6-4 Artificial Enzymatic Synthesis of Cellulose
—Elucidation of Self-Organization Mechanism of Reaction Products by Using Small-Angle Neutron Scattering Method—

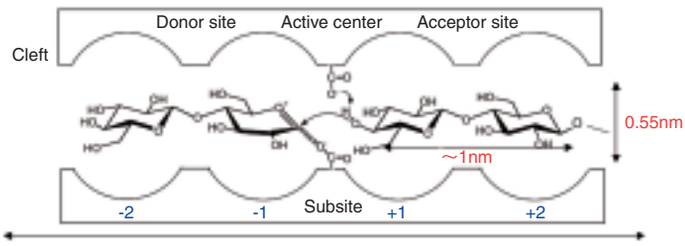

Fig.6-8 Schematic illustration of a so-called cleft, a specific reaction site in an enzyme
It contains a donor site, which recognizes the monomer, a pair comprising an active center and a subsite, which activate the monomer and chemically link the activated monomer into the end of the growing polymer chain, and an acceptor site, which anchors the growing polymer, in a very narrow space of about 3 nm in length and 0.55 nm in cross-sectional width.

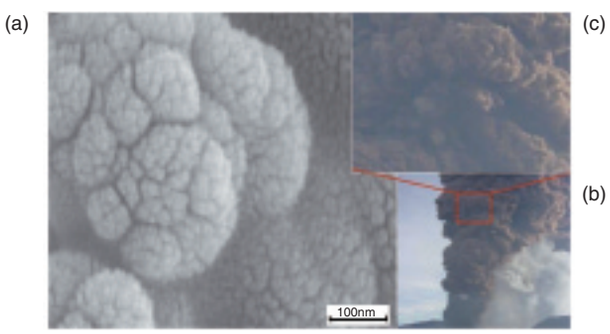

Fig.6-9
Field-emission scanning electron micrograph of a part of the self-similarly rough surface formed by cellulose molecules (a), similar to volcano fumes rising from a crater (b, c). Part c is an enlargement of the part of b encompassed by the red square.

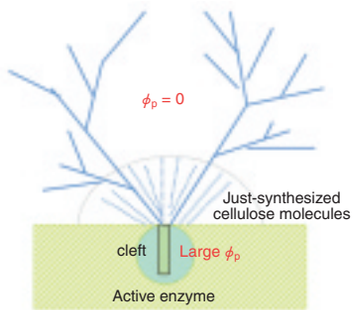

Fig.6-10 Schematic diagram showing the interface between the enzyme aggregate (the shaded green part) and the reaction medium
The cleft in the active enzyme (shown by the blue circle) keeps spurting out polymerized cellulose molecules, as a volcano crater springs out fumes. The cellulose polymers formed are soon aggregated into dendritic fibrils (shown by the blue dendrites) in the reaction medium, and they form a surface-based fractal object as shown in Fig.6-9(a). The large difference between the concentration (ϕ_p) of the cellulose molecules dissolved in the cleft and those in the reaction medium is expected to be a key factor in the spurting out of the cellulose molecules from the cleft and the formation of the fractal structure.

Cellulose, one of the important naturally made polymers, is biosynthesized at specific reaction sites in plants and bacteria. Synthesized cellulose molecules self-organize in-situ into crystalline fibrils. The specific reaction site is the so-called cleft found in active enzymes (Fig.6-8). Artificial syntheses of cellulose have been tried since 1941, based on traditional organic chemistry approaches, but none of them has succeeded. Kobayashi et al. first reported a successful synthesis based on enzymatic polymerization using cellulase as the catalytic enzyme and β-cellobiosyl fluoride as a substrate monomer (J. Am. Chem. Soc., vol.113, 1991, p.3079-3084).

In order to gain basic understanding of complex bioactivities concerning the cellulose synthesis and its self-organization into the cellulose fibrils, we attempted to explore the simple system employed by Kobayashi et al. using the time-resolved small angle neutron scattering spectrometer at the JRR-3 research reactor in JAEA. We elucidated for the first time in the world the following facts. (1) The enzyme molecules form aggregates having sizes greater than 200 nm in the reaction medium. (2) 1 g of active enzymes creates about 14 kg of cellulose during the whole reaction time, which means that one active enzyme creates about 5 cellulose molecules per second. (3) A large number of cellulose molecules, which have sprung out from the cleft assemble themselves into aggregates in the reaction medium and end up completely wrapping the enzyme aggregates. The surface of the cellulose aggregates (Fig.6-9(a)) has a self-similar roughness characterized by surface fractal structure with fractal dimension of 2.3 over an extremely wide length scale ranging from 30 nm to 30 μm, quite unique among the manifestations of fractal geometry. This surface structure is quite analogous to that formed by fumes from a volcano crater (Fig.6-9(b), (c)), except for the difference in length of a factor of 10^9. The similarity of the two patterns shown in Fig.6-9 together with the above fact (2) help us to intuitively understand the enormous energy that the enzymatic reaction potentially has.

References

Hashimoto, T. et al., Chemical Reaction at Specific Sites and Reaction-Induced Self-Assembly as Observed by In-Situ and Real Time SANS: Enzymatic Polymerization to Synthetic Cellulose, Biomacromolecules, vol.7, no.9, 2006, p.2479-2482.

Tanaka, H., Hashimoto, T. et al., Self-Assembly of Synthetic Cellulose during In-Vitro Enzymatic Polymerization Process as Studied by a Combined Small-Angle Scattering Method, Macromolecules, vol.40, no.17, 2007, p.6304-6315.