3D-reconstruction of Cellulose Synthase by Electron Microscopy

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Cellulose is one of the major biomass on the earth, and billions tons of that are produced a year by many biological organisms. This is because cellulose has strong mechanical strength and biological resistance, which are given by its high crystalline fibrous structure rich with hydrogen b onding network, microfibril. The gene of cellulose synthase is identified in many species, but none of them is proven to be the molecule that synthesizes cellulose microfibril. It's because the cellulose synthase is membrane protein and forms complex. As a simple model, vinegar-producing bacterium, *Gluconacetobacter xylinus* is selected. It is shown in this organism that two proteins are necessary to synthesize cellulose *in vitro*: GxCesA and GxCesB. Then, this study focused on these proteins and aimed to analyze these structures.

Experiment

GxCesA and GxCesB protein were coexpressed by *E.coli* expression system, which was designed to have GxCesB with His6 tag fused at carboxyl terminal. Cell membrane was isolated from the grown *E. coli* cells expressing GxCesA and GxCesB by differential centrifugation, and solubilized by detergent to have these two proteins in extract. The solubilized fraction was purified by metal affinity and gel filtration chromatography. In the current protocol, it is not possible to purify both GxCesA and GxCesB as the former was lost during the purification steps, and as a result highly purified GxCesB was obtained. This sample was observed by TEM (Transmission Electron Microscopy) with negative staining. The specimen was micrographed at 0° and 45° as a tilt-pair, and analyzed with SPIDER [1] and EMAN [2] package.

Results

Purified GxCesB protein was estimated to be 1,000 kDa by gel filtration chromatography, indicating that GxCesB is 10-12mer of homo-oligomer. By electron microscopy with negative staining, GxCesB was consistently observed as globular particles of 15 nm. As there has been no structural information about this protein, we tried to reconstruct three dimensional structure of GxCesB by random conical tilt using a series of tilt-pair. When alignment of the particle images was carried out by reference-free method, less features were found in the model as shown in Figure 1A, which may result from incorrect alignment and/or assignment of angular information. On the other hand, heterogeneous features are found in the model reconstructed from the images that were aligned by multi-reference alignment (Figure 1B). Starting from each of these 30 structures, we respectively refined model with 1136 particles using EMAN. The refined model has a kind of heterogeneity, and it became clearer when 2-fold symmetry was applied: the face that has "horns" (top) is found in the opposite to the flat face (bottom). This feature appeared whichever the initial structure was selected for the refinement, suggesting that both purification and reconstruction (image analysis) were reliable enough for further analysis.

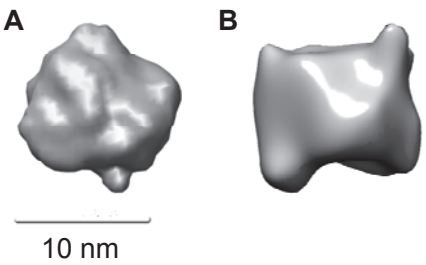

Figure 1. Three dimensional models of GxCesB reconstructed in this study by random-conical tilt: (A) a model when alignment was done by reference-free and (B) multi-reference protocol. Both models are shown with the noise-less level, by which *ca*. 70-80% of the volume expected from 1,000 kDa of MW was explained.

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References

1. **http://www.wadsworth.org/spider_doc/spider/docs/spider.html**
2. **http://blake.bcm.tmc.edu/eman/**