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Structural change of myosin head in skeletal muscle fiber without thin filament

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1. Introduction

In relaxed muscle, diffraction pattern gives information about myosin structure. However, when actin and myosin undergo normal contractile interaction or potentiated contractile interaction facilitated by phosphorylation of myosin light chain, structural information of myosin was dissipated due to intervention of actin structure. The aim of this experiment was elucidating structural change of myosin itself during ATP hydrolysis without intervention of actin-myosin interaction to reveal elementary process of chemomechanical coupling on myosin realizing muscle contraction.

$$M \rightarrow M\text{-}ADP\text{-}Pi \rightarrow M\text{-}ADP \rightarrow M$$

Scheme 1 Proposed ATP hydrolysis cycle on myosin
M:myosin without nucleotide,
M-ADP-Pi: ADP-Pi-bound myosin
M-ADP:ADP-bound myosin

2. Experiments

Skinned skeletal muscle specimens were obtained by treatment of bundles consisting of 3 skeletal muscle fibers from rabbit psoas muscle with 0.5% triton-X to destroy cell membrane. Skinned skeletal muscle specimens were then treated with 3 mg/ml gelsolin purified from bovine serum [1] for 5 hours to remove actin filament from the specimen. X-ray diffraction patterns in relaxing (myosin is mainly in M-ADP-Pi state), ADP-rigor (myosin is in M-ADP state), rigor (myosin is in M state) were obtained using imaging plate system at 20 degrees of Celsius. To remove remnant ATP within the specimen, hexokinase plus glucose system or apyrase were used. P^1,P^5-di(adenosine-5') pentaphosphate (AP_5A) was added to ADP-rigor solution to inhibit myokinase activity [2].

3. Results and Discussion

Myofilament spacing of thin-filament-extracted fibers

By extraction of thin filament, spacing between thick filament reduced by 5nm in relaxing condition. No difference was detected among relaxing, ADP-rigor, and rigor conditions.

Change of myosin layer line by thin-filament extraction

By extraction of thin filament, peak position of myosin layer line in relaxing condition did not change by extraction of thin filament significantly (Fig.1: blue; before extraction, black; after extraction).

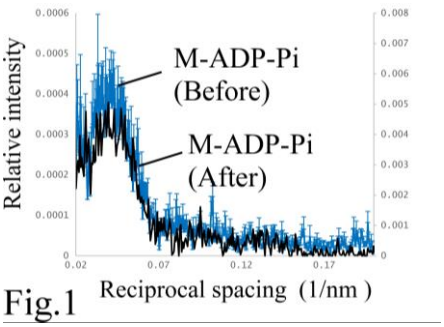

Fig.1

Change of myosin layer line by transition from M-ADP-Pi to M-ADP state

On ADP condition, peak height of myosin layer line decreased and the peak position slightly shifted toward meridian compared with relaxing condition (M-ADP-Pi state).
It indicated that myosin head slightly protruded from backbone and gained increased mobility with transition from M-ADP-Pi state to M-ADP state (Fig.2: blue; M-ADP-Pi, green; M-ADP).

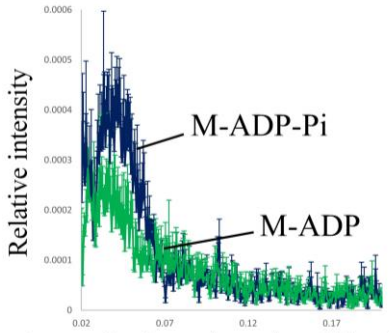

Fig.2

Change of myosin layer line by transition from M-ADP to M state

In rigor state (myosin is in M state), peak position of myosin slightly shifted toward meridian compared with ADP condition (M-ADP state). It indicated that myosin heads slightly protruded from backbone with transition from M-ADP to M (Fig.3: green; M-ADP, magenta; M).

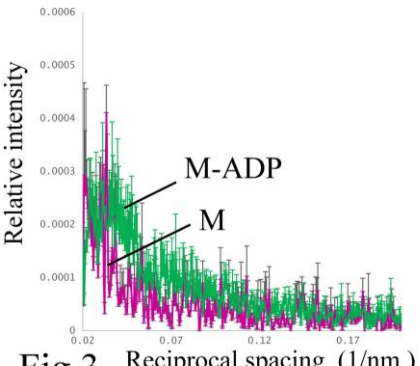

Fig.3

4. Conclusion

1. Myosin head was found to undergo radial movement involved in ATP hydrolysis reaction even without actin.
2. Myosin head further shifted from thick-filament backbone to actin by transition from M-ADP state to M state.
3. This result provide fundamental information for the understanding of structural change of myosin in normal or potentiated contraction by myosin light chain phosphorylation.

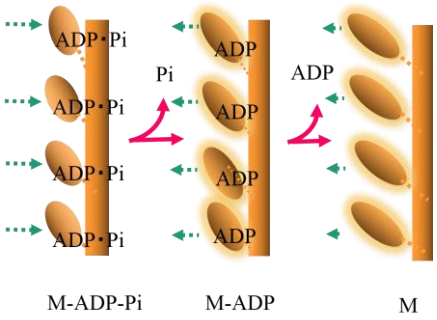

Fig.4

5.Acknowledgement

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6.References

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