Photoinactivation of Photosystem II at low light intensity. Mathematical models

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ABSTRACT   We studied the effect of low light intensity on Photosystem II (PSII) of thylakoid membranes isolated form spinach. The application of low frequency single turnover flashes results in the decreased oxygen evolving activity of PSII. This effect was explained in the framework of a model, which assumes that saturating visible light flashes at low frequency can result in the degradation of the D1 protein, since $S_{2,3}/Q_B^-$ and S_2/Q_A^- recombinations generate singlet oxygen through the intermediate triplet chlorophyll formation. We propose a pathway for the light-induced transitions and dark period processes of the S-states, which results the best fit of our experimental data.   **Acta Biol Szeged 46(3-4):167-169 (2002)**

KEY WORDS

low light effect
Photosystem II
acceptor-side photoinactivation
S-states

In higher plants and cyanobacteria water oxidation to oxygen molecules is an essential part of the photosynthetic electron transport. This occurs at the lumenal side of the thylakoid membrane, at a protein complex closely associated to the reaction centre of PSII. Oxidising agents (Mn ions) and cofactors (Ca^{2+} and Cl^-) are ligated to amino acids of the protein complex. Basic functioning of this complex involves the accumulation of four positive charges on the Mn ions. This is followed by the conversion of a water molecule to oxygen and protons. While protons are pumped to the lumenal side of the thylakoid membrane, electrons are transferred towards the reaction centre chlorophyll to reach the Q_A and Q_B quinones through a pheophytWn molecule. On the acceptor side Q_A is bound by the D2 protein of PSII and normally cannot be removed. In contrast, Q_B is located in a binding niche formed by the D1 protein of PSII and can uptake two electrons. In the semireduced state (Q_B^-) is strongly bound, but can be easily exchanged with plasto-quinones (form the pool) in both the oxidized (Q_B) and fully reduced (Q_B^{2-}) form.

The Kok model

According to the well-known Kok model, the water splitting complex cycles through five charge storing states – which correspond to different redox states of the four catalytic manganese ions – denoted by S_0, S_1, S_2, S_3 and S_4. In this process there are light induced transitions, namely $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_4$, and the spontaneous $S_4 \rightarrow S_0$ conversion. During this last step a molecular oxygen molecule is released. Half-times for the first two transitions are around 0.4 ms, for the third is 0.1 ms and for the last is 2-3 ms. If one exposes photosynthetic apparatus (thylakoid membranes) to continuous photon exposure or to a large number of consecutive light flashes, the distribution of S-states shows an equilibrium ($S_0 : S_1 : S_2 : S_3 = 25\% : 25\% : 25\% : 25\%$). But in dark S_2 and S_3 states relax back to the S_1 state with a half-time of 30-100 s, *i.e.* only S_0 and S_1 states

are stable in dark. Obviously, after 3-5 minutes of dark adaptation the distribution of S-states will be close to $S_0 : S_1 = 25\% : 75\%$.

It is known, that the quantum yield of photoinactivation of PSII at low light intensity may be greater than at high photon exposure (Park Y-I et al. 1995). The role of back electron flow and $S_{2,3}/Q_B^-$ recombination was also studied (Keren N et al. 1997), but the mechanism behind these processes has not been clarified yet. The studied system and the bi-cycle model of S-state transitions (see later) seem simple at first, but contain contradictions, which could not be resolved yet. The aim of the present work was to propose a physiologically possible and most probable model for explaining our experimental data.

Materials and Methods

Preparation of thylakoid membranes

Thylakoid membranes were isolated from spinach with standard methods and were stored in 0.4 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 1mM $MnCl_2$, 2 mM EDTA and 50 mM HEPES (pH 7.5) with 2-6 mg Chl/ml concentration at –80ºC. The samples were stored in room-temperature at a concentration of 30 µg Chl/ml during the treatment and at 12 µg Chl/ml for the measuring processes.

Measurements of the photosynthetic activity of PSII

Measuring the amount of evolved oxygen during the water oxidation process one can reliably calculate the photosynthetic activity of plants. As the default protocol, steady-state rates of oxygen evolution were measured at saturating light intensity on 2 ml of 12 µg Chl/ml thylakoid membranes in the addition of 0.5 mM DMBQ (2,5-dimethyl-p-benzo-quinone) as an electron acceptor, applying a Clark-electrode (Hansatech).

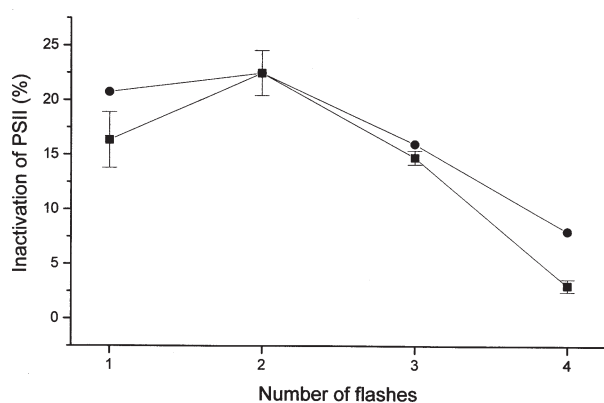*Corresponding author. E-mail: imre@nucleus.szbk.u-szeged.hu

Figure 1. The effect of repetitive light flashes on the oxygen evolving capacity of PSII. The full squares represent experimental data, while the predicted amount of recombining $S_{2,3}/Q_B^-$ pairs are shown as full circles. The two data sets are normalized to the value after the 2nd flash.

