Closing report on the OTKA project (K-101433)
"Development of virtual Photosystem II and photosynthetic electron transport models, and their application for *in silico* investigations of photosynthetic processes"

Background

Photosynthesis is a basic process on Earth, which directly or indirectly provides energy to almost all life forms in our biosphere. The primary steps of light energy conversion take place in pigment-protein complexes, which are embedded in the so called thylakoid membrane. The electrons, which are utilized in the photosynthetic process are extracted by light-induced oxidation of water in the Photosystem II (PSII) complex. Whereas, the light reaction in Photosystem I (PSI) raises the redox potential of the electrons to sufficiently negative level to produce NADPH, which is used in CO_2 fixation and other cell processes. Electron transport between the two photosystems is mediated by the cytochrome b_6f ($cytb_6f$) complex, which receives electrons from PSII via PQ molecules, which diffuse in the in the lipid phase of the membrane, and transfer them towards PSI via plastocyanin (PC) in the thylakoid lumen (Fig. 1).

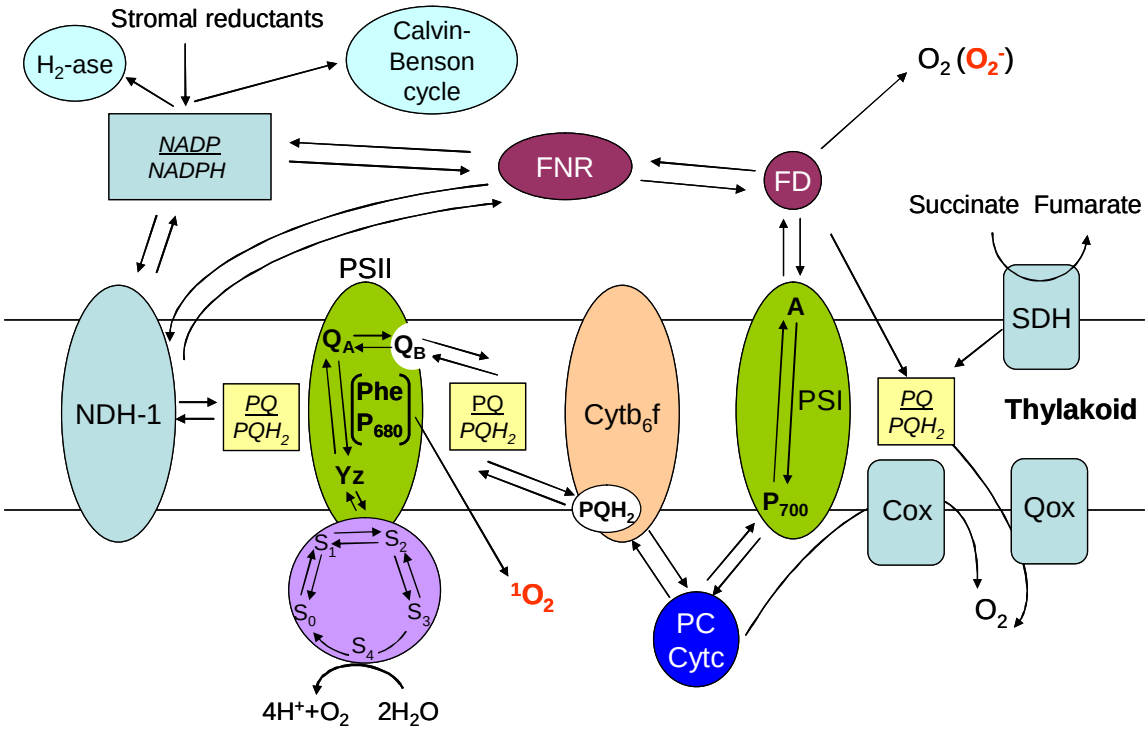

Figure 1. *The electron transport network of the thylakoid memebrane*

In cyanobacteria, as well as in higher plants, the thylakoid membranes contain also complexes of the respiratory electron transfer chain (Fig.1). Among those the most important is the NADPH-dehydrogenase (NDH1) complex, which feeds electrons into the PQ pool via oxidizing NADPH produced by respiratory and metabolic processes. Besides the linear electron transport pathway, that goes from H_2O to NADPH there exists a so called cyclic pathway around PSI. During cyclic electron flow the electrons from the

