THE CONTRACTION OF "GHOST" MYOFIBRILS AND GLYCERINATED MUSCLE FIBERS IRRIGATED WITH HEAVY MEROMYOSIN SUBFRAGMENT-1

Avraham Oplatka, Hanna Gadasi and Julian Borejdo

Department of Polymer Research
The Weizmann Institute of Science, Rehovot, Israel

Received April 8,1974

SUMMARY: "Ghost" myofibrils, i.e. striated rabbit myofibrils from which myosin has been extracted, were irrigated with heavy meromyosin subfragment-1. The addition of MgATP caused contraction. The value of the isometric tension developed by glycerinated muscle fibers at rest length could be increased upon irrigation with HMM S-1. Muscles stretched to such an extent that there was practically no overlap between thick and thin filaments, generated tension after irrigation. The additional tension was probably due to the interaction between HMM S-1 molecules and actin filaments in the I-bands. It is concluded that a mechanochemical force can be generated by the interaction of isolated myosin heads with actin in the presence of ATP.

It is now agreed by most workers in the field that the most likely seat of the force-developing mechanism in striated muscle is the globular part of myosin which undergoes cyclic attachments to the actin filaments (1). A direct proof for the non-necessity of the myosin filament shaft for force generation was recently presented by showing that the water-soluble poly-DL-alanyl-myosin (PAM) can cause the contraction of "ghost" myofibrils, i.e. rabbit muscle fibrils from which myosin has been extracted, upon the addition of ATP; moreover, contraction was observed also when ghosts were irrigated with heavy meromyosin (HMM) which lacks the filament-forming light meromyosin [(2); also Oplatka, Gadasi and Tirosh, submitted to Biochim.Biophys.Acta]. Since HMM is composed of two globular heads, each of which appears to be capable of splitting ATP and of binding actin, it was obvious that repetition of these experiments using isolated heads [i.e. an HMM S-1 (or S-1) preparation] could help in solving the problem whether or not the generation of the mechanochemical force requires the concerted action of two bound together heads. As briefly reported in our previous paper, ghosts were actually found to undergo contraction also after irrigation with various "one-headed" myosin species, including S-1. In the following we shall present a detailed description of these experiments.

On the macroscopic scale, glycerinated muscles, either at their rest length or after being stretched to a point of practically no overlap between thick and thin filaments, were found to develop additional tension after irrigation with S-1.

In another communication we shall describe the contraction of "ghost" fibrils which have been irrigated by other "one-headed" myosin species in which one of the heads (in HMM and myosin) was trinitrophenylated, thus inhibiting its interaction with actin in the presence of ATP.

Copyright © 1974 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS

HMM S-1 was prepared according to Lowey et al. (3) from myosin extracted from white back muscles of rabbits (4). It was further purified by a Sepharose-adipic dihydrazide-ATP (Seph-ADH-ATP affinity chromatography column) [(5); also Lamed and Oplatka, submitted to Biochemistry] which can separate active and inactive myosin and myosin subfragments. 200 mg of S-1 in a solution containing 40 mM KCl, 10 mM Tris-HCl buffer pH 7.6 and 1 mM EDTA (equilibrium buffer) was applied to a 50 ml column. The latter was washed with 150 ml of equilibrium buffer (flow rate 200 ml/hr). Desorption was performed with 100 ml of a 0.7 M KCl, 10 mM Tris, 1 mM EDTA solution. The protein concentration in the 5 ml fractions was determined and only the ascending part of the peak was collected and used. This ascertained no contamination with HMM which, being two-headed, is adsorbed more strongly (Lamed and Oplatka). Its purity was checked (a) by sodium dodecyl sulfate (SDS)-gel electrophoresis; no myosin contamination could be detected (Fig. 1); (b) in an analytical ultracentrifuge, a single, 5.5S, peak was observed (Fig. 2); (c) by adsorption on a DEAE-cellulose column and elution with a KCl gradient - S-1 came out as a single peak at 0.2 M KCl (Fig. 3). The Ca^{2+} and actin-activated ATPase activities were 1.2 and 0.152 μmoles/min/mg (0.4/ml S-1 and 1.25 mg actin in 3 ml), respectively.

Protein concentration was determined by Lowry's method (6).

ATPase activities were measured following H^{+} ions liberated in a pH-stat at pH 7.4 (7).

Myofibrils were prepared from a mixture of rabbit back and psoas muscles (8), kept in 50% glycerol at -18°C and suspended in a "standard salt solution" (s.s.s.) (0.1 M KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer pH 7.0). The fibrils were centrifuged for 15 min at 5,000 g, re-suspended in s.s.s. and centrifuged 3 more times.

