



Kinetic Mechanisms of Biological Regulation in Photosynthetic Organisms

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Abstract. Principles of regulation on different levels of photosynthetic apparatus are discussed. Mathematical models of isolated photosynthetic reaction centers and general system of energy transduction in chloroplast are developed. A general approach to model these complex metabolic systems is suggested. Regulatory mechanisms in plant cell are correlated with the different patterns of fluorescence induction curve at different internal physiological states of the cells and external (environmental) conditions. Light regulation inside photosynthetic reaction centers, diffusion processes in thylakoid membrane, generation of transmembrane electrochemical potential, coupling with processes of CO₂ fixation in Calvin Cycle are considered as stages of control of energy transformation in chloroplasts in their connection with kinetic patterns of fluorescence induction curves and other spectrophotometric data.

Key words: Regulation, Photosynthesis, Fluorescence induction curve, Modeling, Primary photosynthetic reactions, Calvin cycle, Transmembrane electric potential difference.

1. Introduction: Principles of regulation in biological systems

Regulation properties of biological processes are determined by their hierarchical organization in space and time. Thus the hierarchy of processes in plants covers the time interval from 10⁻¹² s (absorption of light quanta, primary charge separation) to seconds in metabolic processes and days which is the characteristic time for the whole plant growth.

On every hierarchy level of their organization biological systems are far from thermodynamic equilibrium and open for energy and matter fluxes. That is the actual reason why kinetic models of biological processes are nonlinear. As a result complex time-space behavior patterns together with relaxation are demonstrated, reflecting multistable, oscillatory and quasystochastic character of biological processes. What type of regulation will take place in a system, depends on the time-space hierarchy of the processes and on the character of the influencing factors.

From a kinetic view point the existence of regulation in a system means, that models parameters (usually the rate constants of reactions) change in response to the action of the internal or external factors. The actual character of the response to

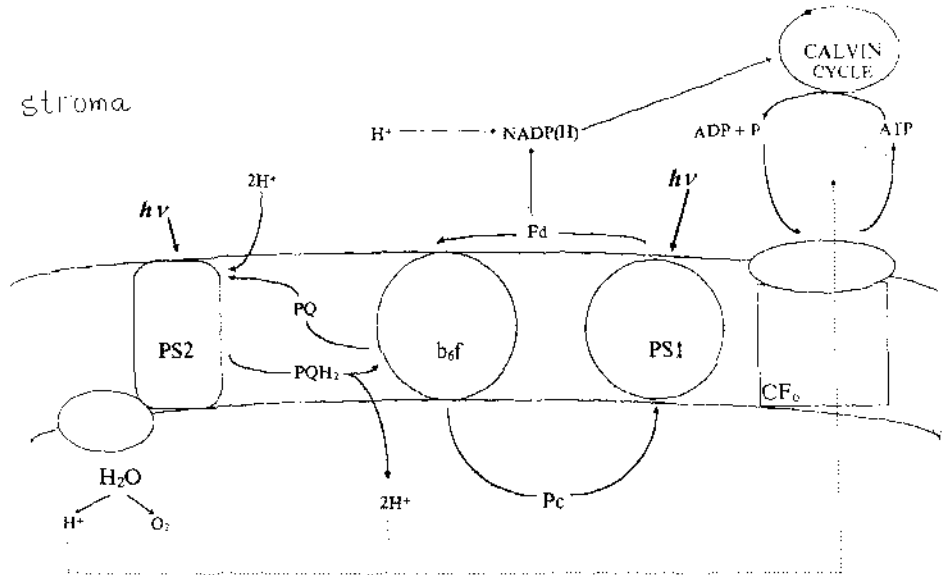


Figure 1. Scheme of primary photosynthetic processes and ATP consumption coupling in chloroplast: PS1, PS2 – photosystems 1 and 2, b_6f – plastocyanin-plastoquinone reductase, CF_o-CF₁ – ATP synthetase, PQ(H₂) – plastocyanin-plastoquinone (plastoquinol), Pc – plastocyanin, Fd – ferredoxin.

external factors is determined by the structure of the system and by the role of the regulated process in cell metabolism. In this paper we consider the system of regulation operating in photosynthetic organisms. The experiments, theoretical study and computer simulations were done at the Biophysical Department of Biological Faculty and A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University.

2. Mathematical Approach to Photosynthetic Electron Transport Processes

The system of primary reactions of photosynthesis schematically presented in Figure 1, is located in chloroplast thylakoids of algae and green plants and in chromatophores of photosynthesizing bacteria. It is one of the most thoroughly investigated complex biological systems. In the last decades the details of its molecular structure and function organization were revealed. New data on freeze-fracture electron microscopy, gel electrophoresis, biochemical studies, x-ray-structural analysis were obtained for the main components of the electron-transport chain. The spectrophotometric measurements were performed to estimate the rate constants of electron transfer at separate steps of the electron transport chain [1, 2].

Most experiments which permit investigation of the structure of molecular components involved in primary reactions of photosynthesis have been performed on separate fragments of photosystems: bacterial photoreaction centers, photosyn-

thetic centers of photosystems 1 and 2. These experiments allowed to establish the relationship between the consequences of electron-transport redox-reactions in photosynthetic chain and electron-conformational and electron-vibrational interactions accompanying electron transport [1-5].

