Motion of Activated Myosin Heads as Detected by Fiber X-ray Diffraction

Muscle contraction is caused by the interaction between the two contractile proteins, actin and myosin. Each of the proteins polymerizes to form filaments, and the contractile force is generated as the myosin head, which sticks out of the myosin filament backbone, exerts a pull on the actin filament (Fig. 1a, 1b). The myosin head contains all the components needed to exert force, including the actin-binding site and the ATP-binding pocket, while the rest of the myosin molecule (myosin tails) forms the backbone of the filament (Fig. 1c). The recent crystallographic results [1] showed that the head is further divided into two parts, *i.e.*, the motor and the lever arm domains (Fig. 1d). The conventional theory for force generation mechanism assumes that the motor domain grabs the actin filament firmly (by making stereospecific interactions at the actin-myosin interface) and the lever arm makes a swing on it (Fig. 1e).

In the conventional theory, therefore, the stereospecific interactions play a crucial role in force generation. The question is how such interactions can be detected. A potent method to achieve this is to record X-ray diffraction patterns from a muscle or muscle fibers under various conditions. A regular array of molecules, such as that in a protein crystal, gives rise to a number of bright spots or lines at specific positions in the diffraction pattern. They are called reflections, and their positions and intensities carry information about the structure of the molecular array. In the case of muscle, the molecules of myosin and actin are arranged periodically in helices. This arrangement gives rise to a number of line-shaped reflections (layer lines) across the long axis of the muscle fibers, as in the patterns recorded at beamline **BL45XU** [2] (Fig. 2). Figure 2a shows the diffraction pattern recorded from stretched muscle fibers, in which the myosin and actin filaments do not overlap and therefore the myosin heads cannot interact with actin. A few, weak layer line reflections are seen, and they are based on actin repeat. The

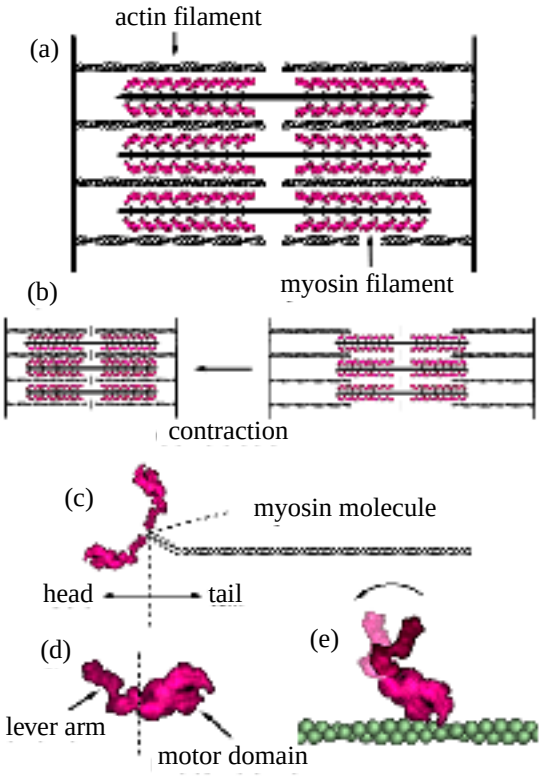

Fig. 1. Structure of the contractile machinery of muscle.
*(**a**) Structure of a sarcomere consisting of two sets of filaments (myosin and actin).*
*(**b**) Mechanism of contraction, which is caused by the sliding of the filaments relative to each other.*
*(**c**) Structure of a single myosin molecule.*
*(**d**) Structure of a myosin head, consisting of motor and lever arm domains.*
*(**e**) Conventional explanation of the mechanism of contractile force production, caused by the swing of the lever arm domain on the motor domain bound to an actin filament in a stereospecific manner.*

pattern in Fig. 2b was taken after myosin heads (prepared by severing the whole molecule with protease) were diffused into the stretched muscle fibers as in Fig. 2a in the absence of ATP. This is the condition equivalent to rigor mortem, in which mitochondria no longer produce ATP, and strong stereospecific interactions are known to be formed between actin and myosin (this makes the muscle very stiff). In the diffraction pattern, the actin-based layer lines, notably the one at 5.9 nm (arrow), are strongly enhanced. The enhancement is caused by the myosin heads, which are bound to the actin filament and now follow the actin repeat. It is also noticed that the peak of the reflection at 5.9 nm has shifted inwards.

Addition of ATP to the muscle fibers creates a condition equivalent to contraction. The heads had been cross-linked to actin so that they would not dissociate. In such muscle fibers the heads hydrolyze ATP at a very high rate, because the two contractile proteins are held in close proximity. It is expected from the conventional theory that in such highly activated muscle fibers, stereospecific interactions are formed substantially and therefore the diffraction pattern would be more or less like that in Fig. 2b. However, the recorded pattern (Fig. 2c) was very similar to that in Fig. 2a, *i.e.*, the pattern from naked actin filaments [3]. The results are explained only if the myosin head is swinging as a whole in this highly activated actin-myosin complex, and little stereospecific interactions are formed. It is probable that the motor domain of myosin plays a more dynamic role than simply providing a scaffold for the lever arm swing.

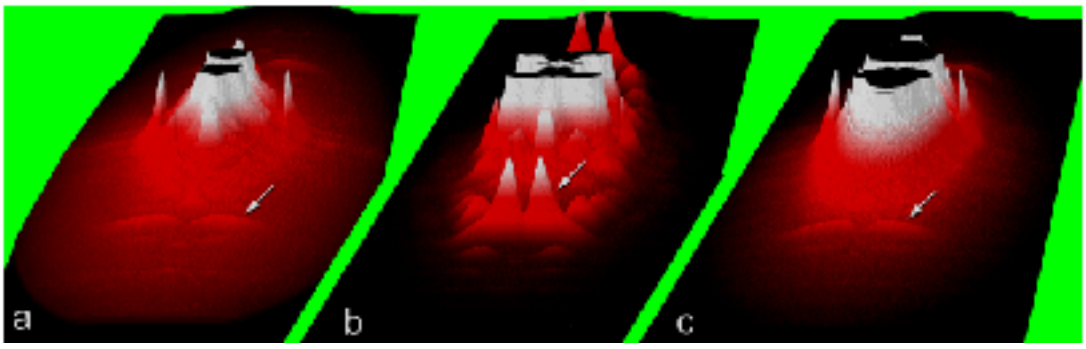

*Fig. 2. Bird's-eye views of the diffraction patterns recorded from an array of overstretched single rabbit skeletal muscle fibers. (**a**) Pattern recorded in the absence of exogenously introduced myosin head. Layer lines typical of bare actin filaments are observed. (**b**) Pattern recorded after myosin heads had been exogenously introduced in the absence of ATP. The actin-based layer lines are strongly enhanced because of the stereospecific labeling of the actin filaments by the myosin heads. (**c**) Pattern recorded in the presence of ATP after exogenously introduced myosin heads had been cross-linked. The actin-based layer lines are as weak as those of bare actin filaments and there is little sign of stereospecific binding. The arrow indicates the layer line indexed to the 5.9 nm repeat of actin monomers.*

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