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Effect of Low Temperatures on Photochemical Activity of PS1 Reaction Centers from *Synechocystis* sp. Frozen under Illumination

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Abstract—After cooling of *Synechocystis* sp. photosystem 1 (PS1) reaction centers (RC) to 160 K under illumination most of the photoactive pigment is fixed for a long time in the oxidized state. The same effect is observed in purple bacteria RC. The dark reduction kinetics of PS1 P700 chlorophyll, which still retains its photochemical activity, in these samples was similar to that in samples cooled in the dark. We suggest that the photoinduced charge separation in PS1 RC, as well as in purple bacteria RC, is accompanied by conformational changes that can be fixed in samples cooled under illumination. As a result, the electrons photomobilized in RC cooled under illumination are unable to return backward the process of electron transfer to $P700^+$ after cessation of actinic illumination. Such irreversible trapping of electrons can take place in different parts of the PS1 RC electron acceptor chain.

Key words: cyanobacteria, photosystem 1, electron transport, conformational changes

It is well known that photoactivation of photosynthetic reaction centers (RC) of purple bacteria is associated with their conformational mobility. It has also been demonstrated that light-induced conformational changes of RC are accompanied by changes in their photochemical activity [1-4]. These conformational changes can be detected by various methods, including X-ray diffraction analysis. For example, according to the X-ray diffraction of *Rhodobacter sphaeroides* RC crystals, light-induced reduction of the secondary (Q_B) quinone acceptors of an electron is accompanied by a 5 Å displacement and reorientation of the quinone ring in the RC protein structure [5]. These changes can be fixed by rapid immersion of illuminated crystals in liquid nitrogen and cooling to cryogenic temperature [5]. The process of the RC (*Rb. sphaeroides*, *Rhodospirillum rubrum*) cooling in the light brings about a state in which most part of photoactive bacteriochlorophyll dimer (P) is fixed oxidized at low temperature for an indefinitely long time interval (at least one day). In the RC fraction that retains photochemical activity under these conditions, the process of electron transfer to photooxidized P from reduced acceptors Q_A and Q_B is characterized by half-time values close to those at room temperature (seconds). It should be noted that in RC preparations frozen in the dark direct electron transfer from Q_A to Q_B is completely blocked, whereas the time of the photomobilized electron transfer from Q_A^- to P^+ at temperatures below 120 K is about one-third of the value at T = 295 K (~100 msec → ~30 msec). Reaction centers of purple bacteria as well as photosystem 2 (PS2) RC of cyanobacteria, algae, and higher plants are reaction centers of so-called quinone type [6]. In these complexes, the tunneling-mediated charge separation within the transmembrane pigment–protein phototransformation complexes composed of photoactive dimer of (bacterio)chlorophyll molecules, (bacterio)chlorophyll monomer, (bacterio)pheophytin, and quinone acceptors is followed by relatively slow temporary (much slower than charge separation) stabilization of the electron on the secondary quinone acceptor. The secondary quinone acceptor is an intermediate in the process of transfer of reduced equivalents to the photosynthetic membrane. This process is controlled by diffusion. Another type of photosynthetic reaction centers, RC of iron-sulfur type [6], is found in green bacteria, PS1 of cyanobacteria, algae, and higher plants. These transmembrane pigment–protein photo-

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transformation complexes contain the following chain of electron transfer cofactors: photoactive dimer of chlorophyll molecules, chlorophyll monomer, a phylloquinone molecule, and iron-sulfur clusters F_X, F_A, and F_B. It was of considerable interest to reveal if cooling of RC of iron-sulfur type in the light was accompanied by the photochemical activity changes similar to those observed in RC complexes of purple bacteria. The objects of the study were RC complexes of PS1 of the cyanobacterium *Synechocystis* sp.

