Development of the maximum isometric force at short sarcomere lengths in calcium-activated muscle myofibrils

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Summary. Studies on the sarcomere length-force relation in Ca^{++}-activated vertebrate muscle myofibrils indicate that the maximum isometric force can be generated even at sarcomere lengths around 1 μm, indicating that the ascending limb of the sarcomere length-force relation is virtually absent.

The relation between sarcomere length and isometric force in striated muscle has been established with electrically-stimulated vertebrate muscle fibers[2]. Though the linear decline of isometric force with increasing sarcomere length above 2.2 μm (descending limb) can well be explained as being due to a decrease in the amount of overlap between the myofilaments, the decline of force with decreasing sarcomere length below 2.0 μm (ascending limb) is not readily accounted for because of an incomplete activation of the myofilaments at short sarcomere lengths[3]. The effect of incomplete activation can be eliminated by use of glycerinated or skinned muscle fibers which can be directly activated by calcium. The results with skinned fibers hitherto reported are, however, contradictory; Schoenberg and Podolsky[4] reported the development of a large relative force even at a sarcomere length of 1 μm, while Moss[5] found no difference in the ascending limb between living and skinned fibers. The present work was undertaken to ascertain whether vertebrate muscle myofibrils activated by calcium could generate large relative forces at short sarcomere lengths, using small myofibril bundles in which the sarcomere spacings were clearly visible along their entire length.

Material and methods. Rabbit psoas muscles were glycerinated by the method of Tanaka, Tanaka and Sugi[6] and bundles of myofibrils (5–20 μm in diameter) were isolated in a relaxing solution containing 100 mM KCl, 0.5 mM $MgCl_2$, 4 mM ATP, 3 mM EGTA and 10 mM histidine (pH 6.8). The myofibril bundle preparation was mounted horizontally in an experimental chamber filled with the relaxing solution by wrapping both ends around 2 parallel glass needles 50–200 μm distant from each other; one glass needle was fixed to the bottom of the chamber, while the other was connected to a 10 cm magnesium lever of the force transducer. The lever was pivoted on a steel spring, so that the force generated by the preparation caused a movement of the vane at the opposite end of the lever which was sensed by a couple of differential light beam-photodiode systems[7]. The force transducer had a fairly large compliance (20–50 μm/mg), so that the preparation shortened progressively as the force it exerted increased, thus providing an auxotonic condition. The preparation was activated by contracting solutions with various pCa-values prepared by adding $CaCl_2$ to the relaxing solution, taking the apparent stability constant of Ca-EGTA complex to be $1.95 \times 10^6/M$[8]. The sarcomere length changes along the length of the preparation during the course of auxotonic contractions were observed under a Zeiss microscope equipped with Nomarski differential interference optical system (objective: Zeiss Plan 40X, n.a. 0.65), and recorded with a Nikon television camera-videotape recorder system and a 35 mm camera for analysis (fig. 1,A). The cross-sectional area of the preparation was calculated from the diameter of the preparation, assuming its circular cross-section. All experiments were performed at room temperature (20–24 °C).

Results and discussion. The mechanical response of the myofibril bundle preparation to contracting solutions was reproducible; after a period of auxotonic shortening, the same amount of steady isometric force was attained many times by repeated application of contracting solutions with a fixed pCa (fig. 1,B). The maximum steady isometric force (2.1–3.3 kg/cm^2) was produced at pCa 5.5, while the half-maximum isometric force was obtained around pCa 6.5 (fig. 1,C). To examine local sarcomere length changes during the course of auxotonic shortening, the entire length of the preparation was divided into consecutive segments, each consisting of 5–10 sarcomeres, and the average sarcomere length was measured for each segment on photomicrographs of the preparation.

A typical result of the experiments, in which the sarcomere spacings were observable along the entire length of the preparation even during the maximum activation with pCa 5.5, is shown in figure 2,A. The sarcomere length of the resting preparation at its slack length was not completely uniform, but always showed some variation (a). When the preparation was activated in a contracting solution of pCa 6.5, the preparation shortened auxotonically by 10–20% to produce a nearly half-maximum steady isometric force (b), the extent of shortening differing from segment to segment. In a solution of pCa 6.5, the sarcomere spacings were always visible along the entire length of the preparation.

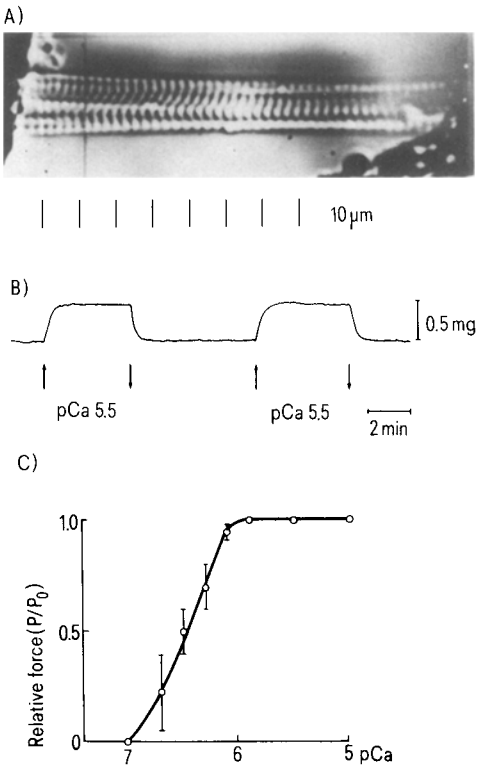

Figure 1. *A* Photomicrograph of a myofibril bundle preparation. *B* Reproducible force development in a contracting solution of pCa 5.5. *C* Relation between the steady isometric force attained after auxotonic contraction and pCa. Forces are expressed relative to the maximum isometric force at pCa 5.5. Each point is the mean, and each vertical bar is the SD for 15 different preparations. The preparations were initially kept at slack length.

