TENSION DEVELOPMENT IN STRETCHED GLYCERINATED MUSCLE FIBERS AND CONTRACTION OF 'GHOST' MYOFIBRILS INDUCED BY IRRIGATION WITH HEAVY MEROMYOSIN

Avraham OPLATKA, Julian BOREJDO and Hanna GADASI
Department of Polymer Research, The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

As we have recently reported [1,2], solutions containing actomyosin and ATP exhibit vigorous streaming, reminiscent in many respects of cytoplasmic streaming, when introduced into glass microcapillaries. We took this as a demonstration of mechanochemical energy transformation in a system in which the actin and myosin filaments are free and do not form immobilized structures as in a striated muscle. Since the mechanochemical force in the latter is believed to be generated as a result of the interaction with actin of the ATP-carrying heavy meromyosin (HMM) subunit of myosin we were not surprised to find that on replacing myosin by HMM in the microcapillaries we still obtained streaming [3]. This result encouraged us to examine the possibility that glycerinated muscles which have been stretched to a point of no overlap between the two sets of filaments will be able to develop tension after irrigation with HMM. We describe here the results of these experiments as well as the contraction of 'ghost' myofibrils, i.e., myofibrils from which the myosin has been extracted, induced by irrigation with HMM followed by the addition of MgATP under the appropriate conditions.

2. Materials and methods

2.1. *Glycerinated muscle fibers*

Strips of rabbit psoas muscle were glycerinated [4] for a period of 6–8 weeks. Small muscle bundles (1–1.5 cm long, less than 200 μ in diameter) were teased from the strips in a solution containing 15% glycerol, 50 mM KCl, 2 mM EGTA. The bundles were allowed to equilibrate to room temperature for at least 15 min before the experiment. The maximum tension at rest length (P_0) was usually in the range 100–200 mg.

2.2. *Myofibrils*

These were prepared from a mixture of rabbit back and psoas muscles [5], kept in 50% glycerol at -18°C and suspended in a 'standard salt solution' (SSS) (0.1 M KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer, pH 7.0). The fibrils were centrifuged for 15 min at 5000 g, re-suspended in SSS and centrifuged 3 more times.

HMM was prepared according to Lowey and Cohen [6] from myosin extracted from white back muscles of rabbits [7]. It was further purified by a Sepharose–adiptic acid dihydrazide–ATP affinity chromatography column ([8]; also Lamed and Oplatka, submitted to Biochemistry) which can separate active and inactive myosin and myosin subfragments. One-hundred milligrams of HMM in 30–40 ml were applied to a 3.5 X 15 cm column after dialyzing against a solution containing 40 mM KCl, 1 mM EDTA and 10 mM Tris–HCl buffer, pH 7.6 (equilibrium solution). Any inactive protein present is washed out by this solution and the active HMM can be eluted as a single symmetrical peak with a KCl gradient. The HMM was eluted stepwise with 0.7 M KCl. The Ca^{2+}-activated ATPase activity was 1.2/μmole/min/mg. Purity was checked by SDS-gel electrophoresis (fig. 1), adsorption on a DEAE-cellulose column (fig. 2) and sedimentation in

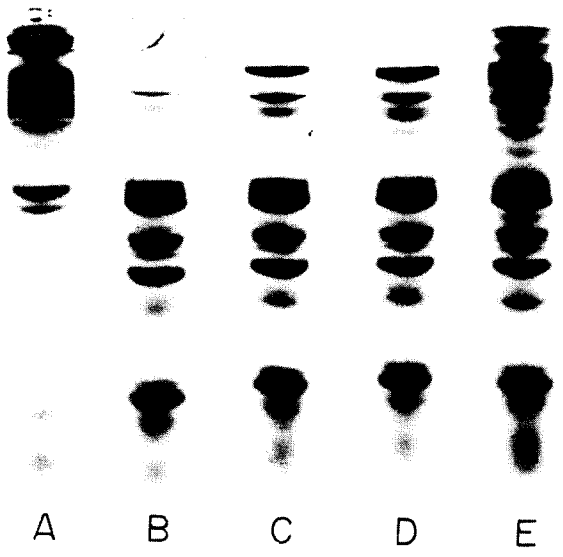

Fig. 1. SDS-gel electrophoresis [9] of HMM and of HMM–myosin mixtures. A: 1.4 mg/ml myosin; B: 1.4 mg/ml HMM; C: 1.4 mg/ml HMM + 0.05 mg/ml myosin; D: 1.4 mg/ml HMM + 0.2 mg/ml myosin; E: 1.4 mg/ml HMM + 0.5 mg/ml myosin.

