Analysis of the Prompt Fluorescence Induction by Means of Computer Simulation of the Primary Photosynthetic Reactions

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The induction phenomenon of prompt fluorescence of the photosynthetic system in green plants reflects the adaptation of the electron transport system from a dark adapted state to a steady state under light condition. The appearance of the P-peak in the prompt fluorescence induction curve is explained by the decay of the electrochemical gradient across the thylakoid membrane in the dark. The decay of the electrochemical gradient in the dark is calculated using measured fluorescence induction curves with different times of dark adaptation. The influence of photophosphorylation inhibitors is discussed and the threshold effect of ATP-formation depending on the electrochemical gradient is explained by the coupling of the ATP-formation with the electron transport in the thylakoid membrane.

Introduction

The intensity of prompt fluorescence from photosystem II in the photosynthetic system of green plants reflects the redox state of the quencher Q, the primary electronacceptor of photosystem II [1 – 4]. The redox state of the quencher Q depends on the state of all the other pools in the electron transport chain from the first electron donor of photosystem II — the water splitting system Z — to the terminal electron acceptor of photosystem I — probably ferredoxin — which reduces $NADP^+$. This process is described by the Z-scheme by Bendall and Hill [5]. The electron transfer causes an electrochemical potential difference across the thylakoid membrane providing energy for the synthesis of ATP from ADP and inorganic phosphate [6 – 8]. ATP and reduced NADP drive the dark reaction, *i. e.*, the reduction of CO_2 to $(CH_2O)_6$ in the Calvin-Benson cycle. The induction phenomena of prompt fluorescence of green plants subjected to a sudden change from dark to light reflect the change of the redox state of the quencher Q during adaptation of the whole system from the dark adapted state to the steady state in the light. This can be described by a computer model in terms of coupled redox pools of the intermediates of the electron transport chain [9, 10]. The change of reduced quencher Q^- shows the characteristics of the fluorescence induction curve as measured *e. g.* in green algae — the Kautsky effect.

The peak P is caused by a pile up of electrons in the chain due to the initial inability to transport electrons, probably because of a lack of ATP necessary to reoxidize $NADPH_2$. As ATP is synthesized by the increasing electrochemical potential difference, $NADPH_2$ and also the other pools are reoxidized due to the increasing electron flow through the chain. Therefore, this high energy state should lead to the decrease of fluorescence intensity from peak P to steady state S (curve 0 in Fig. 5), probably by reoxidating $NADPH_2$ via ATP formation. Thus it may be possible to use the fluorescence induction curve, and especially the peak P, as an indicator of the high energy state in the photosynthetic system. In this paper the decay of the electrochemical potential in the dark is calculated from the measured height of the P-peak in the induction curve after different times of dark adaptation.

Methods and Materials

The photosynthetic sample used was a suspension of the green algae *Chlorella vulgaris* grown in aerated liquid culture illuminated by white fluorescence tubes. The culture medium had the following composition per liter (slightly modified from [24]): 10^{-2} mol KNO_3, 4.52×10^{-3} mol NaH_2PO_4, 5.3×10^{-4} mol Na_2HPO_4, 1.6×10^{-5} mol H_3BO_3, 2.5×10^{-5} mol EDTA (Titriplex III), 10^{-3} mol Mg^{2+}, 10^{-4} mol Ca^{2+}, 2.5×10^{-5} Fe^{2+}, 10^{-6} mol Zn^{2+}, 7×10^{-7} mol Mn^{2+}, 2.4×10^{-8} mol Cu^{2+}, 8.4×10^{-9} mol Co^{2+}, 10^{-3} mol SO_4^{2-}, and 10^{-4} mol Cl^-. pH was about 6. The glass tubes with the culture were flushed with air from the bottom and il-

luminated by 5 white light fluorescence tubes (40 W) in a distance of about 15 cm with a light-dark period of 14:10 hours. The prompt fluorescence is measured in the usual way exciting the sample with the light of a 100 W halogen lamp through a blue filter (BG18 or SFK6, Schott/Mainz) and recording the signal through an interference filter at 650 nm by a photomultiplier (EMI 9683) and a storage oscilloscope. The experimental results were compared with the computer model stored on the IBM/378-168 at KFA Jülich [10].