The kinetics of interactions in the unique photosynthetic pigment-protein complexes can be described by ordinary differential equations. The corresponding mathematical approach to describe the states of the photoactive complex was first suggested by S. Malkin [6] to describe the electron and energy transitions inside photosystem 2. A general mathematical description of the electron transfer processes in molecular complexes was thoroughly developed by A. Rubin, V. Shinkarev, G. Riznichenko [3, 7-9]. It is quickly described as follows. The state of the complex of n components C_1, C_2, \dots, C_n (depicted in square brackets)



is considered as an arrangement of the consecutive redox states of the electron carriers C_i , included in the complex. Transitions between the redox states are described by ordinary differential equations, which are linear in probabilities of the corresponding states.

$$\frac{dp_i}{dt} = \sum_{j=1}^l (p_j k_{ji} - p_i k_{ij}). \quad (2)$$

The initial probabilities are

$$p_i(0) = b_i, \quad i = 1, \dots, l.$$

Or, in vectorial form:

$$\frac{dP}{dt} = K^T P, \quad P(0) = B. \quad (3)$$

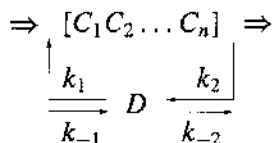
The probability of finding the carrier number q in a certain state G is presented as

$$P_q(G) = \sum_{S_q \in G} p(S_q). \quad (4)$$

Here we sum over all states, where the q -th component is presented in the state G . A photosynthetic complex contains several components, each of them can be presented in oxidized and reduced form, sometimes the components can also be in excited or protonized form. Thus the scheme of the states and their transitions for photosynthetic complex usually includes dozens of states.

The whole electron transport chain (Figure 1) contains photosystem 1 and 2 reaction center complexes, and a cytochrom complex with fixed organization. The interactions of these complexes between each other is performed by means of mobile carriers like Plastoquinone (PQ) and plastocyanin (Pc) (see Figure 1).

These diffusion controlled steps of electron transfer between complex $[C_1 C_2 \dots C_n]$ and the mobile electron carrier D is according to the scheme



and can be described by the mass-action law-type equations

$$\frac{d[D^-]}{dt} = k_2[C_n^-][D^+] - k_{-2}[D^-][C_n^+] - k_1[D^-][C_1^+] + k_{-1}[C_1^-][D^+]. \quad (5)$$

Here $[D^+]$, $[D^-]$ are concentrations of the mobile carrier in the oxidized and reduced forms; $[C_1^+]$, $[C_1^-]$, $[C_n^+]$, $[C_n^-]$ are concentrations of the components of the complex, interacting with the mobile carrier in the oxidized and reduced forms. Each of them could be calculated according to (4). k_i – bimolecular rate constants of the corresponding reactions.

Thus, the whole system of equations, describing redox reaction processes in electron transport chain is nonlinear and contains several dozens of equations. Furthermore electron transport processes are coupled with transmembrane ion fluxes, electro-chemical potential generation, ATP-formation and Calvin-cycle processes. The generalized model describing such a complex system is presented below. The system is essentially nonlinear, and the identification of its parameters presents a special problem.

The problem could be simplified if we consider the processes in isolated photoreaction centers. The model of such a comparatively simple system can be presented as a set of differential equations linear in the probabilities of the states of the complex (see Equation 1–3). For such systems there exist some constructive approaches to conduct computer assisted identification of model parameters according to experimental data [10].

We previously solved the problem of identification of fragments of photosystem 1 and 2 of green plants, chromatophores and fragments of bacterial reaction centers. The models parameters were estimated using the kinetic experimental data obtained by methods of spectrophotometry, EPR, fluorescence, delayed luminescence. The results are summarized in [3–5, 7–9].

3. Two Types of Regulation in Photosynthetic Reaction Centers

Using a mathematical approach, described above, we constructed models for different photosynthetic objects: fragments of photosystems 1 and 2, chromatophores and reaction centers of bacteria of different types. The analysis of the mathematical simulation and model identification allowed to conclude that at least two types of regulation in electron transport chain exist, which are determined by the character of interaction between electron carriers.

