Biological Science

15A/2005G310

Modeling of the Thin Filaments in Resting and Contracting States of Vertebrate Skeletal Muscle Based on X-ray Fiber Diffraction Data

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Introduction

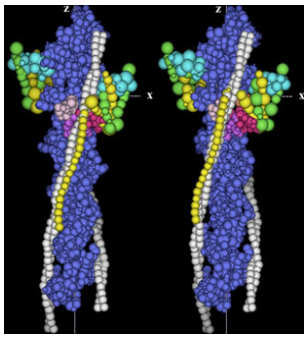
Using X-ray fiber diffraction data obtained from frog skeletal muscles in resting and contracting states, the structure of the thin filament was modeled by introducing troponin molecules. In the modeling calculation, crystal structures of actin, tropomyosin and troponin core-domain were used. Since the crystral structure of troponin-T1 part is unknown, it was approximated as a long helix. Four models were obtained with a low R-factor value in each state.

Experimental

Live frog sartorius muscles were mounted vertically against the X-ray beam. The X-ray diffraction patterns in resting and contracting states were recorded with image plates at the BL15A1.

Results and Discussion

The intensities of the thin filament-based layer lines were measured from the X-ray diffraction patterns and used in the modeling calculation. Firstly, using the high-angle X-ray data which the F-actin contributes dominantly, the F-actin model was constructed by altering the actin subdomain structures. The Holmes' model was used as a starting model. Next, we constructed the model which was composed of F-actin (FA), tropomyosin (TM) and troponin (TN)-T1 using the low-angle X-ray data. Finally, by adding the TN core-domain, the whole thin filament model was searched by minimizing the R-factor to obtain the better fit to all layer-line data. In this approach, we were able to obtain the four models in both states which fitted to the observed data well. The best-fit model revealed the following features. In the resting state, TMs located near the subdomain 1 of actin, where the binding sites of myosin heads reside. The TN core domains also positioned to cover the binding sites of the myosin heads to actin and the TN-T1 part sat along the TM strand. In the contracting state, TM strands together with TN-T1s moved around the FA by ~20° toward the inner domains of actin. The TN core-domains still kept its resting orientation but projected outward from the FA surface by ~7Å. Since the myosin head binding sites are uncovered in this model, it is possible for the myosin heads to interact with the actin strongly.



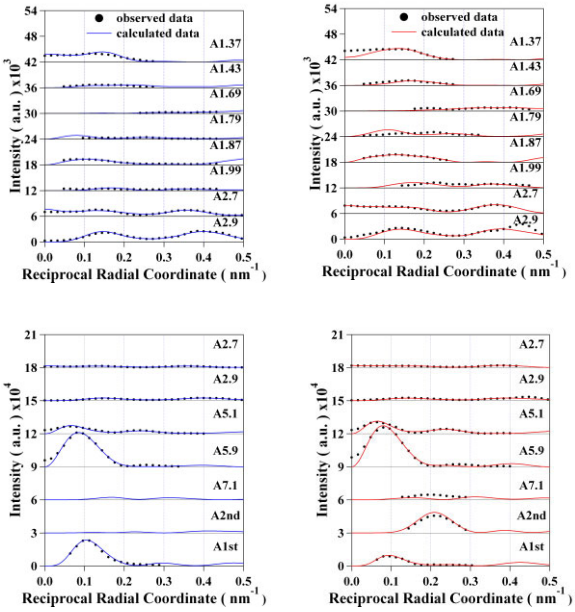


Figure 1. The left and right models denote the best-fit resting and contracting structures of the thin filament, respectively. Actin is represented in blue, TMs are in silver and TN subunits are in yellow, green and light-blue. In the lower figures, the dotted curves denote the observed intensity data, the blue-line ones denote the calculated intensities from the best-fit model (R~0.14) in the resting state and the red ones denote the observed intensity data and calculated ones from the best-fit model (R~0.13) in the contracting state.

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