Analysis of F-actin dynamics

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Actin, highly ubiquitous protein in eukaryotic cells, plays crucial roles in various aspects of cell motility. The actin monomers (G-actin) polymerize into filamentous form (F-actin). This polymerization, controlled through interactions with various proteins, and flexibility of F-actin have been shown to be the key features in the functions of actin. It is therefore important to understand how the dynamics of actin at various levels, from the internal dynamics of G-actin to fluctuation of F-actin, are related to various functions of actin. Here, as one of the projects towards ultimate understanding of the dynamics of actin at various levels, we carried out neutron spin-echo (NSE) experiments on actin. NSE spectroscopy is a quasielastic neutron scattering technique that can study long range relaxation processes in a macromolecule on nano-sec timescales and nano-meter length scales.

Actin was purified from rabbit skeletal muscles. Actin was polymerized by adding KCl to form F-actin. In the preparation of G-actin, actin labelled by the fluorescent probe tetramethyl-rhodamine-5-maleimide was used to block polymerisation (Otterbein, Graceffa, and Dominguez, 2001). Solutions of G-actin and F-actin were prepared in D2O. The NSE measurements on these solutions were carried out on the NSE spectrometer, iNSE, run by the University of Tokyo instruments, installed at the guide hall of the research reactor, JRR-3M, Ibaraki, Japan. From the set-up of these NSE measurements, information on the dynamics of actin on timescales up to 15 nsec and on length scales from 5 to 15 nm should be obtained. Figure 1 shows the normalized intermediate scattering functions of G-actin and F-actin. The functions at several representative Q-values were plotted against Fourier time. Clear differences in the spectra between G-actin and F-actin were observed. While the normalized intermediate functions of G-actin show usual features that the decay of the functions could be fit with single exponentials, the rate of decay of those of F-actin appears very slow. This indicates that there is a large difference in translational diffusion between G-actin and F-actin. To make quantitative comparison of the dynamic behaviours of G-actin and F-actin, detailed analysis of these intermediate functions are currently underway.

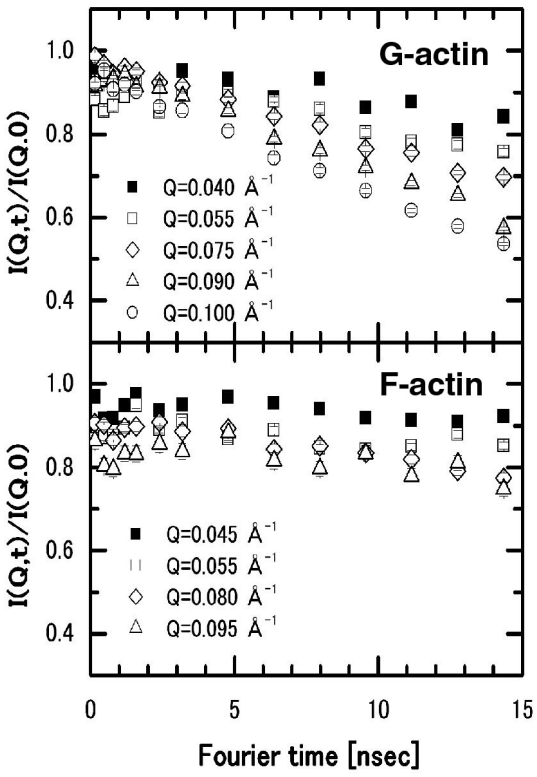

Fig. 1. The normalized intermediate scattering functions of G-actin and F-actin.