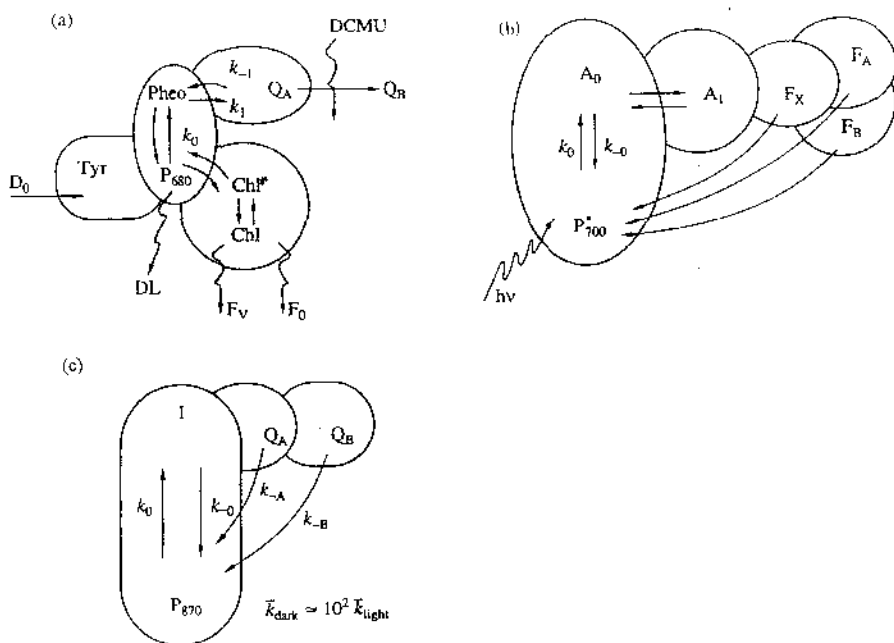


Figure 2. Scheme of light mediated regulation in photosynthetic reaction centers. (a) – scheme of organization of PS 2 reaction center. D_0 – electron donor. Tyr – primary donor of PS 2. P_{680} – photoactive pigment, *Pheo* – primary acceptor of porphyrin nature, Q_A , Q_B – secondary quinone acceptors. $Chl^* \leftrightarrow Chl$ – light harvesting chlorophyll antenna: DL, F_v , F_0 – delayed, variable and basic fluorescence; which are experimentally registered; k_0 , k_1 , k_{-1} – light regulated rate constants; DCMU – inhibitor blocking electron flow from PS2 to PS1. (b) Scheme of photosystem I organization. P_{700} – photoactive pigment, A_0 – primary acceptor of porphyrin nature, A_1 – primary acceptor; F_x , F_A , F_B – prosthetic groups of iron sulphur proteins; k_0 – light regulated rate constant. Arrows indicate reactions which depend on light. (c) Scheme of bacterial photoreaction center organization. P_{870} – photoactive pigment; I – primary acceptor, Q_A , Q_B – secondary acceptors of ubiquinone nature. Arrows indicate light regulated reactions. k_0 – light regulated rate constant of charge separation. The values of rate constants k_0 , k_{-A} , k_{-B} under illumination are two order of magnitude greater than in the dark.

The first type is a light regulation system inside photoactive complexes of photoreaction centers. Such systems optimized by the process of evolution are extremely stable with respect to their functional and structural organization and kinetic parameters under a wide range of environmental conditions. The main regulatory effect here is caused by the absorbed light. The absorption of light quantum leads to charge re-distribution in the primary photosynthetic pair and is followed by conformational changes of protein components, which prevent backward electron transport and energy losses in fluorescence.

The schemes of regulation in PS1, PS2, and bacterial reaction centers are presented in Figure 2. As indicated in 2a, 2b and 2c, in all cases changes of reaction rate constant values under illumination as compared to dark conditions increase the

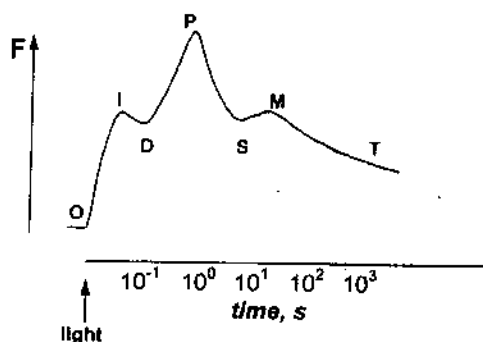


Figure 3. Scheme of a typical chlorophyll fluorescence induction curve with generally accepted designations of its parameters. The arrow indicates the moment of increase in light intensity, F – fluorescence intensity.

efficiency of direct electron flow from the primary electron pair to the electron transport chain. Obviously, the 'light regulation' plays here physiological role increasing the efficiency of energy consumption. It is somewhat similar to allosteric regulation in enzyme catalysis, which is performed via conformational changes of proteins.

The second type of regulation involves mobile carriers. It appears at the level of interaction of a PS2 complex with a cytochrome complex and plastoquinone and at the level of interaction of a cytochrome complex with bacterial reaction centers and PS1 complex with plastocyanin. The range of parameter changes at these stages under different environmental conditions is of several orders of magnitude [4–5].

Reactions, which occur by means of mobile carriers proceed through diffusion mechanisms and are effected by such intracellular factors as viscosity, pH, endogenous inhibitors, metabolites etc. These intracellular parameters could be changed in accordance with changes in physiological state of the organism. From the kinetic point of view, it results in changing the rate constants of interaction of photoreaction complexes with immediate surrounding donors and acceptors. In its turn it means changing constants of influx and efflux in photoreaction centers, which determines the steady state and kinetics of the whole electron transport chain. This fact allows to use characteristics of the processes in photoreaction centers such as fluorescence, as indicators of the whole photosynthetic system and therefore to use a fluorescence induction curve to characterize the state of photosynthetic organisms.

4. Chlorophyll Fluorescence Induction Curves

Fluorescence induction curves registered from plant samples represent the changes in the photosystem 2 fluorescence yield in response to an increase in light intensity. These fluorescence transients reflect the complex sequence of primary and secondary photosynthetic processes, taking place after an increase in illumination and

therefore certain parameters of the induction curves may be used as indicators of a photosynthetic apparatus state. The scheme of a typical fluorescence induction curve is given in Figure 3.

Two main parts in fluorescence induction kinetics are usually distinguished: fast fluorescence rise OI_{DP} in the time interval 0–1 s and a slow relaxation PSMT of fluorescence yield to the steady state level, proceeding in the time scale of minutes.

Fast fluorescence transients OI_{DP} are usually attributed to the primary photosynthetic processes in thylakoid membranes: increase of concentration of quinone acceptor *Q_a*, rise of transmembrane electric potential, redox transformations of the plastoquinone pool and reduction of the PS 1 acceptors (for review see [11]).

