that usage of these models leads to very different simulated FLRs. It is important to stress that the initial conditions and values of the rate constants (except of one; see the Appendix) were always the same in all the simulations presented above. Therefore an ability of some of the models to simulate the O-J-I-P FLR more closely than the other models is a property of the models *per se* (i.e., property of “structure” of the models) and not a property of the values of the model parameters (rate constants and initial conditions).

Except of Models 2 and 4, all other models were able to simulate a FLR. Unusual shapes of the fluorescence transients simulated on the basis of Models 2 and 4 are caused by the fact that S_0 - S_1 -transitions are not realized in these models, that is, the S-states of OEC do not proceed in a circle. However, the unclosed circle of the S-states is primarily caused by an assumption made with respect to number of electron acceptors in the model and not by consideration of only those reactions which follow in logical order, which is a secondary reason. In other words, the models assume only Q_A and Q_B electron acceptors which enable storage of three electrons only accompanied by formation of $P680^+$ three times. Hence, to reduce $P680^+$ by OEC, only three S-state transitions can be realized and not four S-state transitions to form a circle. Therefore, even if Models 2 and 4 do not simulate a standard FLR, they are useful here to demonstrate how assumptions made prior to formulation of model reactions might influence the results.

No differences between the simulated FLRs based on Models 5 and 6 and on Models 7 and 8 demonstrate that a consideration of some reactions in a model does not lead to a better simulation of the FLR. The reason is that accumulations of some model forms are so small (if any) that overall fluorescence signal is unchanged. However, it is not possible to generally determine which reaction might be safely neglected because accumulation or not of some model forms depends on assumed initial conditions. In our simulations we considered the simplest initial conditions (Q_A and Q_B initially oxidized, P680 initially reduced and all OEC in the S_1 -state initially), however, also different initial conditions could occur. Therefore, in dependence on assumed initial conditions, some model forms can accumulate more or less during the course of the FLR.

From all eight explored PSII models (Models 1 – 8), the PSII model where OEC was assumed bound to PSII and the Q_B^{2-}/PQ exchange being described by two subsequent reactions and where either all or only “logical” reactions were considered (Models 8 and 7,

respectively) gave simulated FLR (curve b in Fig. 3) which is very close to experimental FLR (Fig. 1). As there was no effect of the consideration of all reactions (when compared with the consideration of only the “logical” reactions) on shape of the FLR curve, the PSII model where only the reactions which follow in logical order were considered (Model 7; Scheme 5), seems to be the best choice for simulations of the FLR from all PSII models explored here. But this conclusion is not based only on qualitative agreement between the experimental and theoretical FLRs but also because of the approaches used in formulation of this model: from current knowledge of structure and function of PSII (see Wydrzynski and Satoh, 2005) it is clear that OEC is really bound to PSII and that reactions of the Q_B^{2-}/PQ exchange really occur in two subsequent steps.

However, the qualitative agreement of the simulations based on Model 7 with the experimental FLR does not mean that Model 7 is really the correct description of electron transport reactions occurring in PSII: Model 7 is also a simplified description of PSII electron transport reactions (e.g., tyrosine Z between the OEC and P680 and pheophytin between P680 and Q_A were not considered in any model explored here). The agreement between the simulation (curve b in Fig. 3) and the experiment (Fig. 1) also does not mean that Model 7 is really the correct description of the O-J-I-P FLR because, e.g., also other electron transport reactions (beyond the PQ pool; Schansker et al., 2003, 2005) and mechanisms (e.g. changes in transmembrane electric potential; Pospíšil and Dau, 2002) occurring in the thylakoid membrane are known to affect the shape of the FLR. In other words, also Model 7 is a simplification and it was formulated on the basis of restraint assumptions.

