



実験報告書様式(一般利用課題・成果公開利用)

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 Experimental Report 	承認日 Date of Approval 2014/10/2 承認者 Approver Kaoru Shibata 提出日 Date of Report 2014/10/2
課題番号 Project No. 2014A0117 実験課題名 Title of experiment Dynamics of the muscle thin filaments studied by neutron scattering 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation Japan Atomic Energy Agency	装置責任者 Name of Instrument scientist Kaoru Shibata 装置名 Name of Instrument/(BL No.) DNA (BL02) 実施日 Date of Experiment June 20, 2014 ~ June 26, 2014

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) 82 mg/ml Muscle thin filaments in the low-Ca ²⁺ state in D ₂ O (2) 79 mg/ml Muscle thin filaments in the high-Ca ²⁺ state in D ₂ O (3) 115 mg/ml F-actin in D ₂ O (4) Buffer in D ₂ O
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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. <u>Experimental method</u> The neutron scattering spectra of the samples described in section 1 were measured in the energy transfer range of -0.04 meV and 0.1 meV and the momentum transfer range of 0.125 Å ⁻¹ and 1.8 Å ⁻¹ , at the energy resolution of 2.5 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 5 hours and 6 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.
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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 ($-Ca^{2+}$, sample #1) thus correspond to the state where no muscle contraction occurs while those in the high- Ca^{2+} state ($+Ca^{2+}$, 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 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 the "Guinier" plots of the EINS curves.

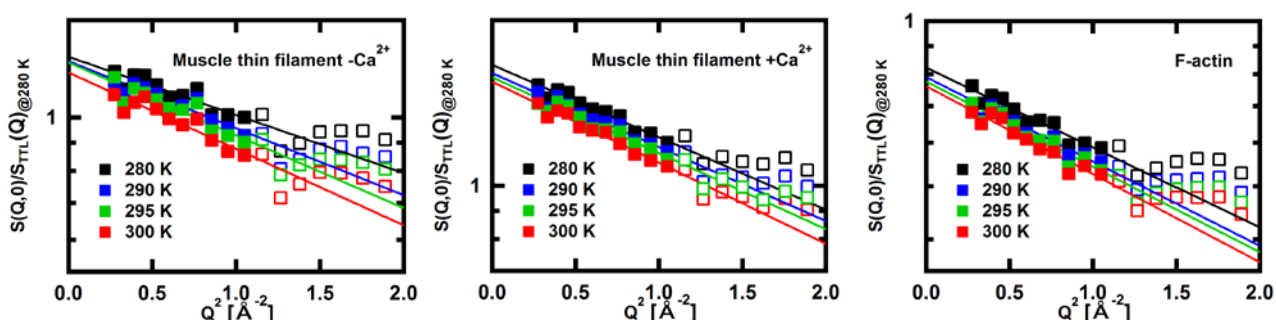


Figure 1. The Guinier plots of the EINS curves. The curves of the muscle thin filament in the low- Ca^{2+} state ($-Ca^{2+}$), the high- Ca^{2+} state ($+Ca^{2+}$), and F-actin are shown. To remove the effects of the form factor, the curves were divided by the curve integrated over the whole energy transfer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

The slopes of these curves provide the values of the mean square displacements (MSD). Figure 2 shows temperature dependence of the MSDs of the muscle thin filament in the low- and high- Ca^{2+} states and F-actin. Since the MSD values here were obtained from the EINS curves divided by the curves integrated for the energy range measured, the relative changes of the MSD values are important. The solid lines in Fig. 2 show the results of the linear fits to the data, from which the effective force constants, $\langle k' \rangle$, are estimated by the equation:

$$\langle k' \rangle = 2k_B / (d\langle u^2 \rangle / dT),$$

where k_B denotes the Boltzmann constant, and T denotes the temperature. This effective force constant reflects the resilience, or flexibility, of the protein. The $\langle k' \rangle$ values were estimated to be 0.12 ± 0.03 N/m, 0.27 ± 0.14 N/m, and 0.28 ± 0.14 N/m for the muscle thin filaments in the low- Ca^{2+} state, those in the high- Ca^{2+} state, and F-actin, respectively. The differences in these values indicate that the muscle thin filaments are more flexible in the low- Ca^{2+} state than in the high- Ca^{2+} state. Similar values in the muscle thin filaments in the high- Ca^{2+} state and F-actin suggest that the flexible region in the muscle thin filaments is 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.

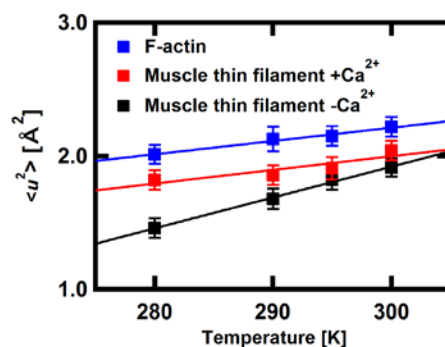


Figure 2. Temperature dependence of the mean square displacements $\langle u^2 \rangle$ of the muscle thin filaments in the low- and high- Ca^{2+} states and F-actin.