Recording X-ray fiber diffraction patterns of amyloid fiber formed by yeast prion Sup35

Aiko Kishimoto[1] (8429), Kazuya Hasegawa*[2] (3342), Keiichi Namba[2,3] (3335)

[1]Chemical Resources Laboratory, Tokyo Institute of Technology
[2]Protonic NanoMachineProject, ERATO, JST
[3]Graduate School of Frontier Bioscience, Osaka University

Prion, which is named after proteinaceous infectious particle, causes several mammalian nerve diseases. It is believed that prion proteins adopting an abnormal conformation self-catalytically induce the normal prions to adopt the abnormal conformation and form amyloid fibers. These fibers deposit in the cell and finally result in the death of cell. Therefore, revealing the structure of the amyloid fiber is a crucial point to understand the mechanism of the self-catalytic conformational changes and the formation of amyloid fiber. Sup35 is an yeast transcription termination factor and has no homology with mammalian prions but it forms amyloid fibers in self-catalytic manner, and abnormal prions are inherited in progeny without obeying Mendel's law. Therefore, it is called yeast prion and serves as a model protein for mammalian prions. We have been studying the structure of amyloid fibers formed by Sup35 using X-ray fiber diffraction.

Fragment comprised of N- and M-domain of Sup35, which are expected to be involved in the filament core, was overexpressed in *E. Coli* and polymerized into amyloid fibers *in vitro*. Oriented fiber specimens were prepared by drying filament solution between the tips of two glass rods placed with a 3mm gap. Their diffraction patterns recorded in our laboratory showed layer line reflections but the S/N-ratio was quite poor except strong reflections at 4.7 Å. Therefore, we decided to use a synchrotron radiation beamline.

Diffraction patterns were recorded at a camera length of 240 mm and a wavelength of 1.0 Å. The orientation of the filament was not so good and therefore the S/N-ratio was not significantly improved, but the diffraction patterns showed some interesting features that have not been observed so far. The most intriguing point was two reflections at 2.4 Å and 2.2 Å on the meridian, which was contrary to our expectation that only one reflection corresponding to the second harmonic of 4.7 Å reflection would appear around this region. We would like to examine in the next machine time using a long camera length whether two reflections at 2.4 Å and 2.2 Å correspond to the second harmonics of two reflections at 4.8 Å and 4.4 Å which are not split in our current data.

Application of Small Angle X-ray Scattering for Predicting of Structure of Protein molecules

Katsuaki INOUE (1222), Toshihiko OKA (1327) and Naoto YAGI (1129)
Life & Environment Division, Spring-8, JASRI, Mikazuki, Sayo, Hyogo 679-5198 JAPAN

BL40B2 is for recording small-angle x-ray scattering from non-crystalline biological materials. The light source is a bending magnet and the white x-rays generated by the bending magnet are monochromatized using a double crystal monochromator and focused by a 1-m-long rhodium coated cylinder mirror. The flux, when the ring current was 100 mA, is estimated to be 1×10^{11} photons/sec at 1 Å (At the other wavelength, the flux is in same order). In this condition, the energy resolution (δE/E) is in the order of 10^{-4}. The beam size is 250 μm (horizontal)×200 μm (vertical) (FWHM) at the detector position. The tunable energy range is 0.7 Å ~ 1.8 Å. Four sets of quadrant slit are set in the experimental hutch. For correcting the beam shape and removing the parasitic scattering, one or two sets of slit will be used. Two fixed-length vacuum paths allow camera lengths of 400 mm and 1000 mm. As a detector, an imaging plate area detector (RIGAKU R-AXIS IV++) is installed. This beamline was designed to be suitable for static measurements with high accuracy and high resolution.

So far, although we tested many conditions of optics, as a result, the collimator whose diameter is about 1.2 mm is very effective to remove the parasitic scattering and it was clear that the minimum q-range of 0.007 $Å^{-1}$ can be achievable. In the case that the wavelength is 1.5 Å and the camera length is 1 m, the measurable q-range is estimated to be 0.007 ~ 0.6 $Å^{-1}$ (900 ~ 10 Å in Bragg spacing). Under these conditions, we measured scattering from GroEL solution, one of the chaperon protein whose molecular weight is 840 kDa. Fig.1 shows the scattering pattern of GroEL. It can be clearly measured down to 0.0014 $Å^{-1}$ in s-range. Moreover, wider s-region can be measured up to 0.08 (equal to 0.5 in q-region) clearly. It strongly reveals that the condition of the optics is very suitable for small-angle scattering measurements.

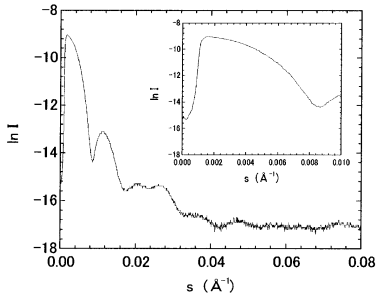

Fig.1 Representative scattering pattern from GroEL solution. Inset shows the magnification of the smaller angle region.