SUPERPRECIPITATION OF HEAVY MEROMYOSIN WITH AGGREGATED F-ACTIN

Julian BOREJDO, Nira GARTY, Tova SHIMON, Stanley HIMMELHOCH and Avraham OPLATKA
Departments of Polymer Research and Biological Ultrastructure, The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

The phenomenon of superprecipitation [1] is the ATP-induced dehydration of actomyosin in suspension which gives rise to a spectacular increase in turbidity [2] and low angle light scattering [3]. This conversion has long been considered a simple model for muscle contraction [1]. Upon addition of MgATP to an actomyosin suspension the two proteins first dissociate, giving rise to what is named a 'clearing phase'. As the substrate concentration decreases due to hydrolysis, a dense network consisting of many actin filaments crosslinked by filamentous myosin is formed from which fluid is extruded. On the other hand it had long been known that the soluble myosin fragment heavy meromyosin (HMM) is incapable of substituting for myosin in superprecipitation [4], presumably because it cannot participate in network formation. However, in view of the recent suggestion [5] that HMM may interact with neighbouring actin filaments in striated muscle, and of our findings that HMM is mechanochemically competent [6,7], we have searched for conditions under which HMM also would superprecipitate with actin. We find that when G-actin devoid of divalent cations is polymerized by acid, an apaque F-actin solution is obtained which will superprecipitate with HMM.

2. Materials and methods

Actin was prepared by a modified procedure of [8]. The acetone-dried powder is extracted at room temperature with 20 vol. distilled water for 1 h, with stirring. The extract is filtered and washed with additional 15 vol. distilled water. The actin in the filtrate is allowed to polymerize in 0.1 M KCl for 1 h. KCl is then added to 0.6 M final conc. and the solution allowed to stand at room temperature for 1 h. The F-actin is sedimented at 176 000 × g for 1 h, the pellet is washed with distilled water and redissolved in 1 mM $MgCl_2$. The protein is again treated with 0.6 M KCl, sedimented and resuspended in a solution containing 0.2 mM ATP, 1 mM DTT and 2 mM Tris-acetate buffer (pH 7.0). After dialyzing overnight against the same solution, 6.0 mM EDTA is added and the pH lowered to 5.4–5.8 by the slow addition of 50 mM acetic acid. The actin solution obtained is opaque and viscous.

Opaque actin can stimulate the ATPase activity of HMM to the same extent as the conventional actin prepared according to [9]. Electron microscopic examination of negatively stained specimen shows the usual F-actin filaments which can form arrowhead complexes with HMM [10]. Zimm plots (cf. [11]) of light scattering data (fig.1) suggest that the molecular size of opaque F-actin is much larger than that of ordinary F-actin: the molecular weights estimated for opaque and conventional actins are 2.5×10^8 and 2.0×10^7, respectively. The corresponding values for the radius of gyration are 378 and 502 Å.

Myosin was prepared as in [6]. HMM and HMM subfragment-1 (S-1) were obtained by the method in [12]. HMM was purified by ammonium sulfate fractional precipitation and further by fractionation on Sepharose 2B–adipic acid hydrazide–ATP (Seph–ADH–ATP) column [13]. The specific EDTA-activated ATPase activity of ammonium sulfate HMM was 1.1 μmol P_i .mg^{-1}.min^{-1} and increased to 1.7 units for the column-purified HMM. The SDS gel electrophoresis patterns of the proteins used are

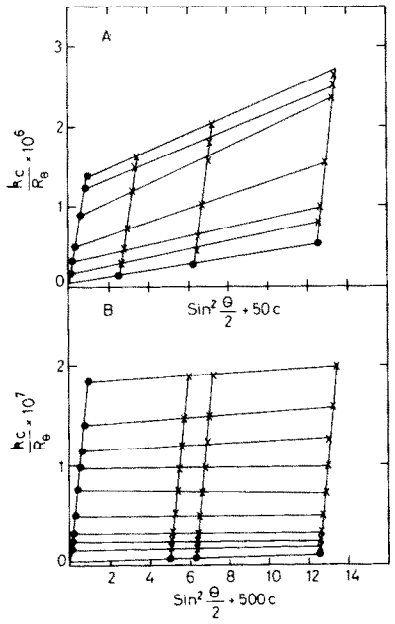

Fig.1. Zimm plots for conventional (A) and opaque (B) F-actins, k, optical constant; c, protein concentration in g/ml; R_θ, Raleigh's ratio; θ, scattering angle.