Glycerinated muscle fibers: Fresh strips of rabbit psoas muscle 2-5 mm wide were tied to glass rods and stored for 24 hrs in 50% glycerol-water at 0°C, then transferred to a fresh solution containing 50% glycerol, 100 mM KCl, 10 mM imidazole buffer at pH 7 and stored for 6-8 weeks at -18°C.

METHODS

Contraction of irrigated "ghost" fibrils:

This was carried out under a phase-contrast microscope (magnification 1,250-3,000) following the procedure employed by Hanson and Huxley (9). 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 fibrils by an Hasselbach-Schneider (H-S) solution (0.47 M KCl, 10 mM sodium pyrophosphate and 0.1 M phosphate buffer pH 6.4). The ghosts obtained were washed out with s.s.s. (addition of ATP caused no contraction) and irrigated with S-1 (1.2-3.5 mg/ml) in 50 mM KCl, 5 mM Tris buffer pH 7.6.

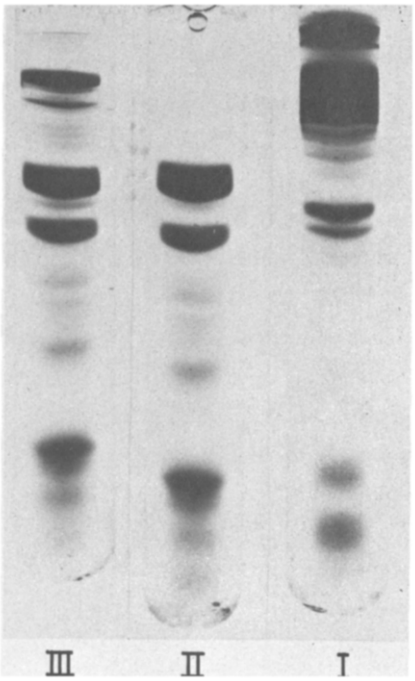

Fig. 1. SDD-gel electrophoresis of HMM S-1:
r. to l.: I - 1.4 mg/ml myosin; II - 1.4 mg/ml S-1; III - 1.4 mg/ml S-1 + 1.4 mg/ml myosin (5% polyacrylamide, 1% SDS, 0.1 M phosphate buffer pH 7.0; gel length 5 cm and current passed 10 μA).

Irrigation made the ghosts darker. A solution containing 0.1-5 mM MgATP and S-1 (at the same concentration as in the irrigating solution) was now added.

Isometric contraction of irrigated glycerinated muscle fibers:

Before the experiment, strips were allowed to equilibrate to room temperature in a solution containing 15% glycerol, 50 mM KCl, 2 mM EGTA and small bundles of fibers were teased from them. The muscle preparation was glued (with Eastman type 910 Adhesive) between an arm of a tension measuring device and a mechanical support mounted on a micromanipulator (Narishige) for adjustment of the initial muscle length. It was then suspended in a test solution filling an experimental cell consisting of a Perspex block in which a shallow recess had been machined. The volume of the recess was 1 ml.

Tension was measured by a Hewlett-Packard type 7DCDT-250 gauge in which transformer core was mounted on a strip of phosphorus bronze metal. The compliance of this system at the point of muscle attachment was about 500 μ/g; with the typical sample length of 1-1.5 cm and the maximum tension usually below 200 mg, the contractions could be considered isometric. The transducer was calibrated against a series of weights and found linear within the range of tensions obtained in the

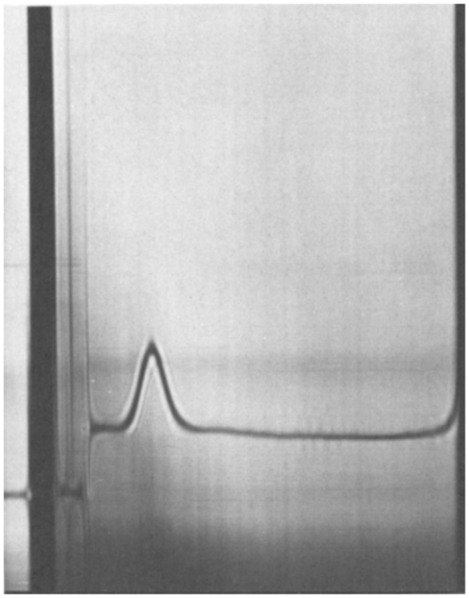

Fig. 2. Sedimentation pattern of HMM S-1:
0.7% S-1, 50 mM KCl, 5 mM Tris-HCl buffer pH 7.6, sedimented in a Spinco Model E analytical ultracentrifuge at 20°C, 56,000 r.p.m.

present work. The output of the transducer was displayed on Moseley type 135 recorder.

