X-ray fiber diffraction from the native thin filament and F-actin sols

Toshiro Oda[1] (0003380), Kouji Makino[1,2] (0003385), Kazuya Hasegawa[1,3] (0003342), Ichiro Yamashita[1] (0003343), Keiichi Namba[1,4] (0003335) & Yuichiro Máeda[1*] (0003383)

[1]International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd., [2]Department of biological science, graduate school of science, Nagoya university, [3]Institute for Protein Research, Osaka University, [4]ERATO Protonic NanoMachine Project

Our group has been studying Ca^{2+}-regulation mechanism of muscle contraction. Sketetal muscle consists of two kinds of filaments - one is the thin filament containing actin, tropomyosin and troponin, and the other is the thick filament containing myosin. Myosin, in the thick filament, hydrolyses ATP to drive the relative sliding between the two filaments. The thin filament has a function of Ca^{2+}-regulation. The purpose of the project is to obtain the atomic structure of the thin filament. As a step leading to this end, we have been trying to solve a high resolution structure of actin filament, the backbone of thin filament. Moreover we are studying the structural change of the thin filament induced by Ca^{2+} binding. We use the X-ray fiber diffraction method, which is suitable for the structural analysis of fibrous macromolecular complexes.

We used a beamline BL41XU to record diffraction patterns from oriented sols of (1) gelsolin-capped actin filaments from rabbit sketetal muscle and (2) native thin filaments from porcine cardiac muscle. We first checked the life time of well-oriented filament sols under the X- radiation of this beamline, by employing flagella filament sols as a model system, in collaboration with Namba's group (1997B0024-NL-np). We determined to set the X-ray exposure time of within 10 sec. For compensation for the short exposure time, multiple diffraction patterns from several spots of the same domain in a F-actin sol were recorded on one IP. Figure shows a diffraction pattern from 10 spots of gelsolin capped F-actin sols in a 0.7mm capillary, 6 sec exposure time for each spot (total 60 sec), using an X-ray wavelength of 1.0Å and a specimen-IP distance of 560mm. Remarkable are the sharp layer lines and the low background noise, compared with the diffraction pattern obtained with our laboratory source. The sharper lines may be attributed to the narrow width of the X-ray beam. The lower background noise must be due to monochromatic beam and low level of scattering from the optics.

Some improvements, however, remain to be introduced in the beamline. The present beam stop casts a large shade on the low angle area (see figure), hiding the reflections below 5.1 nm spacing. We could not observe reflections that are associated with the troponin structure with a repeat distance of 36 nm. We plan to install a smaller beam stop with fine positioning mechanism.

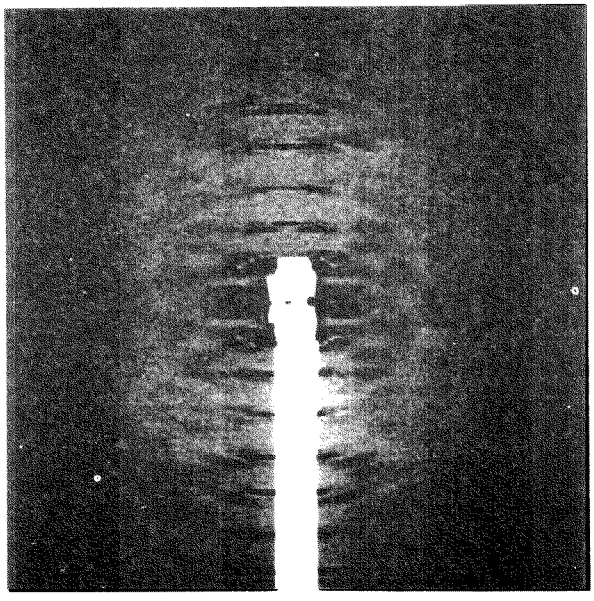

Figure X-ray fiber difraction pattern from Gelsolin- capped F-actin sols