Characterization of *Synechocystis* Flavodiiron Proteins

Pengpeng Zhang, Yagut Allahverdiyeva, Marion Eisenhut, Dalton Carmel and Eva-Mari Aro

Department of Biochemistry and Food Chemistry, Plant Physiology and Molecular Biology, University of Turku, FI-20014, Finland

Introduction

Flavodiiron proteins, previously called as A-type flavoproteins, are molular enzymes involved in electron transfer reactions. Different from most of the enzymes in anaerobic microbes, cyanobacterial flavodiiron proteins additionally have a flavin reductase domain at the C terminus (Fig. 1).

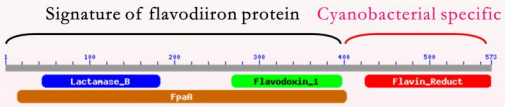


Fig. 1 Conserved domains in flavodiiron proteins

In *Synechocystis* 6803 genome, there are four genes encoding flavodiiron proteins (*flv1*: *sll1521*, *flv2*: *sll0219*, *flv3*: *sll0550*, *flv4*: *sll0217*). The *flv2* and *flv4* genes are organized in one operon, which is highly induced by low CO_2. Sequence analysis shows that cyanobacterial type of flavodiiron proteins are also present in other oxygenic photosynthetic organisms, such as *Chlamydomonas*, *Physcomitrella* and *Selaginella*.

It has been reported that *Synechocystis* Flv3 is capable of reducing oxygen completely to water (Vicente *et al.*, 2002), and Flv1 and Flv3 were suggested to function in Mehler reaction (Helman *et al.*, 2003). However, the physiological roles of Flv2 and Flv4 remain elusive.

Aim: To characterize *Synechocystis* flavodiiron proteins and clarify the role of Flv2 and Flv4 using knockout mutants

Results:

1. Phylogenetic tree of flavodiiron proteins

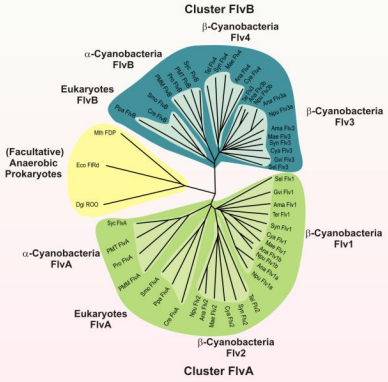


Fig. 2 Phylogenetic analysis of flavodiiron proteins in different organisms (Zhang *et al.*, 2009)

Ana, *Anabaena* sp. PCC 7120 (Flv1a: All0177, Flv1b: All3891, Flv2: All4444, Flv3a: All0178, Flv3b: All3895, Flv4: All4446); Ama, *Acaryochloris marina* MBIC11017 (Flv1: Am1_1384, Flv3: Am1_1386); Cya, *Cyanothece* sp. ATCC 51142 (Flv1: Cce_2580, Flv2: Cce_3835, Flv3: Cce_3635, Flv4: Cce_3833); Cre, *Chlamydomonas reinhardtii* (FlvA: XP_001692916, FlvB: XP_001699345); Dgi, *Desulfovibrio gigas* (ROO: AAG34792); Eco, *Escherichia coli* (FlRd: Q46877); Gvi, *Gloeobacter violaceus* PCC 7421 (Flv1: Glr1776, Flv3: Glr1775); Mae, *Microcystis aeruginosa* NIES-843 (Flv1: MAE61610, Flv2: MAE50820, Flv3: MAE01310, Flv4: MAE50840); Mth, *Moorella thermoacetica* (FDP: AAG00802); Npu, *Nostoc punctiforme* ATCC 29133 (Flv1a: Npun_F4867, Flv1b: Npun_F5838, Flv2: Npun_R0591, Flv3a: Npun_F4866, Flv3b: Npun_F5837); PMM, *Prochlorococcus marinus* MED4 (FlvA: PMM0042, FlvB: PMM0043); PMT, *Prochlorococcus marinus* MIT9313 (FlvA: PMT2165, FlvB: PMT2164); Ppa, *Physcomitrella patens* subsp. patens (FlvA: XP_001759251, FlvB: XP_001756079); Pro, *Prochlorococcus marinus* SS120 (FlvA: Pro0044, FlvB: Pro0045); Smo, *Selaginella moellendorffii* (FlvA: estExt_Genewise1.C_61201, FlvB: estExt_fgenesh2_pg.C_1160033); Sel, *Synechococcus elongatus* PCC 6301 (Flv1: Syc2283, Flv3: Syc2284); Syc, *Synechococcus* sp. CC 9902 (FlvA: Synce9902_2183, FlvB: Synce9902_2180); Syn, *Synechocystis* sp. PCC 6803 (Flv1: Sll1521, Flv2: Sll0219, Flv3: Sll0550, Flv4: Sll0217), Tel, *Thermosynechococcus elongatus* BP-1 (Flv2: Tll1373, Flv4: Tlr1088); Ter, *Trichodesmium erythraeum* IMS 101 (Flv1: Tery_0770, Flv3: Tery_0302).

2. Cellular location of flavodiiron proteins

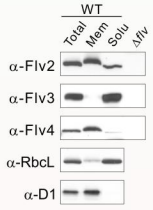


Fig. 3 Localization of flavodiiron proteins in different cellular fractions (Zhang *et al.*, 2009)

Flv2 and Flv4 proteins are present both in membrane and soluble fractions, mainly in membrane, whereas Flv3 is exclusively localized to cytosol.