MATERIALS AND METHODS

Methods of cultivation of the cyanobacterium *Synechocystis* sp. PCC 6803 cells and isolation of thylakoid membranes and preparations of PS1 pigment–protein complexes were described elsewhere [7]. Some experiments were performed using PS1 complexes isolated in the laboratory of Prof. J. Golbeck (Pennsylvania State University, USA) from the *Synechocystis* 7002 mutant *rubA*$^-$ containing no iron-sulfur centers F_X, F_A, and F_B [8]. The resulting PS1 particles were suspended in 50 mM Tris-HCl buffer, pH 8.3, containing 10% glycerol and 5% dodecyl maltoside and stored at −80°C until use.

Photoinduced reactions in PS1 samples were investigated by detecting cation-radical signal of photoactive chlorophyll using the ESR method. These experiments were performed in the laboratory of Prof. J. Golbeck (Pennsylvania State University, USA) using a Bruker ESC-106 spectrometer with 3-cm microwave range. Samples were activated with continuous light (wavelength range, 400-800 nm; intensity, ~1 kW/m^2) through a special window in the spectrometer cavity. To obtain samples frozen in a dark state, an ESR capillary (diameter, ~3 mm) with PS1 preparation was placed in the dark in the spectrometer cavity precooled with liquid nitrogen to 160 K. To measure signals of samples frozen in the actinic light, PS1 preparations were cooled in the illuminated spectrometer cavity. The time of sample cooling to 160 K was 20-30 sec. To remove oxygen from samples, suspension of PS1 preparations was kept for several hours in a gas-tight chamber in a nitrogen atmosphere containing no molecular oxygen.

RESULTS AND DISCUSSION

Both kinetics of electron transfer induced by single short light pulses in dark-adapted PS1 RC and temperature dependence of these kinetics have been studied by many researchers (see [9-11] and references therein). It was found that the process of dark reduction of photooxidized P700 within the temperature range from room to cryogenic temperatures contained several kinetic components with characteristic times ranging from ~200 μsec to tens of milliseconds. The relative amplitudes of the kinetic components depend on temperature. According to results reported in [9], at T < 150 K reaction centers in PS1 cooled in the dark fall into three populations, which can be regarded as evidence that at T below 150 K these complexes are fixed in three different conformational substates. For example, phylloquinone was found to be the terminal acceptor of photomobilized electron in about 45% of reaction center complexes. The time of dark recombination of oxidized chlorophyll P700 and photoreduced phylloquinone at 150 K is about 170 μsec. In ~20% of reaction center complexes electron photomobilized after photoactivation of sample is transferred to the iron-sulfur cluster F_X. The process of dark reduction of photooxidized $P700^+$ in this case is characterized by multiphase kinetics with characteristic times of corresponding components ranging from 5 to 100 msec. In ~35% of reaction center complexes photoactivation of a sample induces electron transfer to the iron-sulfur centers F_A and F_B, where it is stabilized for a time interval larger than the time interval between excitation light pulses used in [9], i.e., 0.5-1 Hz.

In contrast to the kinetic pattern observed after photoactivation of samples with single light pulses, kinetics of $P700^+$ reduction in preparations frozen in the dark and activated with continuous light is substantially slower (Fig. 1a). There are some kinetic components of this

