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Rapid report

Different cardiac myosin isoforms exhibit equal force-generating ability in vitro

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Abstract

We measured forces generated by myosin molecules and a single actin filament using an optical trap system. The force per unit length of actin filament did not differ significantly between cardiac myosin isoforms, V_1 and V_3. This indicates that the ability to generate force is equal between V_1 and V_3, despite their difference in the unloaded sliding velocity past actin.

Keywords: Motility assay, in vitro; Laser optical trap; Myosin; Cardiac myosin

The cardiac adaptation process is known to consist of changes in the relative amount of three cardiac myosin isoforms (V_1, V_2 and V_3) in cardiac muscle [1], as evidenced by a close correlation between the shortening velocity of cardiac muscle and the myosin isoform composition [2]. Recent development of in vitro motility assay systems has made it possible to study the interaction of these isoforms with actin filament under microscopic observation. Using one of these assay systems, in which beads coated with the cardiac myosin isoforms are made to slide on actin filament arrays (actin cables) in giant algal cells, we have already shown that the unloaded velocity of sliding on actin filament for V_1 is definitely larger than that for V_3 [3]. By combining this assay system with a centrifuge microscope, we have also shown that the force–velocity curve for V_1 is different in shape from V_3 [4]. Although these results indicate that the characteristics of interaction with actin filament differ between V_1 and V_3 in many respects, it is not yet clear whether the ability to generate force differs between V_1 and V_3 or not. The present experiments were undertaken to compare the maximum 'isometric' force generated by individual V_1 and V_3 molecules. For this purpose, we measured forces generated by the interaction between myosin molecules fixed on a glass surface and a single actin filament with an optical trap system; the densities of myosin samples were adjusted to make the number of myosin molecules interacting per unit length of actin filament was almost the same among different samples. It will be shown that the maximum 'isometric' force generated by the individual molecules does not differ significantly between V_1 and V_3 isoforms.

Cardiac myosin V_1 isoform was obtained from 3-week-old Wistar rats and V_3 isoform was obtained from 22-week-old Wistar rats in which hypothyroidism had been induced by adding 1-methyl-2-mercaptoimidazole (Methimazole, Chugai Pharmaceutical Co., Japan) (15 mg per day) to drinking water for 10 weeks. Hearts were excised from the animals under diethyl ether anaesthesia, and myosin samples were prepared from the ventricular myocardium by the method of Katz et al. [5] with slight modifications. All procedures were carried out at 4°C in the presence of 5 mM dithiothreitol and 5 μg/ml leupeptin (proteinase inhibitor). At the final stage, trace amount of actin was removed by centrifugation (120 000 × g for 3 h). No procedure for phosphorylation of myosin was added. We also prepared skeletal muscle myosin from the rabbit back muscle in a similar manner. Actin was prepared from rabbit back muscle acetone powder by the method of Spudich and Watt [6] and was incubated at 4°C overnight with a molar excess of rhodamine-phalloidin

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(Molecular Probes, Eugene, OR) in 25 mM KCl to form actin filaments. In vitro force measurement was performed according to the method of Miyata et al. [7]. Actin filaments were bound to gelsolin-coated polystyrene beads (1.0 μm diameter; Polyscience, Warrington, PA) by mixing them overnight at 0°C. Each myosin sample was diluted with high-ionic-strength buffer (0.6 M KCl and 50 mM Tris HCl, pH 7.5) to 250 μg/ml and 60 μl of this was applied to a nitrocellulose-coated coverslip (60 mm × 30 mm, Matsunami Co, Japan) and covered by another smaller cover slip (18 mm × 18 mm). The coverslips were separated by about 100 μm with a layer of silicon grease to form a flow cell. After a 5 min incubation on ice, bovine serum albumin (0.5 mg/ml) was applied to the flow cell to wash out unbound myosin and coat the exposed nitrocellulose surface. At this stage, we evaluated the density of active myosin heads by determining the K-EDTA-ATPase in the flow cell relative to the specific activity of the K-EDTA-ATPase of myosin in solution [8]. Actin filaments with beads attached were suspended in Mg-ATP solution (50 mM imidazole, 25 mM Tris-HCl, 25 mM KCl, 6 mM $MgCl_2$, 1 mM EDTA, 2 mM ATP, 0.2% methylcellulose, pH 7.5) and introduced to this myosin coated surface by perfusion. The force measurement system consists of a fluorescent microscope (Axiovert; Zeiss, Germany, equipped with an oil-immersion objective, 100 ×, NA 1.3) and a Nd-YLF laser (OEM 1047-1000P, wavelength 1047 nm, 1 W; Amoco Laser IL) coupled with a specially designed optical path apparatus. In each experiment, a bead bound with only one actin filament was selected under the fluorescence microscope and captured by the laser trap. The position of the bead was detected by a position sensor comprising a quadratic photodiode (S1557; Hamamatsu Photonics, Japan) [9] and the displacement from the trap center was converted to force by multiplying Hookean constant of the trap. The Hookean constant was determined by the fluctuation analysis [10]

