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Cytochrome c_{6B} of *Synechococcus* sp. WH 8102 – Crystal structure and basic properties of novel c_6-like family representative ☆

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ABSTRACT

Cytochromes *c* are soluble electron carriers of relatively low molecular weight, containing single heme moiety. In cyanobacteria cytochrome c_6 participates in electron transfer from cytochrome b_6f complex to photosystem I. Recent phylogenetic analysis revealed the existence of a few families of proteins homologous to the previously mentioned. Cytochrome c_{6A} from *Arabidopsis thaliana* was identified as a protein responsible for disulfide bond formation in response to intracellular redox state changes and c_{550} is well known element of photosystem II. However, function of cytochromes marked as c_{6B}, c_{6C} and c_M as well as the physiological process in which they take a part still remain unidentified. Here we present the first structural and biophysical analysis of cytochrome from the c_{6B} family from mesophilic cyanobacteria *Synechococcus* sp. WH 8102. Purified protein was crystallized and its structure was refined at 1.4 Å resolution. Overall architecture of this polypeptide resembles typical I-class cytochromes *c*. The main features, that distinguish described protein from cytochrome c_6, are slightly red-shifted α band of UV–Vis spectrum as well as relatively low midpoint potential (113.2 ± 2.2 mV). Although, physiological function of cytochrome c_{6B} has yet to be determined its properties probably exclude the participation of this protein in electron trafficking between b_6f complex and photosystem I.

1. Introduction

In cyanobacterial cells, the interaction between different photosynthetic membrane complexes is possible due to the presence of mobile electrons' carriers. Plastoquinone molecules transport electrons between PSII and cytochrome b_6f complex within phospholipid bilayer. Metalloproteins: cytochrome c_6 and/or plastocyanine are responsible for electron transport between cytochrome b_6f complex and PSI in thylakoid lumen. The oxidized form of these metalloproteins (Cu^{2+}-carrying plastocyanine as well as Fe^{3+}-containing cytochrome c_6) adopts one electron from cytochrome *f*, being a part of the b_6f complex [1]. As a result of this reaction the reduced electron carrier is formed which moves within the lumen towards PSI. For specific targeted traffic of mentioned metalloproteins electrostatic and/or hydrophobic interactions are responsible [2]. After docking to PSI, metalloprotein passes an electron onto photooxidized dimer of chlorophyll $P700^+$ molecules. This event closes the electron transfer from b_6f to PSI [1]. Photosynthetic electron transport is not the only function of cyanobacterial cytochrome c_6 and plastocyanin. In cyanobacteria, photosynthetic and respiratory electron transport chains physically overlap in thylakoid membranes. In opposition, analogous protein complexes localized in the cytoplasmic membranes participate only in respiration process. In cyanobacteria typical cytochromes of type *c* which would pass electrons to cytochrome *c* oxidase (in cyanobacteria - oxidase type aa_3) are absent. Nevertheless, a long time ago hypothesis was formed, that b_6f complex, quinone pool or cytochrome c_6 are elements common to the processes of photosynthesis and respiration in cyanobacteria. Subsequent data seem to confirm the validity of such supposition over the years [3]. The third process, which involves the cyanobacterial cytochrome c_6 is the process of anoxygenic photosynthesis, where hydrogen sulfide is a source of electrons. In this process, cytochrome c_6 can transport electrons between quinones and iron-sulfur centers during the anaerobic oxidation of sulphides [4].

Abbreviations: Cyt, cytochrome; Wat, water.

☆ Database: Atomic coordinates and structure factors for Cytochrome c_{6B} of *Synechococcus* sp. WH 8102 are available from the Protein Data Bank under the accession code 4KMG.

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Bialek and co-workers have identified two new groups of c_6-like cytochromes. Due to the earlier discovery of c_{6A} type cytochromes, these groups are named analogously as c_{6B} and c_{6C} [5]. Family of cyanobacterial c_{6B} cytochromes is quite closely related to chloroplastic c_{6A} cytochromes. However, cytochromes of group c_{6B} and c_{6C} do not include in their primary structure 12-residues-long loop, characteristic for c_{6A} family. Another difference is conserved tyrosine residue at position 61 of cytochrome c_{6B} and c_{6C}, which is analogous to phenylalanine or tryptophan occupying corresponding position in c_6 and c_{6A} cytochromes. In the c_{6B} group sequences are mainly derived from non-binding nitrogen marine cyanobacteria species–*Prochlorococcus* and *Synechococcus*. These species are characterized by about 95% identity to the 16S rRNA sequence, but consists of a number of physiologically and genetically different ecotypes [6–9].

The presence of genes encoding c_6-like cytochromes in genomes of many cyanobacteria provides a basis for extending hypotheses about the role of these proteins in the proper functioning of cells. The diversity of cyanobacteria, in which these genes are present (single-celled green algae, filamentous or nitrogen-fixing) indicate that c_6-like cytochromes appeared relatively early in the history of the evolution of these microorganisms [5]. Compared to enormity of research projects concerning cytochromes in general, c_6-like cytochrome are rarely mentioned in the literature. This fact may be primarily due to their relatively recent discovery. Data presented in this paper extend current knowledge about c_6-like cytochromes. Unfortunately, biological function of these proteins still remains unclear.