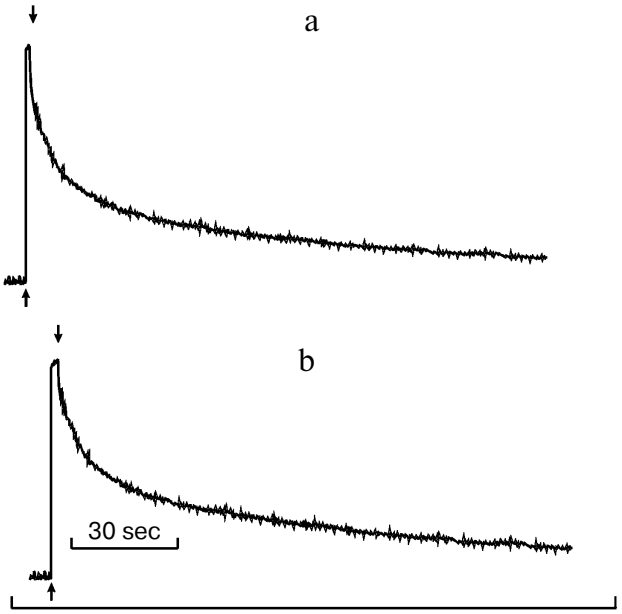

Fig. 1. a) Kinetics of photoconversion of P700 induced by continuous light (3-4 sec) in PS1 RC preparations of *Synechocystis* PCC 6803 cooled in the dark to 160 K; b) kinetics of photoconversion of P700 induced by continuous light in the PS1 RC fraction of the sample cooled in the light to 160 K with retained photochemical activity. Upward and downward arrows indicate moments of actinic light on and off, respectively.

process with characteristic time of up to several minutes and even longer. The characteristic time of the fastest kinetic component of this process is no shorter than one-tenth of one second. It should be noted that we studied kinetics of P700 photoconversion after RC activation with continuous light at 160 K both in aerobic and anaerobic conditions, because it is well known that a fraction of iron-sulfur centers is oxidized in the presence of molecular oxygen. To remove oxygen from samples, suspension of PS1 RC was kept for several hours in a nitrogen atmosphere. Experimental samples in this case also contained a cryoprotector (glycerol, 50% by volume). In spite of this difference in details of experimental conditions, kinetic curves of dark reduction of $P700^+$ after exposure to actinic light at 160 K proved to be very similar in samples of all types. Slow kinetics of dark reduction of $P700^+$, similar to that measured in our experiments, was also observed earlier in preparations of PS1 cooled in the dark to 150 K and exposed to continuous actinic light [12]. The fact that the process of dark reduction of $P700^+$ after activation with continuous light was significantly slower than after pulse photoactivation was interpreted in [12] as a consequence of the light-induced structural transitions in the acceptor site of PS1 RC. This suggestion was confirmed by conformational changes of centers F_A and F_B observed under these conditions using EPR [12].

Cooling of the *Synechocystis* PCC 6803 PS1 preparations to 160 K under exposure to actinic light in our experiments fixed the majority of the RC complexes of the sample in the state with "irreversibly" oxidized P700. The magnitudes of the level of oxidation measured in samples cooled in the light to 160 K and subjected to 10- or 40-min dark relaxation (actinic light off) differed from each other by no more than 2-3%. This level of "irreversibly" oxidized P700 in aerobic samples without or with cryoprotector was 80 or 80-85%, respectively. In anaerobic samples without or with glycerol, this level was 70 or 75%, respectively.

In other words, cooling of PS1 RC preparations in the light and cooling of RC preparations from purple bacteria in the light causes similar effects of fixation of a large fraction of photochemically active pigment in the oxidized state. On the other hand, the kinetics of the dark reduction of P700 oxidized by continuous light in the PS1 RC fraction with retained photochemical activity was essentially indistinguishable from the kinetics of the dark reduction of P700 in samples cooled in the dark (Fig. 1b). It was noted above that RC from purple bacteria cooled in the dark or in the light were characterized by different patterns of kinetics of dark reduction of photooxidized bacteriochlorophyll, which corresponded to pathways of electron transfer to P^+ from Q_A^- or Q_B^-, respectively.

Perhaps, similarity between the kinetic pattern of dark reduction of $P700^+$ in the PS1 RC fraction with retained photochemical activity after cooling in the light with such pattern of dark reduction of $P700^+$ in the PS1 RC cooled in the dark can be regarded as evidence that "irreversible" trapping of electrons in a fraction of reaction centers cooled in the light occurs with different rates at different sites of the acceptor side of PS1 RC. Indeed, it was demonstrated in [9] that freezing in the dark gave rise to stratification of reaction centers and formation of three populations corresponding to three conformational substates with different terminal acceptors of electrons. Therefore, the similarity between the kinetic patterns discussed above can be explained assuming that electrons are "irreversibly" trapped in three populations.