Description of the Computer Simulation of the Photophosphorylation

The Z-scheme of the electrontransport in the thylakoid membrane can be described in terms of coupled redox pools of the intermediates. Electrons are pumped by the two photosystems PSII and PSI (Fig. 1) from the first water splitting electron donor Z of PSII to the terminal electron acceptor of PSI, Ferredoxin (FD) and $NADP^+$, from where the dark reactions start. According to the chemi-osmotic theory ATP is synthesized from ADP using the energy stored in the electrochemical potential difference across the thylakoid membrane. This electrochemical potential difference across the membrane is built up by H^+-transport across the membrane coupled to electron transport f_e in the membrane [11, 12]. The water splitting process results in a release of one H^+-ion at the inside of the membrane for each electron transported to Z. The reduction of PQ at the outside of the membrane is as-
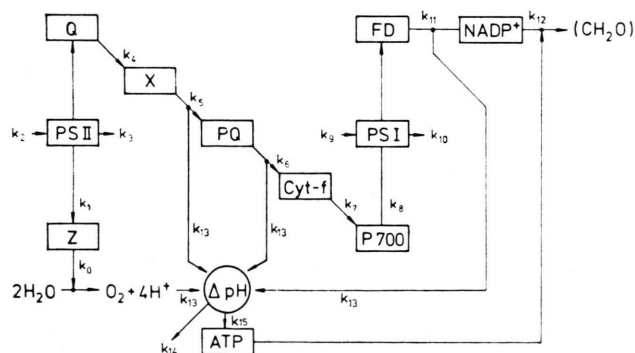

Fig. 1. Z-scheme of the photosynthetic electron transport chain from water to $NADP^+$ represented as a compartment model of electron pools. PSII and PSI, photosystem II and I; Z, water splitting system of PSII (primary donor); Q, quencher of fluorescence (primary acceptor of PSII); X, PQ (plastoquinone), and Cyt-f, intermediate pools; P700, reactioncenter chlorophyll a_{700} of PSI; FD, ferredoxin.

sociated with an uptake of one H^+-ion from the stroma for each electron transported from X to PQ. The reoxidation of PQH_2 at the inside of the membrane releases one H^+-ion into the interior of the thylakoids for each electron transported from PQ to Cyt-f. Finally, the reduction of $NADP^+$ in the stroma results in an uptake of one H^+-ion from the stroma for each electron transferred from FD to $NADP^+$. Altogether this means that two H^+-ions are taken up from the stroma outside and two H^+-ions are released to the inside of the thylakoids, *i. e.* a net transport of two H^+-ions through the membrane for each electron transferred through the whole transport chain from Z to $NADP^+$. As a consequence pH decreases inside the thylakoids and increases outside the thylakoids. The simplest way to describe this is to assume that the pH difference is proportional to the electron flow rate. The pH-difference also leads to a backflow of H^+-ions through the membrane. Therefore we can describe the change of ΔpH with time by

$$\frac{d}{dt} \Delta pH = k_{13} \cdot (f_0 + f_5 + f_6 + f_{11}) - k_{14} \cdot \Delta pH. \quad (1)$$

Due to the pH-difference across the membrane ATP is synthesized from ADP. On the other hand 3 ATP molecules are used to bind one CO_2-molecule, *i. e.* for every four electrons transported from $NADPH_2$ to the intermediates of the dark reaction. Therefore we can describe the ATP-formation by

$$\frac{d}{dt} [ATP] = k_{15} \cdot \Delta pH \cdot [ADP] - \frac{3}{4} \cdot f_{12}. \quad (2)$$

f_i are the electron transport rates and k_i are the transport coefficients according to Fig. 1.

For the stationary state we have $f_0 = f_5 = f_6 = f_{12} = f_e$ and

$$\Delta pH_s = \frac{k_{13}}{k_{14}} \cdot 4 \cdot f_e \quad (3)$$

and

$$[ATP]_s = [ADP_0] - \frac{3}{16} \cdot \frac{k_{14}}{k_{13} \cdot k_{15}}. \quad (4)$$

The stationary electron flow rate f_e is determined by the chosen poolsize of the intermediates and by the experimentally known reduction-time and reoxidation-time of plastoquinone [13 – 15]. The poolsize of plastoquinone is set to be $[PQ_0] = 500$, which implies that the sizes of the other pools (Z, Q, X, Cyt-f, P700, FD, PSII, and PSI) are 100, *i. e.* we consider 100 electron transport chains.

