実験報告書様式(一般利用課題・成果公開利用)

( ※本報告書は英語で記述してください。ただし、産業利用課題として採択されている方は日本語で記述していただいても結構です。 )

| CROSS TOKAI **Experimental Report** J-PARC | 承認日 Date of Approval 2013/9/18<br>承認者 Approver Kaoru Shibata<br>提出日 Date of Report 2013/6/26 |
|---|---|
| 課題番号 Project No.<br>2012B0136<br>実験課題名 Title of experiment<br>Dynamics of the muscle thin filaments studied by neutron scattering<br>実験責任者名 Name of principal investigator<br>Satoru Fujiwara<br>所属 Affiliation<br>Japan Atomic Energy Agency | 装置責任者 Name of Instrument scientist<br>Kaoru Shibata<br>装置名 Name of Instrument/(BL No.)<br>DNA (BL02)<br>実施日 Date of Experiment<br>February 15, 2013 ～ February 22, 2013 |

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。(適宜、図表添付のこと)
Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

(1) 130 mg/ml Muscle thin filaments in the low-$Ca^{2+}$ state in $D_2O$

(2) 170 mg/ml Muscle thin filaments in the high-$Ca^{2+}$ state in $D_2O$

(3) 175 mg/ml F-actin in $D_2O$

(4) Buffer in $D_2O$

2. 実験方法及び結果 (実験がうまくいかなかった場合、その理由を記述してください。)
Experimental method and results. If you failed to conduct experiment as planned, please describe reasons.

Experimental method

The neutron scattering spectra of the samples described in section 1 were measured in the energy transfer range of −0.5 meV and 0.5 meV and the momentum transfer range of 0.125 $Å^{-1}$ and 1.775 $Å^{-1}$, at the energy resolution of 12 μeV, using the instrument BL02 (DNA), run at 300 kW. The measurements were done at several temperature points between 280 K and 300 K. Exposure times of the measurements were between 6 hours and 10 hours. The obtained spectra were corrected for the vanadium standard, and the contribution of the empty cell was subtracted. The spectra of the background (the sample # 4 in section 1) were then subtracted from those of the samples. These difference spectra contain information on the internal dynamics of the protein complexes measured. The spectra thus obtained were then integrated over the region corresponding to the energy resolution around the elastic peak at each Q. These integrated curves are the elastic incoherent neutron scattering (EINS) curves.

2. 実験方法及び結果（つづき） Experimental method and results (continued)

Results

The samples used here were purified from bovine cardiac muscles, and retain the ability to control muscle contraction in a [$Ca^{2+}$]-dependent manner. The muscle thin filaments in the low-$Ca^{2+}$ state (sample #1) thus correspond to the state where no muscle contraction occurs while those in the high-$Ca^{2+}$ state (sample #2) correspond to the state where the contraction can occur. Comparison of the dynamics in these states should therefore provide insights into the role of the dynamics in the regulatory mechanism of muscle contraction. The measurements on F-actin were done as a standard for comparison, because F-actin lacks the regulatory proteins, troponin and tropomyosin, existed in the muscle thin filaments. The EINS curves of each sample were analyzed by the "Guinier" plots to estimate the mean square displacement arising from thermal fluctuations of the atoms in the protein complexes. Figure 1 shows examples of the "Guinier" plots of the EINS curves.

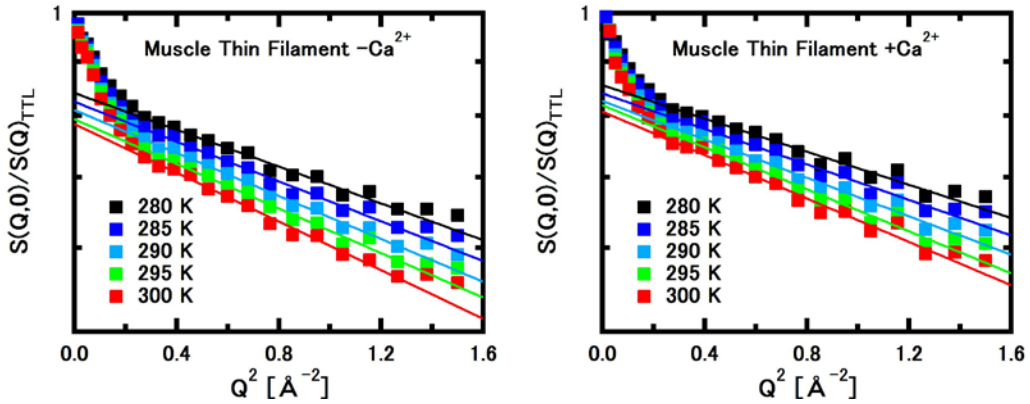


**Figure 1. Examples of the EINS curves. The curves of the muscle thin filament in the low-$Ca^{2+}$ state (-$Ca^{2+}$) and the high-$Ca^{2+}$ state (+$Ca^{2+}$) are shown. To remove the effects of the form factor, the curves were divided by the curve integrated over the whole energy transfer range measured at 280 K.**

The slopes of these curves provide the values of the mean square displacements. Figure 2 shows temperature dependence of the mean square displacements of the muscle thin filament in the low- and high-$Ca^{2+}$ states and F-actin. Differences in the values of the mean square displacements are clearly observed. The large values of the mean square displacements of the muscle thin filaments in the low-$Ca^{2+}$ state than those in the high-$Ca^{2+}$ state indicate that the muscle thin filaments are dynamically more disordered in the low-$Ca^{2+}$ state than in the high-$Ca^{2+}$ state. It should also be noted that the muscle thin filaments are more disordered than F-actin. Taking account of the difference in the components in the muscle thin filaments and F-actin, it appears that the components which are dynamically disordered are the regulatory proteins, troponin and tropomyosin. The changes in the dynamics of these regulatory proteins suggest that the dynamics of these proteins plays an important role in the regulatory mechanism of muscle contraction.

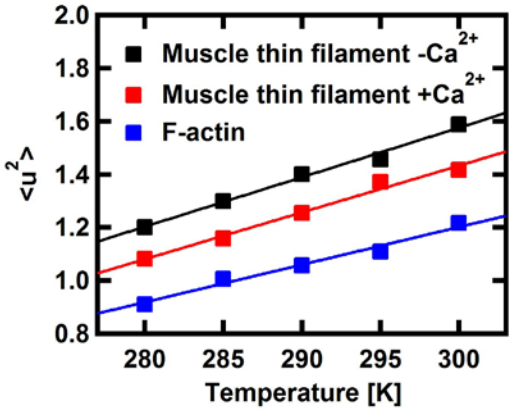


**Figure 2. Temperature dependence of the mean square displacements $\langle u^2 \rangle$ of the muscle thin filaments in the low- and high-Ca2+ states and F-actin.**