acceptor side of PSI are transferred back to the PQ pool, and then to the PSI donor side via $cytb_6f$ and PC. The first part of the cycle, i.e. from PSI to the PQ pool is not fully clarified, but can take place via the ferredoxin-NDPH oxido-reductase (FNR) and NDH1, or directly via ferredoxin (Fd) to the PQ pool. Oxidation of reduced PQ (PQH_2) can take place not only via the linear $Cytb_6f$ – PC – PSI – NADPH pathway, but also via various oxidases. Functioning of different elements of this complex electron transport network is reasonably well known. However, the connection of these elements, especially the interaction of the photosynthetic and respiratory electron transport is not yet clarified in details. In addition, functioning of the PSII electron transport also needs further clarification, especially regarding the process of water oxidation and the interpretation of the transition parameters of the S-state turnovers.

For the detailed description of PSII and of the whole photosynthetic electron transport network very efficient solution could be provided by the up to date methods of bioinformatics, and especially the network level approach. This makes possible the modeling of the interconnected photosynthetic electron transport network, as well as the functioning of its certain components, especially of PSII. During development of such a model system first the optimization of the model parameters can be done using the available literature and own experimental data. Then the model can work as a virtual photosynthetic system, which can be used to perform virtual experiments that yield new information about the system. The great advantage of the virtual photosynthetic system is that it makes possible testing large number of various experimental conditions, as well as following redox components, whose direct measurement is difficult. The important predictions of virtual experiments can then be checked by targeted measurements.

Aims

The primary aim of the project was the construction of mathematical models of PSII electron transport and of the whole photosynthetic electron transport network, as well as application of the models for the interpretation of already existing experimental results, performing virtual experiments and validation of their predictions by targeted real measurements:

1. The aim of PSII modeling is the description of the kinetics of all redox components after excitation with either flash, or continuous illumination considering the forward as well as backward processes.
2. The main aim of modeling the photosynthetic electron network is the description of the kinetics of linear, cyclic and alternative electron transfer pathways after excitation with either flash, or continuous illumination. Special emphasis is devoted to the investigation of electron transfer routes factors, which affect the PQ pool and the Q_A redox state.
3, The main targets of the application of the model was the interpretation of experimental data in the following specific areas:

- Functioning of the water oxidizing complex with special emphasis on the on the interpretation of the transition parameters of the Joliot-Kok model.
- The role of linear and cyclic electron transport pathways in the recently described wave phenomenon of the Q_A reduction state under conditions of flash excitation.
- Further verification of the charge recombination model of the photoinhibition of PSII, with special emphasis on the role of light intensity and PQ reduction state in singlet oxygen production.

Results

Due to the large number of the involved components we have developed various models of different complexity. The models are based on a set of coupled linear differential equations, which are solved by using a Matlab based software.

PSII functioning

One of our aims was the in depth understanding of the functioning of electron transport in the PSII complex. The catalytic site of water oxidation in PSII consists of 4 Mn and 1 Ca ions, which accumulates 4 positive charges during 4 subsequent light reactions in its so called S oxidation states (S_0, S_1, S_2, S_3, S_4). The cyclic conversion of the S-states results in the extraction of 4 electrons and 4 protons from 2 H_2O molecules, and leads to release of an O_2 molecule. When short, saturating light pulses are applied the amount of the produced oxygen undergoes a period four oscillation. Explanation of this phenomenon requires the assumption that in a certain fraction (10-15 %) of the PSII centers light absorption does not yield advancement of the S-states, instead a so called miss event (miss: $S_i \rightarrow S_i$) occurs. The interpretation of this phenomenon is not yet clear, and the appearance of miss events can be due to either backward electron flow between the flashes, or other phenomena may also take place. In order to deal with this problem we constructed a model that contains all possible redox states of 10 PSII electron transport components (Q_B, Q_A, Phe, P680, Y_Z as well as the S0,…,S4 states). Our data obtained for the S-state cycle show that the so called "miss" phenomenon can be fully explained by charge recombination reactions between oxidized donors and reduced acceptors, which are formed during the light induced charge separation and stabilization. The model can also explain the "uneven miss" phenomenon, i.e. the dependence of the "miss" factors on the S-state transitions [1].