This results in $f_e \sim 9000$, *i. e.* the absolute value of the stationary electron transport is ~ 90 e/sec in one chain.

This is in agreement with reported values [22], if we take into account that one chain has about 500 chlorophyllmolecules (250 in PSII and 250 in PSI). Since four electrons are transported for each O_2-molecule released, this implies that we have an oxygen evolution of about $20-25$ O_2-molecules/sec in one chain. This is twice the value reported in ref. [23].

The stationary rate of ATP-formation is calculated to be $60-75$ ATP-molecules/sec per chain.

Of course, these values are sensitive to light intensity, to the stationary ΔpH-value, and to the values of the coefficients chosen in the model.

From the experimentally known values of ΔpH_s [13] then the ratio k_{13}/k_{14} is also determined (*e. g.* for $\Delta pH_s = 2.2$ is $k_{13}/k_{14} \sim 6 \times 10^{-5}$). On the other hand we also know the decay time of ΔpH in the dark when electron flow stops [13]. The time constant is measured roughly to be $k_{14} = 1.0$ sec^{-1}, *i. e.* $k_{13} = 6 \times 10^{-5}$. The coefficient k_{15} determines the ATP-formation and thus the position of the P-peak in the fluorescence induction curve. If k_{15} is too small then the P-S-decrease will occur too late in the model compared with the experimental curve. If k_{15} is too high then the P-S-decrease will occur too early or even cause the P-peak to disappear if the electron flow in the model is increased by the reoxidation of the quencher Q before the pile up process dominates.

From this a value of $k_{15} \sim 1.5 \times 10^3$ is evaluated from the model comparing with the experimental fluorescence induction curve.

Using these values the computer model shows that the ΔpH-value changes relatively slowly — the characteristic time is about 1 sec, whereas the ATP-value almost immediately follows the ΔpH-value.

We have to be aware of the fact that the ΔpH-value of the model corresponds to the total electrochemical potential difference across the thylakoid membrane, *i. e.* the real pH-difference and also the potential difference $\Delta\psi$ across the membrane [13, 16], which is also designated "proton motive force" (pmf) [8].

Results and Comparison with the Model

Increasing the time of dark adaptation prior to the measurement results in an increase of the peak P

in the fluorescence induction curve. At short times of dark adaptation (seconds) a fluorescence induction curve without the peak P is obtained. Fig. 2 shows the P-S-difference in dependence on the time of dark adaptation. Since the peak P is suggested to result from the low energy state and since the ATP level decreases in the dark in some species in the same time scale as the peak P occurs [17] we may suggest that the increase of peak P with time of dark adaptation is caused by a decrease of the ATP-level due to a decay of the electrochemical potential leading to a stop of ATP-synthesis. This assumption was checked with the computer model — in order to check the consistency of the model. All the pools were set to have the state of the dark adapted system, since the electron transfer between the pools takes very short time, about ms, compared to the dark adaptation time considered here. Only the initial ΔpH-value was set to different values. Fig. 3 shows the calculated P-S-difference in dependence on the initial ΔpH-value of the system.
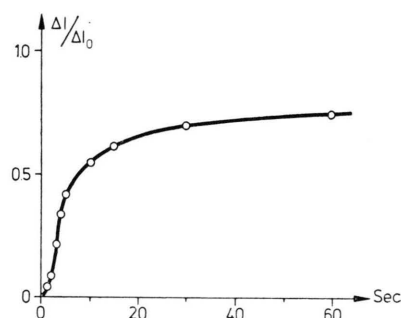

Fig. 2. Difference ΔI between the intensity of the fluorescence of the P-peak and the fluorescence of the quasisteady S-level divided by ΔI_0 of a fully dark adapted system, measured with *Chlorella vulgaris* in dependence on the time of dark adaptation.
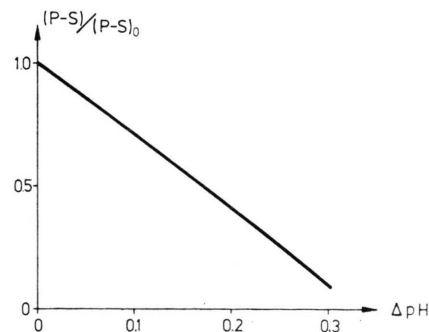

