RECENT RESEARCH ACTIVITIES

Visualization of cellulose molecules in synthesis with time-resolved SAXS

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Purpose of the study

Cellulose is one of the sustainable materials to be used in near future given its high renewability. The major reason why cellulose could be such a useful material is that cellulose has a solid structure with fiber morphology, in which polymeric straight molecules of cellulose are crystallized (cellulose microfibril). Such a molecular assembly together with its molecular conformation provides the toughness with cellulose microfibril. The protein that produces such an excellent super-molecular structure is cellulose synthase, an enzyme embedded in cell membrane. We have not yet successfully reconstituted the native cellulose synthase activity *in vitro* despite many trials for as long as 10 years. In those experiments, cellulose polymer was successfully synthesized. However the synthesized product was no longer microfibril as found in nature, but globular aggregation as reported previously [1]. For clarifying this error in the reaction, we tried to understand what happens in this "abnormal" *in vitro* reaction with small angle X-ray scattering (SAXS), which allowed cellulose molecular seembling process to be visualized *in situ* in the reaction.

Experiments and results

In vitro reaction by crude enzyme of cellulose synthase, which was prepared from a bacterium *Komagataeibacter xylinus* [1], was observed by time-resolved SAXS experiment at BL40B2 in SPring-8 (Hyogo, Japan): details of the experiments are found in our published paper [2]. Briefly, SAXS pattern was recorded from the *in vitro* reaction every 5 min after starting the reaction, and azimuthally integrated to convert the scattering pattern to 1-D data with scattering vector q (= $(4\pi \cdot \sin\theta)/\lambda$) and scattering intensity I: θ is a half of scattering angle and λ is X-ray wavelength. Scattering signal from the cellulose synthesized by the reaction was approximately visualized by subtracting the 1st frame data of a time-resolved measurement (Figure 1). The scattering at q = 0.05 nm^{-1} started being apparent after 16 min of the reaction, while the one at q = 0.25 nm^{-1} was found already at very beginning of the reaction. Given that the former represents larger structure, it was shown that the synthesized cellulose molecules formed smaller structure at first, and subsequently those primitive aggregations were packed to form larger aggregates. This is the behavior of cellulose molecules synthesized by partially denatured cellulose synthase in our *in vitro* system.

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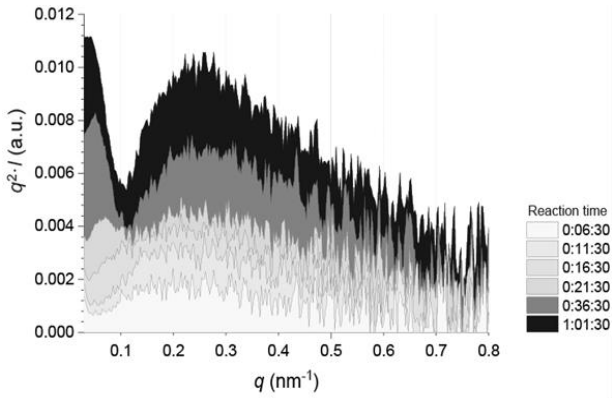

Figure 1.Typical time-resolved SAXS data for the *in vitro* synthesis of cellulose represented by Kratky plot: q vs q^2I. Scattering intensity indicates the existence of structure at the corresponding q, inversely indicating the size of structure.

References

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[2] Tajima, H., Penttilä, P.A., Imai, T., Yamamoto, K., Yuguchi, Y. "Observation of *in vitro* cellulose synthesis by bacterial cellulose synthase with time-resolved small angle X-ray scattering", *Int.J.Biol.Macromol.* **130,** 765-777, 2019.