Another version of the model was developed for the investigation of the functioning of the Q_A-Q_B two-electron gate. This model includes PQ binding to the Q_B site and its 2-step reduction followed by the release of PQH_2, as well as the possibility of applying competitive inhibitors of the Q_B sites, such as DMCU. This model also has all other components of PSII with the exception of P680 and Phe, i.e. the electron from Y_Z goes directly to Q_A. By using a curve fitting procedure for the analysis of the Q_A^- relaxation kinetics, measured by flash-induced variable Chl fluorescence yield, it is possible to determine the rate of forward and backward electron transport between Q_A and Q_B, as well as the binding rates of PQ and its inhibitor homologues to the Q_B site [1].

Interaction of stromal and thylakoid bound electron transport components

In order to describe the highly important interaction electron transport components located in the thylakoid membrane and in the stroma compartment we developed a model that contains all electron transport components shown in Fig. 1. using a simplified inner PSII model (charge separation between Y_Z and Q_A). The model provides an excellent tool to simulate electron transport processes under a wide range of conditions and can be used to perform *in silico* experiments, whose predictions can be verified by measuring the abundance and kinetics of various electron transport components. We have recently observed that relaxation of flash-induced fluorescence can

exhibit a wave phenomenon in cyanobacteria. We have used our *in silico* PSII model to interpret this phenomenon in parallel with measurement of fluorescence relaxation in the presence of various electron transport inhibitors. In *Synechocystis* PCC 6803 fluorescence yield decays in a monotonous fashion under aerobic conditions. However, under microaerobic conditions the decay exhibits a wave feature showing a dip at 30-50 ms after the flash followed by a transient rise, reaching maximum at ~1 s, before decaying back to the initial level. The wave phenomenon can also be observed under aerobic conditions in cells preilluminated with continuous light. Illumination preconditions cells for the wave phenomenon transiently: for few seconds in *Synechocystis* PCC 6803, but up to one hour in *Thermosynechocystis elongatus* BP-1. The wave is eliminated by inhibition of PQ(H_2) binding either to the Q_B site of Photosystem-II or the Q_o site of cytochrome b_6f complex by DCMU or DBMIB, respectively. The wave is also absent in mutants, which lack PSI or the NDH-1 complex. It is concluded that the unusual wave feature of fluorescence relaxation reflects transient oxidation of the PQ pool by PSI followed by its re-reduction from stromal components via the NDH-1 complex, which is transmitted back to the fluorescence yield modulator Q_A^- via charge equilibria [2].

Photodamage of the PSII complex

Light is not only the basic driving force of photosynthesis, but also an important damaging factor of the photosynthetic apparatus. According to our previously developed mechanistic model charge recombination processes play a highly important role in photodamage both as the precursors of ^{3}P680 that sensitizes production of highly damaging singlet oxygen ($^1O_2$), and also as a safety mechanism that compete with ^{3}P680 formation. By using the computer model of the inner electron transport processes of PSII, shown in Fig. 2, we simulated the light intensity dependence of $^1O_2$, as well as the photo-protective effect of enhanced non-radiative recombination processes. The in silico studies were supplemented with direct determination of singlet oxygen production in site directed mutants of the cynobacterium *Synechocystis* 6803 in which the Gln residue at the 130th position of the D1 reaction center subunit was changed to either Glu or Leu, which affect the efficiency of nonradiative charge recombination from the primary radical pair. We found that the D1-Gln130Glu mutant showed decreased $^1O_2$ production concomitant with decreased rate of photodamage relative to the WT, whereas both $^1O_2$ production and photodamage were enhanced in the D1-Gln130Leu mutant. The data support the key role of ^{3}P680 mediated $^1O_2$ production in PSII photodamage, and the photoprotective role of nonradiative charge recombination pathways [3,4].

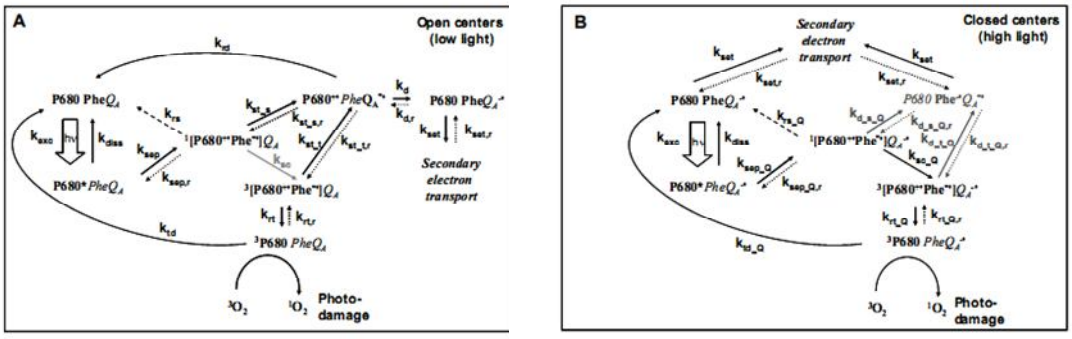

Fig. 2. Electron transport processes in the inner core of the PSII complex.