Fig. 3. The calculated P-S-difference in dependence on the initial ΔpH-value divided by the P-S-difference at $\Delta pH_{init} = 0$.
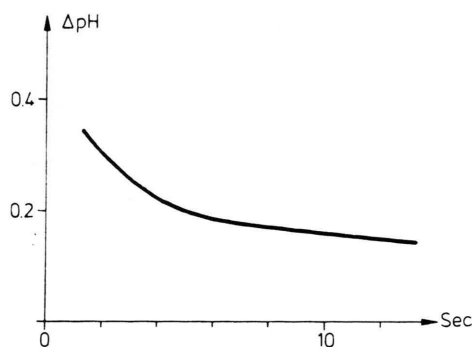
Fig. 4. Decrease of the hypothetical electrochemical potential ΔpH during dark adaptation of the photosynthetic system.

Comparison of the Figs 2 and 3 yields the decrease of the hypothetical electrochemical potential ΔpH in the dark (Fig. 4).

If we compare this with the measured decay of the real pH-gradient according to Witt [13] with a decay time of 1 sec, then we see that the hypothetical electrochemical potential ΔpH, although the time scale is the same, decreases more quickly in the first few seconds and than decreases more slowly than the experimental pH-gradient.

The P → S depression seems to be determined mainly by the pH-gradient, but also influenced by some other factors with a slower decay than the pH-gradient, perhaps by some residual ATP.

Discussion

The P-S-decrease in fluorescence intensity has been related to an increase of electron transport and reoxidation of the quencher due to starting of dark reaction, uptake of CO_2, and Calvin-cycle, or due to an increase in PSI activity [18]. Slow fluorescence induction phenomena in the time range up to minutes which are not considered in this paper, have been related to the photophosphorylation process and to structural change in the thylakoid membrane [2, 19]. The increase of PSI activity during the P-S-decrease or the starting of the Calvin-cycle, probably needs some sources of energy. This may be the membrane potential, or the pH-difference across the membrane of the thylakoids built up by the proton pumping effect of the light driven electron transport or by ATP. Therefore, also a correlation between the P-S-decrease and a high energy state production is suggested as described earlier [2]. As explained above the simple model of the

reoxidation of the intermediates of the electron transport chain roughly describes the P-S-decrease if the hypothetical parameter ΔpH in the model is considered to describe the high energy state.

Therefore, the uncoupler NH_4Cl which is believed to collapse the pH-gradient [8] should inhibit the P-S-fluorescence quenching. Fig. 5 shows the influence of NH_4Cl on the fluorescence induction curve. In fact, the S-line is rised with NH_4Cl without affecting the P-peak very much [2].
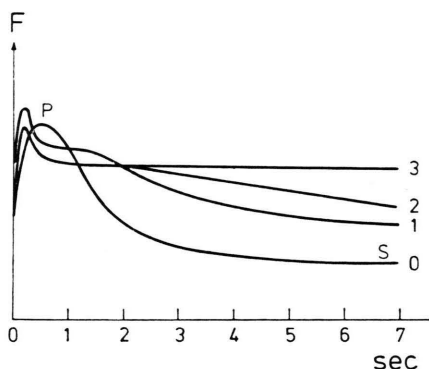
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Fig. 5. Influence of NH_4Cl on the fluorescence induction curve of *Chlorella vulgaris*. 0 ... Control; 1 ... 20 mM/1 NH_4Cl; 2 ... 40 mM/1 NH_4Cl; 3 ... 80 mM/1 NH_4Cl. P ... P-peak of the control curve; S ... Steady state of the control curve.

