E204 Preparation and Characterization of Photosystem II Abundant Thylakoid Membranes from Green Algae, *Chlamydomonas reinharditii*

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Chlamydomonas reinharditii cc400 is a very useful material to manipulate genes, because it is possible to transform genes and has no cell walls. Thus several species of mutant for photosynthesys were manufactured. However, preparative method is not confirmed to determine biophysical properties yet. Here, I developed a simple method to prepare a photosystem II abundant thylakoid membranes. Cells are broken by prench press at 1000 kg/cm^2 . Broken cells were centrifuged at 50,000 x g for 30 min. Attained membranes evolved oxygen at 300 ~ 360 μm O_2/mg Chl and contained less photosystem I components. Although the press is higher than other cases, it does not affect any chracteristics of thylakoid membranes. But yield of the membranes were decreased. The thylakoid membranes are consisted of all components of photosystem II, several unidentified polypeptides and a few components of photosystem I. The membranes show high yield of photosystem II fluorescence, but fluorescecne of photosystem I decreased to 25% at 715 nm compared to cells. The preparation method can be applied to all types of *Clhamydomonas sp.*. These prepared membranes are also useful for characterization of photosystem II kinetics containing water splitting mechanisms.

E205 Biosynthesis of Brassinosteroids in Suspension Cultured Cells of *Marchantia polymorpha L.* : From Teasterone to Typhasterol

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Brassinosteroids(BR) are steroidal phytohormones which regulate plant growth and differentiation. In order to elucidate the biosynthesis of BR in *M. polymorpha*, a feeding experiment of $[d_0+d_6]$ teasterone in the suspension cultured cells was undertaken. The metabolites of $[d_0+d_6]$ teasterone fed to suspension cultured cells of *M. polymorpha* were purified by open column chromatographies and HPLC. After analyzing the metabolites by HPLC and TLC, two major candidates were selected. They were characterized to be $[d_0+d_6]$ 3-dehydroteasterone and $[d_0+d_6]$ typhasterol by full scan GC-MS analysis. The identification of 3-dehydroteasterone and typhasterol as main metabolites from the cell suggests that C3-epimerization from C3β- hydroxylated teasterone to C3α-hydroxylated typhasterol is present in the cell, and that the C3-epimerization is intermediated by C3-keto BR, 3-dehydroteasterone. Thus the two-step reaction for C3-epimerization by dehydrogenation and hydrogenation is proposed as follow : teasterone→ 3-dehydroteasterone→typhasterol.