IMAGING MOLECULAR MOTION of MUSCLE MOTOR DYNAMICS

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The kinetics of an isolated molecular motor of the skeletal and cardiac muscles is studied here by image analysis of the motility assay of an isolated actin filament sliding over the isolated myosin heads, which perform as linear motors. Image analysis allows to study the dynamics of the interactions between the actin molecule and the myosin heads, and explore the kinetics of the biochemical processes that supply the energy for the molecular motor.

INTRODUCTION

Cardiac muscle contraction and mechanical function are generated by the sarcomere, the intracellular contractile element, and determined by the interaction between the actin –myosin filaments. Myosin interacts with actin, creating cross-bridges (XBs), which convert biochemical to mechanical energy, generate force and contract the sarcomere by filament sliding. The intracellular mechanical activity is based on XB cycling between a "weak", non-generating force, conformation and a "strong", force generating ,one (1). The head of the myosin, is much smaller than all the human-made nano-motors: it is 19nm long and 5nm. The isolated myosin heads creates a unitary force of about 2pN and a stroke step of 5nm. Each cubic mm of muscle tissue contains $40 \cdot 10^{12}$ motor units. The main interesting issues are: how does the muscle regulate this huge density of motor units and what are the mechanisms that yield the outstanding efficiency of the muscle motors, which is around 70%. The contractile filament function (Sarcomere), at the sub-cellular, organelle level, is regulated by two main feedback mechanisms that regulate XBs recruitment (2) and provide the linear relationship between the energy consumption (ATP hydrolysis), and the mechanical energy (3,4).
The motility assay technique allows to study the biophysics of the isolated molecular motor, and to measure the interactions between isolated actin and myosin molecules: i.e.,the displacement (nm) and force (pN) generated by the XB motor unit (5-8). The rate of biochemical energy consumption is evaluated from the kinetics of XB cycling between the strong and the weak states (8). Motility assay thus allows to study the molecular basis of XB function and malfunction and to explore the pathogenesis of various heart diseases.

MATERIALS & METHODS

Experimental. The actin filaments are fluorescently labeled (9). The myosin head molecules are attached to a microscope cover slip. The thickness of the actin filament is 5nm. To observe actin filament sliding, the filaments are fluorescently stained with Rhodamine-phalloidin. Motor unit activation is initiated when skinned fiber solution, which contains the intracellular electrolytes and ATP, is added. The actin filaments are propelled by the myosin heads and are observed with an inverted epi-fluorescent microscope. The fluorescent images of the actin motion are obtained using an image intensifier CCD camera with double MCP.
Image Analysis. *Our software* analysis the velocity of the actin segments (10) proceeds in the following steps: 1) Object identification in each frame. 2) Recognition of the same filaments

(objects) in the successive frames. 3) Detection of filament backbone. 4) Identification of object trajectory. 5) Calculation of the filament length at the various frames. 6) Calculating the trajectory velocity and acceleration of each point along the filament, at the various frames.

RESULTS

Figure 1 presents 33 successive frames taken in 1 sec. The image analysis implies that while the energy for filament sliding is provided by the myosin heads, the direction of the actin filament trajectory if determined by the polarity of the actin filament. One side of the filament with always remain ahead and the other will be the tail, although the myosin head are randomly scattered over the slide. The lengths of the actin filament remain constant during the filament sliding, within the spatial resolution of the optical system. The length of the actin filament shown in Fig.1 was 23.32±0.34 μm at the various frames. This allows to quantify the velocity of motion of each point along the actin filament. The speed of the center of gravity of each filament was 0-10 μm/s. However, the speed of as individual marker point along the actin filament was 12-20 μm/s.

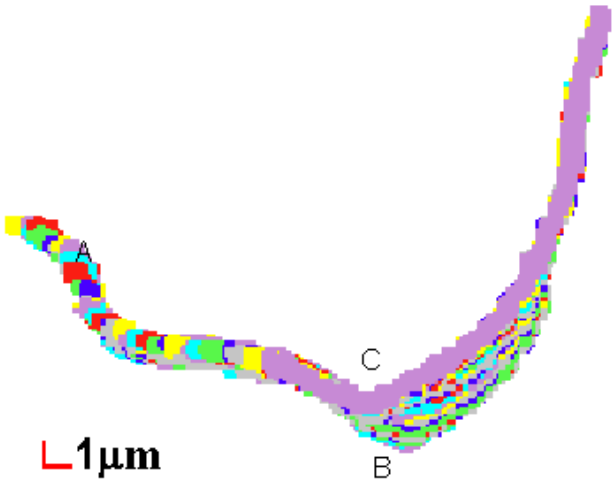

Figure 1. Trajectory of an actin filament during 1 second (33 frames).

DISCUSSION AND CONCLUSIONS

The image analysis allows to verify and void the various hypotheses and theories of muscle function. The sliding velocity defines the minimal rate of XB attachment-detachment. The stroke step of the myosin head is determined by the structure of the actin filament. The actin filament is composed of double strand polymers of actin. The size of actin monomer is 5nm, and indeed most of the studies suggest that the myosin head motion is composed of multiple steps of 5nm each (5-8). The classical Huxley's theory of muscle contraction (11) suggests that the XB stoke steps is limited to 10 nm. The classical theory of muscle function also suggests that the myosin head consume one molecule of ATP per stroke step and that the rate of ATP consumption and XB attachment-detachment is low (order of 40 s^{-1} at 25^0C). The finding that the sliding velocity of a marked point along the actin filament is of the order of 20μm/s suggests that the myosin heads have to perform at least 2000 stroke steps per second, each step about 10nm long, to allow the actin filament to slide at 20 μm/sec.

The data and the consequent analysis characterize the physical kinetics of "attachment/detachment" (11). The kinetics of attachment-detachment is orders of magnitude

faster (2000 1/sec) than the kinetics of XB cycling between the weak and strong biochemical conformations (less than 100 s^{-1}). The image analysis verifies that multiple steps of XB attachment/detachments occur per hydrolysis of a single ATP molecule (12,13). It supports the hypothesis that XB dynamics involves two distinctive kinetics: A fast one (attachment-detachment) and a slow one (XB cycling between strong and weak conformations) kinetics.

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