In a solution of pCa 5.5, the preparation further shortened auxotonically by 20–30% from the slack length, to result in the development of the maximum steady isometric force (c); since the development of the maximum isometric force was preceded by a considerable auxotonic shortening, the sarcomere length in some segments decreased to as short as 1.2 μm. In many other preparations, the striations tended to become invisible in some segments during the maximum activation with pCa 5.5[9], though their average sarcomere length could be calculated to be 1.1–1.4 μm. In all the preparations examined, the extreme sarcomere shortening to less than 1.4 μm always took place progressively after the force had risen above the half-maximum value to approach the maximum value, but was never observed at the beginning of Ca-activation when the force was still very small, indicating that the sarcomeres can shorten actively against an increasing auxotonic load even at lengths below 1.4 μm. The sarcomere length changes terminated as soon as the steady isometric force was reached, and no appreciable changes in striation pattern were observed so long as the steady force was maintained, indicating the balance in force between the sarcomeres along the entire length of the preparation. The segmental length changes were reproducible; on returning to the relaxing solution, each segment restored its initial sarcomere length, and reactivation caused the length changes similar to those in the previous activation.

If the preparation was previously stretched beyond its slack length, both the distance of auxotonic shortening and the final steady isometric force at pCa 5.5 decreased with increasing degree of the previous stretch. Figure 2, B shows the relation between the steady isometric force at pCa 5.5 and the shortest average sarcomere length during isometric force generation, when the preparation was activated at various initial lengths. The maximum isometric force was found to be constant over a wide range of sacromere lengths from 1.2 to 2.5 μm. The variation of sarcomere length along the length of the preparation tended to be more marked as the initial length was decreased, and the location within the preparation of the segment with the shortest average sarcomere length differed from preparation to preparation.

Meanwhile, it was noticed in the present study that, at pCa 6.5, the force-generating ability of the sarcomeres appeared to decrease at short lengths. This suggests that the results of Moss[5] may derive from an incomplete activation, since he activated skinned fibers in solutions with a pCa-value of above 6.0 to obtain clear striation patterns. This view is supported by a recent report that the isometric force in intact frog fibers markedly increase at short sarcomere lengths in the presence of potentiating agents[10].

In conclusion, the present results indicate that, in maximally Ca-activated vertebrate muscle myofibrils, the ascending limb of the sarcomere length-isometric force relation is virtually absent. This implies that the ability of the sarcomeres to generate the maximum isometric force may not be impaired by double overlap of the thin filaments, collision of the thick filaments against the Z-bands or disordering of the filament lattice at extremely short sarcomere lengths.

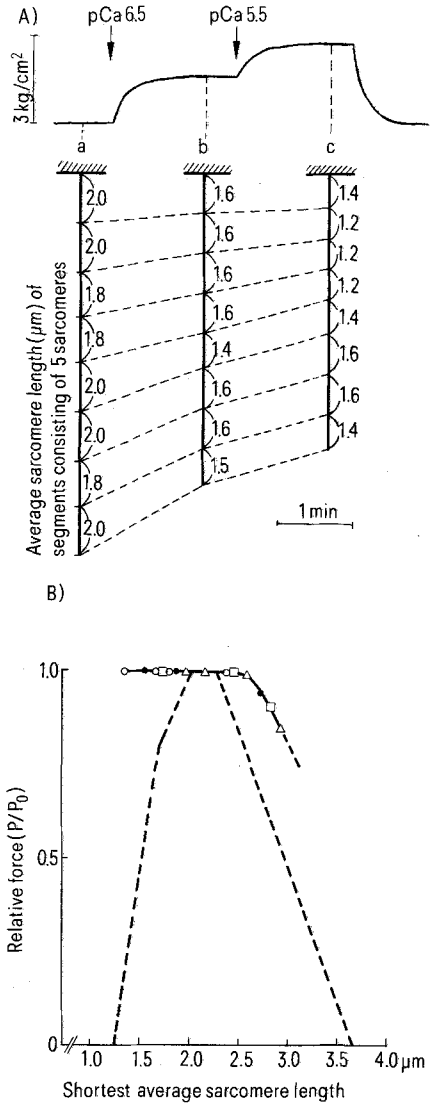

Figure 2. *A* Typical example of sarcomere length changes in each elementary segment along the entire length of the myofibril bundle preparation during the course of auxotonic shortening in contracting solutions of pCa 6.5 and pCa 5.5. The average length of 5 sarcomeres are shown for each segment in relaxed state (a) and in contracted states at pCa 6.5 (b) and pCa 5.5 (c). In a, b and c, the top and the bottom sements represent the segment nearest the fixed end and that nearest the other end connected to the force transducer respectively. The force development during auxotonic contraction is also shown at the top of the figure. *B* Relation between the maximum isometric force at pCa 5.5 and the shortest average sarcomere length during the isometric force generation, when the preparation was previously stretched at various degrees beyond slack length. Each symbol represents results obtained from the same preparation. Broken line is the sarcomere length-force relation obtained by Gordon et al.[2] on intact muscle fibers.

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