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„Cytochromes of cyanobacteria – structures and properties”.

### Abstract.

In the cells of photosynthetic organisms, the presence of mobile electrons' carriers allows interaction between key membrane complexes of the photosynthetic apparatus. Within the phospholipid bilayer, plastoquinone molecules are responsible for the transport of electrons between photosystem II and the cytochrome  $b_6f$  complex. In thylakoid lumen, between cytochromes  $b_6f$  complex and photosystem I, electrons are transported by metalloproteins: cytochrome  $c_6$  and/or plastocyanine. This particular step begins the flow of electrons when the electron is passed from the cytochrome  $f$  (which is part of the complex  $b_6f$ ) on the oxidized form of metalloproteins (cytochrome  $c_6$  associated with the  $Fe^{3+}$  cation or plastocyanine associated with  $Cu^{2+}$  cation). As result, a reduced electron carrier is produced, which moves in the lumen toward photosystem I. This cycle closes when the electron is passed from metalloproteins to photooxidized dimer of  $P700^+$  chlorophyll molecules.

Photosynthetic electron transport is not the only function of cyanobacterial cytochrome  $c_6$  and plastocyanine. Within the thylakoid membranes, respiratory and photosynthetic electron transport chain physically overlap with each other. Analogous protein complexes located in the cytoplasmic membrane, in turn, are only responsible for the respiratory electron transport chain. Thus, the complex of cytochromes  $b_6f$ , quinones pool and cytochrome  $c_6$  were considered as common elements of cyanobacterial processes of respiration and photosynthesis. The third process, which involves the cyanobacterial cytochrome  $c_6$  is anoxygenic photosynthesis in which the source of electrons is hydrogen sulfide. In this process, cytochrome  $c_6$  transfers electrons from quinones pool onto iron-sulfur centers during the anaerobic oxidation of sulphides.

Aside from a fairly well-established and characterized group of cytochromes  $c_6$ , in the genomes of many species of cyanobacteria sequences encoding cytochromes similar to  $c_6$  (called cytochromes  $c_6$ -like) can be found. In contrast to the conventional cytochromes  $c_6$ , cytochromes  $c_6$ -like are group of proteins of unknown function and poorly defined properties.

Research conducted in last few years in the Department of Biophysics, led to the isolation of two new groups of cytochromes similar to  $c_6$ . Accordingly to earlier discovery of plant of cytochromes  $c_{6A}$ , these groups were named analogous as  $c_{6B}$  and  $c_{6C}$ . Chloroplast cytochromes  $c_{6A}$  are quite closely related to the family of cyanobacterial cytochromes  $c_{6B}$ . The genes encoding cytochromes  $c_{6B}$  are mostly present in the genomes of marine species of cyanobacteria which are unable to fix nitrogen - *Prochlorococcus* and *Synechococcus*. In turn, genomes of nitrogen-fixing cyanobacteria, unicellular or producing heterocysts, often contain sequences coding cytochromes of  $c_{6C}$  group. An interesting exception in this group is cytochrome PetJ2 of cyanobacterium *Synechococcus sp.* PCC 7002, not having *nif* genes.

The presence of genes encoding cytochromes  $c_6$ -like in the genomes of many cyanobacteria provides a basis to extend the hypotheses regarding the function of these proteins in the biochemical processes of cyanobacterial cells. Species diversity of cyanobacteria having genes encoding cytochromes  $c_6$ -like indicates that this family of proteins appeared relatively early in the history of the evolution of cyanobacteria. In comparison to the enormity of scientific papers dealing with cytochromes in general, cytochromes  $c_6$ -like are almost imperceptible in the literature. Accordingly, the author of this essay during research undertook work to expand knowledge of the cytochromes  $c_6$ , particularly of cytochromes  $c_6$ -like. The main goal was to obtain crystal structures of these of cytochromes and relating them to the basic biochemical properties.

This work is devoted mainly to three cytochromes: PetJ1 (representative of the family of cytochromes  $c_6$ ), PetJ2 (representative of the family of cytochromes  $c_{6C}$ ) of *Synechococcus sp.* PCC 7002 and cytochrome  $c_{6B}$  of *Synechococcus sp.* WH 8102.