The origin of the slow fluorescence transients PSMT is still unclear. Fluorescence decay PS is assumed to result from the generation of pH gradient on the thylakoid membrane. Further increase of fluorescence yield SM is attributed to the activation of the enzyme Ferredoxin – NADPH reductase (FNR) or to the redox transformations of the NADPH pool [12, 13]. ST decay may result from activation of Calvin cycle enzymes or from the increase of ATP/ADP ratio [11].

So the fluorescence induction kinetics reflects the dynamics of interactions between different photosynthetic processes and there are different hypotheses about the origin of its separate phases. To understand actual regulatory mechanisms of photosynthesis resulting in the complexity of fluorescence induction, mathematical models are often used.

5. A Model of Photosynthetic Processes in Chloroplast

The models of fluorescence kinetics usually consider its rising part OI_{DP} and include the description of the fast (nano- and microsecond) processes of light absorption, transfer of excited states, charge separation and/or the slower (millisecond) processes of electron transfer through the two-electron gate (redox transformations of quinones in acceptor part of photosystem 2 [14–18]). In our model [19] we consider the whole system of primary and secondary photosynthetic reactions (Figure 4). This scheme is the detailed presentation of the electron and ion fluxes in the system presented in Figure 1. It allows us to give a description both of the fast and the slow phases of fluorescence kinetics. The model takes into account strict compartmentalization of photosynthetic processes. We consider photosynthetic events in three different chloroplast compartments: the thylakoid membranes, intrathylakoid space (lumen) and stroma of the chloroplast.

First let us consider the primary photosynthetic events, proceeding in the time scale of 10^{-9} – 10^{-3} s, which determine the pattern of OI_{DP} section of fluorescence induction curve. There are different hypothesis concerning the origin of OI_{DP} fluorescence kinetics (for review see [11]). Here we focus on the investigation of the effects of transmembrane electric potential formation and decreasing luminal pH on the parameters of fluorescence induction curve.

