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Conformational Changes of Smooth Muscle Heavy Mero Myosin Related to Regulatory Mechanism

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Introduction

In smooth muscle, myosin molecule plays an important role in the regulatory mechanism as well as in the responsibility for force generation. Heavy mero-myosin (HMM) is a proteolytic fragment of myosin containing two myosin heads and short coiled-coil tail but lacking the light mero-myosin (LMM). Actin-activated ATPase of HMM is markedly accelerated by phosphorylation of the regulatory light chain (RLC). It is also known that upon phosphorylation in the presence of ATP, HMM undergoes a structural transformation similar to the so-called 6S-10S transition of the whole myosin molecule while the single-headed myosin (S1) does not. In order to understand the mechanism of smooth muscle myosin function of the force generation and the regulation, we have done X-ray solution scattering on the smooth muscle HMM.

Experiments

HMM was prepared from chicken gizzard muscle myosin. The X-ray solution scattering experiments were done at the BL15A1 using small-angle diffractometer at a camera length of 2.4m and 1.2m. All X-ray scattering data were collected as a function of scattering vector length with a 1D-PSD. The protein concentration was varied in the range of 2 to 8 mg/ml and X-ray scattering was measured at 20 °C. Structure models were built from the scattering curves of dephosphorylated and phoshorylated HMM with and without ATP solution, by using *ab initio* 3-D structure prediction program DAMMIN.

Results and Discussion

The radius of gyration (Rg) value of the dephosphorylated HMM in the absence of ATP was caluculated from the Guinier plot of intensity data and it was ~10nm. The Rg value was very large compared to the Rg of single myosin head of ~4.8nm. The p(r) function showed distinct two peaks. The predicted 3-D model calculated from scattering intensity showed that these observed features were come from the conformation with two heads widely separated in opposite directions. On the other hand, in the presence of ATP, the p(r) functions had a single peak and an Rg value smaller thanHMM without ATP. The structure model for HMM with ATP solution had a conformation with two heads closely sat.

The Rg value of phosphorylated HMM was similar to the value of dephosphorylated HMM both in the presence and absence of ATP. While, the p(r) function of phosphorylated HMM did not show two peaks but single peak. The structural model of phosphorylated HMM had a conformation with close two heads and elongated coiled-coil tail.

These conformational changes of HMM related to phosphorylation and ATP may explain the regulatory mechnism of smooth muscle.

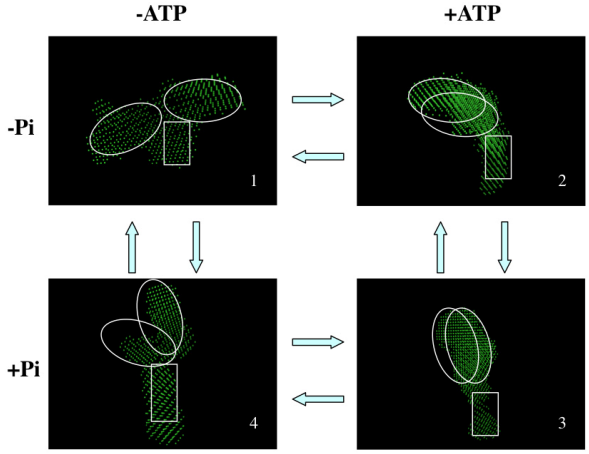

Figure 1
3-D structure models of dephosphorylated and phoshorylated HMM with and without ATP solution.

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