The main goal of this work is not to say which from all here explored PSII models is correct and which is not but to clearly demonstrate that different approaches applied to describe the “same things” can lead to different results. But if Model 7 is considered as the best PSII model, as for applied approaches, then a short review of PSII models recently used

for simulations of the FLRs can be made (it is indicated in the brackets if the applied approach is “justified” or “unjustified” with respect to the results of this work). The PSII model used in Belyaeva et al. (2005) described the Q_B^{2-}/PQ exchange by the two subsequent reactions (“justified”) but their model did not consider the S_i -states of OEC at all (“very unjustified”). Vredenberg et al. (2001) consider OEC to be bound to PSII (“justified”) but the exchange of Q_B^{2-} with PQ molecule from the PQ pool was not considered at all (“very unjustified”) in their model. Stirbet et al. (1998) considered OEC bound to PSII (“justified”) but they described the Q_B^{2-}/PQ exchange by the second order kinetics (“unjustified”) in their model. Lázár (2003) and Zhu et al. (2005) described the Q_B^{2-}/PQ exchange by the second order kinetics (“unjustified”) and considered OEC as “virtually” separated from PSII (“unjustified”) in their models. Further, Zhu et al. (2005) considered that energy transporters of PSII form separated model forms and electron carriers at the PSII acceptor side form model forms consisting of two adjacent electron carriers (e.g., P680Pheo is one model form and $Q_A Q_B$ is another model form; Pheo means pheophytin). This approach leads to second order kinetics which are not justified for energy/electron transport in PSII. Although this approach was not studied in this work, Stirbet and Strasser (1996) already showed that consideration of the second order kinetics for electron transport in PSII gives different simulated FLR when compared to the FLR simulated on the basis of correct approach (i.e., using first order kinetics).

It implies from the above that there are a lot of differences in approaches applied in formulation of PSII models to be subsequently used for simulations of FLRs. As demonstrated in this work, application of different approaches to describe the same electron transport reactions occurring even only in PSII can lead to very different results. Therefore, this work can help the readers to better understand models (and their possible weaknesses) published elsewhere in the literature. The existence of the differences in approaches applied in formulation of PSII models also raises a question whether formulations of more complex

models where reactions occurring beyond the PQ pool are considered (Lebedeva et al., 2002; Kroon and Thoms, 2006; Laisk et al., 2006) is not premature in the case of simulations of the FLRs. Even if electron transport reactions occurring beyond the PQ pool were experimentally shown to affect shape of the O-J-I-P FLR, namely the I step (Schansker et al., 2003, 2005), incorporation of these reactions into complex models should be done with care with respect to applied approaches to describe these reactions, in analogy with PSII reactions, as demonstrated in this work. When the electron transport reactions occurring beyond the PQ pool are considered in a complex model, a kind of control analysis, as applied for simulations of fluorescence signals e.g. in Lazár et al. (2005a, 2005b), should be made to clearly determine to what extent particular reactions affect particular features of the FLR (i.e. the high of the I step).

Conclusions

Using simple PSII models, always with the same electron carriers, and same type of reactions, it was shown that different approaches applied in formulations of the models lead to different chlorophyll *a* fluorescence transients simulated on the bases of these models: the different models simulated FLRs with either one or two steps between F_0 and F_M as well as completely unusual shapes of fluorescence transients were obtained. These results demonstrate that approaches themselves applied in formulation of PSII model can significantly affect the shape of resulted fluorescence transient. Therefore, a care must be taken to approaches applied in formulation of any model used for simulation of the chlorophyll fluorescence transient and generally of any photosynthetic quantities. To avoid formulation of a “wrong” model, a combination of theoretical modeling with suitable experiments (e.g. treatments of samples by suitable chemicals) is suggested.

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Appendix

Reactions, values of rate constants and initial conditions considered in all formulated models

As it is well known that RCIIIs that cannot reduce Q_B , the so called Q_B -nonreducing RCIIIs, are present in a photosynthetic sample (e.g. Lavergne and Briantais, 1996) and because it was shown that consideration of the Q_B -nonreducing RCIIIs is necessary for simulation of a good shape of the O-J-I-P FLR (e.g. Strasser and Stirbet, 1998), this basic PSII heterogeneity (Q_B -reducing/ Q_B -nonreducing) was also considered here in all formulated models. Only reactions i, ii, and iii (see below) were assumed to occur in the Q_B -nonreducing RCIIIs in all formulated models. Following reactions were considered for the Q_B -reducing RCIIIs in all formulated models:

