SL336

Generation and Characterization of Photoheterotrophic mutants for *psaB* gene of Cyanobacterium *Synechocystis* sp. PCC 6803

Soohyun Kim and Young-Mok Park
Biomolecule Group, Korea Basic Science Institute, Taejon, 305-333 Korea

The cyanobacterium *Synechocystis* sp. PCC 6803 performes oxygenic photosynthesis in a manner to that in higher plants. The reaction center of (photosystem I) PSI is composed of a heterodimer of homologous polypeptides, PsaA and PsaB. To investigate the relationship between structure and function of PSI, cartridge mutagenesis technique was used to inactivate the *psaB* gene which encodes subunit Ib (PsaB protein) of PSI. Mutant strains carrying mutations at the subunit Ib were generated by transforming wild type cells with cloned DNA, pOV300, in which *psaB* gene of *Synechococcus* 7002 was interrupted by a gene conferring chloramphenicol resistance. Unexpectidly, mutants were shown to exhibit various colors, other than blue-green, displayed in wild type cells. They were divided into five groups according to their colors; yellow-green, orange, dark-green, green and greenish-brown. Mutant cells did not grow photoautotrophically. Moreover, biochemical analysis revealed that mutants have reduced the amount of chlorophyll *a*, even though they possess normal amount of carotenoids. To investigate the composition and the amount of pigments in wild type and mutants more quantitatively, pigments were extracted with 90 % methanol from whole cells, followed by reverse phase liquid chromatography. In case of wild type, chlorophyll *a* occupied at least 75 % of total pigment of cell. The other major pigments were revealed as two carotenoids, β-carotene and zeaxanthin, which occupies 9 % and 11 % of. total pigments, respectively. Photosynthetic rate of mutant cells measuring by oxygen evolution also decreased to 26-60 % compared to that of wild type cells, when normalized to equal cell number. 77K fluorescence emission spectra were measured upon 590 nm excitation (exciting phycobilin) to determine efficiency of energy transfer, cells of wild type and mutants clearly showed three fluorescence emission maxima. But energy transfer between phycobilisomes and PSI in mutants appeared to be less efficient than that in wild type. Interestingly, immunodetection analysis for the several components of PSI showed that mutants had reduced amount of core polypeptide, PsaA/PsaB, but a normal range of peripheral protein, PsaC and PsaD. It appears that mutation results in reducing the amount of PsaB protein in mutants, which in turn reducing the activity of electron transport of photosystem I complex. The photoheterotrophic mutants, although generated in an attempt similar to those in previous works, seems to have a subtle mutation at the *psaB* locus, thus provide a very unique model system for future study of photosystem I.