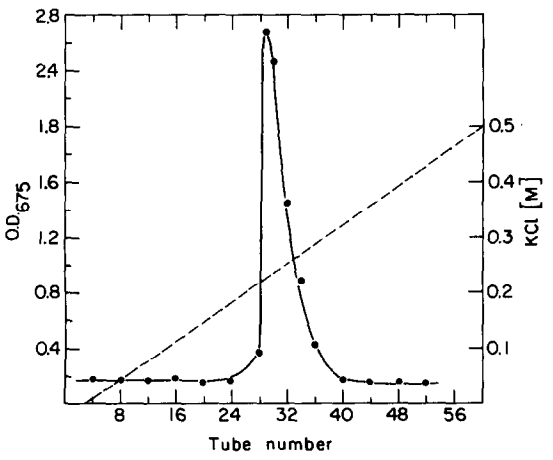

Fig. 2. Elution of HMM from a DEAE-cellulose column: 20 mg HMM in a 10 ml solution containing 50 mM KCl, 5 mM Tris–HCl buffer, pH 7.6, were absorbed by a 1.4 × 11 cm DEAE-cellulose (Whatman DE 32) column and eluted with a 0–0.5 M KCl gradient. The fractions collected were 1.9 ml each. Ordinate: optical density of protein as determined by Lowry's method [10].

an analytical ultracentrifuge (0.7% HMM, 50 mM KCL, 10 mM phosphate buffer pH 7.0, 0°C, 56 000 rpm in a Spinco Model E ultracentrifuge), which gave a major peak of 6.3 S and a minor peak of 3.45.

The muscle preparation was glued with Eastman 910 Adhesive to a pair of bright platinum supports, one of which was mounted on a shaft of Hewlett Packard type 7DCDT-250 gauge for the measurement of tension. The compliance of the mechanical system was about 500 μ/g at the point of muscle attachment giving the maximum deviation from isometric conditions of 1%. The free oscillation frequency of the transducer was 20 Hz. The fibers were suspended in a test solution filling a recess (volume 1 ml) machined in a block of Perspex. Change of muscle bath was achieved by sucking the solution out and replacing it rapidly with a new solution leaving the fibers exposed to air for less than 10 sec.

Rigor and relaxation were assumed to exist in fibers bathed in the following solutions: 50 mM KCl, 10 mM imidazole buffer, pH 7, 1 mM $CaCl_2$ (rigor); 50 mM KCl, 10 mM imidazole pH 7, 4 mM ATP, 4 mM $MgCl_2$, 2 mM EGTA (relaxation). Contraction was induced by applying a concentrated solution of MgATP to the rigor solution (final concentration 5 mM), while stirring continuously. The degree of penetration of MgATP into the muscle was judged by the rate of tension development [11]. Only the fibers which developed full tension in less than 1 min were used.

Contraction of myofibrils was carried out under a phase-contrast microscope (magnification 1250–3000) following the procedure employed by Hanson and Huxley [12]. Changes in composition were performed by the addition of a few drops of the appropriate solution from a glass capillary and 'sucking' liquid at the opposite side with a piece of blotting paper. Myosin was extracted from suspended myofibrils by a Hasselbach–Schneider solution (0.47 M KCl, 1 mM $MgCl_2$, 10 mM sodium pyrophosphate, 0.1 M phosphate buffer, pH 6.4). The ghosts obtained were washed out with SSS (addition of MgATP at this stage caused no contraction) and irrigated with HMM (2–6 mg/ml, 50 or 100 mM KCl, 2 mM Tris buffer pH 7.6). As a result the I-band regions became optically denser. A solution containing 2–6 mg/ml/HMM and either 0.1 or 1.0 mM MgATP was now added and contraction followed.