There are still some difficulties with this picture concerning the effect of other uncouplers of phosphorylation. Also the uncoupler CCCP is believed to colapse the transmembrane pH-difference. This should prevent ATP production, *i. e.* the P-S-decrease should be inhibited. However, this seems not to be the case. CCCP has no effect on the fast fluorescence induction curve at low concentrations, where it acts as an uncoupler of phosphorylation ($\sim 10^{-7} - 10^{-6}$ mol/l) [4, 20]. That might be due to the fact that there exists a threshold effect in the ATP formation in dependence on the electrochemical gradient. If the electrochemical gradient is not collapsed totally there might be enough energy, *e. g.* in the transmembrane potential itself which is not collapsed by neutral molecules penetrating the membrane, as for example NH_3, to produce ATP leading to a P-S-decrease.

The threshold effect is explained by Gräber and Witt [16] by a different fractional dependence of a basal proton flux and a phosphorylation proton

flux. In this computer model the threshold effect is explained by the coupling of the electron transport through the intermediates and the phosphorylation according to Eqns (1) and (2). Although Eqn (2) does not explicitly exhibit any threshold effect the coupling by the second term to the electron transport f_{12} leads to a threshold effect.

The electron transport f_{12} due to reoxidation of $NADPH_2$ is coupled to the pools by the simple assumption

$$f_{12} = k_{12} \cdot [NADPH_2] \cdot [ATP] \qquad (5)$$

and the $[NADPH_2]$-pool is coupled to the other pools with electron transport by similar equations [9, 10].

If the steady state values are calculated for different constant ΔpH-values, which corresponds to the case that ΔpH has a certain value in the S-state depending on the H^+-leak due to uncouplers present, $i.\,e.$ depending on k_{14} in Eqn (1), then we obtain for $e.\,g.$ $[ATP]_s$, $[NADPH_2]$, electron transport rate f_e, and the quencher $[Q^-]_s$ the dependence in Fig. 6.

$[ATP]_s$ is very small below the threshold value of $\Delta pH \sim 0.4$. Below that value also the redoxstate of the quencher Q depends on ΔpH. With decreasing ΔpH $[Q^-]_s$ is increased which means an inhibition of the P-S-decrease in the fluorescence induction curve. Above the threshold value the $[ATP]_s$ value increases rapidly and the $[NADPH_2]_s$ value decreases leading to a value of $[Q^-]_s$ which is not influenced by the ΔpH. That means that an uncoupler only can inhibit the P-S-decrease in the fluorescence induction curve if the hypothetical electrochemical parameter ΔpH is suppressed below the threshold value of about 0.4. That may not be the case with some inhibitors especially at low concentrations if they only collaps the real pH-gradient and not the membrane potential $\Delta\psi$. Thus it may be possible that still some ATP is produced by the potential difference [16, 21] which may be the
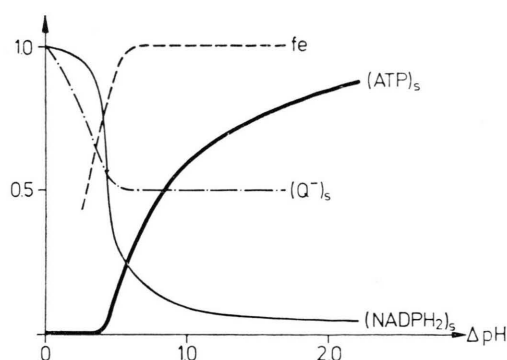

Fig. 6. Calculated steady state values of ATP, $NADPH_2$, and reduced quencher Q^- divided by their total poolsize, and calculated steady state value of the electron transport rate divided by the ΔpH independent value above $\Delta pH = 0.5$ in dependence of the ΔpH value. In the computer model was $\Delta pH = \text{const.}$ set by $k_{13} = 0$ and $k_{14} = 0$ and by different initial values of ΔpH. With these values the induction curves of ATP, $NADPH_2$, and Q^- were calculated. The steady state was obtained after a time of calculation corresponding to about 0.3 sec.

reason that the P-S-quenching is not totally inhibited with NH_4Cl (Fig. 5).

We have used the simple linear relation between ΔpH and ATP-formation [Eqn (2)]. Using an exponential relation according to Gräber and Witt [16] only leads to a slight change in the curves in Figs 3 and 4. Also the threshold effect in Fig. 6 is insensitiv to the special relationship between ΔpH and ATP-formation. The threshold effect is much more a consequence of the coupling between the ATP-formation and the electron transport.

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