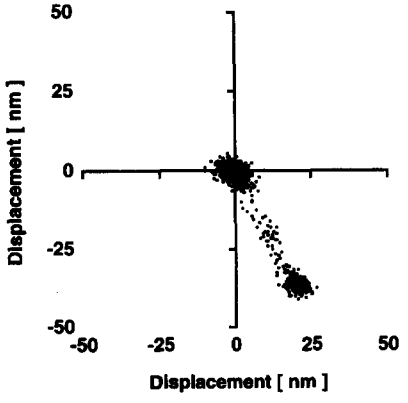

Fig. 1. *X-Y* plot showing the position of a bead through the course of an experiment. When an actin filament-bound bead was trapped in the medium, it showed Brownian motion around the trap center. However, as the actin filament started interacting with myosin molecules, it pulled the bead from the trap center toward the edge of the trap zone.

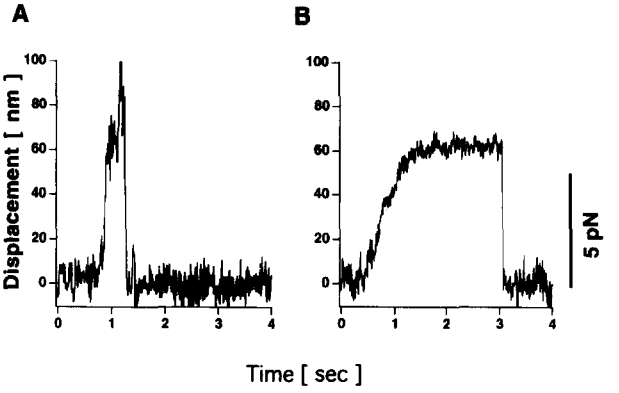

Fig. 2. Time course of the force development by cardiac myosin V_1 isoform (A) and V_3 isoform (B). The rate of force development is higher for V_1.

and ranged from 0.07 to 0.12 pN/nm. Although the position sensor was linear up to 500 nm, the linear range of the trap force was confined to 250 nm from the trap center. At the same time we recorded the image of the actin filament and measured its length with an image processor (Argus 10; Hamamatsu Photonics, Japan). We also measured the sliding velocity of freely moving actin filaments (not bound to beads). Experiments were done at room temperature (25°C).

The densities of active myosin molecules on nitrocellulose-coated glass surface were estimated to be 1.47 ± 0.19 (mean ± S.D., $n = 5$) for V_1, 1.57 ± 0.13 ($n = 5$) for V_3, and 1.60 ± 0.20 ($n = 5$) ng/mm^2 for skeletal muscle myosin. Because these values were not significantly different, we considered that the number of myosin molecules that can interact with unit length of actin filament did not differ significantly among the three different myosins. Assuming a band model [11], we estimated the number of myosin heads per μm of actin filament at about 95.2 ± 12.4 for V_1, 102.1 ± 8.5 for V_3, and 104 ± 13.0. Without the laser trap, most actin filaments on the myosin-coated surface were observed to move smoothly with the maximum unloaded velocity of 3.9 ± 0.7 ($n = 45$) for V_1, 1.7 ± 0.4 ($n = 45$) for V_3, and 5.1 ± 1.0 ($n = 50$) μm/s for skeletal muscle myosin. These values are comparable with those in the previous reports [12,13].

In each experiment, we trapped an actin filament-bound bead floating in the medium and brought it to the myosin coated glass surface. Although the bead first exhibited Brownian motion in the trap, it was pulled from the center towards the edge of the trap zone as the actin filament started interacting with myosin molecules (Fig. 1). As shown in Fig. 2 the bead was pulled to a point where it stayed for a few seconds and then quickly moved back to the trap center. The transient stoppage of the bead movement is due to a balance between the maximum 'isometric' force generated by the actin-myosin interaction and the trapping force. We also noted that V_1 myosin has higher rate of force development than V_3. In Fig. 3, the maximum