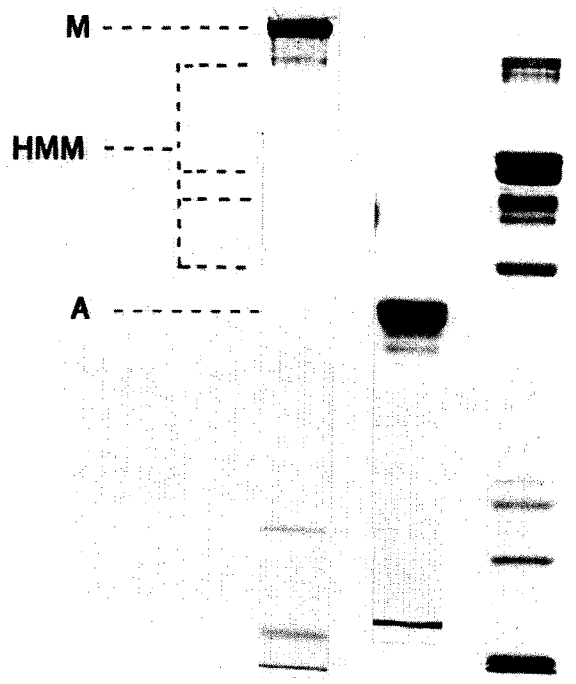

Fig.2. (a) Slab gel electrophoresis of proteins used in the superprecipitation experiments. 10% polyacrylamide, 0.1% sodium dodecylsulfate, 0.2 M Tris–glycine buffer (pH 8.5). The cells were overloaded to expose protein contamination. The gels were loaded with (from left to right): 8.7 μg myosin, 13.1 μg actin prepared by the procedure described in this paper, 14.3 μg HMM purified only by ammonium sulfate fractionation. M indicates the heavy chain of myosin and A denotes actin.

presented in fig.2. Superprecipitation was followed by recording the turbidity change at 660 nm [2] and by electron microscopic observation of the negatively stained specimen [10]. The reaction mixture (2 ml) contained varied amounts of actin and HMM, 50 mM KCl, 10 mM imidazole or phosphate buffer (pH 6.7) and 0.5 mM MgATP. Superprecipitation was carried out at 31°C.

3. Results and discussion

The time course of the turbidity change monitored when MgATP was added to a solution containing various concentrations of actin and HMM is shown in fig.3. The initial transient turbidity decline, which indicates dissociation of the acto–HMM complex, is followed by a gradual increase over a period of several minutes, depending on the actin or HMM concentration. Figure 4A shows that the extent and rate of superprecipitation increase with increasing actin concentration at fixed HMM concentration. Figure 4B shows a similar behavior for increasing HMM concentration at fixed actin. The final level of the absorbance obtained at 0.14 mg actin/ml and 0.28 mg myosin/ml was 0.27, not in great disparity with the final level reached with the same concentration of HMM (cf. fig.4B).

We have also checked the influence of MgATP and KCl concentration on the rate and extent of superprecipitation. Increasing MgATP concentration decelerates the rate and enhances the extent while KCl slowly decreases both. By combining high actin, HMM and MgATP together with low KCl concentrations we have observed spectacularly high turbidities reaching A 0.5.

It is important to note that superprecipitation does not occur when the turbidity is monitored at

