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DETECTION OF GPI-ANCHOR MOLECULE IN CELL WALL ACID PHOSPHATASE

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In this experiments a crude cell wall NaCl-extract was used as cell wall APase. A cytoplasmic APase was used as the precurser protein of cell wall APase(Pro-cwAPase). Strategy for the identification of the GPI-anchor molecule by TLC-immuno blotting failed to use because the antibody against the cell wall APase as well as the sweet potato APase had recognized none of the seven glyco lipids on TLC-immunoblotting. The cell wall APase and the Pro-cwAPase were mixed with PL-C to release phosphatidyl-inositol molecule from GPI-anchored protein. The change in molecular mass of the PL-C treated GPI-anchored protein might be detected on HPLC or on native PAGE. After the Pro-cwAPase was treated with PL-C, the decrease of the molecular mass was observed on HPLC.The mobilty of the PL-C treated Pro-cwAPase increased on native-PAGE, while that of the cell wall APase however showed no change. In order to remove phospholipid derived from cell membrane debris adsorbed to the Pro-cwAPase, the microsome fraction was treated with 1.0% TritonX-100. After the treatment, the fraction was applied to DEAE-cellulose column and the enzyme was eluted with NaCl to obtain three active fractions. The active fractions I and II analyzed by native-PAGE were determined to contain the Pro-cw APase.They are to be examined to detect GPI-anchor molecule.

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GLYCOSYLPHOSPHATIDYLINOSITOL (GPI)-ANCHORED PROTEINS OF *MARCHANITIA POLYMORPHA* CULTURE CELLS

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Since the nitrate reductase of *Chlorella saccharophila* was found to be a GPI-anchored protein in 1995, GPI-anchored proteins have been reported in plants such as duckweed, tobacco, rose, and *Arabidopsis thaliana*. Thus it is considered that GPI-anchored proteins are not rare also in plants. We describe here the screening of GPI-anchored proteins in a moss using *Marchanitia polymorpha* culture cells.

For the screening of GPI-anchored proteins, the *M. polymorpha* culture cells were labeled with [^{3}H]ethanolamine (EA) and [^{3}H]myristic acid (14:0), precursors of GPI, for 18 hr. Proteins extracted from cells and medium were electrophoresed and stained by CBB. Gels were sliced to 1mm-pieces and dissolved in H_2O_2 and then their radioactivity was measured. The cellular protein sample contained many bands which were consistently labeled with [^{3}H]EA and/or [^{3}H]14:0, whereas the secreted protein sample contained only one major band with molecular mass of 50 kDa, the protein which was consistently labeled with [^{3}H]EA but not with [^{3}H]14:0. From amino acid composition analysis of the purified 50 kDa protein, it was shown that the protein contained 1 mol of EA and 4 mol of glucosamine per mol. Thus the 50 kDa secreted protein of *M. polymorpha* was concluded to be a GPI-anchored protein. This protein would lose the lipid moiety of the GPI when it was removed from the plasma membrane.

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MOLECULAR CLONING AND CHARACTERIZATION OF PROTEIN PHOSPHATASE 2A IN GUARD CELLS FROM *VICIA FABA* L.

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Recent pharmacological studies showed that protein phosphatase 2A (PP2A) is involved in the regulation of inward-rectifying K^+-channels and slow-type anion channels in plasma membrane of stomatal guard cells. In this study, we isolated three full-length cDNAs (*vfPP2A-I, vfPP2A-II, vfPP2A-III*) encoding catalytic subunit of PP2A in guard cells of *Vicia faba* L. The deduced amino acid sequences of *vfPP2A-I, vfPP2A-II, vfPP2A-III* were exhibited strong similarities to previously described PP2Ac isoforms from animals and plants. RT-PCR analysis showed that the transcripts were expressed in guard cells, root and leaves. Recombinant vfPP2A-I purified from *E. coli* had protein phosphatase activity, and the activity was inhibited by okadaic acid and calyculin A, inhibitors of PP2A. These results indicate that vfPP2A encodes catalytic subunit of PP2A in guard cells.

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Characterization and gene cloning of ferredoxin-NADP oxidoreductase from thermophilic cyanobacterium *Synechococcus elongatus*

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Many types of ferredoxin-NADP oxidoreductase (FNR) have been purified and characterized in the relationship between enzyme activity and their structures, especially an active site and a substrate binding site according to X-ray crystalography and site-directed mutagenesis. However, there are few report on the possibility whether regions apart from active site of FNR are involved in its activity.

We measured the activity of FNR partially purified from thermophilic cyanobacterium, *S. elongatus*, cloned the gene and determined the DNA sequence. Thermophilic FNR was compared with mesophilic FNRs to elucidate the structure-function relationship. We will discuss some properties of thermophilic FNR in relation to an adaptation to high temperature.