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# Reduction of Photosystem I Reaction Center by Recombinant DrgA Protein in Isolated Thylakoid Membranes of the Cyanobacterium *Synechocystis* sp. PCC 6803

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**Abstract**—To study the function of soluble NAD(P)H:quinone oxidoreductase of the cyanobacterium *Synechocystis* sp. PCC 6803 encoded by *drgA* gene, recombinant DrgA protein carrying 12 histidine residues on the C-terminal end was expressed in *Escherichia coli* and purified. Recombinant DrgA is a flavoprotein that exhibits quinone reductase and nitroreductase activities with NAD(P)H as the electron donor. Using EPR spectroscopy, it was demonstrated that addition of recombinant DrgA protein and NADPH to DCMU-treated isolated thylakoid membranes of the cyanobacterium increased the dark re-reduction rate of the photosystem I reaction center ($P700^+$). Thus, DrgA can participate in electron transfer from NADPH to the electron transport chain of the *Synechocystis* sp. PCC 6803 thylakoid membrane.

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*Key words*: cyanobacteria, *drgA* gene, NAD(P)H:quinone oxidoreductase, cyclic electron transport around photosystem I, EPR spectroscopy

The thylakoid membrane of cyanobacteria contains both the photosynthetic electron transport chain (ETC) including two photosystems (PSI and PSII), plastoquinone (PQ) pool, cytochromes $b_6f$ complex, a number of soluble electron carriers, and the whole respiratory ETC including the NDH-1 complex, succinate dehydrogenase, and cytochrome and quinol oxidases. The PQ pool, cytochromes $b_6f$ complex, and soluble electron transporters are a common part of the photosynthetic and respiratory ETC [1]. Electrons can move to the PQ pool from both PSII and cytoplasm substrates, and transfer from plastoquinol to both PSI and terminal oxidases, depending on which acceptor is the most oxidized under certain conditions of cell growth [2].

The NDH-1 complex, which is a proton-translocating NADPH:quinone oxidoreductase, takes part in both respiration and photosynthetic cyclic PSI-dependent transport of electrons [3]. The subunit composition of cyanobacterial complex significantly resembles that of the *E. coli* NDH-1 complex, but it lacks three subunits corresponding to the NuoE, F, and G subunits of the *E. coli* NDH-1 complex. These subunits of NDH-1 bacterial complexes contain NAD(P)H and FMN (flavin mononucleotide) binding sites as well as a number of Fe-S clusters essential for catalytic activity of the complex [4].

The nature of the catalytic domain of the cyanobacterial NDH-1 complex is still unclear. A number of proteins (or protein complexes) with NAD(P)H dehydrogenase activity including ferredoxin:$NADP^+$ oxidoreduc-

*Abbreviations*: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol indophenol; decylplastoquinone, 2,3-dimethyl-6-decyl-1,4-benzoquinone; dinoseb, 2-*sec*-butyl-4,6-dinitrophenol; duroquinone, tetramethyl-1,4-benzoquinone; ETC, electron transport chain; Fd, ferredoxin; FMN, flavin mononucleotide; FNR, ferredoxin:$NADP^+$-oxidoreductase; IPTG, isopropyl-β-D-thiogalactopyranoside; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; PQ, plastoquinone; PS, photosystem; $Q_0$, 2,3-dimethoxy-5-methyl-1,4-benzoquinone (ubiquinone $Q_0$); SOD, superoxide dismutase.

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tase (FNR) and the 23 kDa protein encoded by the *drgA* gene was found in a membrane fraction of the cyanobacterium *Synechocystis* sp. PCC 6803 (further *Synechocystis* 6803) [5]. The *drgA* gene was first cloned as a gene responsible for resistance to the nitrophenolic herbicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) (*d*inoseb *r*esistance *g*ene) [6, 7]. The gene encodes soluble NAD(P)H: quinone oxidoreductase [8], which also has nitroreductase activity [7, 9]. According to the Pfam (http://pfam.sanger.ac.uk/family/) and InterPro (http://www.ebi.ac.uk/interpro/) databases, the DrgA protein belongs to the nitroreductase family (PF00881, IPR000415) of enzymes able to reduce the $NO_2$ group of nitroaromatic compounds. Nitroreductase activity is typical for oxygen-insensitive NAD(P)H nitroreductases (EC 1.6.99.7), for a number of NADH dehydrogenases (EC 1.6.99.3), and several oxidoreductases (e.g. DT-diaphorase; EC 1.6.99.2). DrgA reduces quinones and nitroaromatic compounds (e.g. dinoseb) by a two-electron mechanism using NADPH and, with lower efficiency, NADH as the electron donor [7-10].

As we demonstrated earlier, the *drgA* gene product influences electron transport in thylakoid membranes of cyanobacteria. In the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which inhibits transport of electrons from PSII, the re-reduction rate of the light-oxidized PSI reaction center (P700$^+$) was significantly lower in the *drgA* mutant cells than in wild type cells. These data suggest that the DrgA may participate in regulation of electron transport in thylakoid membranes of cyanobacteria, transferring electrons from NADPH to the PQ pool [11].