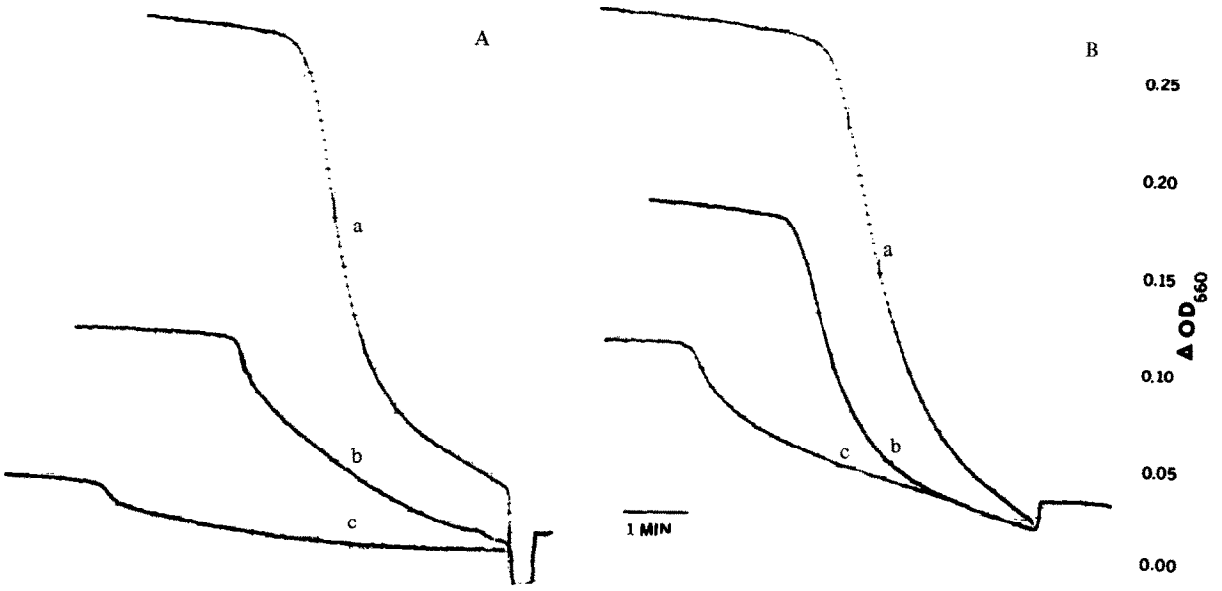

Fig.3. (A) Time course of turbidity changes when MgATP is added to a solution containing actin and HMM. Curves a, b and c show superprecipitation when HMM concentration is fixed at 0.4 mg/ml and actin concentration is 0.3, 0.2 and 0.14 mg/ml, respectively. (B) Superprecipitation when actin concentration is held fixed at 0.25 mg/ml and HMM concentration is varied between 0.5 mg/ml (curve a), 0.4 mg/ml (curve b) and 0.25 mg/ml (curve c).

pH 7.0 instead of 6.7 or 6.85. This striking pH sensitivity might have a physiological relevance in connection with actin bundles (cf. [14]) in non-muscle cells.

When the pH of a conventional (Spudich-type) F-actin is lowered by acetic acid to 4.8, isoelectric precipitation occurs and such actin does not superprecipitate with HMM.

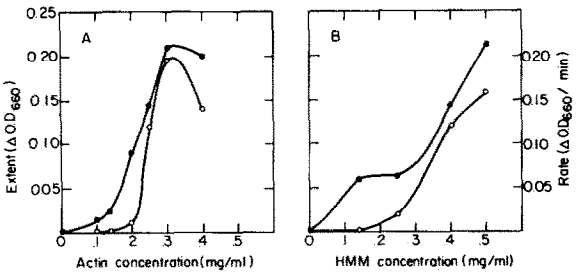

Fig.4. Summary of results similar to those presented in fig.1. (A) The effect of actin concentration on the extent (•) and rate (○) of superprecipitation. HMM concentration held fixed at 0.4 mg/ml. (B) The effect of HMM concentration on the extent (•) and rate (○) of superprecipitation. Actin concentration held fixed at 0.25 mg/ml.

In agreement with [4], no superprecipitation occurs when the Spudich-type F-actin is mixed with HMM and ATP. This was true for any ratio of actin and HMM tested at any pH. This very fact makes it improbable that (possible) residues of myosin in the HMM preparation are responsible for the superprecipitation. In spite of this we have made an effort to positively exclude such a possibility. SDS slab gel electrophoresis of purified HMM (fig.2) shows that myosin contamination (if any) is $< 1\%$ of the weight of HMM. If one adds this amount of myosin to opaque actin in the presence of MgATP no superprecipitation occurs. As a further test we have determined the minimum concentration of myosin required to give observable superprecipitation. At 0.14 mg actin/ml, $\geqslant 0.015$ mg myosin/ml was necessary to observe turbidity increase after adding MgATP. It took 0.025 mg HMM/ml to give the same turbidity increase; thus HMM would have to be contaminated with $> 60\%$ (by wt) of myosin to explain the turbidity change as due to myosin.

HMM subfragment-1 was totally ineffective in inducing superprecipitation even at the highest actin

and S-1 concentrations. HMM S-1, has been found to be active mechanochemically in other systems [6,7] even though to a smaller extent than HMM. Consequently, the absence of superprecipitation when S-1 was substituted for HMM suggests that the bifunctional nature of HMM is necessary in the presently described system. The very fact that HMM can substitute for myosin in superprecipitating actin in its opaque form leads us to the conclusion that HMM can participate in network formation, presumably by crosslinking neighbouring actin filaments, corroborating the suggestion in [5]. In superprecipitating acto–HMM, such crosslinking can apparently only occur when the actin filaments are already aggregated, as is probably the case in opaque actin, the molecular weight of which is 12.5-times larger than that of conventional actin. Such aggregates may become denser during superprecipitation due to the extrusion of water by the mechanochemically active HMM [7,15] and by interfilamentous rigor complex formation with the double-headed HMM following the depletion of ATP.

Our results provide a particularly rigorous confirmation of our contention [6,7] that HMM is mechanochemically competent. We had shown that HMM is capable of causing the contraction of myofibrils or muscle fibers in which myosin has been either extracted or inactivated. This left open a possibility that the residual myosin, though chemically inactive, might have been somehow involved in the contraction. These experiments, involving solely actin and myosin-free HMM, set this possibility to rest. The ability of F-actin to interact mechanochemically with a soluble myosin species once again opens the possibility that such interactions may play an important role in non-muscle motility.

Acknowledgement

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