Rigor and relaxation were induced by exposing muscle fibers to the following solutions: 50 mM KCl, 1 mM $CaCl_2$, 10 mM imidazole buffer at pH 7 (rigor) and 50 mM KCl, 10 mM imidazole buffer pH 7, 4 mM ATP, 4 mM MgCl, 2 mM EGTA (relaxation). Contraction of fibers was induced by addition of MgATP (final concentration 5 mM) to the rigor solution, while stirring.

RESULTS

a) "Ghost" myofibrils:

Out of about 100 experiments performed with 5 mM MgATP+ 3.5 mg S-1/ml, contraction occurred in about 90. In these cases all the irrigated ghosts underwent contraction (Fig. 4). In isolated cases only part of the myofibrils exhibited contraction. When the MgATP concentration was only 1 mM, contraction took place in about 70% of the cases. If the concentration was lowered to 0.1 mM, no contraction could be detected; however, the ghosts became less dense, probably due to the dissociation and escape of the S-1. If the irrigated ghosts were washed out with s.s.s., the addition of 1 mM MgATP (+ S-1) did not cause any contraction while the ghosts became less opaque.

b) Glycerinated muscle fibers:

The isometric tension developed by glycerinated muscle fibers decreases in each

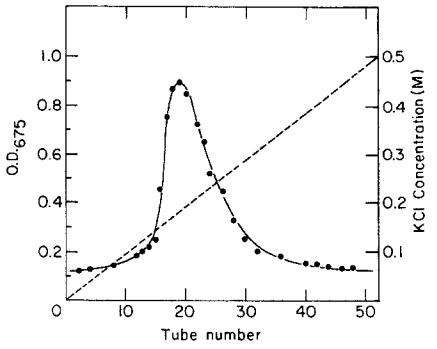

Fig. 3. Elution of HMM S-1 from a DEAE-cellulose column: 20 mg S-1 in a 10 ml solution containing 50 mM KCl, 5 mM Tris-HCl buffer pH 7.6 were absorbed by a 1.4x11 cm DEAE-cellulose (Whatman DE32) column and eluted with a 0-0.5 M KCl gradient. The fractions collected were 1.9 ml each. Ordinate: Optical density of protein as determined by Lowry's method (6).

successive contraction-relaxation cycle. Since the additional tension due to the interaction of S-1 with actin was expected to be small, it was essential to find conditions under which the difference between successive tensions was as little as possible. It was found that if a muscle rigor-contraction cycle was repeated 3-5 times with muscle kept at its rest length, the difference between successive contractions was less than 5% of the maximum tension in the first contraction.

Fig. 5 illustrates a typical experiment in which a muscle preparation has been subjected to 3 rigor-contraction cycles. Curves A and B represent 2nd and 3rd contractions, respectively. The initial drop in tension following addition of MgATP arises because the resting rigor tension is initially greater than the active contraction tension. The decline in isometric force in successive contractions is clearly illustrated. Following 3rd contracture, the muscle was exposed to a solution containing 3.5 mg/ml S-1 in 50 mM KCl for 3 min. Addition of MgATP induced an immediate contraction (curve C) with the final level of tension 2.5 times greater than in the previous contraction. The same results were obtained when the muscle was initially stretched by 5-10% of its rest length.

The presence of the additional tension arising from actin-S-1 interactions was demonstrated further in a muscle in which overlap between thin and thick filaments was nearly completely abolished. Under such conditions the length of the thin filament available to S-1 is maximal and the tension due to the filamenteous myosin cross-bridges is very small, suggesting that the effect of the S-1 interaction should be especially pronounced. Indeed, when the muscle preparation was stretched in relaxing solution to 70% of its rest length, giving the active tension of only about 1% of the maximum tension developed at rest length, 2 min irrigation with S-1