Model experiments with purified protein are essential for proving that DrgA does participate in photosynthetic or respiratory electron transport in *Synechocystis* 6803. For this goal, the *drgA* gene was expressed in *E. coli* using the pTrc99A plasmid, and recombinant DrgA protein carrying 12 histidine residues on the C-terminal end was purified. The influence of the purified protein on the dark re-reduction rate of the PSI reaction center (P700$^+$) after its oxidation with a white light pulse was studied in isolated *Synechocystis* 6803 thylakoid membranes.

## MATERIALS AND METHODS

**Strains, plasmids, and growth conditions.** Strains *E. coli* DH5α (F$^-$ *gyrA96* (Nal$^r$) *recA1 relA1 endA1 thi-1 hsdR17* ($r_k^- m_k^-$) *glnV44 deoRΔ (lacZYA argF) U169* [ϕ80d Δ(*lacZ*) *M15*]) and BL21(DE3) (F$^-$, *omp*T, *hsd*$S_B$, ($r_B^- m_B^-$), *gal*, *dcm* (DE3)) (Novagen, USA) and wild type strain and the *drgA* mutant (Ins2) [6] of the cyanobacterium *Synechocystis* sp. PCC 6803 taken from the collection of the Department of Genetics of Moscow State University were used. *Escherichia coli* cells were cultivated for 15-18 h at 37°C in LB medium [12]. Bacteriologically pure cultures of the cyanobacteria were incubated for 5 days at 30°C and 40 μE·m$^{-2}$·sec$^{-1}$ of continuous lighting in BG-11 liquid mineral medium [13]. The Ins2 mutant cells were cultivated in the presence of kanamycin (100 μg/ml). Clones of the *E. coli* transformants were cultivated in LB medium containing ampicillin (100 μg/ml). The pTrc99A plasmid was used as a vector for cloning and expression of the *drgA* gene [14].

**Genetic engineering.** Plasmid DNA was purified using a QIAprep Spin Miniprep Plasmid Kit (Qiagen, USA). Molecular cloning, determinations of the nucleotide sequences and PCR (polymerase chain reaction) were carried out by standard methods [12]. The PCR products were separated in 1% agarose gel. *Escherichia coli* cells were transformed using plasmid DNA by a standard method [14]. Chromosome DNA was extracted from the wild type *Synechocystis* 6803 by a method described earlier [15].

**Construction of pTrc-DrgA12His recombinant plasmid.** A fragment of the chromosomal DNA of the wild type *Synechocystis* 6803 carrying the *drgA* (*slr1719*) gene was amplified by PCR using the following primers: Fw1 (5′-CCA CCC ATG GAC ACC TTT GAC GCT ATT TAC C-3′) and Rev1 (5′-CCA CTC GAG GGC AAA GGA GTT TTC CCA GAC C-3′). The Fw1 primer was used for insertion of the *Nco*I site before the *drgA* template, and the Rev1 primer was used for insertion of the *Xho*I site after the *drgA* gene. A 620-bp PCR product was separated from gel and purified using the QIAquick Gel Extraction Kit (Qiagen).

Synthetic oligonucleotide duplex encoding 12 histidine residues was joined to the 3′-end of the *drgA* gene at the first stage of the cloning. For this goal, the purified PCR fragment was cleaved by the *Bam*HI and *Xho*I endonucleases. The reaction products were separated in agarose gel, 448 bp band of the *drgA* 3′-end region was separated from the gel and ligated with the pTrc99A plasmid DNA treated with *Bam*HI and *Hind*III together with the synthetic oligonucleotide 12HisF–12HisR duplex (12HisF: 5′-TCG AGC ATC ACC ATC ACC ATC ACC ATC ACC ATC ACC ATC ACT AAA-3′; 12HisR: 5′-AGC TTT TAG TGA TGG TGA TGG TGA TGG TGA TGG TGA TGG TGA TGC-3′) carrying *Xho*I and *Hind*III sites. The resulting recombinant plasmids were amplified in *E. coli* DH5α and the insertion was sequenced.