- i) Light induced charge separation between P680 and Q_A leading to $P680^+$ and Q_A^- (rate constant k_L). The rate of charge separation is limited by amount of excitations coming to RCII which was for the intensity of excitation light used for measurement of the O-J-I-P FLR and usual concentration of chlorophylls in a sample calculated to be ~ 4000 excitation per RCII per second (e.g. Lazár, 2003). Therefore $k_L = 4000 \text{ s}^{-1}$.
- ii) Charge recombination between $P680^+$ and Q_A^- with rate constant $k_{\text{rec}} = 10000 \text{ s}^{-1}$ (e.g. Lazár, 2003).
- iii) Electron donation from S_i -state ($i = 0, 1, 2, 3$) of OEC to $P680^+$ leading to formation of S_{i+1} -state and P680 with rate constants $k_{0,1} = 20000 \text{ s}^{-1}$ (for S_0 - S_1 transition), $k_{1,2} = 10000 \text{ s}^{-1}$ (for S_1 - S_2 transition), $k_{2,3} = 3330 \text{ s}^{-1}$ (for S_2 - S_3 transition), and $k_{3,0} = 1000 \text{ s}^{-1}$ (for S_3 - S_0 transition) (e.g. Lazár, 2003).
- iv) Electron transport from Q_A^- to oxidized or singly reduced Q_B with rate constants $k_{AB1} = 3500 \text{ s}^{-1}$ and $k_{AB2} = 1750 \text{ s}^{-1}$, respectively (e.g. Lazár 2003). Both these reactions were

considered reversible with rate constants $k_{BA1} = 175 \text{ s}^{-1}$ and $k_{BA2} = 35 \text{ s}^{-1}$, respectively (e.g. Lazár, 2003).

v) Exchange of doubly reduced (its protonation is not considered explicitly but it is assumed implicitly) Q_B with oxidized PQ molecule from the PQ pool. The exchange was described by either reversible second order reactions or two subsequent reversible reactions as described in the section Defining of explored problems and applied approaches. In the case of description of the exchange by one second order reaction, the forward and backward rate constants were $k_{\text{exch}}^f = 250 \text{ s}^{-1}$ and $k_{\text{exch}}^b = 250 \text{ s}^{-1}$, respectively (e.g. Lazár, 2003). In the case of description of the exchange by two subsequent reactions (unbinding of Q_B^{2-} followed by binding of PQ), the forward and backward rate constants were $k_{\text{unbind}}^f = k_{\text{bind}}^f = 500 \text{ s}^{-1}$ and $k_{\text{unbind}}^b = k_{\text{bind}}^b = 500 \text{ s}^{-1}$, respectively. The two-times increase in the values of the rate constants in the later case is because it is assumed that to keep the same overall time necessary for whole exchange in both approaches, the two subsequent reactions must each of them proceed with a twice rate when compared to the case when the exchange is described only by one second order reaction. However, it was also checked that when the k_{unbind} and k_{bind} equal 250 s^{-1} , the second step of FLR appearing at about 10 ms (curve c in Fig. 2 and curve b in Fig. 3) is only a little bit higher but shapes of the FLRs are qualitatively retained (data not shown).

vi) Reversible reoxidation of reduced PQ molecules from the PQ pool with time constant $k_{\text{ox}} = 10 \text{ s}^{-1}$ and $k_{\text{red}} = 10 \text{ s}^{-1}$ for forward and backward reactions, respectively (e.g. Lazár, 2003).

The initial conditions were in all considered models and simulations always the same. That is, 25% of the Q_B -nonreducing and 75% of the Q_B -reducing RCII was assumed, OEC being all in the S_1 -state, Q_A and O_B fully oxidized and no empty Q_B -pocket initially, P680 fully reduced, and a half of PQ molecules reduced and a half of them oxidized. The 75% of the Q_B -reducing RCII were served by 5 PQ molecules. For more details, see the descriptions of particular schemes.

The Q_A and Q_B were assumed initially fully oxidized and all OEC was assumed initially in only S_1 -state because of simplicity: if Q_A or Q_B or both were initially reduced to some extent as well as if OEC was partially also in another S-state than in the S_1 -state, it would lead to much more complicated models because there would be more initial states from which all reactions in the models would started. Therefore, not to complicate model schemes and interpretations of the results, the simplest initial conditions, as mentioned above, were assumed.