'isometric' force is plotted against the actin filament length for the three types of myosin. In all cases, the data points distributed around a linear regression line drawn by the least square method. The slope of the regression lines with a constraint at the origin for V_1 and V_3 are much less steep than that for rabbit skeletal muscle myosin, the slope being 2.8 ± 0.2, 3.1 ± 0.2, and 9.7 ± 0.7 pN per 1 μm of actin filament for V_1, V_3 and skeletal muscle myosin respectively. These results may be taken to indicate that the ability of a single myosin head to generate force is almost equal between V_1 and V_3, while it is much larger in skeletal muscle myosin than in cardiac myosins. Based on the above estimation for the number of myosin heads per 1 μm of actin filament, the maximum 'isometric' force generated by a single myosin head is calculated as 0.03 pN for V_1, 0.03 pN for V_3, and 0.1 pN for skeletal muscle myosin. Although the value for skeletal muscle myosin is much smaller than that obtained by direct measurement of single force transient [14], it is comparable to that estimated in a similar experiment using a glass microneedle [11]. Since myosin molecules are randomly oriented on the glass surface, the above values are obviously smaller than the forces generated by the myosin head correctly oriented with respect to the actin filament, though such underestimation does not affect the present result that V_1 and V_3 have an almost equal force-generating ability. The present result that the cardiac myosin isoforms having different rate of interaction with actin filament generate equal time-averaged 'isometric' force can be explained as follows. In the contraction model of Huxley, the isometric force (P) generated in an active muscle is proportional to $f/(f+g)$, where f and g are rate constants for making and breaking actin-myosin linkages. On this basis, the present result suggests that both f and g are about twice as large for V_1 as for V_3, since the ATPase activity is about twice as large for V_1 as for V_3 [15].

The present result shows a clear contrast to the recent work by Harris et al. [16] and VanBuren et al. [17] demonstrating about two-fold difference in average force between the two cardiac myosin isoforms. Their conclusion is consistent with the number of earlier studies using muscle preparation [18–20]. However, we can also find papers showing that the maximum force per unit area does not differ between ventricular papillary muscle preparations obtained from hyperthyroid animals (containing predominantly V_1 myosin isoform) and those obtained from control animals (predominantly V_3 isoform) [21,22]. The reason for the different results observed in VanBuren's study [17] and ours is not clear, but the one possibility is that higher viscosity in their assay buffer (0.375% methylcellulose compared to 0.2% in ours) may impose an extra load on the faster-moving V_1 isoform.

Harris et al. [16] also showed that V_3 myosin and skeletal muscle myosin generate comparable force which again contradicts our finding. In addition to the possible effect of species difference (chicken vs. rabbit), this discrepancy can be explained from a structural point of view. It is well known that the slow skeletal muscle myosin is composed of β-myosin heavy chains (MHC). However, Harris et al. compared the fast skeletal muscle (chicken pectoralis) myosin and V_3 myosin the heavy chains of which are different (MHCf and β-MHC, respectively) [23]. Accordingly, their result can be taken to suggest that the heavy chain structure may not be a determinant of force generating capacity of myosin. Alternatively, considering the recent findings suggesting the importance of light chains in the force generating process [24], the identical light-chain structure (ventricular type) of these two cardiac

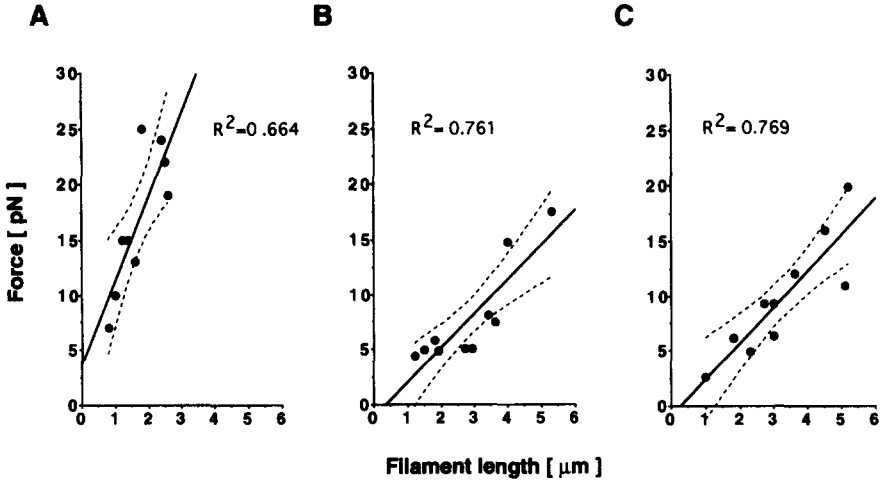

Fig. 3. Relationships between the force and the actin filament length for rabbit skeletal muscle myosin (A), rat cardiac myosin V_1 isoform (B), and V_3 isoform (C). Linear regression lines (solid lines) are drawn with 95% confidence limits (dashed lines). When data are constrained through the origin, the regression for skeletal muscle myosin is about 3-times steeper than that for cardiac myosin. However, the difference between the two isoforms (V_1 and V_3) is not significant (see text).

myosin isoforms (V_1 and V_3) may account for the equal force generating ability observed in this study.

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