To separate the *drgA* 5′-end region, the PCR fragment carrying the *drgA* gene was cleaved by *Nco*I and *Bam*HI endonucleases. The reaction products were separated in agarose gel and a 155 bp band was eluted. Since the *drgA* 3′-end region contains two supplementary *Nco*I sites, insertion of the 5′-end *Nco*I/*Bam*HI fragment of the *drgA* was carried out in two stages. One DNA portion of the pTrc99A recombinant plasmid carrying the *drgA* 3′-end region and the 12HisF–12HisR duplex was cleaved by *Nco*I and *Mlu*I, and a 1036 bp fragment (not carrying

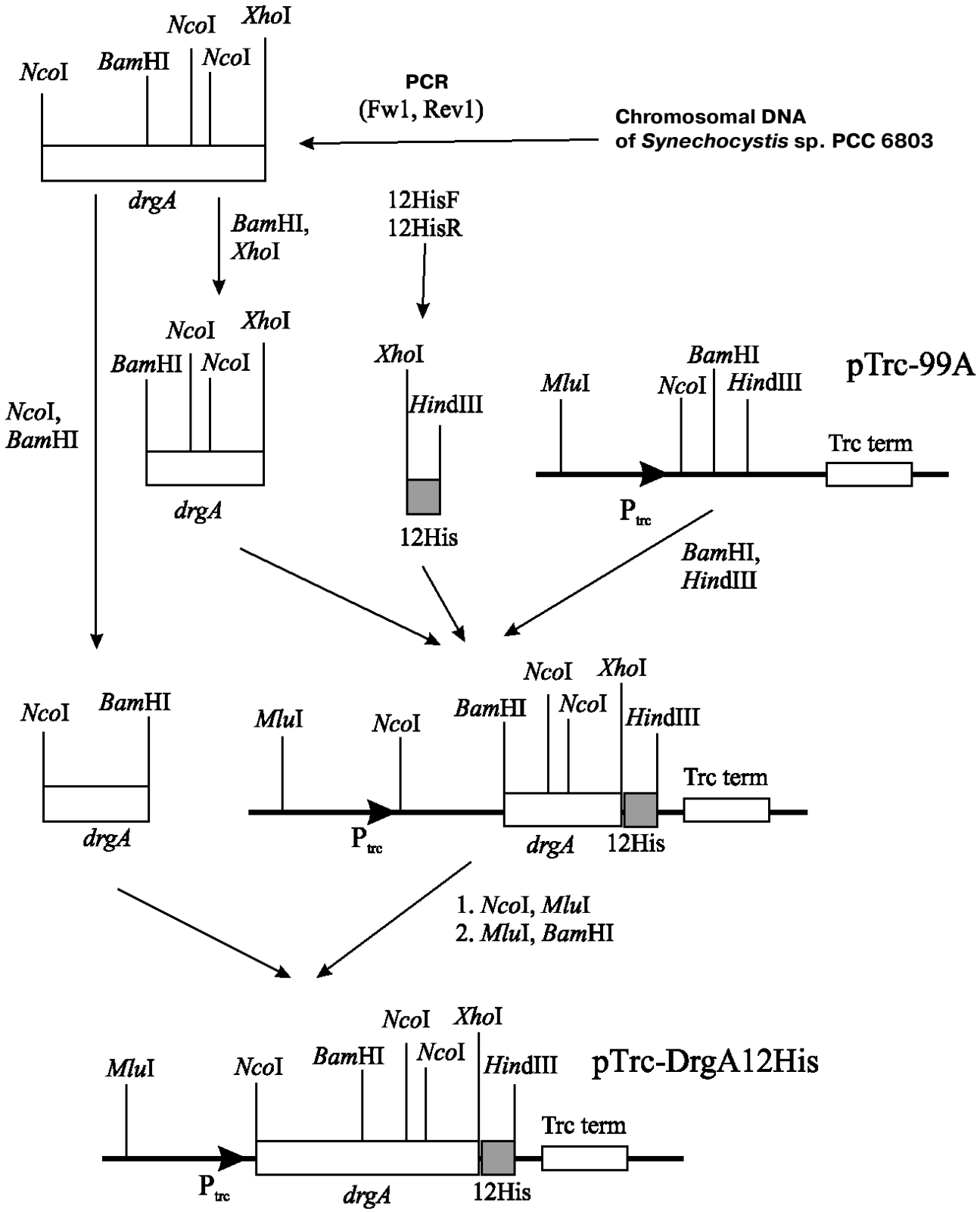


**Fig. 1.** Construction of pTrc-DrgA12His recombinant plasmid. $P_{trc}$, promoter; Trc term, transcription terminator; 12His, synthetic oligonucleotide duplex encoding 12 histidine residues.

supplementary sites for *Nco*I) was eluted; another portion was cleaved by unique *Mlu*I and *Bam*HI sites, and a 3770 bp fragment was eluted from the gel. Both fragments were ligated with the 5′-end *Nco*I/*Bam*HI fragment of *drgA*. The resulting recombinant plasmids were amplified in *E. coli* DH5α, and the insertion was sequenced. Construction of pTrc-DrgA12His recombinant plasmid is demonstrated in Fig. 1.

**Heterologous expression and purification of recombinant DrgA protein.** For preparative protein purification competent cells of *E. coli* BL21(DE3) were transformed with the sequenced plasmid. Single colonies of the transformants were cultivated in LB medium (5 ml) containing ampicillin. After overnight cultivation, the cells were transferred into 250 ml of fresh medium and incubated at 37°C until the optical absorption reached $A_{560}$ = 0.8. Expression was induced adding 0.2 mM IPTG (isopropyl-β-D-thiogalactopyranoside). After 16 h incubation with IPTG, the cells were collected by centrifugation and frozen at –70°C.

