NON-LINEAR ELASTICITY OF SINGLE SKELETAL MYOSINS IS AN ESSENTIAL MECHCANAL PROPERTY OF MUSCLE CONTRACTION

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INTRODUCTION

Muscle contraction is caused by a sliding of the thin past thick myofilaments [1,2]. A fundamental problem in the muscle mechanics field is how the modulation of myosin motors attached to actin is achieved, and this might be addressed by measuring displacements and forces generated by single myosins. It has been generally accepted that the elasticity of myosins is linear beased on single muscle fibers experiments [3]. However in this case, the compressive (resistance) force generated by negatively strained myosins is expected to be substantially high, which is disadvantage for active force generation. Furthermore, the resistance forces of single myosins cannot be measured in single fibers experiments. Thus, more careful investigation is necessary to evaluate the elasticity of sinlge myosins. The purposes of this study are to measure the elasticity of single skeletal myosins for a range of strain covering both negative and positove directions by using the novel optical trap and fluorescence imaging techniques, and to discuss how the elasticity of myosins is designed for effective force generation.

METHODS

Myosins and fluorescently-labeled rods (myosins without head) are synthesized into cofilaments. In the absence of ATP, single myosins in cofilaments were tightly bound to the actin filament, which both ends were attached to the beads maintained by optical traps (Fig. 1a). Then the positions of the two trapped beads were moved triangularly along the longitudinal axis of the cofilament at 5 Hz for 10 s to stretch and compress myosins (Fig. 1a). The total force exerted by a single myosin were calculated as the sum of the forces exerted on the two trapped beads. The displacement of myosins are calculated by tracking the centroid positions of fluorescence intensity profiles of quantum dots attached to actin filaments and those of cofilaments relative to the glass surface. The stiffness of single myosins was obtained by fitting the non-linear regression line [4] with the force-displacement plots (Fig. 1b).

RESULTS AND DISCUSSION

In contrast to the previous studies, the force-displacement curves for single myosins clearly show the non-linear elasticity (Fig. 1b, top). The stiffness dramatically increases up to 2.8 pN/nm when myosins are stretched (displacement > 0), compared to when they are compressed (~0.02 pN/nm, displacement < 0). When myosins are further compressed below the displacement of -80 nm, the stiffness increases again (Fig. 2b, bottom). The low stiffness of 0.02 pN/nm appeared to be similar to the bending stiffness of the S2 portion of myosin. Interestingly, the range of 80 nm displacement associated with low stiffness is similar to the double length of the S2 portion of myosin head (Fig. 1b). In contrast, the high stiffness of ~2.8 pN/nm is more consistent with either the stretching or bending stiffness of S1 portion. Therefore, the high stiffness observed in either positive displacements or below -80 nm represents the elasticity of myosin S1 when the S2 portion is fully stretched in either the positive or negative direction. The low stiffness in compression of myosins suggests that the resistance force generated by either the post-power stroke or rigor myosins can be minimal when they are compressed by the sliding of myofilaments powered by the working stroke of active myosins.

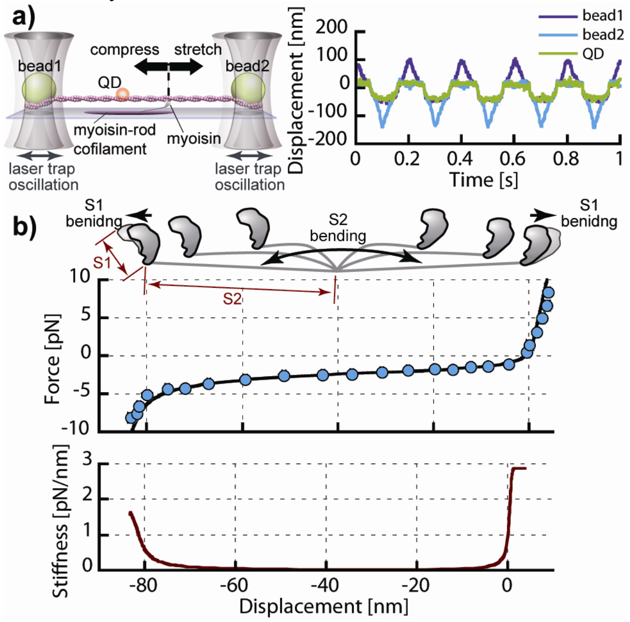

Figure 1: a) Schematic diagram of optical trap system and time-course of beads and quantum dot (QD) displacements during the oscillation of optical traps. b) Force-displacement curve of single myosins (top) and the corresponding stiffness calculated as the slopes of the fitting curves (bottom).

CONCLUSIONS

The elastic property of single myosins was found to be non-linear by measuring the elasticity for both the negative and positive strain directions. This property is essential to generate force effectively in the assembly of myosin motors by minimizing the resistance force generated by the rigor-like myosin heads.

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REFERENCES

1. Huxley AF and Niedergerke R, *Nature* **173**:971-973, 1954 .
2. Huxley HE and Hansen J, *Nature* **173**:973-976, 1954
3. Huxley AF and Simmons RM, *Nature* **23**:533-538, 1971.
4. Higuchi H and Goldman YE, *Biophys J.* **69**:1000-1010, 1995.