ABSTRACTS (MASTER THESIS)

Quantitative analysis of cellulose synthase activity

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Cellulose is one of the major biomass on the earth, and billions ton of that is produced a year by many biological organisms. This is because cellulose has been in general quite beneficial for those organisms by virtue of strong mechanical strength and biological resistance, which are given by its high crystalline fibrous structure with hydrogen bonding network, microfibril. The gene of cellulose synthase is identified in many species, and molecular and cell biological aspects of cellulose synthase is well understood. On the other hand, its enzymatic mechanism is just partially understood, and there are a lot of questions to be answered. Quantitative description of cellulose-synthesizing activity, for example enzyme kinetics, is very important for this purpose, and in my thesis, cellulose-synthesizing activity of a vinegar-producing bacterium *Gluconacetobacter xylinus* is quantitatively analyzed in vitro.

Experiments

Gluconacetobacter xylinus ATCC53524 was grown in SH-medium at 28°C until OD_{660} reaches to 0.5-0.7. Cells were ruptured by French-Press at 20,000 psi and then ultracentrifuged by 100,000g for 1 h to collect cell membrane fraction. Crude enzyme was solubilized from cell membrane by several detergents and the cellulose-synthesizing activity was measured with the ^{14}C-labeled substrate, UDP-D-[U-^{14}C]-glucose: the amount of ^{14}C incorporated into cellulose (ethanol insoluble fraction) was measured by liquid-scintillation counting. The specific activity was calculated and plotted as a function of substrate concentration to figure out substrate-saturation curve (S-V curve).

Results and Discussion

More than 30 conditions of solubilization (detergent molecule and its concentration) were tried and the synthesizing activity in detergent-extract was measured for 1h of reaction (Figure 1). It is noticeable that alkylmaltoside gave substantial activity in the extract. This type of detergent is one of the most successful ones in structural biology of membrane protein, implying less unfavorable interferes with protein. However, the activity in the extract is higher than that of cell membrane before solubilization, indicating activation of the enzyme by solubilization. Thus the enzyme might be substantially modified, and it is quite consistent with that this in vitro reaction produces cellulose II [1], a non-native form of cellulose. It will be supposed that cellulose synthase must be embedded in cell membrane for synthesizing cellulose I microfibril.

Using this protocol for counting the activity, kinetic analysis was carried out with the initial rate of the extract of decyl-β-maltoside. The concentration dependency of the activity about UDP-glucose and c-di-GMP was analyzed. As shown previously, c-di-GMP shows sigmoid S-V curve, indicating that the binding of c-di-GMP is cooperative about cellulose synthesis. Interestingly, S-V curve of UDP-glucose also shows sigmoidal feature in detergent extract, whereas it is hyperbolic before solubilization. K_m for UDP-glucose is 10^{-3} M while c-di-GMP has 10^{-6} M, and they do not change before and after solubilization. Although careful interpretation is necessary, these observations are very insightful.

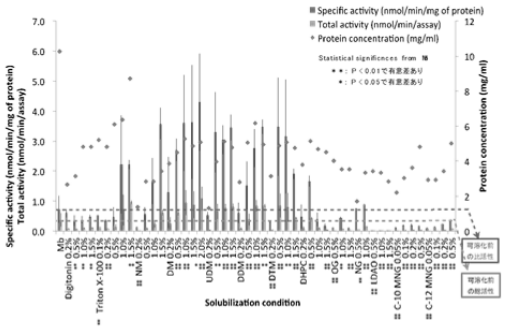

Figure 1 Total activity (green bars) and specific activity (red bars) of detergent-extracts. * and ** stands for significant difference from membrane (Mb) with $p<0.05$ and $p<0.01$, respectively.

References

[1] Hashimoto, A.; Shimono, K.; Ichikawa, T.; Horikawa, Y.; Wada, M.; Imai, T; Sugiyama, J. *Carbohydr. Res.* vol. 346, pp.2760-2768, 2011.