DOCTORAL THESIS

Biochemical Studies on Ferredoxin-$NADP^+$ Oxidoreductase (FNR) and Thermostable Glucosidase in Cyanobacteria.

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Abstract

Ferredoxin-$NADP^+$ oxidoreductase (FNR) catalyzing the terminal step of the linear photosynthetic electron transport was purified to homogeneity from the cyanobacterium *Spirulina platensis* and the red alga *Cyanidium caldarium*. FNR of *Spirulina* consisted of three domains (CpcD-like domain, FAD-binding domain and $NADP^+$-binding domain) with a molecular mass of 46 kDa and was localized in either phycobilisomes or thylakoid membranes. The membrane-bound FNR with 46 kDa was solubilized by NaCl and the solublized FNR had an apparent molecular mass of 90 kDa. Degradation of *Spirulina* 46 kDa FNR *in vitro* produced the intermediate size FNRs with molecular masses of 37 kDa, 36 kDa, 35 kDa and 34 kDa and these cleavages of FNR protein specifically took place in the hinge region, which is the interspace region between the CpcD-like domain and the catalytic domains according to the N-terminal amino-acid sequences of these intermediate size FNRs. These results suggest that the cyanobacterial two-domains FNR is a proteolytic product of the three domains cyanobacterial FNR. FNR of *Cyanidium* consisted of two domains (FAD-binding domain and $NADP^+$-binding domain) with a molecular mass of 33 kDa. In *Cyanidium*, FNR was found on thylakoid membranes but there was no FNR on phycobilisomes. The membrane-bound FNR of *Cyanidium* was not solublized by NaCl, suggesting the enzyme is tightly bound in the membrane. All forms of FNR under investigation in this study were biochemically similar and showed a highly active quinone dependent cytochrome C reduction activity indicating that they have a quinone reductase activity as well as ferredoxin-$NADP^+$ oxidation reduction activity. Although both cyanobacteria and red algae are photoautotrophic organisms bearing

phycobilisomes as the light harvesting complexes, FNR localization and characteristics of binding to the membrane were different. These results suggest that FNR binding to phycobilisomes is not characteristic for all phycobilisome retaining oxygenic photosynthetic organisms, and that the rhodoplast of red algae had possibly originated from a cyanobacterium ancestor with FNR lacking the CpcD-like domain.

The cyanobacterium *Nostoc commune* is adapted to the terrestrial environment and forms a visible size of colony in which the cells are surrounded by extracellular polysacharides (EPS). The EPS play a crucial role for extreme desiccation tolerance of this organism while the EPS-depleted cells are sensitive to desiccation. When the natural colonies immersed in water at room temperature, breakdown of colonies was observed within 2 days and fully vital *N. commune* cells with photosynthetic O_2 evolution and respiratory O_2 consumption were released to the water. In this study, a new method for massive isolation of the external polysaccharides (EPS) from cells of the terrestrial cyanobacterium *Nostoc commune* colonies was developed using high concentration phosphate buffer. The EPS-depleted cells of *N. commune* represented 4.7 % of the total fresh mass of *N. commune* colonies and around 95 % of the total fresh mass of *N. commune* was in the extracted water hydrated external polysaccharides. Around 96 % of the total protein and almost no EPS were recovered in the EPS-depleted cells. The activities to hydrolyze the glycoside bonds using artificial nitrophenyl-linked sugars as substrates were examined. A thermostable beta D-glucosidase activity was detected in the water soluble fraction of the EPS and the enzyme was resistant to boiling for 20 minuets. The beta D-glucosidase was purified to homogeneity. The molecular mass of the purified protein was 20 kDa and its N-terminal amino-acid sequence was identical with the cyanobacterial secreted and surface fasciclin protein. The beta D-glucosidase activity of the purified protein had a

neutral optimum pH value of 7 and showed a lower affinity towards other glycoside bonds. The gene encoding the fasciclin protein was amplified and sequenced from *N. commune* strain KU002, *N. punctiforme* strain M15 and *N. commune* (D-type), and the deduced amino-acids sequence showed a high similarity with the protein of other cyanobacterial species. The calculated molecular mass of the deduced protein in *N. commune* starin KU002, *N. punctiforme* strain M15 and *N. commune* (D-type) were 13.55 kDa, 13.58 kDa and 13.6 kDa respectively. The slow electrophoretic migration of the native protein (20 kDa) might indicate a possible posttranslational modification in the native protein. The glucosidase activity of the fasciclin protein in *N. commune* might play a role in partial hydrolysis of the EPS surrounding the *N. commune* cells possibly for growth of the colony and cell expansion.

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