3. Results and discussion

The effect of HMM on tension development was demonstrated in a muscle in which the interaction between myosin and actin was nearly completely abolished by the applied stretch. The result of a typical experiment is illustrated in fig. 3a; the fibers were stretched slowly (~ 3%/min) in relaxing solution to 165% of their rest length. The relaxing solution was then replaced by the rigor solution; addition of MgATP resulted in the development of a residual tension represented by the curve A. After washing with rigor solution, the fibers were irrigated with 3.1 mg/ml HMM in 50 mM KCl, 10 mM phosphate buffer at pH 7.2 for 1 min. Addition of MgATP resulted in a pronounced contraction (B); after replacing the irrigating solution with the rigor solution, addition of MgATP induced no detectable tension rise (C). The tension associated with HMM–actin interaction was in this experiment 36 mg, i.e. 18.5% P_0 (P_0 = 195 mg).

The same interaction could be demonstrated in the fibers at rest length. We found that the isometric tension developed under such conditions decreased in each successive contraction. This decrease was initially larger than the additional tension developed by the fibers after irrigation with HMM but it could be reduced by subjecting the muscle to a series of 2–3 rigor–contraction cycles. After the third contraction, the tension generated by the HMM–actin interaction was generally greater than the decrease. This is illustrated in fig. 3b where A and B represent the second and third cycles; the first contraction (not shown) gave about P_0 = 100 mg force with resting tension = 0. Following B the fibers were irrigated with 2.2 mg/ml HMM in 50 mM KCl, 10 mM phosphate buffer at pH 7.2 for $1\frac{1}{2}$ min. Addition of MgATP induced a strong contraction (C) with the active tension nearly twice the value obtained in the preceding contraction. When the fibers were washed with the rigor solution, addition of MgATP resulted in a residual contraction (D) due probably to myosin–actin interaction. The tension due to HMM can thus be evaluated as 28% of P_0; this is probably an overestimation due to a simultaneous decrease in tension associated with each successive contraction cycle.

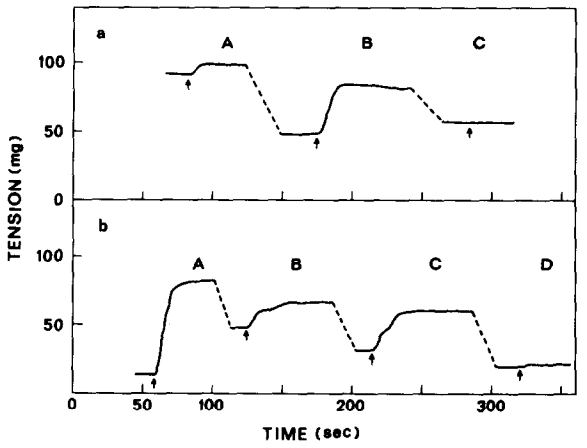

Fig. 3. Tension developed (at 20°C) by glycerinated muscle fibers upon irrigation with HMM. Arrows mark the time of addition of MgATP: a) Fibers stretched to 165% of the rest length P_0 = 195 mg; b) fibers at rest length.

Addition of 1.0 mM MgATP (with or without additional HMM) caused contraction of all HMM-irrigated ghost myofibrils (fig. 4). This was found to be the case in hundreds of experiments. No contraction could be observed if the MgATP concentration was 0.1 mM or less or if its addition was preceded by washing out with SSS. It thus appears that the contraction of HMM-irrigated ghosts requires well-defined conditions. This may account for Hanson and Huxley's failure to induce contraction of ghosts by HMM.

Since it has been reported by Hanson and Huxley [12] that ghost myofibrils irrigated with myosin contracted upon adding MgATP, we checked the minimal concentration of *myosin* required for contraction and found it to be more than 0.1 mg/ml. On the other hand, the supernatant obtained after dialyzing a solution of myosin in 0.5 M KCl against the solution used for dialyzing HMM prior to its application to the ATP column contained only 0.03 mg/ml of myosin, while the SDS-gel electrophoresis pattern of HMM (fig. 1) shows that if any myosin impurity is present, its concentration should be less than 0.01 mg/ml. Moreover, the DEAE-cellulose elution pattern (and probably also the sedimentation) does not indicate the presence of myosin.