2. Materials and methods

2.1. Protein expression and purification

Escherichia coli strain DH5α was co-transformed with pUC_c6B and pEC86 plasmids. The former harbors a gene encoding mature cyt c_{6B} from *Synechococcus* sp. WH8102, whereas the latter the heme maturation gene [10]. 5 mL of overnight culture were used to inoculate 1.75 L of TB medium in a 2 L flask. Cultures were grown for 8 h at 37 °C with vigorous agitation and then IPTG was added to final concentration of 0.75 mM. Subsequently, cultures were grown for 72–96 h at 30 °C with agitation at 80 rpm. Cells were harvested at 5000*g*, 4 °C, washed in 30 mM Tris, pH 8.0, 0.1 M NaCl, 20% sucrose, 1 mM EDTA. After centrifugation and resuspension in the same buffer the periplasmic proteins were released by lysozyme treatment (0.2 mg/mL) at RT for 20 min with shaking and then centrifuged at 25,000*g* for 25 min at 4 °C. The supernatant, which contains the periplasmic protein fraction, was incubated with ammonium sulfate (45% saturation, 30 min, 4 °C) and centrifuged at 20,000*g* for 20 min at 4 °C, and the pellet was discarded. Ammonium sulfate was added to the supernatant to 95% saturation and treated as described above. The red pellets were resuspended in 20 mM phosphate buffer pH 6.2, 1 mM PMSF and dialyzed against the same buffer, overnight at 4 °C. Solution after dialysis was loaded onto a HiTrap SP HP column (GE Healthcare) connected to AKTA Purifier system and equilibrated with the same buffer. Proteins were eluted with linear 0–200 mM NaCl gradient. Fractions collected from the first column were dialyzed against 50 mM ethanolamine pH 9.0. After overnight dialysis the sample was applied to a HiTrap Q HP column (GE Healthcare) equilibrated with the same buffer. Proteins were eluted with linear 100–500 mM NaCl gradients. Purified cyt c_{6B} was characterized by SDS–PAGE and heme staining as described in [5]. The A_{557}/A_{280} ratio of purified cyt c_{6C} was 0.623. The proteins were aliquoted and stored at −20 °C.

2.2. Cytochromes absorption spectroscopy and redox titrations

All spectroscopic measurements were carried out using a Beckman DU800 spectrophotometer. Measurements were conducted at room temperature using a 1 cm path-length cuvette. Cytochrome was diluted to final concentration of 5 μM in 10 mM Tris pH 7.5 containing 1 mM potassium ferricyanide (spectra of oxidized cytochromes) or 1 mM sodium dithionate (spectra of reduced cytochromes). Redox titrations were performed as described in [11]. Procedure was repeated three times in both directions in a custom-made anaerobic cuvette, containing platinum electrode and calomel reference electrode, under argon flow, in 50 mM MOPS pH 7.0 and 100 mM KCl in the presence of redox mediators: tetrachlorohydroquinone (TCHQ, $E_{m,7}$ = 350 mV), 2,3,5,6 tetramethyl-p-phenylenediamine (DAD, 260 mV), 1,2-naphthoquinone-4-sulfonate (NQS, $E_{m,7}$ = 210 mV), 1,2-naphthoquinone (NQ, 130 mV), phenazine methosulfate (PMS, 80 mV), phenazine ethosulfate (PES, 55 mV), duroquinone (DQ, 5 mV). All redox mediators were at concentration of 45 μM and cytochrome at 5 μM. 50 mM Potassium ferricyanide was used as an oxidant and 50 mM sodium dithionate as a reductant. Spectra (range 400–600 nm) were recorded in intervals of 10–30 mV. Midpoint potentials were obtained from cytochromes α-band absorbance plotted against corresponding voltage.

2.3. Crystallization

Initial screening for cytochrome c_{6B} crystallization conditions was performed at the HTX facility, EMBL-Hamburg using the Classic, Classic 2, PACT and AmSO4 Qiagen suites [12]. The sitting-drop vapour-diffusion technique was used at 292 K, by mixing 0.3 μl protein (7.5 mg/mL in 20 mM sodium citrate, pH 3.2) and 0.3 μl reservoir solution. The optimization of crystallization conditions was carried out using the hanging-drop vapor-diffusion method at 292 K, by mixing 1 μl protein solution and 1 μl reservoir solution. Red crystals suitable for X-ray analysis were obtained from

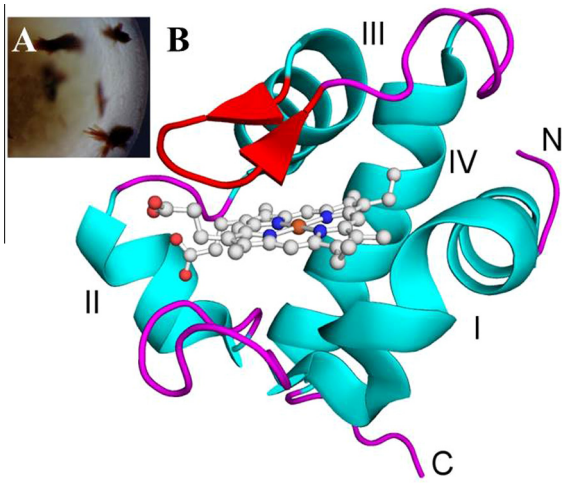

Fig. 1. Crystal structure of cytochrome c_{6B} from *Synechococcus* sp. WH 8102. (A) Crystals of cytochrome c_{6B} from *Synechococcus* WH 8102 obtained in 2.2 M sodium malonate, pH 7.0, 19 °C, over a period of 10 days. Average dimension of 200 μm. (B) Overall structure of cytochrome c_{6B} from *Synechococcus* sp. WH 8102 (PDB:4KMG). I–α-helix I; II–α-helix II; III–α-helix III; IV–α-helix IV. The structure resembles characteristic features of cytochromes of class c_6. Polypeptide chain wraps around the heme moiety (grey). Secondary structure is described by four α-helices (cyan), several loops (magenta) and one β-hairpin (red). The N- and C-terminus are indicated.