Definition of fluorescence during the O-J-I-P transient

Fluorescence signal during the FLR was calculated from time courses of model forms. A sum of all model forms with reduced Q_A , $\Sigma[Q_A^-](t)$, was used for calculation of unquenched fluorescence signal, $F_{unq}(t)$, with respect to assumed energetic connectivity between PSII (Joliot and Joliot, 1964) as

$$F_{unq}(t) = ((1 - p) \times \Sigma[Q_A^-](t)) / (1 - p \times \Sigma[Q_A^-](t)),$$

where p ($= 0.55$) is the connectivity parameter. From the unquenched fluorescence, fluorescence quenched by oxidized PQ molecules (Vernotte et al., 1979), $F_q(t)$, was calculated as

$$F_q(t) = F_{unq}(t) / (1 + ((a + (\Sigma[Q_A^-](t) \times b)) \times [PQ](t))),$$

where $[PQ](t)$ is amount of oxidized PQ molecules and the constants a ($= 1/45$) and b ($= 4/63$) were calculated (assuming 10% and 30% quenching of F_0 and F_M , respectively, and 5 PQ molecules) as described in details in Stirbet et al. (1998). Time courses of the quenched fluorescence signal ($F_q(t)$) are presented in Figs. 2 and 3. However, we note that existence of the fluorescence quenching by oxidized PQ molecules in leaves was questioned (Tóth et al., 2005), though it can be observed in thylakoid preparations of different integrity (Kurreck et al., 2000).

FLR presented in the inset of Fig. 2 and simulated on the basis of the PSII model of Lazár (2003) was simulated in the same way as any FLR coming from heterogeneous PSII as described in that work (for more details, see Lazár, 2003). The initial conditions for simulation of this FLR were the same as mentioned above.

Schemes of the models

Particular models used for the simulations of the chlorophyll fluorescence transients and some model forms as shown in Figs. 2 – 4, are presented below in Schemes 2 – 5.

Scheme and figure legends

Scheme 1 A sketch summarizing approaches used in particular models.

Scheme 2 Scheme of Models 1 and 2. Parts A and B describe reactions occurring in the Q_B -reducing and Q_B -nonreducing RCIIIs, respectively. P, A, B, N, S_i , PQ, PQH₂ mean P680, Q_A , Q_B , the Q_B -nonreducing RCIIIs, the S_i -states of OEC, oxidized and reduced PQ molecules, respectively. The models assume OEC “virtually” separated from PSII and the Q_B^{2-}/PQ exchange described by the second order reaction. All possible reactions are considered and shown as for electron transport between P680, Q_A and Q_B . The “i” subscript in the S_i -states in both parts of the scheme equals 0, 1, 2, 3 when all possible S_i - S_{i+1} -transitions are considered (Model 1) and “i” equals only 1 when only those S_i - S_{i+1} -transitions are considered which follow in logical order (Model 2). Model forms in dashed rectangles are the model forms of PSIIIs with non-zero initial amounts ($[PAB]_0 = 0.75$, $[PAN]_0 = 0.25$). Further initial conditions are: $[S_1]_0 = 1$, $[PQ]_0 = 2.5$ and $[PQH_2]_0 = 2.5$. Meaning and values of used rate constants are described above in the Appendix.

Scheme 3 Scheme of Models 3 and 4. Parts A and B describe reactions occurring in the Q_B -reducing and Q_B -nonreducing RCIIIs, respectively. P, A, B, E, N, S_i , PQ, PQH₂ mean P680, Q_A , Q_B , empty Q_B -pocket of the Q_B -reducing RCIIIs, the Q_B -nonreducing RCIIIs, the S_i -states

of OEC, oxidized and reduced PQ molecules, respectively. The models assume OEC “virtually” separated from PSII and the Q_B^{2-}/PQ exchange described by two subsequent reactions. All possible reactions are considered and shown as for electron transport between P680, Q_A and Q_B . The “i” subscript in the S_i -states in both parts of the scheme equals 0, 1, 2, 3 when all possible S_i - S_{i+1} -transitions are considered (Model 3) and “i” equals only 1 when only those S_i - S_{i+1} -transitions are considered which follow in logical order (Model 4). Model forms in dashed rectangles are the model forms of PSII with non-zero initial amounts ($[PAB]_0 = 0.75$, $[PAN]_0 = 0.25$). Further initial conditions are: $[S_1]_0 = 1$, $[PQ]_0 = 2.5$ and $[PQH_2]_0 = 2.5$. Meaning and values of used rate constants are described above in the Appendix.