In conclusion: It is very probable that the 'tail-less' HMM can generate a mechanochemical force upon interacting with actin and ATP, similarly to myosin aggregated into filaments. Thus the relative sliding of actin and myosin *filaments* does not appear to be

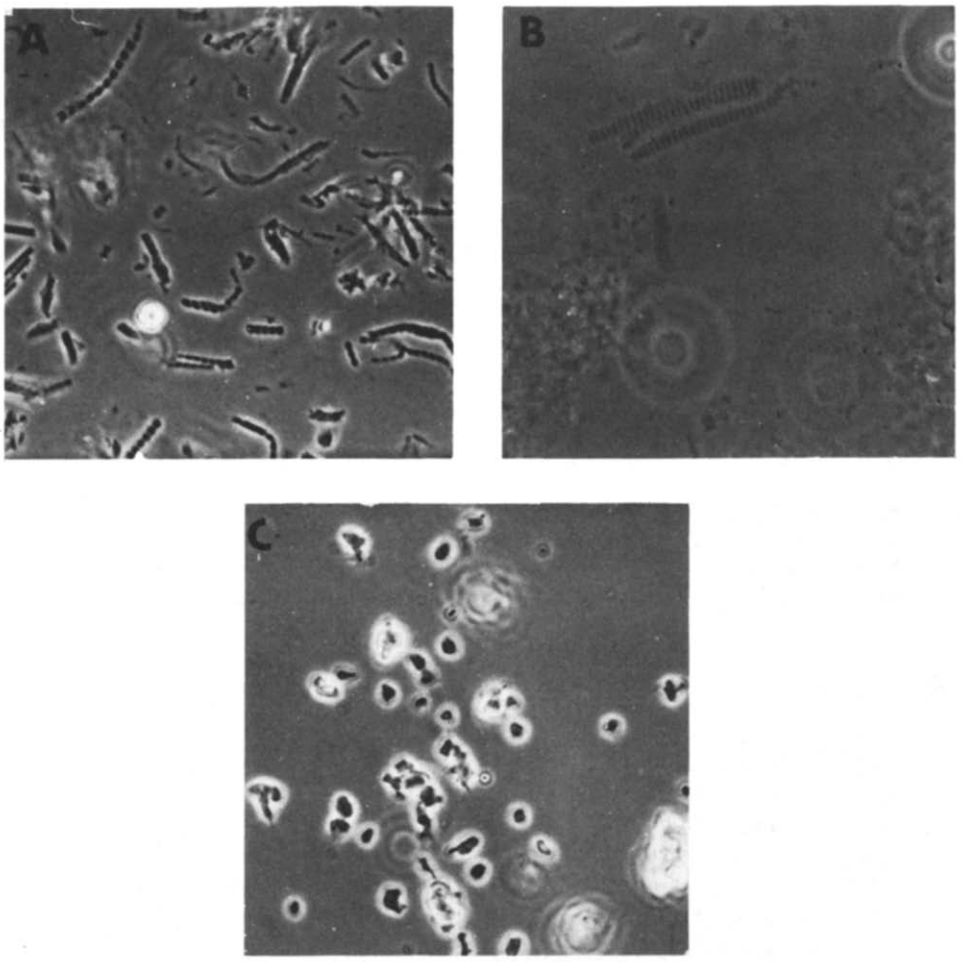

Fig. 4. Contraction of HMM-irrigated ghost myofibrils under a phase-contrast microscope (magnification 1700): A – intact fibrils; B – ghost fibrils after washing with SSS; C – contracted 'reconstituted' fibrils.

absolutely necessary for mechanochemical transformation in actomyosin systems.

In another communication we shall report similar observations with HMM subfragment-1-irrigated myofibrils and glycerinated muscle fibers.

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