THE DYNAMICS
OF TRANSPORT PROCESSES

Alternative Pathways of Photoinduced Electron Transport
in Chloroplasts

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Abstract—The influence of the alternative pathways of electron transport in photosynthetic systems of the oxygen type on the kinetics of the photoinduced redox transitions of P$_{700}$, ferredoxin, NADP, pH of the intrathylakoid space or lumen, and relative concentration of ATP was studied. The oxygen effect on the kinetics of photooxidation of P$_{700}$ was analyzed. The retardation of the photooxidation of P$_{700}$ at low oxygen concentrations can be explained by the “over-reduction” of the acceptor side of PS1 as a result of a decrease in the electron outflow from PS1 to oxygen during hypoxia. The results of numerical experiments are in good agreement with known experimental data that the withdrawal of electrons from PS1 (on the ferredoxin–NADP segment of the chain) can be the limiting stage in the noncyclic electron transport chain. The functioning of the cyclic electron transport chain provides additional synthesis of ATP molecules and weakens the excess reduction of the acceptor segment of PS1. The alternative pathway of electron transport, namely, electron outflow from PS1 to oxygen also favors the optimum conditions for the functioning of the photosynthetic electron transport chain.

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INTRODUCTION

One of the characteristic features of all living organisms is being open systems capable of extracting, transforming, and utilizing the energy of the environment in the form of organic nutrients (chemotrophs) or solar radiation energy (phototrophs). Synthesis of ATP from ADP and orthophosphate (P$_i$) is the key process in bioenergetics. It is endergonic and initiated by the external sources of energy. In phototrophs, this is the energy of solar light, which is absorbed by higher plants, cyanobacteria, and photosynthetic bacteria [1]. Photosynthetic organisms of the oxygen type (plants, algae, and cyanobacteria) have two photosystems, 1 (PS1) and 2 (PS2). They use the energy of the absorbed light quanta for transferring two electrons from the water molecule (electron donor in PS2) to the NADP$^+$ molecule (the final electron acceptor in PS1). The energy donating reactions of electron transport are accompanied by the generation of the transmembrane difference in the electrochemical potentials of hydrogen ions, providing the functioning of ATP-synthase complexes. The NADPH and ATP molecules, formed during the light stages of photosynthesis, are used for the synthesis of carbohydrates at the dark stages of photosynthesis (Calvin cycle) [2–5].

Studies of the regulatory mechanisms of electron transport in photosynthetic systems and their adaptation to the environmental conditions is the central problem in biophysics of photosynthesis [6–10]. Chloroplasts and cyanobacteria have several mechanisms for regulating photosynthetic electron transport. One of these is pH-dependent regulation of the electron transfer from PS2 to PS1 due to the photoinduced acidification of the intrathylakoid space. This regulatory mechanism is associated with the retardation of the oxidation of plastoquinol by the cytochromic $b_{6}f$ complex (photosynthetic control [11–14]) and the increase in the energy dissipation in the light-harvesting antenna of PS2 [15–20]. Phosphorylation of the proteins of the mobile light-collecting complex is another type of electron transport regulation in chloroplasts, which affects the optimum light distribution between photosystems [21]. Moreover, the consumption of NADPH and ATP is regulated due to the photoinduced activation of the enzymes of the Calvin cycle [22–29].

As is known, photosynthetic systems of oxygenic type involve alternative pathways of electron transport. One of these is cyclic electron transport around PS1 via ferredoxin quinone reductase (FQR) and NADPH dehydrogenase (NDH-1), which is possible in addition to the linear electron transport from water to NADP$^+$ [7–10, 22, 23]. Another alternative pathway is pseudocyclic electron transport from PS1 to oxygen, forming a water molecule [24–26]. The cyclic electron transport can serve for activating the synthesis of ATP and increasing the ATP:NADPH ratio. The electron flow to oxygen plays the role of a by-pass, preventing the overreduction of the acceptor side of PS1 [30]. The optimum balance between the different pathways of electron transport enhances the efficiency of the functioning of both photosystems and the electron transport chain in general.
The measurements of photosynthetic redox transformations of P$_{700}$, which is the primary donor of electrons to PS1, play an important role in the in vivo and in situ studies of the regulatory mechanisms of electron transport in intact photosynthetic systems [1, 31]. Here we analyze the complex kinetics of the photooxidation of P$_{700}$ in photosynthetic systems of oxygenic type (chloroplasts of higher plants and cells of cyanobacteria) using a generalized mathematical model of electron and proton transfer. A distinction of the suggested model is consideration of the functioning and interactions of photosynthetic and respiratory chains of electron transport. In addition, the model considers the alternative pathways of the photosynthetic electron transfer (linear and cyclic electron transport), reduction and oxidation of NADP, synthesis of ATP catalyzed by ATP synthase, consumption of ATP and NADPH in the Calvin cycle, oxygen evolution during the irradiation of PS2, and its consumption by cytochrome oxidases. An analysis of the numerical experiments allowed us to reveal the factors responsible for the complex multiphase kinetics of electron transport.