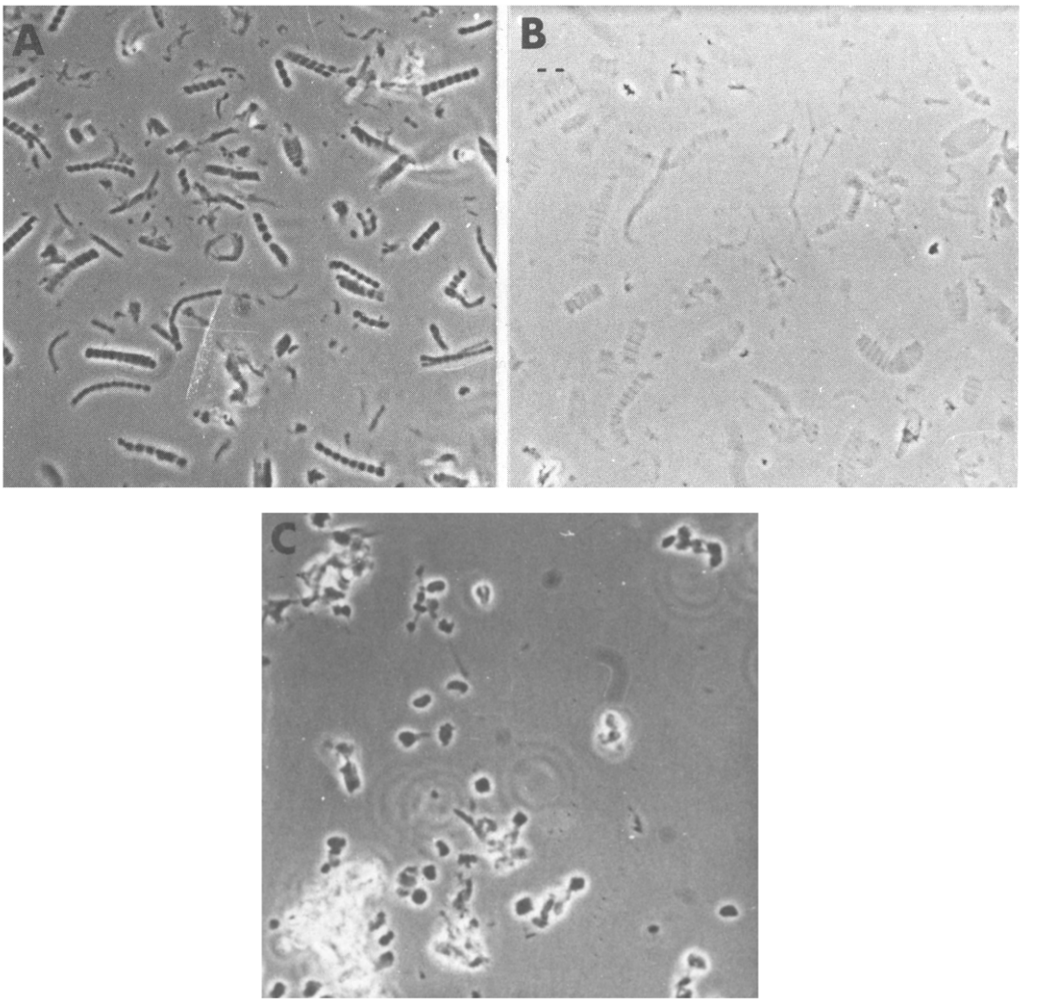

Fig. 4. Contraction of S-1 irrigated "ghost" fibrils under the microscope (phase contrast):
A - intact myofibrils; B - "ghost" myofibrils after washing with s.s.s.;
C - contracted "reconstituted" myofibrils.

increased the value of active tension of stretched muscle to 9% of the tension at rest length.

Similar results have been obtained when muscle preparations at different degrees of stretch, as well as the fibers from which myosin has been extracted with an H-S solution, were irrigated with either HMM under the same conditions, or with myosin at 0.4 M KCl, followed by washing with s.s.s. (Borejdo and Oplatka, in preparation).

D I S C U S S I O N

As we have shown in our previous work (2), the minimal concentration of myosin (at 0.4 M KCl) required in order to reconstitute ghost myofibrils into contractile

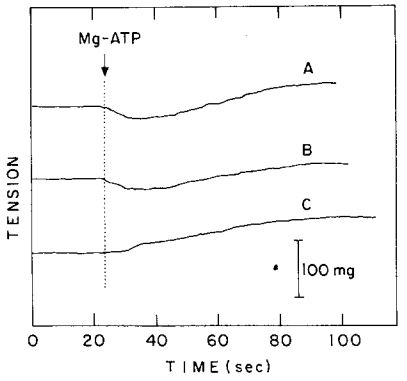

Fig. 5. Isometric tension development in HMM S-1 irrigated glycerinated muscle fibers:
A & B: contraction of glycerinated fibers upon addition of MgATP (broken line); C: contraction after irrigation with S-1; 3-fibers preparation at about 20°C.

elements (9) was more than 0.1 mg/ml. On the other hand, the supernatant obtained after dialyzing a solution of myosin (3 mg/ml) in 0.5 KCl against the solution used for dialyzing HMM S-1 prior to its application to the ATP column (see MATERIALS) was found to contain only 0.03 mg/ml of myosin, while the SDS-gel electrophoresis pattern of S-1 (Fig. 1) shows that if any myosin impurity is present, its concentration should be less than 0.01 mg/ml. Moreover, both the sedimentation and the DEAE-cellulose-elution patterns do not show the presence of either myosin or HMM. We therefore find it difficult to believe that trace amounts of myosin could be responsible for the contraction induced by irrigation with the S-1 preparations.