Scheme 4 Scheme of Model 5. Parts A and B describe reactions occurring in the Q_B -reducing and Q_B -nonreducing RCII, respectively. P, A, B, N, S_i , PQ, PQH_2 mean P680, Q_A , Q_B , the Q_B -nonreducing RCII, the S_i -states of OEC, oxidized and reduced PQ molecules, respectively. The model assumes OEC bound to PSII, the Q_B^{2-}/PQ exchange described by the second order reaction and the model considers only those reactions which follow in logical order with respect to the initial model forms (redox states) which are in dashed rectangles. The initial conditions are: $[S_1PAB]_0 = 0.75$, $[S_1PAN]_0 = 0.25$, $[PQ]_0 = 2.5$ and $[PQH_2]_0 = 2.5$. Meaning and values of used rate constants are described above in the Appendix.

Scheme 5 Scheme of Model 7. Parts A and B describe reactions occurring in the Q_B -reducing and Q_B -nonreducing RCII, respectively. P, A, B, E, N, S_i , PQ, PQH_2 mean P680, Q_A , Q_B , empty Q_B -pocket of the Q_B -reducing RCII, the Q_B -nonreducing RCII, the S_i -states of OEC, oxidized and reduced PQ molecules, respectively. The model assumes OEC bound to PSII, the Q_B^{2-}/PQ exchange described by two subsequent reactions and the model considers only those reactions which follow in logical order with respect to the initial model forms (redox states) which are in dashed rectangles. The initial conditions are: $[S_1PAB]_0 = 0.75$, $[S_1PAN]_0$

= 0.25, $[PQ]_0 = 2.5$ and $[PQH_2]_0 = 2.5$. Meaning and values of used rate constants are described above in the Appendix.

Figure 1 Chlorophyll *a* fluorescence rise (the O-J-I-P transient) measured with control dark-adapted pea leaf using PEA fluorometer upon $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of red light. Particular steps of the transient are denoted and the minimal fluorescence (O level) was normalized to 1. The transient is shown on a logarithmic time-axis.

Figure 2 Fluorescence transients simulated on the basis of models with OEC “virtually” separated from PSII (Models 1 – 4) and where: all possible reactions (S_i - S_{i+1} -transitions) are considered and the Q_B^{2-}/PQ exchange is described by the second order reaction (curve a; Model 1), only “logical” S_i - S_{i+1} -transitions are considered and the Q_B^{2-}/PQ exchange is described by the second order reaction (curve b; Model 2), all possible reactions (S_i - S_{i+1} -transitions) are considered and the Q_B^{2-}/PQ exchange is described by two subsequent reactions (curve c; Model 3), only “logical” S_i - S_{i+1} -transitions are considered and the Q_B^{2-}/PQ exchange is described by two subsequent reactions (curve d; Model 4). The inset shows fluorescence transients simulated on the basis of the PSII model of Lazár (2003). All the transients are shown on a logarithmic time-axis.

Figure 3 Fluorescence transients simulated on the basis of models with OEC bound to PSII (Models 5 - 8 in the text) where only reactions which follow in logical order are considered and where: the Q_B^{2-}/PQ exchange is described by the second order reaction (curve a; Model 5) and the Q_B^{2-}/PQ exchange is described by two subsequent reactions (curve b; Model 7). All the transients are shown on a logarithmic time-axis.

Figure 4 Time courses of model forms (redox states) with reduced Q_A , which mostly accumulate during the fluorescence transients shown in Fig. 3 as curve a (panel A; Model 5) and as curve b (panel B; Model 7). All time courses are shown on a logarithmic time-axis.











