Changes in picosecond dynamics of muscle thin filaments and their hydration water caused by Ca^{2+}-activation studied by quasielastic neutron scattering.

National Institutes for Quantum and Radiological Sciences and Technology, Tatsuhito Matsuo

1. Introduction

Thin filaments are protein complexes involved in the regulation of cardiac muscle contraction in a Ca^{2+}-dependent manner. They consist of actin filament (F-actin), Troponin (Tn), and Tropomyosin (Tm) (Fig. 1), among which Tn plays a central role in the regulatory function of the thin filaments. As the Ca^{2+} concentration inside muscle cells rises, Ca^{2+} binds to Tn. This triggers a series of structural changes within the thin filaments, which in turn promotes the interaction between myosin and actin, leading to force production.

Elucidation of the molecular mechanism of the regulatory function of thin filaments has been one of the major subjects in muscle physiology. Much attention has been paid to the dynamical aspects of thin filaments. A recent study by molecular dynamics simulation has shown that the root-mean-square fluctuation of protein atoms in thin filaments changes upon Ca^{2+}-binding to Tn (Manning et al., Biochemistry, 2011). Furthermore, it has been demonstrated that cardiomyopathy-causing mutations in Tm changes the profile of its energy landscape on the F-actin surface (Orzechowski et al., ABB, 2014), implying that modulation of Tm dynamics causes the disruption of the regulatory function of thin filaments. These studies strongly suggest that the regulatory function is controlled by the dynamical properties of thin filaments. Since the atomic fluctuations in protein molecules are driven by the interaction between the protein atoms and the hydration water surrounding the proteins, it is essential to characterize the details of the dynamical behavior of not only the thin filaments but also their hydration water for ultimate understanding of the molecular mechanism of the regulatory function.

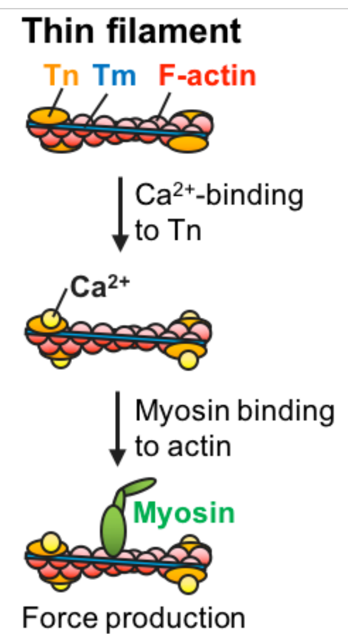

Fig. 1 Schematic diagram of thin filaments

In this proposal, we investigated the changes in atomic fluctuations of thin filaments caused by Ca^{2+}-activation at picosecond time scale and angstrom length scale using quasielastic neutron scattering.

2. Experiment

In this experiment, the following solution samples were prepared:

1) 150 mg/ml thin filament in the $-Ca^{2+}$ state in the H_2O buffer
2) 150 mg/ml thin filament in the $-Ca^{2+}$ state in the D_2O buffer
3) 150 mg/ml thin filament in the $+Ca^{2+}$ state in the H_2O buffer
4) 150 mg/ml thin filament in the $+Ca^{2+}$ state in the D_2O buffer
5) H_2O buffer (20 mM MOPS pH 7.0, 100 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.5 mM DTT, 1 mM NaN_3)

6) D_2O buffer (20 mM MOPS pD 7.0, 100 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.5 mM DTT, 1 mM NaN_3)

These samples were put in an aluminum flat cell and sealed with indium wire. The QENS measurements were done using BL14 AMATERAS at 300 K. The exposure time was ~12 hours for each sample. In addition to the samples listed above, an empty cell and a vanadium slab were measured for correcting background and obtaining the instrumental resolution function.

3. Results

The QENS spectra arising from only proteins were obtained by subtracting the spectra of a D_2O-buffer from those of a D_2O-sample. All these spectra (scattering intensity: S(Q,ω)) were integrated along the ω-direction to obtain the S(Q) (Fig.2, *left*). Whereas an intensity peak arising from the coherent scattering of solvent molecules is observed at around Q = 1.9 [$Å^{-1}$] for the D_2O-sample and the D_2O-buffer, the S(Q) of proteins is almost flat in Q > 0.4 [$Å^{-1}$], demonstrating that the contribution of the solvent to the spectra of proteins is negligible. The increase in S(Q) in Q < 0.4 [$Å^{-1}$] is due to the coherent scattering of proteins, and hence these Q regions will be omitted from a further analysis. Representative spectra of proteins are shown in Fig.2, *middle*.

Next, in order to extract the spectra of the hydration water, the spectra of proteins were subtracted from those of a H_2O-sample using a factor determined by the scattering cross-section. This provides the spectra of the sum of the bulk water and the hydration water (Fig. 2, *right*). From these spectra, the contribution of the bulk water (the spectra of a H_2O-buffer) was subtracted assuming the hydration ratio of proteins of 1.6 g H_2O/g proteins, which corresponds to about two layers of the hydration shell.

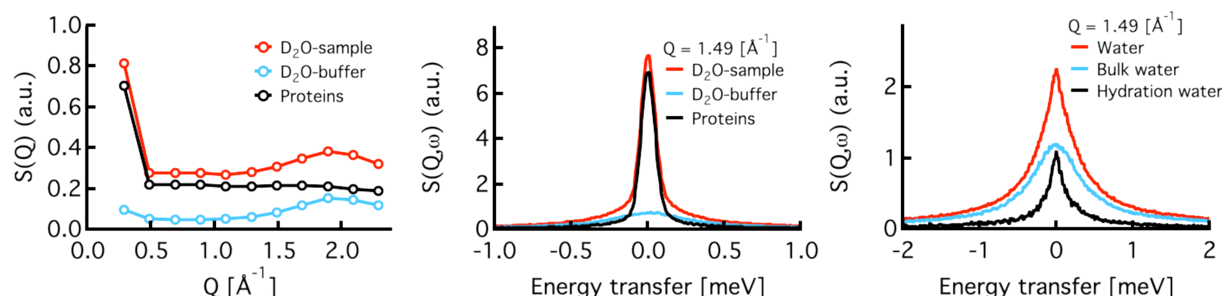

Fig. 2. Analysis of the QENS spectra. (*Left*) The S(Q) of the proteins with D_2O-buffer, the D_2O-buffer, and the proteins are denoted by red, cyan, and black solid lines, respectively. (*Middle*) The S(Q,ω) of the proteins with D_2O-buffer (red line), the D_2O-buffer (cyan), and the proteins (black) at Q = 1.49 [$Å^{-1}$]. (*Right*) The S(Q,ω) of the sum of the bulk and the hydration water (red line), the D_2O-buffer (cyan), and the hydration water of thin filaments (black) at Q = 1.49 [$Å^{-1}$].

4. Conclusion

The QENS spectra arising from cardiac thin filaments were extracted successfully both in the absence and presence of Ca^{2+}. Furthermore, the spectra arising from their hydration water were also obtained in spite of its small scattering cross-section. The detailed analysis of these spectra is currently underway to extract both the internal and the global motions of the cardiac thin filaments, and their hydration mobility.