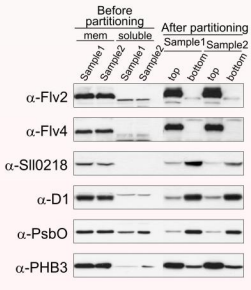


Fig. 4 Localization of Flv2 and Flv4 into plasma membrane

Total membrane was further purified by aqueous two phase partitioning (Norling *et al.*, 1998). The plasma membrane is enriched in top fraction, and the thylakoid membrane is enriched in bottom fraction.

Flv2 and Flv4 proteins are largely enriched in the top phase during partitioning, which indicates that they are plasma membrane proteins. However, Sll0218 is enriched in the bottom phase, similar to D1 and PsbO, indicating that it is a thylakoid membrane protein.

3. Flavodiiron protein complexes

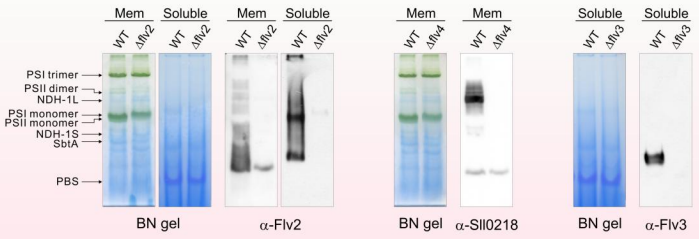


Fig. 5 Flv complexes presented in membrane and soluble fractions

Flv3 forms a protein complex of about 130 kDa, which might be a homo/hetero dimer in the cytosol. Besides dimeric complexes, Flv2 seems also to form a much larger complex, which might be a tetramer or association of Flv2 with other proteins.

4. Phenotype of Flv knock-out mutants

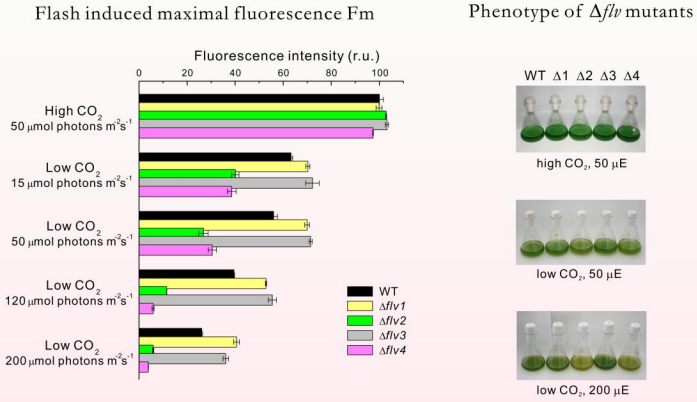
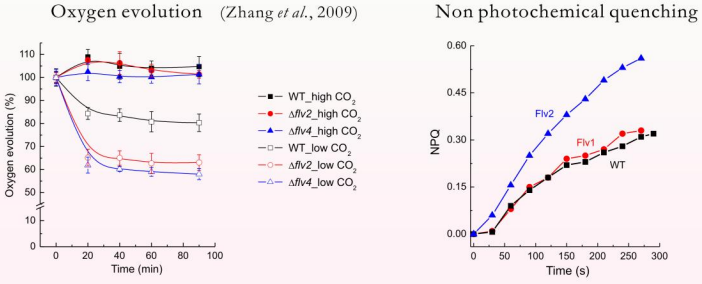


Fig. 6 PSII fluorescence and phenotype of Flv mutants (Zhang *et al.*, 2009)

Δ*flv2* and Δ*flv4* mutants show apparently yellowish phenotype by losing PSII activity under low CO_2, the higher light intensity, the more loss of active PSII centers.

5. Flv2 and Flv4 are involved in photoprotection



Conclusions and future perspectives

- There are four distinct flavodiiron proteins (Flv1, Flv2, Flv3 and Flv4) involved in different functions in *Synechocystis* 6803.
- Flv3 is located in cytosol and involved in Mehler reaction, which reduces oxygen to water without forming reactive oxygen species.
- Flv2 and Flv4 are associated with plasma membrane, and play an important role in photoprotection of PSII.
- We will clarify whether Flv2 and Flv4 function together as a heterodimer, and find out the interaction partners of Flv2 and Flv4 to elucidate the mechanism of their function in photoprotection.

Reference

Helman Y, Tchernov D, Reinhold L, Shibata M, Ogawa T, Schwarz R, Ohad I, Kaplan, A. (2003) Genes encoding A-type flavoproteins are essential for photoreduction of O_2 in cyanobacteria. Curr. Biol. 13: 230-235.

Norling B, Zak E, Andersson B, Pakrasi H. (1998) 2D-isolation of pure plasma and thylakoid membranes from cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett.* 436: 189-192.

Vicente JB, Gomes CM, Wasserfallen A, Teixeira M. (2002) Module fusion in an A-type flavoprotein from the cyanobacterium *Synechocystis* condenses a multiple-component pathway in a single polypeptide chain. *Biochem. Biophys. Res. Commun.* 294: 82-87.

Zhang P, Allahverdiyeva Y, Eisenhut M, Aro E-M. (2009) Flavodiiron proteins in oxygenic photosynthetic organisms: photoprotection of photosystem II by Flv2 and Flv4 in *Synechocystis* sp. PCC 6803. *PLoS ONE* 4: e5331. doi:10.1371/journal.pone.0005331

March 8-9th, 2010