The recombinant DrgA protein was purified by metal chelate chromatography. The frozen cells were thawed in an ice bath, resuspended in cold buffer I (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.001% phenylmethylsulfonyl fluoride (PMSF)) adding 10 ml of buffer I per gram of cells, and incubated on ice for 15 min. The cells were centrifuged for 10 min at 8000*g*, and then supernatant cell debris was centrifuged at 15,000*g* for 15 min. For purification under native conditions, supernatant containing DrgA protein labeled with 12 histidine residues was incubated for 1 h at 25°C with Ni-Sepharose. Sepharose together with bound protein were transferred onto a column (Bio-Rad, USA) and flushed with two volumes of buffer I and with two volumes of the buffer containing 50 mM $NaH_2PO_4$ (pH 7.5), 50 mM NaCl, 20 mM imidazole, and 0.001% PMSF. The protein was eluted with four volumes of the buffer containing 50 mM $NaH_2PO_4$ (pH 7.5), 50 mM NaCl, 300 mM imidazole, and 0.001% PMSF. Fractions of 0.5 ml were collected, and protein content was analyzed by SDS-PAGE [16]. After the analysis, the fractions containing DrgA protein were dialyzed from buffer containing 50 mM $NaH_2PO_4$ (pH 7.5), 50 mM NaCl, and 0.001% PMSF and concentrated by ultrafiltration using Amicon PM10 membranes (Millipore, USA). The purified protein was kept at –70°C.

***Synechocystis* 6803 membrane fraction** was isolated by a method described earlier [11].

**Enzyme activity measurements.** *NAD(P)H oxidase activities* of recombinant DrgA protein were measured at 30°C estimated by NAD(P)H decrease in medium containing 0.1 mM NAD(P)H, 20 mM Tris-HCl, pH 7.5, 40 nM of the DrgA protein, and electron acceptors—0.1 mM $Q_0$ (2,3-dimethoxy-5-methyl-1,4-benzoquinone (ubiquinone 0)), 0.1 mM duroquinone (tetramethyl-1,4-benzoquinone), 0.05 mM decylplastoquinone (2,3-dimethyl-6-decyl-1,4-benzoquinone), 0.05 mM nitrofurazon, or 0.1 mM potassium ferricyanide (spectrophotometric detection at 340 nm, $\varepsilon_{mM}$ = 6.2 $mM^{-1}\cdot cm^{-1}$). *NAD(P)H:dinoseb reductase activity* was measured in the same medium containing 0.05 mM dinoseb estimated by optical absorption increase (spectrophotometric detection at 470 nm, $\varepsilon_{mM}$ = 6.9 $mM^{-1}\cdot cm^{-1}$). *NAD(P)H: cytochrome c reductase activity* was measured in the same medium containing 0.05 mM cytochrome *c* in the presence and in the absence of superoxide dismutase (SOD; 3 U/ml) estimated by optical absorption increase (spectrophotometric detection at 550 nm, $\varepsilon_{mM}$ = 20.0 $mM^{-1}\cdot cm^{-1}$). *The rate of superoxide radical generation* was determined by the difference between total measured NAD(P)H:cytochrome *c* reductase activity and activity in the presence of superoxide dismutase. *NAD(P)H:DCPIP reductase activity* was estimated by optical absorption attenuation in the same medium containing 0.05 mM DCPIP (2,6-dichlorophenol indophenol) (spectrophotometric detection at 600 nm, $\varepsilon_{mM}$ = 20.0 $mM^{-1}\cdot cm^{-1}$).

**EPR spectroscopy.** The method for analyzing P700 redox transformations was described earlier [11]. A 0.035-ml sample of the membrane preparation was loaded into a 0.1 ml quartz cell at chlorophyll concentration about 30 μg/ml. EPR signal I was generated by a white light pulse (2000 $\mu E\cdot m^{-2}\cdot sec^{-1}$, 80 msec). After the illumination, the cell was kept in the dark for 11 sec and then illuminated again. This protocol for P700$^+$ signal registration was repeated, and the final signal was the sum of 30-50 individual light signals. P700$^+$ concentration was determined by comparison of the signal I amplitude with the amplitude of the signal of 0.02 mM nitroxide radical TEMPO (Sigma, USA) [17]. DCMU (20 μM), NADPH (100 μM), and purified DrgA protein were added to the cell suspension 5 min before measuring the EPR spectra. Data averaging and exponential analysis of the dark attenuation curves of the EPR signal were performed using OriginPro 7.5 software.