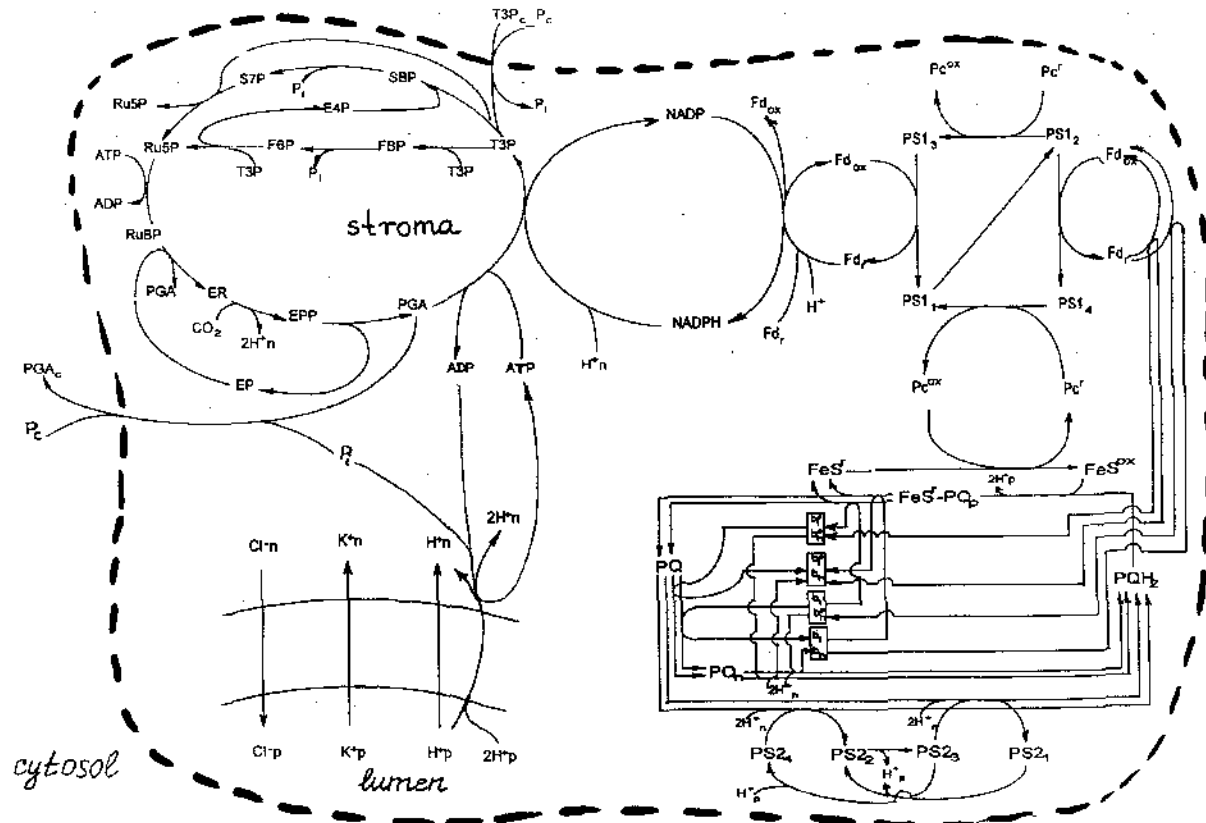


Figure 4. General scheme of the photosynthetic processes in chloroplast. PS2₁, PS2₂, PS2₃, PS2₄ and PS1₁, PS1₂, PS1₃, PS1₄ – basic states of photosystem 2 and photosystem 1 (obtained after reduction of initial system of differential equations); b_l , b_h and b_l^* , b_h^* – oxidized and reduced states of low and high potential hemes of cytochrom b; PQ(H₂) – plastouquinone (plastouquinol), Pc^r and Pc^{ox} – reduced and oxidized plastocyanin; FeS^r , FeS^{ox} and FeS^r-PQ – reduced, oxidized and bound to PQ prosthetic groups of iron sulphur protein; Fd_r , Fd_{ox} – reduced and oxidized ferredoxin; T3P, SBP, S7P, Ru5P, F6P, FBP, PGA, EPP, ER, RuBP, EP – intermediates of Calvin cycle. Subscript characters p , n and c stand for lumen, stroma and cytosol compartments.

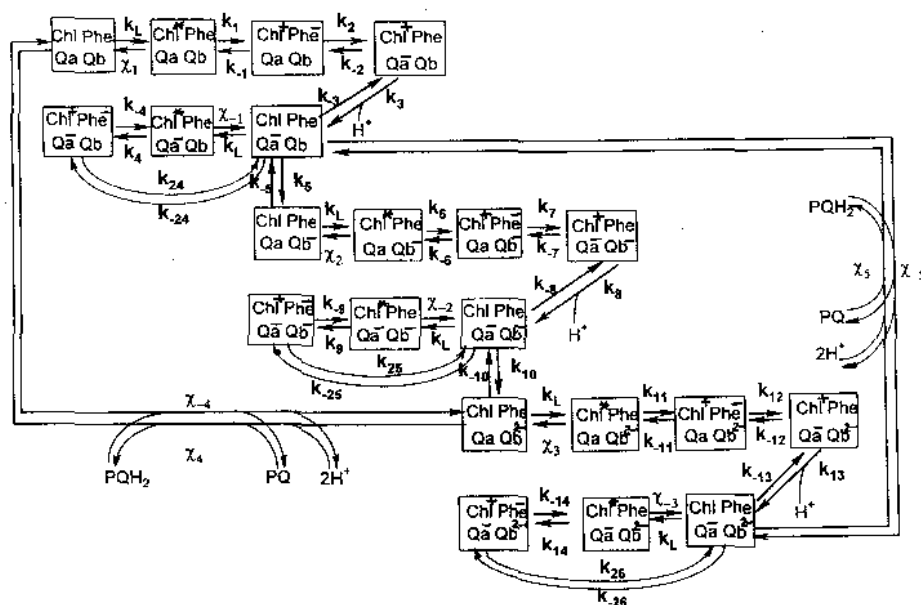


Figure 5. Scheme of the photosynthetic processes in photosystem 2. Transitions between different states of PS2 reaction center are shown. *Chl* – all PS2 chlorophyll, including pigments of light harvesting complex and P680; *Phe* – primary electron acceptor pheophytin; *Q_a* – primary quinone acceptor of electrons *Q_A*; *Q_b* – secondary quinone acceptor of electrons *Q_B*; k_L – rate constant of the light reaction; k_{+i} – rate constants of corresponding electron transfer reaction; $\chi_{\pm i}$, $i = 1, 2, 3$ – rate constants of deactivation of RC excited states for different states of *Q_b*: *Q_b* oxidized ($\chi_{\pm 1}$), *Q_b⁻* ($\chi_{\pm 2}$) and *Q_b²⁻* ($\chi_{\pm 3}$); χ_{+4} and $\chi_{\pm 5}$ – rate constants of electron exchange with the molecules of the mobile plastoquinone pool (PQ). It is taken into account in the scheme that excitation of RC pigments and primary charge separation occur both in open (*Q_a* oxidized) and closed (*Q_a* reduced) RC states.

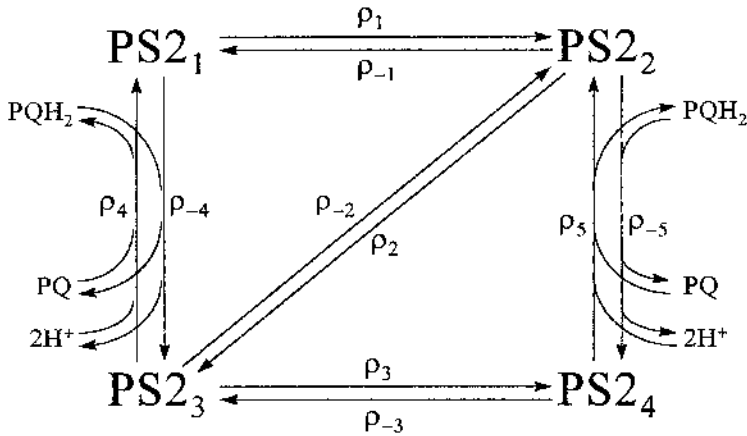
The light-driven electron transfer in the thylakoid membranes is catalyzed by the complexes of photosystem 2, cytochrome b6/f and photosystem 1. The pool of the mobile electron carrier plastoquinone located in the membrane and plastocyanin in the lumen also are components of the electron transfer chain (Figure 4). The complex sequence of photochemical reactions in ETC results in the generation of $\Delta\mu H$ on the thylakoid membrane due to transmembrane electron transfer and the coupled uptake of protons into the thylakoid lumen. In the model the dependence of certain electron transfer stages on electric potential $\Delta\psi$ is introduced.