MATERIALS AND METHODS

The objects of research were the leaves of Chinese rose Hibiscus rosa-sinensis. For kinetic studies of the redox transformations of P$_{700}$, we used rectangular (3 × 30 mm) cut-off pieces of leaves. The samples were placed in a transparent plastic cell. The cell was fixed at the center of a quartz tube placed in a rectangular resonator of an E-4 Varian EPR spectrometer (USA). Air or gas mixtures of a given composition containing oxygen and atmospheric air in different proportions were passed through the tube. The cell design provided contact between the leaf and the atmosphere inside the tube. The samples placed in the resonator of the EPR spectrometer were illuminated with white light of a 100 W tungsten incandescent lamp. IR radiation was trimmed with a water filter 5 cm thick. The intensity of active light on the sample surface was 320 W/m$^2$.

The state of the primary electron donor of PS1 (P$_{700}$) was monitored through EPR signal I from the oxidized P$_{700}^+$ centers [31]. For kinetic measurements of photoinduced redox transformations of P$_{700}$, the magnetic field was fixed at the low-field extremum of EPR signal I. The kinetic measurements were carried out according to the following protocol. To standardize the experimental conditions, the sample was preliminarily illuminated with white light for 1 min. Then the sample was adapted for some time ($t_{ad}$) to the darkness and reilluminated (generally, for 1 or 2 min). After the second illumination, the sample was again adapted to the dark (0.5 min) and then illuminated. The kinetics of photoinduced changes in the magnitude of signal I was recorded beginning with the second cycle of illumination. This protocol makes it possible to exclude the indeterminacy of the initial state of the leaf, which could affect the kinetic behavior of signal I [30].

For oxygen concentration measurements, we used the deuterated oxygen-sensitive spin label 4-amino-2,2,5,5-tetramethyl-3-imidazoline-N-oxyl with the $^{15}$N isotope substituted in it. A 10$^{-3}$ M solution of the label was placed in an oxygen-passing plastic capillary (Zeus, inner diameter 0.35 mm) placed in a resonator of the EPR spectrometer. The procedure for oxygen concentration measurements using spin labels is described in [32].

RESULTS AND DISCUSSION

This section describes the influence of the prehistory of illumination and aeration conditions on the kinetics of the photooxidation of P$_{700}$, which is the primary electron donor of PS1, in the leaves of Chinese rose. Figure 1 shows the typical kinetic curves of photoinduced changes in EPR signal I from the oxidized P$_{700}^+$ centers obtained for different times of sample adaptation to the dark. After sufficiently long adaptation ($t_{ad}$ ≥ 1 min), the kinetics of the photooxidation of P$_{700}$ became multiphase. The relatively small initial abrupt change in the concentration of P$_{700}^+$ (phase A) was followed by the lag phase and relatively slow the second stage of P$_{700}$ oxidation (phase B). The lag phase became longer when the adaptation time increased. We can also distinguish the third, slower stage of the increase in the concentration of P$_{700}^+$ (phase C). The difference between phases A, B, and C observed after sufficiently long adaptation of the leaf to the dark (≥2–5 min) disappeared when illumination started after a short dark pause (≤0.5 min).

As is known, the kinetics of redox transformations of P$_{700}$ in photosynthetic systems of oxygenic type depends on the presence of oxygen because oxygen is one of the acceptors of an electron from PS1 [33]. Figure 2 shows the typical kinetic curves of photoinduced changes in EPR signal I in the leaf of Chinese rose measured at different oxygen contents in atmosphere: (1) air ([O$_2$] = 21%), (2) oxygen at a high concentration ([O$_2$] = 90 ± 10%), and (3) oxygen at a lower concentration ([O$_2$] = 9.5 ± 0.5%). An excess of oxygen did not strongly affect the behavior of signal I, and we observed only relatively small retardation of the photooxidation of P$_{700}$ (curve 2). In contrast, after the adaptation of the sample to the dark in the atmosphere with a low concentration of oxygen (curve 3), it took much more time for signal I to reach the stationary level. The retardation of the photooxidation of P$_{700}$ at low oxygen concentrations can be explained by a weakening of electron outflow from PS1 to oxygen at hypoxia, which can lead to “over-reduction” of the acceptor side of PS1 [34]. Similar data on the oxygen effect on the kinetics of photoinduced redox transformations of P$_{700}$ were obtained for intact cells of cyanobacteria [35].