Structure analysis of bacteral flagellar filament by X-ray fiber diffraction

Kazuya Hasegawa[1], Toshio Oda[1], Koji Makino[1], Katsumi Imada[2], Shigehiro Nagashima[2], Fadel Samatey[2], Ichiro Yamashita[1], Yuichiro Maeda[1], Keiichi Namba[1,2]*

[1]International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd., [2]ERATO Protonic NanoMachine Project

Bacterial flagellar filaments are supercoiled assembly of single protein flagellin. The filaments undergo dynamic transformation of its shape between left and right handed supercoiled forms, in order for bacterial cells to swim and tumble. The structures of the two types of straight filaments, which represent the two states of subunit interactions in supercoiled filaments, have been studied by electron cryomicroscopy and X-ray fiber diffraction, which revealed that the concentric double tubular structure of the filament core plays important roles in the structural formation and switching. However, to resolve the structure at atomic details, fiber diffraction patterns of exceptionally high quality are essential. The relatively long repeat distances of the two types of the filament structures (1016 Å and 425 Å) make accurate measurement of the layer line intensities very difficult. Our new method for orienting filaments in liquid crystalline sols can produce extremely good orientation with the distribution angle as small as 0.6 degree, and therefore a narrow and strong X-ray beam at SPring8 was expected to produce superb fiber diffraction pattern with very sharp layer lines.

We carried out our experiment using the beam line BL41XU to collect fiber diffraction patterns of exceptionally good quality. We first surveyed the stability and life time of the oriented liquid crystalline phase of the sample sols against radiation damage by the intense X-ray beam. We found that the interference color observed under a polarization microscope starts diminishing after 10 second exposure within a portion of the oriented sol that the beam has gone through. After 30 second exposure, the color disappeared completely, leaving a black mark of square column that goes through the capillary diameter from one side to the other. The column size was about 80 μm by 80 μm, the same size and shape as those of the collimator. The column edge was very sharp and this clearly showed that the beam is highly parallel as expected. The figure shows a diffraction pattern collected from a sol of the R-type filaments in a 0.3 mm capillary tube, using an X-ray wavelength of 1.0 Å, a specimen-IP distance of 560 mm, an exposure time of 7 seconds. Sharp layer lines are clearly separated from each other and visible out to 3 Å resolution. The recorded intensities are equivalent to those that can be obtained by 10 hours exposure with our lab source, which has the highest possible brilliancy as a lab source. This means that the beam from BL41XU is more than 1,000 times brilliant, even at the present trial operation at a ring current of 20 mA.