**Spectra of absorption and fluorescence** were registered at room temperature using modified spectrophotometers Hitachi-557 and Hitachi-850 (Hitachi, Japan), respectively. The spectral width of the absorption spectrophotometer was 4 nm and that of the fluorimeter was 5 nm for both excitation and emission.

**Internet resources.** For amino acid sequence analysis, CyanoBase (http://genome.kazusa.or.jp/cyanobase), BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi/), Pfam (http://pfam.sanger.ac.uk/family/), and InterPro (http://www.ebi.ac.uk/interpro/) databases were used.

## RESULTS

**Cloning and expression of *Synechocystis* 6803 *drgA* gene in *E. coli* cells.** To study function, the *drgA* gene was

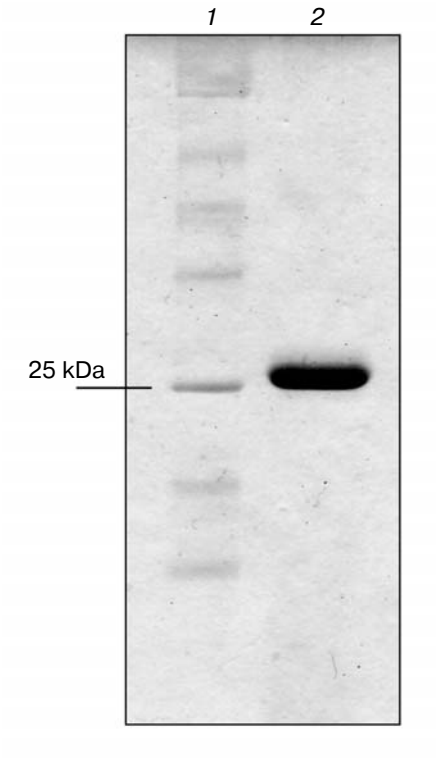


**Fig. 2.** SDS-PAGE analysis of recombinant DrgA protein purified by metal chelate chromatography: *1*) marker of protein molecular mass (SM0431; Fermentas, Lithuania); *2*) recombinant DrgA protein.

expressed in *E. coli* cells. A chromosomal DNA fragment of the cyanobacterium carrying the whole *drgA* gene was cloned in pTrc99A expression vector. For the subsequent purification of expressed protein, the sequence encoding 12 histidine residues was ligated to the 3′-end of the *drgA* gene (see "Materials and Methods" and Fig. 1).

The cyanobacterial protein was expressed in different *E. coli* strains; the expression maximum was reached after induction by 0.1 mM or higher concentrations of IPTG (data not shown). Recombinant DrgA protein carrying 12 histidine residues on the C-terminal end was obtained after purification by nickel affinity chromatography (Fig. 2).

**Properties of recombinant DrgA protein.** The absorption spectrum of purified DrgA protein showed wide absorption bands typical for flavoproteins in the near UV and visible regions of the spectrum near 350 and 460 nm (Fig. 3a). The spectra of fluorescence (Fig. 3b) and fluorescent excitation (Fig. 3c) of the DrgA protein are also similar to those measured in flavoprotein solutions [18, 19]. It was shown earlier by HPLC analysis that the DrgA isolated from *Synechocystis* 6803 contains FMN as a prosthetic group [9]. Recombinant DrgA expressed in *E. coli* as well as native DrgA seems to contain FMN.

Recombinant DrgA shows similarity in substrate specificity to both endogenous DrgA and recombinant DrgA(6His) described earlier [8, 9]. The protein uses NADPH and NADH as electron donors, showing higher activity with NADPH. Quinones are preferred electron acceptors. Moreover, recombinant DrgA as well as the endogenous protein [9] is able to reduce nitroaromatic compounds (dinoseb, nitrofurazon) as well as ferricyanide and DCPIP (Table 1). The enzyme also catalyzes the formation of superoxide radical at a rate of 0.2 μmol/min per mg (Table 1, determined by the difference between cytochrome *c* reduction rates in the presence and in the absence of SOD).

Maximal activity of DrgA ($A_{max}$) and Michaelis constants ($K_m$) for NADH and NADPH were determined in the NAD(P)H:$Q_0$-reductase reaction at 100 μM $Q_0$ (saturating concentrations). Using NADH