We also considered a number of processes, describing $\Delta\mu H$ consumption: ATP synthesis and ion fluxes of H^+ , K^+ and Cl^- through the thylakoid membrane.

In developing the model it was taken into account that dynamics of all membrane complexes (photosystems 1, 2 and cytochrome b6/f) could be described in terms of the dynamic probabilities of its possible states. Their interaction with mobile electron carriers is described according to the approach presented in the above section (for details see [8–9]). For example, the detailed scheme of the processes

in PS 2 complex is presented in Figure 5. The simple sequence of events in PS 2 is depicted in Figure 2a.

After the proper reduction of the system shown in Figure 5 according to the hierarchy of time scales (all $k_{\pm i} \gg k_L, \chi_j; i = 1, \dots, 26; j = 4, 5$) the processes in Photosystem 2 could be presented as following:



Here every state $PS2_i$ corresponds to the summarized concentration of several states of the PS II complex, presented in the Figure 5. Namely:

$$PS2_1 = \begin{bmatrix} Chl & Phe \\ Qa & Qb \end{bmatrix};$$

$$PS2_2 = \begin{bmatrix} Chl^* & Phe \\ Qa & Qb \end{bmatrix} + \begin{bmatrix} Chl^{\cdot} & Phe^- \\ Qa & Qb \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe \\ Qa^- & Qb \end{bmatrix} \\ + \begin{bmatrix} Chl & Phe \\ Qa^- & Qb \end{bmatrix} + \begin{bmatrix} Chl^* & Phe \\ Qa^{\cdot} & Qb \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe^- \\ Qa^- & Qb \end{bmatrix} + \begin{bmatrix} Chl & Phe \\ Qa & Qb^- \end{bmatrix}$$

$$PS2_3 = \begin{bmatrix} Chl^* & Phe \\ Qa & Qb^- \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe^- \\ Qa & Qb^- \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe \\ Qa & Qb^- \end{bmatrix} \\ + \begin{bmatrix} Chl & Phe \\ Qa^- & Qb^- \end{bmatrix} + \begin{bmatrix} Chl^* & Phe \\ Qa^- & Qb^- \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe^- \\ Qa^- & Qb^- \end{bmatrix} + \begin{bmatrix} Chl & Phe \\ Qa & Qb^{2-} \end{bmatrix}$$

$$PS2_4 = \begin{bmatrix} Chl^* & Phe \\ Qa & Qb^{2-} \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe \\ Qa & Qb^2 \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe \\ Qa^- & Qb^{2-} \end{bmatrix} \\ + \begin{bmatrix} Chl & Phe \\ Qa^- & Qb^{2-} \end{bmatrix} + \begin{bmatrix} Chl^* & Phe \\ Qa^- & Qb^{2-} \end{bmatrix} + \begin{bmatrix} Chl^+ & Phe^- \\ Qa^- & Qb^{2-} \end{bmatrix}.$$

The following set of equations describes the processes in the reduced scheme of PS 2:

$$\text{PS2}_1 : \dot{y}_1 = \rho_{-1}[H_p^+]y_2 + \rho_4y_3[PQ][H_n^+]^2 - (\rho_1 + \chi_{-4}[PQH_2])y_1;$$

$$\text{PS2}_2 : \dot{y}_2 = \rho_1y_1 + \rho_5y_4[PQ][H_n^+]^2 + \rho_{-2}y_3[H_p^+] - (\rho_{-1}[H_p^+] + \rho_{-5}[PQH_2] + \rho_2)y_2;$$

$$\text{PS2}_3 : \dot{y}_3 = \rho_2y_2 + \rho_{-4}y_1[PQH_2] + \rho_3[H_p^+]y_4 - (\rho_{-2}[H_p^+] + \rho_4[PQ][H_n^+]^2 + \rho_3)y_3;$$

$$\text{PS2}_4 : \dot{y}_4 = \rho_3y_3 + \rho_{-5}[PQH_2]y_2 - (\rho_{-3}[H_p^+] + \rho_5[PQ][H_n^+]^2)y_4;$$

The rate constants $\rho_{\pm i}$ are the functions of the rate constants of 'slow stages' ($k_{l,j}$, $\chi_{\pm k}$; $j = 1, \dots, 6$; $k = 4, 5$) and the functions of equilibrium constants $K_i = \frac{k_i}{k_{-i}}$ of the 'fast stages'. All designations here correspond to those depicted in Figure 5:

$$\rho_{-1} = \frac{\chi_1/R_1}{K_1K_2K_3K_5}; \quad \rho_1 = k_{L1};$$

$$\rho_{-2} = \frac{\chi_2/R_2}{K_6K_7K_8K_{10}}; \quad \rho_2 = k_{L3}/R_1;$$

$$\rho_{-3} = \frac{1/R_3}{K_1K_2K_3} \cdot \frac{\chi_1\chi_5\chi_{-4}k_{L5}}{\chi_{-5}\chi_4k_{L1}}; \quad \rho_3 = k_{L5}/R_2;$$