The first project was to determine the features of  $K_{44}$ - $S_{49}$  loop, unique for cyanobacterial cytochromes  $c_6$ , located between II and III  $\alpha$  helix of cytochrome PetJ1 of *Synechococcus sp.* PCC 7002. Cyanobacterial cell transformation with *petJ1* gene with deleted sequence coding unique loop showed that cyanobacterium cells are unable to function properly if the cytochrome PetJ1 loop  $K_{44}$ - $S_{49}$  will be removed. Using heterologous expression in *E. coli* cells cytochrome PetJ1  $\Delta 6$  – with removed loop, was obtained. Further research allowed to determine the loop effect on the biochemical properties . Removal of the loop results in a decrease in the redox

potential from +333.4 mV (wild-type PetJ1 ) to +235.8 mV (deletion mutant PetJ1 dl6). There is no significant change to the isoelectric point and the spectral properties (absorbance in the UV-Vis) . Despite many attempts, failed to obtain crystals of the protein, which could be used to solve the crystal structure and further - to a fair explanation of the features of unique loop.

Another element of the thesis was to compare the structures and properties of cytochrome PetJ1 from *Synechococcus sp.* PCC 7002 with a point mutant PetJ1 Q57V. Studies have shown that the substitution of glutamine to valine within the heme pocket (position 57) results in a significant reduction of the redox potential: from +333.4 mV (wild-type PetJ1) to +239.4 mV (PetJ1 Q57V). In addition bathochromic effect was observed (shift of absorption maximum of reduced cytochrome  $\alpha$ -band toward the red light: from 553.2 nm to 555.9 nm). Isoelectric point remained unchanged. Crystallographic structure of mutant PetJ1 Q57V (PDB: 4EID, resolution 1.13 Å) and comparing it with the structure of wild-type cytochrome PetJ1 helped to clarify the nature of these changes.

The third part of the dissertation is a comparison of the structures and properties of cytochrome PetJ2 from *Synechococcus sp.* PCC 7002 and its point mutant PetJ2 L50Q. In this case again it was confirmed that the presence of a polar amino acid side chain within the heme pocket has a significant effect on the biochemical properties . Replacement of the hydrophobic side chain of leucine into hydrophilic chain of glutamine at position 50 of cytochrome PetJ2 effects in increasing the redox potential from +150.2 mV to +202.9 mV. There is also hipsochromic effect (shift of the absorption maximum of reduced cytochrome  $\alpha$  -band toward the blue light - PetJ2 WT: 556.1 nm; PetJ2 L50Q: 553.5 nm). Further work enabled the solution of crystal structures of cytochrome PetJ2 wild-type (PDB: 4EIE, resolution 1.03 Å) and point mutant L50Q (PDB:4EIF, resolution of 1.04 Å). Comparison of the structures has shown that the point mutation does not affect the overall tertiary structure, but only the network of hydrogen interactions within the heme pocket, which explains the change in the biochemical properties. The structures of cytochrome PetJ2 wild-type and point mutant PetJ2 L50Q are the first structures of cytochromes  $c_{6C}$  published in the PDB database.

The last, most important part of the dissertation was to determine the properties and structure of cytochrome  $c_{6B}$  of *Synechococcus sp.* WH 8102. The structure of the cytochrome, refined to 1.40 Å resolution, has been deposited in the PDB database under code 4KMG. It is the world's first protein structure of the family of cytochromes  $c_{6B}$  deposited in the PDB database. Unique for this cytochrome is  $\beta$ -hairpin structure, following third  $\alpha$  helix. Comparison of the properties of this cytochrome to cytochrome PetJ1 of *Synechococcus sp.* PCC 7002 showed a number of differences. Primarily, low redox potential: 113.2 mV (PetJ1: 333.4 mV), different spectral properties (absorption maximum of reduced cytochrome  $\alpha$ -band at 557.0 nm) and a very high dipole moment (1025 D compared to 204 D of cytochrome PetJ1).

In addition to the above main components of thesis, the author also undertook attempts to obtain crystallographic structures of cytochrome  $c_M$  from *Synechococcus sp.* PCC 7002 and cytochrome *f* of *Thermosynechococcus elongatus* BP-1. Despite many attempts, the cytochrome  $c_M$  failed to grow crystals, while for the cytochrome *f* crystals were grown in a number of conditions, but no diffraction data could be registered.

Part of the results of that thesis is presented in the form of structures deposited in the PDB database (access codes: 4EID, 4EIE, 4EIF, 4KMG), press runs (36th Annual Midwest / Southeast Photosynthesis Meeting, Marshall, USA, 10.2010), posters (DGK-AK1 Workshop - Diffraction Data Collection Using Synchrotron Radiation, Berlin, Germany, 07.2011) and publications (Zatwarnicki et al, "Cytochrome C6b of *Synechococcus sp.* WH 8102 - Crystal structure and basic properties of novel c6-like family representative", accepted in BBRC). Other research results are being prepared for publication.