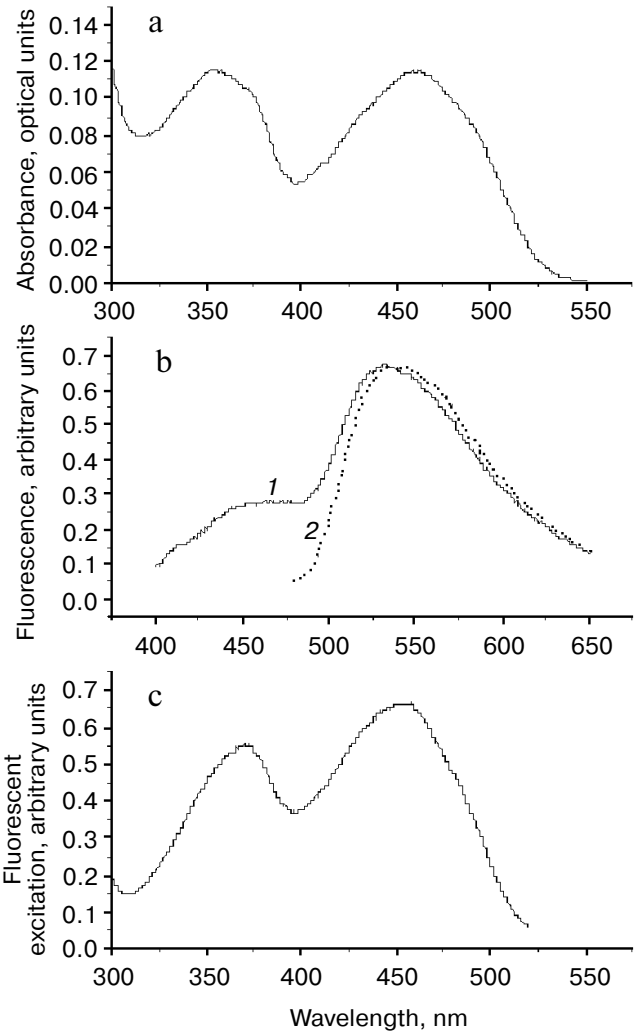


**Fig. 3.** Spectra of aqueous solution of DrgA protein: a) absorption spectrum; b) fluorescence spectrum with (*1*) 360 nm excitation and (*2*) 450 nm excitation; c) fluorescent excitation spectrum of aqueous solution of DrgA protein at 550 nm emission wavelength. All measurements were carried out in a 5 × 5 × 40 mm quartz cell at room temperature.

**Table 1.** Specificity of recombinant DrgA toward electron acceptors

| Acceptor | Activity, μmol/min per mg protein* | |
|---|---|---|
| | NADH (100 μM) | NADPH (100 μM) |
| Decylplastoquinone, 50 μM | 7.2 | 12.8 |
| Ubiquinone $Q_0$, 100 μM | 7.4 | 10.6 |
| Duroquinone, 100 μM | 7.0 | 10.3 |
| DCPIP, 50 μM | 1.4 | 0.4 |
| Cytochrome *c*, 50 μM | 0.4 | 0.4 |
| Cytochrome *c*, 50 μM, in the presence of SOD** | 0.2 | 0.2 |
| Dinoseb, 50 μM | 1.3 | 3.0 |
| Nitrofurazon, 50 μM | 8.4 | 6.2 |
| Ferricyanide, 100 μM | 1.9 | 2.0 |

* Activities were determined as described in "Materials and Methods".
** SOD concentration in measuring medium was 3 U/ml.

and NADPH as electron donors, $A_{max}$ magnitudes were 17 and 63 μmol/min per mg DrgA protein, respectively. $K_m$ values for NADH and NADPH were about 60 μM.

**Influence of recombinant DrgA on dark re-reduction rate of PSI reaction center (P700$^+$) after its oxidation with a white light pulse in isolated thylakoid membranes of *Synechocystis* 6803.** Purified DrgA was used in experiments studying light-induced redox transformations of P700 in isolated thylakoid membranes of *Synechocystis* 6803 by EPR spectroscopy. To remove an influence of endogenous DrgA, membrane fraction was isolated from Ins2 mutant cells with defective *drgA* gene [6].

Time-dependent redox transformations of the P700 induced by white light pulses in isolated membranes of *Synechocystis* 6803 are represented at Fig. 4. Rapid increase of the P700$^+$ signal amplitude was observed on illumination, indicating P700 photooxidation. The P700$^+$ concentration (signal I amplitude compared with amplitude of TEMPO nitroxyl radical) was 0.5 μM. Due to the P700$^+$ dark reduction by electron transfer from the PQ pool to PSI, the P700$^+$ signal is progressively attenuating after turning off the light. The reduction of P700$^+$ after illumination in isolated *Synechocystis* 6803 thylakoid membranes was well described by a sum of two exponentially decreasing curves with time constants of 60 msec ($\tau_1$) and 1.5 sec ($\tau_2$) (Fig. 4, curve *1*). The relative contributions of the amplitudes of the rapid ($A_1$) and slow ($A_2$) phases of the reduction were 57 and 43%, respectively (Table 2).