Figure 2. Model for the forward and backward transitions during cycling of the interacting S-states and Q_B quinone molecules. In this scheme of the proposed mechanism solid arrows represent forward S-state transitions, dashed arrows count for decay reactions in dark, and dotted arrows represent the equalization of the amount of Q_B and Q_B^- molecules.

Light treatment

We used a Stroboslave Xe flash lamp (General Radio, 3 µs, 0.5 J) to produce saturating single turnover flashes. Different flash protocols were used to investigate the effect of low light intensities on PSII. The samples were exposed to 80 flash packages separated by 100 s, and all of the flash-packages comprised 1-4 light pulses with the given frequency 1 Hz.

Results and Discussion

Modifying the Joliot-Kok model

Usually the matrix formalism of Lavorel can be applied for simulating the S-state transitions. One has to drop the short-lived intermediate S_4 state, and may use the formula: $S^{(i+1)} = K * S^{(i)}$, where $S^{(i)}$ describes the distribution of S-states after i flash(es) and the matrix K contains the Kok-parameters. As usual we denoted the miss, single hit and double hit parameters with α, β and γ. This is can be seen below.

$$S = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix}, K = \begin{bmatrix} \alpha & 0 & \gamma & \beta \\ \beta & \alpha & 0 & \gamma \\ \gamma & \beta & \alpha & 0 \\ 0 & \gamma & \beta & \alpha \end{bmatrix}$$

Moreover, the S-states interact with the Q_B quinone molecules, which has to be taken into account, as a consequence a slightly modified version of the Joliot-Kok model was applied. Firstly, we used the

$$S = \begin{bmatrix} S_0 Q_B & S_0 Q_B^- \\ S_1 Q_B^- & S_1 Q_B \\ S_2 Q_B & S_2 Q_B^- \\ S_3 Q_B^- & S_3 Q_B \end{bmatrix}, K = \begin{bmatrix} \alpha & 0 & \gamma & \beta \\ \beta & \alpha & 0 & \gamma \\ \gamma & \beta & \alpha & 0 \\ 0 & \gamma & \beta & \alpha \end{bmatrix}, S^{(i+1)} = K * S^{(i)}$$
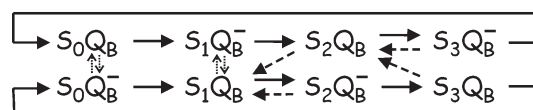
expressions for the forward reactions, which describe the bi-cycle model of S-states Q_B transitions. Secondly, in order to calculate the S-state distributions following different flash protocols an extended version of the shown model was used, which considers the decay of higher S-states during the dark period between the flash packages. For this model the initial distribution of the S_0 and S_1 states (25% and 75%, respectively), the miss (15%) and double hit (3%) parameters were obtained from flash-induced oxygen evolution measurement using a home-built flash-oxygen electrode as described earlier (Vass I et al. 1990). The room temperature decay half-lifetimes of the S_2 ($t_{S2} \approx 25$ s) and S_3 state ($t_{S3} \approx 35$ s) were calculated from the same work.

Possible mechanisms of the flash induced damage to PSII

The application of saturating single turnover flashes resulted in the damaging of oxygen evolving activity as shown in Figure 1. This effect was explained by a model assuming that saturating visible light flashes at low frequency can result in the damage of PSII and the degradation of the D1 protein, since $S_{2,3}/Q_B^-$ and S_2/Q_A^- recombinations generate – with a defined probability – $^3P_{680}$, through the primary recombining $P_{680}^+/Pheo^-$ pairs. The triplet form of chlorophyll molecules can interact with molecular oxygen resulting in the formation of the short-lived singlet oxygen, which damages the PSII reaction centre (Vass et al. 1992; Keren et al. 1995a,b). However, one assumption of this model *i.e.* only S_2/Q_B^- and S_3/Q_B^- back reactions may occur, cannot be justified with full knowledge of the facts that hardly any of the water oxidizing complexes remain in S_2 or S_3 states after dark-adaptation.

It is known from thermoluminescence measurements that in normal conditions the amount of oxidized and reduced Q_B molecules are equally distributed in dark-adapted samples and that the yield of S_3/Q_B^- recombination is 1.5-2 times higher than that of S_2/Q_B^-. The first phenomenon was taken into account as the equalization of the amount of $S_0 Q_B$ with $S_0 Q_B^-$ and $S_1 Q_B$ with $S_1 Q_B^-$ pairs after decay. In our model, the second phenomenon accounts for the 1.5 times higher probability of generating $^3P_{680}$ and hence the higher

probability of the formation of damaging singlet oxygen molecules via the S_3/Q_B^- recombination. In order to explain the experimental data we proposed a pathway for the light-induced conversion and dark decay of the S-states, as shown in Figure 2. This model made possible the best fit of the experimental data shown in Figure 1, considering the S_2/Q_B^- and S_3/Q_B^- pairs as damaging states.

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