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Redirecting Photosynthetic Electron Flux in the Engineered Cyanobacterium synechocystis Sp. Pcc 6803 by the Deletion of Flavodiiron Protein Flv3

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Abstract : Photosynthetic cyanobacteria have been recognized as potential future biotechnological hosts for the direct conversion of CO_2 into chemicals of interest using sunlight as the solar energy source. However, in order to develop commercially viable systems, the flux of electrons from the photosynthetic light reactions towards specified target chemicals must be significantly improved. The objective of the study was to investigate whether the autotrophic production efficiency of specified end-metabolites can be improved in engineered cyanobacterial cells by rescuing excited electrons that are normally lost to molecular oxygen due to the cyanobacterial flavodiiron protein Flv1/3. Natively Flv1/3 dissipates excess electrons in the photosynthetic electron transfer chain by directing them to molecular oxygen in Mehler-like reaction to protect photosystem I. To evaluate the effect of flavodiiron inactivation on autotrophic production efficiency in the cyanobacterial host Synechocystis sp. PCC 6803 (Synechocystis), sucrose was selected as the quantitative reporter and a representative of a potential end-product of interest. The concept is based on the native property of Synechocystis to produce sucrose as an intracellular osmoprotectant when exposed to high external ion concentrations, in combination with the introduction of a heterologous sucrose permease (CscB from Escherichia coli), which transports the sucrose out from the cell. In addition, cell growth, photosynthetic gas fluxes using membrane inlet mass spectrometry and endogenous storage compounds were analysed to illustrate the consequent effects of flv deletion on pathway flux distributions. The results indicate that a significant proportion of the electrons can be lost to molecular oxygen via Flv1/3 even when the cells are grown under high CO_2 and that the inactivation of flavodiiron activity can enhance the photosynthetic electron flux towards optionally available sinks. The flux distribution is dependent on the light conditions and the genetic context of the Δflv mutants, and favors the production of either sucrose or one of the two storage compounds, glycogen or polyhydroxybutyrate. As a conclusion, elimination of the native Flv1/3 reaction and concomitant introduction of an engineered product pathway as an alternative sink for excited electrons could enhance the photosynthetic electron flux towards the target endproduct without compromising the fitness of the host.

Keywords : cyanobacterial engineering, flavodiiron proteins, redirecting electron flux, sucrose
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