In the presence of DCMU, blocking electron transfer from the PSII to the PQ pool, the rates of both the rapid and slow phases of the P700$^+$ signal decay significantly decreased ($\tau_1 > 0.1$ sec, $\tau_2 > 6$ sec) and the contribution of the rapid phase in total amplitude decreased (Fig. 4, curve *2*). Addition of DrgA or NADPH to thylakoid membranes treated with DCMU did not increase the rate of the P700$^+$ reduction after its light-induced for-

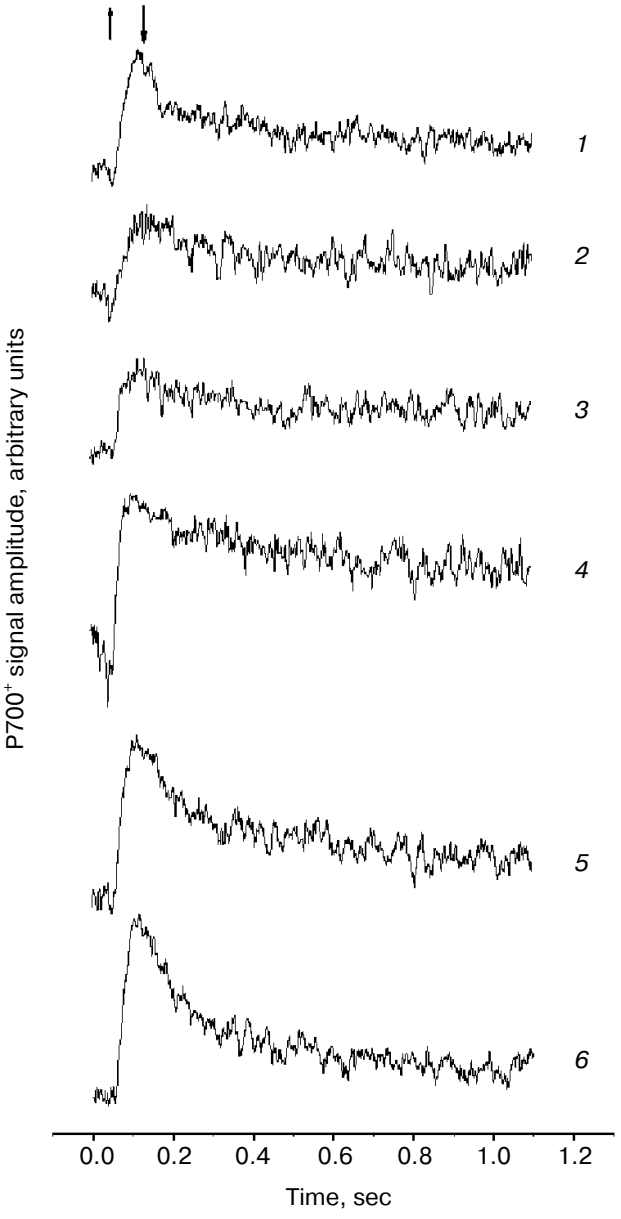


**Fig. 4.** Time-dependent redox transformations of P700 induced by white light pulses in isolated membranes of Ins2 mutant: *1*) without DCMU; *2*) 20 μM DCMU; *3*) 20 μM DCMU, 5 μM DrgA; *4*) 20 μM DCMU, 100 μM NADPH; *5*) 20 μM DCMU, 100 μM NADPH, 2.5 μM DrgA; *6*) 20 μM DCMU, 100 μM NADPH, 5 μM DrgA. EPR signal I was generated by white light (2000 μE·m$^{-2}$·sec$^{-1}$) pulses (80 msec). Turning off and on of the light are designated by vertical arrows.

**Table 2.** Influence of DCMU, NADPH, and DrgA on amplitude ($A$) and time ($\tau$) of $P700^+$ signal attenuation after illumination in isolated thylakoid membranes of *Synechocystis* 6803 (estimated by the results of averaging and biexponential fitting of the signal attenuation curves after illumination from Fig. 4 using OriginPro 7.5 software)*

| Experiment type | $A_1$, arbitrary units | $\tau_1$, sec | $A_2$, arbitrary units | $\tau_2$, sec |
|---|---|---|---|---|
| Control | 0.57 ± 0.02 | 0.06 ± 0.003 | 0.43 ± 0.01 | 1.48 ± 0.08 |
| DCMU | 0.50 ± 0.05 | 0.12 ± 0.03 | 0.50 ± 0.06 | 6.2 ± 1.6 |
| DCMU, DrgA (5 μM) | 0.42 ± 0.04 | 0.1 ± 0.02 | 0.58 ± 0.03 | 6.3 ± 3.1 |
| DCMU, NADPH | 0.42 ± 0.01 | 0.31 ± 0.03 | 0.58 ± 0.01 | >6 |
| DCMU, NADPH, DrgA (2.5 μM) | 0.52 ± 0.02 | 0.09 ± 0.005 | 0.48 ± 0.01 | 1.23 ± 0.07 |
| DCMU, NADPH, DrgA (5 μM) | 0.67 ± 0.01 | 0.08 ± 0.004 | 0.33 ± 0.01 | 1.1 ± 0.08 |

* Curves of $P700^+$ signal attenuation after illumination shown in Fig. 4 are represented as a sum of two exponentials where $A_1$ and $A_2$ are the amplitudes of the rapid and slow phases given in relative units ($A_1 + A_2 = 1$); $\tau_1$ and $\tau_2$, attenuation time (in seconds) of rapid and slow phases of $P700^+$ signal decay, respectively.

mation (Fig. 4, curves *3* and *4*). The total amplitude of the $P700^+$ signal in the presence of NADPH, however, significantly increased, apparently due to the presence of the complementary electron acceptor $NADP^+$ (Fig. 4, curve *4*).

