ABSTRACTS (MASTER THESIS)

A preliminary study for purifying the full complex cellulose synthase

**(Graduate School of Agriculture,
Laboratory of Biomass Morphogenesis and Information, RISH, Kyoto University)**

Kaho Mitani

Cellulose is an abundant biopolymer on Earth, and it is synthesized at cell membrane by cellulose synthase complex (CSC), a complicated protein complex composed of several different subunits. In a famous cellulose-producing bacterium, *Gluconacetobacter xylinus*, it is thought that CSC is an assembly of at least four different polypeptide subunits: CesA, CesB, CesC, and CesD. Recently X-ray crystallographic analysis [1] revealed a three-dimensional structure of CesA/CesB complex, which allowed for a big progress of understanding the polymerization mechanism of cellulose in cellulose synthase. Yet it will be necessary to reveal the three dimensional structure of full complex for understanding another important mechanism of cellulose synthase, crystallization/spinning mechanism to produce cellulose into an apparent single crystal of cellulose microfibril.

To this end, we tried to purify cellulose synthase full complex by using *E. coli* expression system.

Experiments and Results

CesA, CesB, CesC, and CesD proteins from *G. xylinus* were co-expressed in *E. coli* by using a conventional expression vector. A detailed procedure how to prepare the expression vector will be described elsewhere. Hexa-histidine tag was fused at the C-terminus of CesD protein (CesD-His6).

Total membrane (mixture of inner and outer membrane of a gram-negative bacterium like *E. coli*) was isolated from *E. coli* cells expressing the four proteins by a conventional protocol of differential centrifugation. Solubilized fraction of membrane proteins with detergent was processed with immobilized metal affinity chromatography (IMAC) with a nickel agarose to trap CesD protein and any other proteins, if any, which interact with CesD protein. Eluted fraction from IMAC was analyzed by western analysis with SDS-PAGE.

Western analysis showed that CesA protein was found together with CesD-His6 protein in the IMAC elute while CesB and CesC were not found. This indicates a direct interaction between CesA and CesD protein. Given that CesA is known to form a complex with CesB protein [1], CesA, CesB, and CesD are included in the bacterial CSC. The other subunit CesC, which is supposed to be anchored in outer membrane, was not even solubilized by detergent as far as we have tried. Compiling our result and the previous knowledge together, we putatively hypothesize that CesA protein has an interaction with each of CesB and CesD (Figure 1): CesD is supposed to interact with CesA outside the cell given the interaction with cellulose [2].

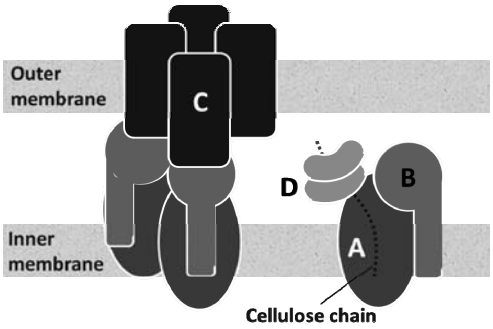

Figure 1. A putative model for the CSC of *Gluconacetobacter*: A, B, C, and D stand for CesA, CesB, CesC, and CesD subunits, respectively.

Acknowledgements

A part of the data was obtained by LC-IT-TOF analysis at DASH/FBAS in CER/RISH, Kyoto University.

References

[1] Morgan, J. L. W., Strumillo, J., and Zimmer, J., "Crystallographic snapshot of cellulose synthesis and membrane translocation", *Nature*, 493, 181-186, 2013.

[2] Hua, S.-Q., Gao,Y.-G., Tajima, K., Sunagawa, N., Zhoua, Y., et al. "Structure of bacterial cellulose synthase subunit D octamer with four inner passageways", *PNAS*, 110, 17957-17961, 2010.