

## Alteration of the Helical Twist Associated with the Extensibility of the Actin and Myosin Filaments during Force Generation of Skeletal Muscles

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### Introduction

Twisting changes of the actin and myosin helical filaments associated with their extensibility in the force generating state of frog skeletal muscles were investigated by the X-ray diffraction techniques.

In the first layer-line complex, the actin and myosin filament-based components were well resolved in the radial region close to the meridian by a Gaussian deconvolution method [1]. We applied the external stretch to contracting muscles and measured the axial spacing change of the actin- and myosin-based first layer-line components in the X-ray diffraction pattern.

### Experimental

Living sartorius muscles of the bullfrog were used for this study. We measured 2D X-ray diffraction patterns from muscles. Length change experiments were performed in the same way as in the previous study [2]. The 2D diffraction patterns of the resting, isometric contraction, and stretch phases were recorded on image plates using the image plate exchanger system. The specimen-to-detector distance was 2.1 m.

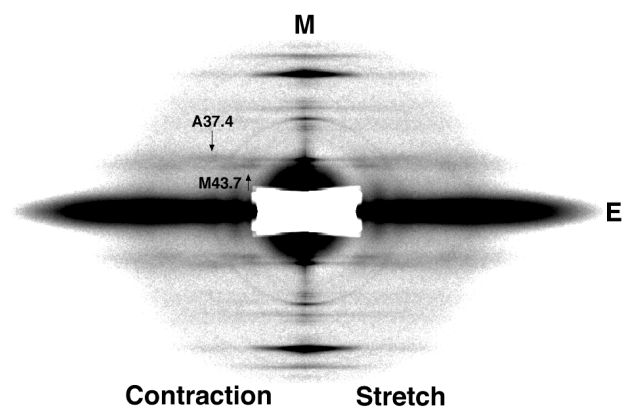
### Results and Discussion

As shown in Fig. 1, during the isometric contraction, in the inner radial region ( $0.039 \leq R \leq 0.063 \text{ nm}^{-1}$ ) the axially sharp reflection existed at  $\sim 1/37.4 \text{ nm}^{-1}$ . This 37.4-nm component seemed to come mainly from the actin first layer-line. Also, the layer-line reflection was observed at  $\sim 1/43.7 \text{ nm}^{-1}$  in the very inner radial region ( $0.021 \leq R \leq 0.032 \text{ nm}^{-1}$ ), which was assigned to the remnant of the myosin first layer-line. We inspected the axial spacing change of the 37.4-nm actin component and the 43.7-nm myosin one, together with those of the 5.1-nm and 5.9-nm actin layer lines and those of the 14.5-nm-repeat myosin meridional reflections. The 37.4-nm actin component corresponding to half pitch of the right-handed 2-start helices increased in axial spacing by  $\sim 0.90\%$  over the isometric value. The 5.1-nm and 5.9-nm layer lines, corresponding to the pitches of the right- and left-handed genetic helices of the monomers in the actin filament, changed in axial spacing by  $\sim 0.13\%$  and  $\sim 0.04\%$ , respectively. These observations confirmed that the extension of the actin filament caused by force generation was closely associated with its helical twisting change;  $\sim 0.36\%$  extension and an untwisting of  $\sim 0.24^\circ$  of the right-handed genetic helix between the monomers under the maximum force. This is consistent with the previous result [2]. The 43.7-nm myosin component

corresponding to the one-third pitch of the right-handed 3-start helices increased in axial spacing by  $\sim 0.41\%$  over the isometric value, while the 14.5-nm and 7.2-nm myosin meridional reflections coming from the axial repeat of the myosin molecules increased in axial spacing by  $\sim 0.13\%$  and  $\sim 0.11\%$ , respectively. These observations indicated that the extensibility of the myosin filament also accompanied its helical twisting change, similarly to that occurring in the actin filament;  $\sim 0.40\%$  extension and an untwisting of  $\sim 0.39^\circ$  of the right-handed 3-start helices between the monomers under the maximum force. Twisting motions of the actin and myosin filaments relate to the mechanics of their intermolecular contacts, as well as the possible torsional forces applied by actomyosin interaction. These findings reveal that the twisting changes of both the filaments closely couple to each other to generate force in muscle contraction.

### References

- [1] Takezawa et al., *J. Mus. Res. and Cell Motil.*, **22**, 205 (2001).
- [2] Wakabayashi et al., *Biophys. J.*, **67**, 2422 – 2435 (1994).



**Fig. 1** A comparison of X-ray diffraction pattern in the small angle region from frog skeletal muscles in the contracting state and during stretching of contracting muscles. The meridional axes are coincided with each other.

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