

## 590(S557)

CHANGES IN PHOTOSYNTHETIC ACTIVITIES DURING REWETTING PROCESS IN A TERRESTRIAL CYANOBACTERIUM, *NOSTOC COMMUNE*

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A terrestrial cyanobacterium, *Nostoc commune*, shows high tolerance to drought and recovers its photosynthetic activities during the hydration process.

When enough water was supplied, the hydration process showed three phases, and the amounts of water absorbed were usually constant for each phase.

However, there are two factors in the hydration process; amounts of water absorbed and time after initiation of hydration. We succeeded to separately observe the two factors and found that water equal to the dry cell aggregate in weight was enough to recover the activities of the cells.

## 591(S558)

## REGULATORY MECHANISMS OF THE CALVIN CYCLE IN CYANOBACTERIA

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We reported that thiol-modulated enzymes involved in the Calvin cycle are not regulated by light/dark condition via the ferredoxin/thioredoxin system (FTS) in cyanobacteria and lack potential redox-sensitive Cys residues involved in the FTS (BBB 62, 374, 1998, BBA 1383, 232, 1998). The occurrence of the gene encoding CP12 in cyanobacterial cells suggested that the CP12 protein might regulate the activities of phosphoribulokinase (PRK) and NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the reversible dissociation of PRK/CP12/GAPDH complex mediated by NADP(H). The size exclusion chromatography and the immunoblot analysis of crude extract of *Synechococcus* PCC 7942 revealed the existence of a 520-kDa PRK/CP12/GAPDH complex in the presence of NAD<sup>+</sup>. Incubation of the complex with NADPH or NADP<sup>+</sup>, in contrast to NAD<sup>+</sup> or NADH, causes its dissociation. We constructed the expression system of *Synechocystis* PCC 6803 CP12 in *E. coli* and purified the recombinant CP12 protein. *In vitro* reconstitution assays with the recombinant *S. 6803* CP12 showed that the recombinant CP12 bound PRK and GAPDH in the presence of NAD<sup>+</sup>. The growth rate of *S. 6803* CP12-disrupted mutant (SsACP12) was almost the same as that of wild-type cells under continuous light condition. Under 12-h light/12-h dark conditions, the wild-type cells grew synchronously, but the SsACP12 cells did not. These data suggest that the CP12 protein serves as one of the important factors to regulate the Calvin cycle in cyanobacteria.

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## TWO REACTION PATHWAYS IN THE FORMATION OF PYROPHEOPHORBIDE

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Demethoxycarbonyl reaction of chlorophylls in plants and algae was investigated. We found that there were two reaction pathways in the formation of pyropheophorbide. One pathway proceeds by two reaction steps; first, the enzyme designated "pheophorbidease" catalyzes the conversion of pheophorbide *a* to a precursor of pyropheophorbide *a* by demethylation, and then, the precursor is decarboxylated non-enzymatically to yield pyropheophorbide *a*. The other pathway is direct conversion of pheophorbide *a* to pyropheophorbide *a* by demethoxycarbonylation dependent on NADPH. This enzyme was termed "NADPH-pheophorbide demethoxycarbonylase (NPD)". Pheophorbidease was purified from cotyledons of radish (*Raphanus sativus*), and NPD from the mutant cells of *Chlamydomonas reinhardtii*. Both enzymes consisted of two types, senescence-induced and constitutive enzymes. Molecular weight and *K<sub>m</sub>* value for pheophorbide *a* for pheophorbidease were 113,000 and 15  $\mu$ M, and for NPD were 170,000 and 283  $\mu$ M.

## 593(S560)

PHOTOSYNTHETIC CONTROL OF PROTOCHLOROPHYLLIDE REDUCTASE GENE EXPRESSION IN *Marchantia paleacea* var. *diptera*. Saeko EGUCHI<sup>1</sup>, Hiroyoshi TAKANO<sup>2</sup>, Kanji ONO<sup>2</sup>, Susumu TAKIO;<sup>2</sup> <sup>1</sup>Grad. Sch. of Sci. & Tech. Kumamoto Univ., <sup>2</sup>Dept. Biol. Sci., Fac. Sci., Kumamoto Univ., Kumamoto 860-8555.

A cell line of suspension culture from the liverwort *Marchantia paleacea* var. *diptera* showed high level of chlorophyll in the light but much reduced level in the dark. The *por* and *chlL/N/B* genes encoding protochlorophyllide reductases were expressed in a light-dependent manner. Under the photoautotrophic conditions, the photosynthetic electron transport was indicated to regulate the *por* expression.

In this study, we investigated the expression pattern of POR protein by using anti-liverwort POR antibody. 1) The antibody reacted with a protein of thylakoid membranes, which had a molecular mass of about 37kDa on a denatured gel. 2) The POR protein was not detected in dark-adapted cells grown in the organic medium. When the photoautotrophic cells were transferred to darkness, the amount of *por* mRNA was reduced but the protein level was not changed. 3) When the photoautotrophic cells were treated with the photosynthetic electron transport inhibitor DCMU, the amount of *por* mRNA was greatly reduced, while the protein amount was not affected. These facts indicate that the photosynthetic electron transport is involved in the *por* expression at transcriptional or posttranscriptional level but it does not affect the stability of POR protein.