The role of electron transport components in photodamage was also studied in under inhibition of the Calvin-Benson cycle, which leads to the accumulation of reduced PQ under illumination. We have shown that Calvin-Benson cycle inhibition by KCN and glycolaldehyde induces enhanced photodamage in the coral symbiotic dinoflagellate algae *Symbiodinium* [5].

The role of the PSII electron transport component cytb559 in photoinhibition was studied in a *Chlamydomonas reinhardtii* mutant in which the His ligand to the haem, provided by the alpha subunit, has been replaced by a Cys residue (mutant PsbE-H23C). The mutant was unable to grow photoautotrophically but could assemble oxygen-evolving PSII supercomplexes to 15-20% of WT levels. Analysis of PSII activity in cells indicated that electron transfer is perturbed in the mutant on the acceptor side between plastoquinones Q_A and Q_B. Oxygen-evolving PSII complexes in mutant cells are more prone to high-light damage than WT with repair of PSII also impaired at the level of D1 synthesis. Importantly we provided evidence that Cyt *b*-559 also protects PSII during the critical stage of assembling the Mn_4CaO_5 cluster [6].

Importance of the project:

Although detailed models of PSII and photosynthetic electron transport would be highly useful for the interpretation of existing experimental results and for performing virtual experiments such models were not available at the start of our work. We have successfully developed computer assisted models of Photosystem II as well as thylakoid-bound and stroma located electron transport components. These models make possible to perform virtual experiments and thus testing a high variety of experimental conditions, which are often difficult to realize in real life. In addition, virtual experiments decrease drastically the time, which is needed for lengthy organism growing (plants, algae, cyanobacteria), as well as for sample preparation. Thus the total duration of real life experiments, which can last for days can be shortened to few minutes or even seconds.

Future plans:

Our research has already obtained further support in the framework of an international collaborative OTKA grant (NN-110960), which started in March 2014. Within this new project we will merge the partial models, which were developed in the here reported project, into one unified model, which will be used to study alternative electron transport pathways of cyanobacteria. We will also launch a publicly accessible website, which makes possible to run the model for by other scientists as well. This work has already been performed mostly in the current project, but the implementation of the web server requires some additional work.

Publications:

[1] Imre Vass, László Sass, Zsuzsanna Deák (2014) In silico photosynthesis: Computer assisted simulation of electron transport through Photosystem II and Photosystem I. Gordon Research Conference on Photosynthesis, Mount Snow Resort, USA, August 8-15

[2] Zsuzsanna Deák, László Sass, Éva Kiss and Imre Vass (2014) Characterization of wave phenomena in the relaxation of flash-induced chlorophyll. Biochim. Biophys. Acta , 2014 DOI: 10.1016/j.bbabio.2014.01.003

[3] Ateeq Ur Rehman, Krisztián Cser, László Sass, Imre Vass (2013) Characterization of singlet oxygen production and its involvement in photodamage of Photosystem II in the cyanobacterium Synechocystis PCC 6803 by histidine-mediated chemical trapping. Biochimica et Biophysica Acta 1827 (2013) 689–698

[4] László Sass, Zsuzsanna Deák, Imre Vass (2014) In silico photosynthesis: Computer assisted simulation of electron transport processes in Photosystem II. International Meeting on Photosynthesis for Sustainability, Puschino, June 2-7

[5] Ross Hill1, Milán Szabó, Ateeq ur Rehman, Imre Vass, Peter J. Ralph and Anthony W. D. Larkum (2014) Inhibition of photosynthetic CO_2 fixation in the coral *Pocillopora damicornis* and its relationship to thermal bleaching. The Journal of Experimental Biology (2014) 217, 2150-2162

[6] Mary Hamilton, Emanuel Franco, Zsuzsanna Deák, Eberhard Schlodder, Imre Vass and Peter J. Nixon (2014) Investigating the photoprotective role of mutant with altered ligation of the haem. *Plant Cell Physiol* (2014) 55 (7): 1276-1285

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