Reconstitution of cellulose synthesizing activity *in vitro* with algal and bacterial model

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Extracting cellulose-synthesizing activity is well known to be very difficult because cellulose synthase is highly complex machinery of membrane protein complex. Until now, few model organisms have successfully provided *in vitro* cellulose synthesizing activity after extraction from cell membrane. This study aims to newly establish *in vitro* system to synthesize cellulose I microfibril. Two models are selected: *Micrasterias crux-melitensis* (a green algae) and *Gluconacetobacter xylinus* (a gram-negative bacterium). The former is unicellular and taxonomically categorized Zygnematale, which is considered to be the direct ancestor of land plants. Therefore it is likely a good model for studying cellulose biosynthesis in plants. The latter is a famous model that has been reported already, but the product is cellulose II of a non-native form. Then improving conditions was conducted for the purpose of synthesizing cellulose I microfibril *in vitro* by using this bacterial model.

Experiments

M. crux-melitensis was grown in *Volvox* medium. *G. xylinus* was grown in SH-medium including 0.1% celluclast (commercial cellulase, Novozyme Inc.). Cells were harvested and disrupted by French Press at 20,000 psi. Microsome prepared by differential centrifugation was solubilized by detergent to extract cellulose synthesizing activity. UDP-glucose (substrate) was added to this detergent-extract for *in vitro* synthesis of cellulose; c-diGMP (cyclic-diguanyl monophosphate, allosteric effector of cellulose synthase in *G. xylinus*) was included together when *G. xylinus* was used. The synthesized product was examined by TEM (Transmission Electron Microscopy) and IR (InfraRed) spectroscopy. c-diGMP was enzymatically synthesized by recombinant DGC (diguanyl cyclase) protein [1].

Results

When *M. crux-melitensis* was used, two types of fibers were observed by TEM: short and long fibers (Figure 1A). The former was more frequently observed than the latter, and looks like those reported as callose [2]. The latter was likely cellulose microfibril when judged by appearance. Actually IR spectra suggested that the product might be a mixture of callose and cellulose. Further examination is in progress.

For *G. xylinus*, two nonionic detergents (*n*-decyl-β-D-maltoside (DM) and *n*-dodecyl-β-D-maltoside (DDM)) were newly tried for solubilzing cell membrane. Electron micrographs by negative staining showed that the product is not microfibril but aggregation in which about 40 nm of particles get together (Figure 1B). Electron diffraction and IR spectra indicated that the product is cellulose II as well.

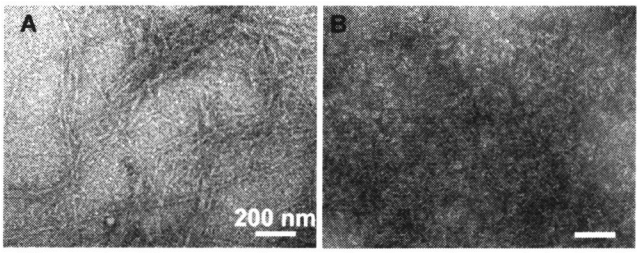

Figure 1. Electron micrographs (negative staining) of the *in vitro* product by *M. crux-melitensis* (A; digitonin used as detergent) and *G. xylinus* (B; DDM used).

Acknowledgements

The author appreciates Prof. Kazufumi Yazaki (RISH, Kyoto University) for the use of ultracentrifugation.

References

[1] Tsukasa Ichikawa "Enzymatic synthesis of c-di-GMP by recombinant protein" *Sustainable Humanosphere*, no.6, in this issue (2010)

[2] for example, Joséphine Lai-Kee-Him, Henri Chanzy, Martin Müller, Jean-Luc Putaux, Tomoya Imai, Vincent Bulone. "*In vitro* versus *in vivo* cellulose microfibrils from plant primary wall synthases: structural differences" *J. Biol. Chem.* vol. 277, no.40, pp 36931-36939, 2002.