Simultaneous addition of DrgA and NADPH to membranes treated with DCMU significantly increased the rates of both phases of the $P700^+$ reduction after its oxidation by light; at the same time the contribution of the rapid reduction phase in total amplitude of the $P700^+$ signal appreciably increased. The maximal effect was reached at 5 μM DrgA (Fig. 4 (curves *5* and *6*) and Table 2). Further increase in DrgA concentration did not lead to any change of a rate of the $P700^+$ signal decay after its photooxidation.

Our data show that recombinant DrgA in the presence of electron donor NADPH does participate in reduction of PSI reaction center oxidized by light. Thus, the properties of the recombinant protein indicate that NADPH:quinone oxidoreductase encoded by the *drgA* gene is able to transfer electrons from NADPH to the PQ pool of the cyanobacterium thylakoid membrane.

The results of changes in amplitude and time of dark decay of the $P700^+$ signal, calculated using OriginPro 7.5 software, are summarized in Table 2.

## DISCUSSION

In the present work we expressed in *E. coli* the DrgA protein of the cyanobacterium *Synechocystis* 6803 carrying 12 histidine residues on the C-terminal end. In its properties and substrate specificity, the recombinant DrgA does not differ from the DrgA protein isolated earlier [9]: it contains flavin as a prosthetic group and has quinone reductase and nitroreductase activities.

We demonstrated earlier that a mutation in the *drgA* gene suppresses the ability of *Synechocystis* 6803 cells to oxidize NADPH with concomitant decrease of the $P700^+$ re-reduction rate in the presence of DCMU in intact cells of the cyanobacterium, as well as decrease in the ability of the cells to reduce the $P700^+$ with electrons from exogenous glucose [11]. These results indicate a significant role of DrgA in the regulation of $NADP^+$/NADPH ratio in the cyanobacterial cells and indirectly show the possible transfer of electrons to the PQ pool of thylakoid membrane by this protein. In the present work, we have demonstrated that there is a recombinant DrgA-mediated electron transfer from NADPH to the ETC of isolated *Synechocystis* 6803 thylakoid membrane. Thus, NAD(P)H:quinone oxidoreductase encoded by the *drgA* gene can directly participate in respiratory and/or cyclic photosynthetic electron transport around PSI.

The pathways by which electrons are transferred from the acceptor part of PSI to the PQ pool of thylakoid membrane in cyclic transport through PSI are poorly studied. A pathway that is ferredoxin (Fd)-dependent and inhibited by antimycin and in which the hypothetical enzyme ferredoxin:plastoquinone oxidoreductase (FQR) functions is thought to occur in cyanobacteria and chloroplasts [20]. The *pgr5* mutant, in which Fd-dependent cyclic electron transport through PSI inhibited by antimycin is interrupted, was isolated from *Arabidopsis thaliana* [21]. A protein with homologous amino acid sequence encoded by the *ssr2016* gene was found in *Synechocystis* 6803. The Ssr2016 protein, as well as PGR5, is supposed to be essential for FQR activity [22].

The NADPH-dependent pathway of PQ pool reduction, in which NDH-1 complex participates, is known in cyanobacteria and chloroplasts along with Fd-dependent cyclic electron transport through PSI. Respiratory and cyclic NADPH-dependent electron transport around PSI are interrupted in the M55 mutant with defective *ndhB* gene [3]. Despite significant homology of the NDH-1 complex subunits of cyanobacteria and *E. coli*, the

NAD(P)H oxidizing domain of the cyanobacterial complex has not been identified yet. The data obtained so far suggest that NAD(P)H:quinone oxidoreductase encoded by the *drgA* gene, which is able to transfer electrons from NADPH to the ETC of thylakoid membranes, can function as the NAD(P)H oxidizing domain in *Synechocystis* 6803. At the same time, the properties of purified DrgA protein indicate its ability to transfer electrons independently to quinone acceptors as well as, by all appearances, to the PQ pool.

It should be noted that DrgA has nitroreductase activity and belongs to the nitroreductase family. These proteins are found in various organisms. A BLAST search showed that the most homologous to the DrgA proteins are found in bacteria and archaea. Besides phototrophic cyanobacteria, these organisms include heterotrophic bacteria, which, like *E. coli*, must contain an NADH oxidizing domain in their NDH-1 complexes. The function of the nitroreductases in these organisms remains unclear. Thus, the problem of the nature of the NAD(P)H oxidizing domain of the cyanobacterial NDH-1 complex remains unsolved.

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