ESRF	**Experiment title: X-ray fibre diffraction from ABP-F-actin complex sols – dolastatin 11.**	**Experiment number**: LS-1847
Beamline: ID02A	**Date of experiment**: from: 04-juni-01 7:00 to: 07-juni-juni-01 7:00	**Date of report**:
Shifts: 6	**Local contact(s)**: Dr. Stephanie FINET	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Kennth C.Holmes

*Dr.Toshiro Oda

*Dr.David Popp

Max Plank Institute for medical research

Jahnstr 29 Heidelberg D-69120 Heidelberg Germany

Collaboration with Prof. Robert B. Bates (Univ. of Arizona)

Report:

1. Introduction

Actin is one of the most abundant and conserved proteins in a cell. One of the most important functions is a formation of cytoskeleton that consists of actin network. To know the molecular mechanism of actin network dynamics, we studied the mechanism of some drugs that influence the actin assembly from viewpoint of structural biology. In this experimant, we focused on dolastatin 11 and phallodin. Dolastatin11 inhibits actin depolymerization like phalloidin. However, these binding positions to F-actin are different because dolastatin11 and phalloidin-rodamine do not compete with regard to F-actin binding. Therefore, the different mechanisms for the depolymerization inhibition have been expected (Bai et al., 2001).

2. Sample preparation

Actin was extracted from aceton powder using a Spudich & Watt (1971). The G-actin was polymerized by adding of KCl without $MgCl_2$ (Ca-actin). Ionic condition of the resulting F-actin solution was changed by dialysis for the solution containing 30mM KCl, 1mM $CaCl_2$, 0.5mM ATP, 1mM DTT, 1mM NaN_3 and Tris-acetate (pH=8.0). After the dialysis, phalloidin or dolastalin 11 was added. F-actin sols were made of the F-actin solution. First, F-actin filaments were collected by low-speed centrifugation and were sucked into the capillary. Second, F-actin filaments were oriented in the sol along the capillary using the strong magnetic field of 18 Tesla and 6 Tesla – normal NMR machine (Oda et al., 1998). Six Tesla was not enough to oriente F-actin filament.

3. Recording of diffraction patterns

At ESRF, we obtained x-ray fibre diffraction patterns from F-actin oriented sols with and without dolastatin 11 or phalloidin. Diffraction patterns were obtained using imaging plates with a beam size of 100μm × 100μm in ID2A beam-line. Typical espouse time was 4-5 seconds and the specimen-film distance was set to be ca 75

cm or ca 57cm. We recorded the patterns at the resolution limit of 8 Å. We also recorded the diffraction patterns from 3 degree tilted capillaries to record the meridian reflections clearly.

4. Suggestion from the diffraction patterns

1) Layer-line positions in three kinds of diffraction patterns were similar. This suggests that the symmetry change is small, if any, by binding of phalloidin or dolastation 11.

2) Major difference between the three kinds of diffraction patterns is intensity on 27Å layer-line. The intensity was enhanced by binding of phalloidin and was weakened by binidng of dolastation 11. This suggests that the binding positions are different along the filament axis.

3) Second peak along 51 Å layer-line was changed only by binding of phalloidin. Maybe this suggests that the radial binding position of phallodin and dolastation 11 from F-actin axis are also different.

5. Conclusion

Our diffraction experiment supports the biochemical experimant of competition between phallodin and dolastation 11 – the binding sites are different.

Reference

Bai et al., (2001) Dolastatin 11, a marine depsipeptide, arrests cells at cytokinesis and induces hyperpolymerization of purified actin Mol.Pharmacol 59: 462-469

Spudic & Watt (1971) The regulation of rabbit skeletal muscle contraction I. Biochemical studies of interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. J.Biol.Chem. 246: 4866-4871

T.Oda, K.Makino, I.Yamashita, K.Namba & Y.Maeda (1998) Effect of length and effective diameter of F-actin on the filament orientation in Liquid crystalline sols measured by X-ray fiber diffraction Biophy.J 75: 2672-2681.