

## Chemical Reaction at Specific Sites and Reaction-induced Self-assembly: Enzymatic Polymerization of Cellulose As a Problem in Open-Nonequilibrium Phenomena

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非平衡開放系の散逸構造形成過程についての一研究として、本研究では酵素反応により溶液中で人工合成されるセルロース系を研究対象として用いた。そして系が形成するメゾスケールの自己秩序構造の形成過程・機構についての研究成果を議論する。

In this work, we would like to present our recent experimental results concerning chemical-reaction-induced self-assembly of reaction products as observed by time-resolved small-angle neutron scattering (SANS) as a probe to unveil mesoscopic-scale structure formation. Although the particular system to be reported here is a system comprised of cellulose molecules artificially synthesized via enzymatic polymerization, this type of research is considered to be concerned with one of general problems in macromolecules and to be interdisciplinary in nature, involving both chemistry (chemical reaction at a specific site of enzyme) and physics (reaction-induced self-assembly of reaction products) and biology as well, if we extend the study to biosyntheses of cellulose.

In this work we prepared separately two stable solutions A and B, as will be detailed below. Mixing the two solutions provides energy required for the enzymatic polymerization reaction and the reaction-induced self-assembly. In this sense our system is considered to belong to an open non-equilibrium system. We especially focus on self-assembling processes, mechanisms, and structures in mesoscopic scale, because they have not been well explored, despite its general importance for understanding pattern formation in nature, including biological systems.

The reaction was started by mixing two kinds of the solutions A and B; A is a solution of  $\beta$ -cellobiosyl fluoride (a substrate monomer, 10.3 mg,  $2.9 \times 10^{-5}$  mol) and deuterated acetate buffer (pD = 5.0, 0.05 M, 170  $\mu$ L); B is 30  $\mu$ L of deuterated acetate buffer (pD = 5.0, 0.05 M) solution containing unpurified cellulase (originated from *Trichoderma viride*; 0.52 mg, 5 wt %). The mixed solution (A + B) was rapidly transferred into a quartz cell with 2 mm thickness, and the cell was set into a chamber controlled precisely at 30 °C. Then time-resolved SANS measurements were started with SANS-J spectrometer installed at research reactor JRR-3 of Japan Atomic

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Energy Agency (JAEA) at Tokai. We applied a combined small-angle scattering (SAS) method of USANS (ultra-small angle neutron scattering), SANS, and USAXS (ultra-small angle X-ray Scattering) to the system in which the polymerization reaction was terminated at 18 hours after onset of the polymerization. The SAS results obtained after the termination are shown in Figure 1 which clearly elucidates that the differential scattering cross-section is given by the power law with the exponent  $\alpha = 3.7$ , corresponding to the surface fractal dimension of  $D_s = 6 - \alpha = 2.7$ , extending over 3 orders of magnitude in the length scale (from  $\sim 30$  nm to at least  $\sim 30$   $\mu\text{m}$ ). The same system was explored also under field-emission electron microscopy (FE-SEM). The FE-SEM observation under varying magnification also elucidated self-similar rough surface with upper and lower cutoff lengths of  $\sim 30$   $\mu\text{m}$  and  $30$  nm, respectively [1]. The inset to Figure 1 shows a typical FE-SEM micrograph obtained under the highest magnification, elucidating the lower cut-off length of the fractal surface of  $\sim 30$  nm.

The unique self-assembled structure will be interpreted as a consequence of (i) formation of a numerous number of cellulose molecules at a specific site existing in a narrow space (so called cleft of  $\sim 3$  nm long and  $\sim 0.55$  nm in cross-sectional area) within each active enzyme. We estimate 1g of active enzymes create 14 kg of cellulose; (ii) The numerous number of cellulose molecules created by the active enzyme spring out from a narrow space of the cleft in the enzyme into a reaction medium where they form the self-assembled structure as they are not soluble in the medium. The self-assembled structure is surprisingly and interestingly analogous to structures of fumes of a volcano rising from a crater.

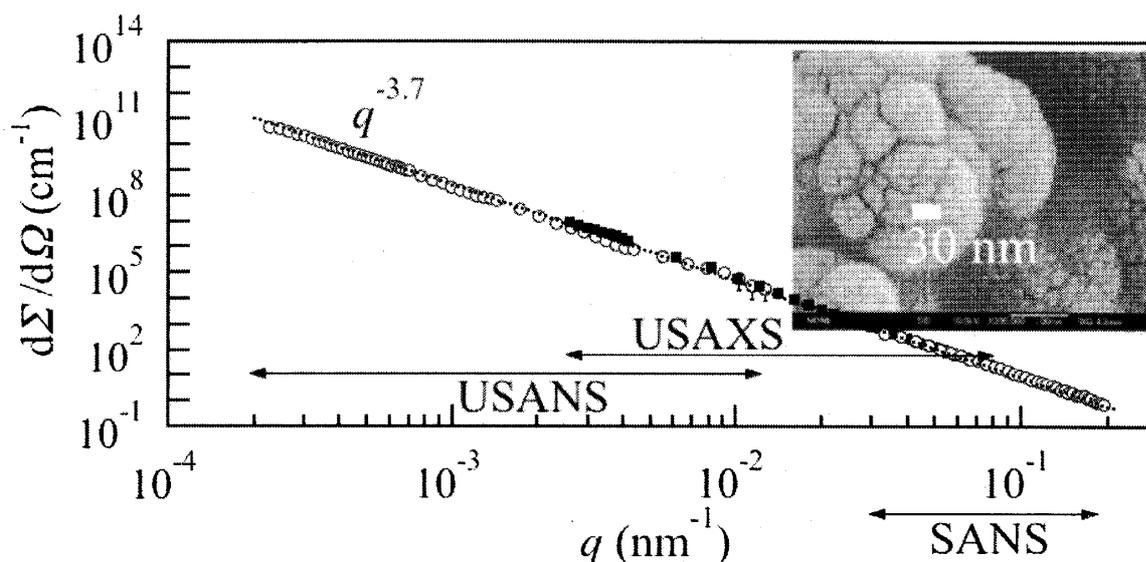


Figure 1 Combined SAS scattering profiles (USANS, USAXS, and SANS) for the self-assembly of the synthetic cellulose. SANS profile was corrected for incoherent scattering. The inset shows a typical FE-SEM micrograph.