$$\rho_{-4} = \chi_{-4}; \quad \rho_4 = \chi_4/R_2;$$

$$\rho_{-5} = \frac{\chi_{-5}/R_1}{K_5}; \quad \rho_5 = \chi_5/R_3;$$

where

$$R_1 = 1 + \frac{1}{K_5} + \frac{1}{K_3K_5} + \frac{1}{K_2K_3K_5} + \frac{1}{K_1K_2K_3K_5} + \frac{k_{L2}(1 + K_4)}{\chi_{-1}K_5}$$

$$R_2 = 1 + \frac{1}{K_{10}} + \frac{1}{K_8K_{10}} + \frac{1}{K_7K_8K_{10}} + \frac{1}{K_6K_7K_8K_{10}} + \frac{k_{L4}(1 + K_9)}{\chi_{-2}K_{10}}$$

$$R_3 = 1 + \frac{1}{K_{13}} + \frac{1}{K_{12}K_{13}} + \frac{k_{L6}(1 + K_{14})}{\chi_{-3}} + \frac{1}{K_1K_2K_3} \frac{\chi_1\chi_5\chi_{-4}k_{L5}}{\chi_3\chi_{-5}\chi_4k_{L1}}$$

To describe the processes in photosystem 1 we used similar approach as for PS 2.

The processes corresponding to electron and proton transport across the membrane generate the electric potential $\Delta\Psi$, which in turn inhibits these processes. This results in such a change of equilibrium constants so as to decrease the rate of charge transfer [20, 21]:

$$K_{eq}(\Delta\Psi) = \exp(-\alpha \cdot \Delta\Psi/(RT/F)) \cdot K_{eq},$$

$$k_+(\Delta\Psi) = \exp(-\delta \cdot \alpha \cdot \Delta\Psi/(RT/F)) \cdot k_+,$$

$$k_-(\Delta\Psi) = \exp((1 - \delta) \cdot \alpha \cdot \Delta\Psi/(RT/F)) \cdot k_-.$$

Here $\alpha \cdot$ indicates the contribution of each electrogenic step to $\Delta\Psi$ generation, δ – the part of membrane potential, which influences the rate constant of the direct reaction (k_+).

Since our model takes into account both $\Delta\Psi$ generation (PS 2, b6/f complex, PS 1) and $\Delta\Psi$ consumption (ATP synthesis, H^+ -leak, K^+ -leak, Cl^- leak) processes $\Delta\Psi$ is a variable of the model and its time dependence is determined by following equation:

$$\frac{C_{thyl}}{F} \frac{d(\Delta\psi)}{dt} = \sum (\text{rate of } \Delta\psi \text{ production}) - \sum (\text{rate of } \Delta\psi \text{ consumption}).$$

Here C_{thyl} is the apportioned capacity of the thylakoid membrane (we assume C_{thyl} is equal to $10^{-2} \frac{C}{V \cdot R(\text{protein})}$), F is Faraday constant.

$\Delta\mu H$ generating (b6/f complex) and $\Delta\mu H$ consuming processes (ATP synthesis and ion transfer across the membrane) were modeled in accordance with [21–23].

The intensity of fluorescence was assumed to be proportional to the sum of concentration of PS 2 excited states multiplied by the corresponding rate constants of their inactivation by fluorescence emission:

$$F = \chi_1 \begin{bmatrix} Chl^* & Phe \\ Q_A & Q_B \end{bmatrix} + \chi_{-1} \begin{bmatrix} Chl^* & Phe \\ Q_A^- & Q_B \end{bmatrix} + \chi_2 \begin{bmatrix} Chl^* & Phe \\ Q_A & Q_B^- \end{bmatrix} \\ + \chi_{-2} \begin{bmatrix} Chl^* & Phe \\ Q_A & Q_B^- \end{bmatrix} + \chi_3 \begin{bmatrix} Chl^* & Phe \\ Q_A & Q_B^{2-} \end{bmatrix} + \chi_{-3} \begin{bmatrix} Chl^* & Phe \\ Q_A^- & Q_B^{2-} \end{bmatrix}.$$

Contribution of the items with non reduced Q_A to the total fluorescence was shown to be much less than contribution of the items with Q_A^- .

The evaluation of the model parameters (about 100) was carried out. Some of them were estimated from the experimental data published in the literature [1], while others were calculated or identified by comparing model simulation and experimental data (presented on the web site <http://www.biophys.msu.ru/personal/riznich/modphot/par.htm>). The curves of fluorescence induction were obtained [24], including the details of fast millisecond kinetics of fluorescence emission (Figure 6).

In the dark all the PS2 and PS1 complexes are in the ground state and no excitation and charge separation processes occur. Plastoquinones Q_A , Q_B and all the mobile electron carriers of plastoquinone pool PQ and plastocyanin Pc were assumed to be fully oxidized in the dark. Concentrations of K^+ and Cl^- in the

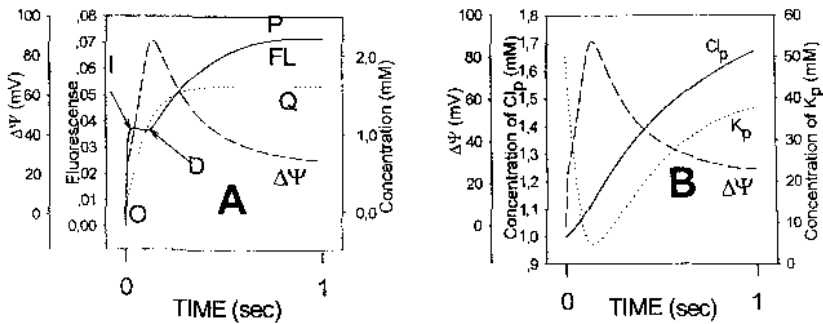


Figure 6. A. Simulated fast fluorescence induction (FI) and time courses of electric potential ($\Delta\psi$) and total concentration of PS II states with non-oxidized $Q_A Q_B(Q)$. B. Calculated kinetics of luminal ion concentration (K_p , Cl_p) and transmembrane electric potential $\Delta\psi$.

stroma and lumen have an equal value. In these conditions the level of fluorescence is minimal (0 in our model). Transmembrane electric potential $\Delta\psi$ and proton gradient ΔpH are also equal to zero.

