RECENT RESEARCH ACTIVITIES

Start-up Experiment for Biochemical/Biophysical Study of Cellulose Biosynthesis

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Living organisms produce 100 billion tons of cellulose each year. Such massive cellulose production on earth means that the ability to synthesize cellulose has been beneficial to the organisms that acquired this ability through evolution, and that it has been "SUSTAINABLE" material in biosphere. The most striking example will be seen in the plant cell walls, in which cellulose plays an important role to support a tall tree such as 100-m. This is because cellulose has strong mechanical strength and biological resistance by virtue of its high crystalline fibrous structure with hydrogen bonding network, microfibril.

In spite of such ubiquitousness on earth, the enzymatic mechanism of cellulose biosynthesis is not well understood since most of researches focus on the molecular and cellular biological aspect of cellulose biosynthesis. This is probably because cellulose synthase is membrane protein and hetero-subunit complex, and extremely difficult to analyze the enzyme itself. Thus now, biochemical/biophysical analyses are absolutely demanded for understanding how cellulose microfibril is synthesized by cellulose synthase in lipid bilayer of cell membrane. For this purpose, cellulose synthase and its enzymatic activity must be directly analyzed in more detailed. I am challenging it using a cellulose-producing bacterium *Gluconacetobacter xylinus*. This is only one organism from which cellulose-synthesizing activity can be extracted without enzymatic contamination, allowing the direct analysis of cellulose synthase.

Enzyme assay paves the way for clarifying biochemical/biophysical aspects of cellulose biosynthesis

Previously reported protocol to extract the activity [1] uses PEG (polyethylene glycol) in buffer, which is unfavorable for many of biochemical analyses. Furthermore there is room for testing detergents to solubilize the activity from cell membrane, because many new detergents have been introduced since the previous protocol was often used in 1980's. Then two points are considered for establishing a new protocol: (i) removing PEG from buffer and (ii) solubilization by mild detergent. A new protocol is thus successfully established with no PEG and solubilization by detergent that has not been tested, like alkylmaltoside [2].

The access to enzymatic assay by this protocol allows biochemical analyses to be carried out (Figure 1). As well, the formation of cellulose microfibril will be investigated in a view of biophysics. These together will lead us to comprehensive understanding of cellulose biosynthesis.

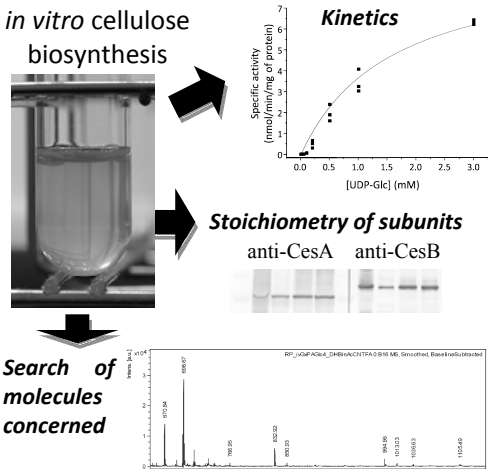

Figure 1. Established *in vitro* cellulose biosynthesis and the researches derived from it. These are actually in progress.

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References

[1] Aloni, Y., Cohen, R., Benziman, M., Delmer, D. "Solubilization of the UDP-glucose: 1,4-β-glucan-4-β-D-glucosyltransferase (cellulose synthase) from *Acetobacter xylinum*. A comparison of regulatory properties with those of the membrane-bound form of the enzyme," *J.Biol.Chem.* **258**, 4419-4423, 1983.

[2] Hashimoto, A., Shimono, K., Horikawa, Y., Ichikawa, T., Wada, M., Imai, T., Sugiyama, J. "Extraction of cellulose-synthesizing activity of *Gluconacetobacter xylinus* by alkylmaltoside." *Carbohydr.Res.* **346**, 2760-2768, 2011.