The fact that the non-aggregating S-1 was found to be capable of forming "contractile complexes" with actin filaments in ghost myofibrils or glycerinated muscle fibers provides further support to the conclusion derived from our previous experiments with HMM-irrigated ghost myofibrils, namely, that aggregation into filaments by the myosin molecules is not obligatory for manifestation of mechano-chemical transformation. Another manifestation is the active streaming in micro-capillaries of acto-HMM, and probably also acto-HMM S-1, in the presence of MgATP (2,10,11). Our findings may be explained on the basis of an hydrodynamic mechanism which is common to both muscle contraction and cytoplasmic streaming (Tirosh, Liron and Oplatka, in preparation).

In order to be able to understand the reason for the existence of two heads in the myosin molecule, it is essential that we know whether each of these heads can or cannot generate a mechanochemical force in the absence of its "twin" subfragment. An average of about one nucleotide molecule has been found to bind per "head" [in myosin, HMM or S-1, cf. (12)] and the ratio of the various molar ATPase activities

of myosin, HMM and HMM S-1 is 2:2:1 (12,13). Using immobilized ATP columns, we have recently demonstrated (Muhlrad, Lamed and Oplatka, in preparation) that all heads can bind to immobilized ATP (apparently with the same affinity) and split it (with practically the same activity). The fact that each of the separate heads (i.e. in an S-1 preparation) can hydrolyze ATP does not necessarily imply that it should also be able to generate a mechanochemical force when interacting with actin in the presence of ATP: as we show in a subsequent communication, the ATPase of S-1 which has been trinitrophenylated at its active site is activated by Mg^{2+} to match the actin activation of the unmodified S-1; at the same time, it is not capable of inducing contraction of ghost myofibrils. Since the actin activated ATPase activity of HMM appears to be twice as large as S-1 under the same conditions (13), it is probable that in the contraction experiments reported above, all the S-1 molecules take part in mechanochemical transduction.

Our findings still do not solve the problem "why two heads?" and might make it more difficult to answer. It may be, however, that the presence of two heads is rather coincidental: if it were important for the myosin "tails" (which form the shaft in the myosin filaments of a striated muscle) to be rigid and for this purpose they "were made" double-helical, then the number of protruding "loose ends" of the two heavy (and equivalent) polypeptide chains which were supposed to interact with ATP and actin would also have to be two.

This work was supported in part by a grant from the Muscular Dystrophy Associations of America (to A.O.) and by a Katzir-Katchalsky fellowship (J.B.).

REFERENCES

1. Huxley, H.E. (1969) Science 164, 1356.
2. Oplatka, A., Gadasi, H., Tirosh, R., Lamed, Y., and Liron, N. (1974). J. Mechanochem.Cell Motility 2, in press.
3. Lowey, S., Slayter, H.S., Weeds, A.G., and Baker, H. (1969) J.Mol.Biol. 42, 1.
4. Azuma, N. and Watanabe, S. (1965) J.Biol.Chem. 240, 3847.
5. Lamed, R., Levin, Y., and Oplatka, A. (1973) Biochim.Biophys.Acta 305, 163.
6. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951) J.Biol.Chem. 193, 265.
7. Bárány, M. (1967) J.Gen.Physiol. 50, No.6 part 2, 197.
8. Bendall, J.R. (1961) Biochem.J. 81, 520.
9. Hanson, J. and Huxley, H.E. (1955) Symp.Soc.Expt.Biol. 9, 228.
10. Oplatka, A. and Tirosh, R. (1973) Biochim.Biophys.Acta 305, 684.
11. Tirosh, R., Oplatka, A., and Chet, I. (1973) FEBS Letters 34, 40.
12. Ebashi, S. and Nonomura, Y. (1973) in: Structure and Function of Muscle (G.H. Bourne, ed.) 2nd edition, vol. III, Academic Press, New York, p.285.
13. Margossin, S and Lowey, S. (1973) J.Mol.Biol. 74, 301.