Time-resolved small-angle x-ray scattering studies on the formation of Bacterial Cellulose

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バクテリアセルロース (BC) の形成過程を時分割小角・超小角 X 線散乱測定し、X 線による菌体の大きさの見積もりと、菌の増殖過程の測定に初めて成功した。また、BC は $0.01\mathrm{nm}^{-1}$ 程度の波数領域においては 2.5 次元のフラクタル構造を有することが分かった。

1 Introduction

Cellulose is one of the most abundant biomass, and has received much attention due to its biodegradability. Some bacteria, such as acetic acid bacteria, are known to produce cellulose, which is called bacterial cellulose (BC). The characteristics of the BC are its purity and its structure of fine and thin microfibrils. This structure cause special characters of BC, such as high Young's modulus and high sound velocity, which are important for the practical use. Although a large number of studies have been made on the structure of BC, little attempt has been made on the *in-situ* observation of its polymerization process[1]. To our knowledge, there are no studies on the *in-vivo* polymerization process by use of time-resolved scattering methods.

Our method of time-resolved small-angle X-ray scattering (SAXS) measurement of BC is particularly interesting from the viewpoint of bacterial-size measurement. Although there have been studies on the bacterial size by time-resolved light scattering[2], the method has its limit when the sample is not sufficiently transparent. X-ray scattering has the advantage in such a case, like our time-resolved measurement on BC where the sample become clouded when the BC is produced enough.

Although we have performed time-resolved SAXS and time-resolved ultra-small-angle X-ray scattering (USAXS) measurements, here we restrict ourselves to describe the results on USAXS.

2 Results and Discussion

Time-resolved USAXS measurements were performed on the BL20XU USAXS station at SPring-8. Figure 1 (b) shows the scattered intensity profiles up to 20 minutes of the culture time. The solid line shows the initial state when the bacteria were put into the nutrient medium, and the

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dashed and dotted lines show the profiles of 10 minutes and 20 minutes of the culture time, respectively. The temperature was kept at 29°C where the bacteria are known to be the most active. Figure 1 (a) shows the culculated curve for ellipsoidal model where the length of minor and major axes are 0.4μm and 1.2μm, respectively. These values were taken from the works of transmission electron microscope (TEM) observation[1], and we also confirmed the values by our TEM measurements of the bacteria. One can see the peak positions of (b) agree with that of (a), that shows the bacterial size was measured by USAXS.

Figure 1 (c) shows the intensity profile of BC without bacteria. One can see that the cellulose itself has a mass fractal structure of the dimension around 2.5. We thus found that BC has the fractal structure, and contributes to the intensity of the baseline slope of the scattering profiles. The separation of the contributions of bacteria and BC and the evolution of each contribution will be presented in the workshop.

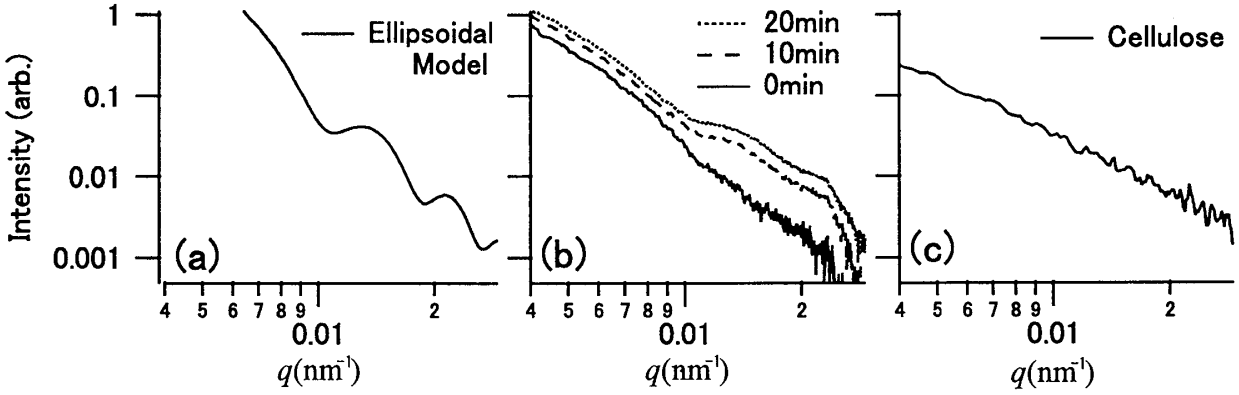

Figure 1: (a) The theoretical scattering curve obtained by the ellipsoidal model. (b) Time-resolved USAXS spectra for the polymerization of BC and the multiplication of the bacteria. (c) The experimental scattering curve of BC without bacteria.

3 Conclusion

We have succeeded in observing the bacterial size and multiplication process by time-resolved USAXS for the first time. We also found that the structure of bacterial cellulose in the range around 0.01nm^{-1} is characterized by the fractal dimensionality of 2.5.

References

[1] For the real-space studies on the production processes of BC, see, for e.g., A. Hirai, *et al.*, Cellulose **9** (2002) 105, **5** (1998) 201, **4** (1997) 239, and references there in.

[2] See, for e.g., W.P.V.D. Merwe, *et al.*, Biophys. J. **73** (1997) 500.