After the beginning of illumination the light-driven electron transfer across the thylakoid membrane is activated, which is coupled to the uptake of protons into the thylakoid lumen. This results in the fast (about 100 ms) increase of $\Delta\psi$ and ΔpH (not shown). The $\Delta\psi$ increase leads to the deceleration of electron transfer process due to the dependence of a number of its stages on electric potential. This results in the fast fluorescence rise OI. The maximal rate of $\Delta\psi$ increase corresponds to the appearance of the local maximum I on the fluorescence induction curve.

The H^+ uptake observed in the thylakoids in the light is possible due to the counterflow of other ions across the membrane. In the framework of our model we may suggest the following mechanism of this process. The $\Delta\psi$ increase results in the acceleration of the ion transfer across the thylakoid membrane and leads to the K^+ - efflux and influx of Cl^- (Figure 6B). In turn, these ion fluxes lead to the deceleration of $\Delta\psi$ rise and subsequent decrease of $\Delta\psi$ (Figure 6A). Deceleration of $\Delta\psi$ rise facilitates in some extent electron transfer processes and leads to a fall in fluorescence yield (ID transition). Further fluorescence increase DP is caused by the electron back-pressure due to the reduction of quinone acceptors Q_A and Q_B (Figure 6A). When $\Delta\psi$ starts to decrease the influx of K^+ may be observed.

Thus, the model seems to be realistic in describing the time course of chlorophyll fluorescence after the beginning of illumination: nonmonotonous behaviour of the fast fluorescence rise OI and the subsequent decrease in fluorescence yield PT (Figure 6). The process of the transmembrane electric potential formation as well as initiated ion fluxes play a significant role in the determination of the fast fluorescence rise pattern.

For simulation of the slow phase of fluorescence induction curve the model needs further refinements. It is necessary to give a proper description of ΔpH formation and to take into account the processes of ATP and NADPH consumption

in the Calvin cycle of CO_2 fixation. The pattern of this slow phase is actually the most variable component of the fluorescence induction curves registered from the different photosynthetic samples.

6. Conclusions

Comparative analysis of isolated photosynthetic complexes and photosynthetic processes in chloroplast, where they are coupled to energy supplying processes and CO_2 fixation cycle, their mathematical modeling and computer simulation leads to the conclusion that regulatory mechanisms have a hierarchical organization at different levels of photosynthetic organisms. While it is true for all complex systems, in photosynthetic processes this thesis is based on the analysis of concrete experimental data and model simulations.

To quantify regulatory properties of the presented model one can employ a metabolic control analysis approach earlier developed in [25–29]. To interpret a large number of experimental data in terms of response coefficients, which show percentage variation of steady state flux at 1% change in external parameters, it is easy to see that the regulation in photosynthetic centers is quite independent on external conditions. This means that steady-state response coefficients are near zero. The absorption of light quanta initiates the sequence of processes, the redistribution of charges and conformational changes, resulted in the rapid electron outflow from the primary photosynthetic pair. Different photosynthetic centers are to the great extent standardized and similar in their organization. Kinetic parameters in PS1, PS2 and bacterial centers almost do not depend on pH, viscosity etc. Kinetic patterns of the components involved in these processes usually could be described by a type of simple exponential relaxation.

At the level of interaction between photosystems the regulation depends on external conditions, i.e. response coefficients differ from zero. Indeed, rate constants of diffusion stages involved here are drastically dependent on pH, redox potential, viscosity. It makes possible the regulation of these stages at the cellular level under altering environmental factors and during the cell growth. Corresponding kinetic patterns are more complex, they may have several maxima. This is manifested in special fluorescence induction patterns.

On the level of the interaction between primary reactions and the Calvin Cycle, more refined control mechanisms are switched on. Here oscillating kinetics are possible, which depend on the existence of pools and feedback relations. This allows photosynthetic CO_2 fixation in the dark as well as under illumination using the energy accumulated in the primary processes of photosynthesis as well as the energy produced by respiration.

All these types of regulation are reflected in the pattern of fluorescence induction curve. The rapid nano and micro-second reactions provide the information about the processes inside the photoreaction centers of photosystem 2. Nonmonotonous rise in millisecond range (simulated in Figure 6) strongly depends on ion

fluxes across the membrane of thylakoid [19, 24]. Patterns of slow stages (seconds range) of decrease of emission are quite different for different algae cells and reflect the physiological status of individual algae organisms [30, 31]. The distribution of these patterns changes during the population growth.

The development of the detailed model, including the mechanisms of interaction of photosynthetic processes permits the use of fluorescence induction curves to reveal the stages of photosynthetic apparatus which are under the control of internal cellular and external environmental factors.

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