The results obtained in our experiments with the mutant *Synechocystis* rubA$^-$ can also be regarded as evidence of the possibility of "irreversible" trapping of photomobilized electron in different fractions of the acceptor site of reaction centers cooled in the light. The reaction centers of this mutant contain no iron-sulfur centers F_X, F_A, and F_B. The terminal electron acceptor in the mutant RC is phylloquinone. Our experiments revealed the following results. In anaerobic samples of *Synechocystis* rubA$^-$ RC cooled in the dark to 160 K, there was no light-induced signal of P700 cation-radical after excitation with continuous light at 160 K (Fig. 2). Perhaps the intensity of the actinic light flux in these experiments was insufficient to maintain the extent of oxidation of P700 at a stationary level (during illumination) because of very fast recombination between photoreduced phylloquinone and $P700^+$. Indeed, even if the incident light intensity at the level of the spectrometer cavity and wavelength 700 nm (absorption maximum of P700) is 1 kW/m^2, some ~10^{18} photons are incident per 1 cm^2 per 1 sec. At RC concentration ~1 μM, the volume of 1 cm^3 of RC prepa-

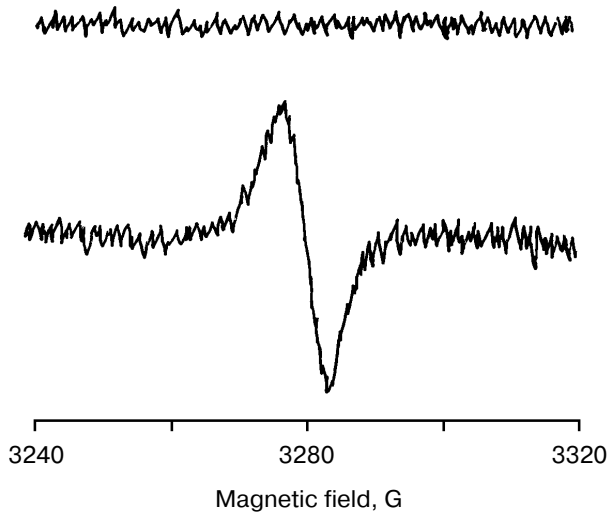

Fig. 2. Record of cation-radical signal of P700 in the PS1 RC preparations of the mutant *Synechocystis* rubA$^-$ cooled to 160 K. Top curve corresponds to sample cooled in the dark and then exposed to continuous actinic light. Bottom curve corresponds to sample cooled under exposure to continuous actinic light.

ration contains ~10^{15} molecules. Even if all quanta were absorbed by RC, one RC molecule would be able to trap 10^3 photons per 1 sec. On the other hand, the characteristic time of electron transfer from reduced phylloquinone to $P700^+$ in the dark is ~100 μsec. However, it is interesting to note that signal of cation-radical P700 was detected in sample cooled to 160 K under exposure to actinic light (Fig. 2). The amplitude of the signal was maintained at a constant level for about 10 min after illumination at 160 K had been turned off. In other words, cooling of the mutant *Synechocystis* rubA$^-$ RC samples in the light brought at least a fraction of the RC complexes to a certain conformational state in which the electron was unable to return back to $P700^+$ after the actinic light was turned off.

Thus, it follows from the results obtained in this work that the process of light-induced charge separation in PS1 RC, like in RC of purple bacteria, is also accompanied by conformational changes that can be observed under special experimental conditions. Such a similarity is thought to represent evolutional closeness between the two types of RC. According to the presently accepted viewpoint, reaction centers of all photosynthetic organisms originate from one common precursor. In particular, this view is consistent with the results of the X-ray diffraction analysis, which indicate that the structure of the PS1 RC core (helical protein fragments associated with electron transfer cofactors) is very similar to the structure of subunits D1 and D2 of PS2 RC, which are evolutional homologs of subunits L and M in RC of purple bacteria [13].

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