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## Effect of the H-meromyosin *plus* ATP system on F-actin

In the absence of ATP myosin accelerates the polymerization of G-actin and stabilizes the state of F-actin[1]. On the other hand, during superprecipitation induced by myosin and ATP, a loosening of the F-actin structure is suggested by the increased exchangeability of nucleotides and divalent cations bound to F-actin[2–4]. Thus, myosin exerts a dual action on actin; it regulates both the formation and the loosening of bonds between actin molecules, depending on whether or not ATP is present. To elucidate this dual action, the effect of a soluble fragment of myosin, H-meromyosin, on F-actin in the presence of ATP has been analysed.

G-Actin was extracted at low temperatures from acetone-dried powder of rabbit skeletal muscle and polymerized in 30 mM KCl. The F-actin obtained was washed 3 times with the same salt solution and stored in the form of pellets. G-actin was obtained by dissolving the pellets into solvents at very low salt concentrations. H-meromyosin was prepared from myosin of rabbit skeletal muscle by limited digestion with trypsin.

H-Meromyosin in the absence of ATP can induce polymerization of G-actin at low salt concentrations where G-actin can not polymerize spontaneously[1]. When F-actin is put into a solvent of a sufficiently low salt concentration, depolymerization takes place. If H-meromyosin is added to this solution in the absence of ATP, production of G-actin is suppressed. We confirmed that even at very low salt concentrations where sonic vibration can not induce polymerization but depolymerizes F-actin, H-meromyosin can induce polymerization or stabilize F-actin. In the absence of ATP H-meromyosin shifts the polymerization equilibrium to F-actin and lowers the critical actin concentration for polymerization.

In the presence of ATP, on the other hand, we found that H-meromyosin accelerates depolymerization of F-actin without shifting the polymerization equilibrium.

In solvent conditions where F-actin is stable, for example, at 0.1 M KCl or 0.6 M KCl, H-meromyosin was added to an F-actin solution in the presence of a high concentration of ATP. The degree of flow birefringence was not changed by the addition of H-meromyosin. (After ATP was eliminated by the ATPase activity of this system, the degree of flow birefringence was dependent on the amount of H-meromyosin[5].)

In the next experiment, a salt solution of F-actin of 5.7 mg/ml in KCl (55 mM) was diluted in 5 vol. of a buffer solution containing a high concentration of ATP (1.3 mM) at a low temperature (2°) to final concentrations of 1.15 mg/ml F-actin and 11 mM KCl, as indicated in Fig. 1. F-actin slowly but spontaneously depolymerized to G-actin, tending toward an equilibrium state in which G- and F-actins coexist. At the time of dilution various amounts of H-meromyosin were added. The degree of flow birefringence immediately after the addition was not changed; however, the decrease of the birefringence with time was hastened by H-meromyosin. Depolymerization of F-actin was accelerated by the H-meromyosin *plus* ATP system. Actually the sedimentation pattern showed that in the course of depolymerization, the amount of G-actin was larger in the presence of H-meromyosin than in its absence. The final value of birefringence in Fig. 1 seems to be independent of the presence of

H-meromyosin, although the true final state in ATP could not be defined accurately due to the ATPase activity of this system.

The results of a series of similar experiments are shown in Fig. 2. In these experiments F-actin pellets were dissolved in a buffer solution containing ATP to various final concentrations of F-actin, and the rate of depolymerization was measured in the presence and absence of H-meromyosin. The relative rate of depolymerization, defined as described in the legend, increased with increasing amounts of H-meromyosin and also increased with decreasing concentrations of F-actin. Under conditions

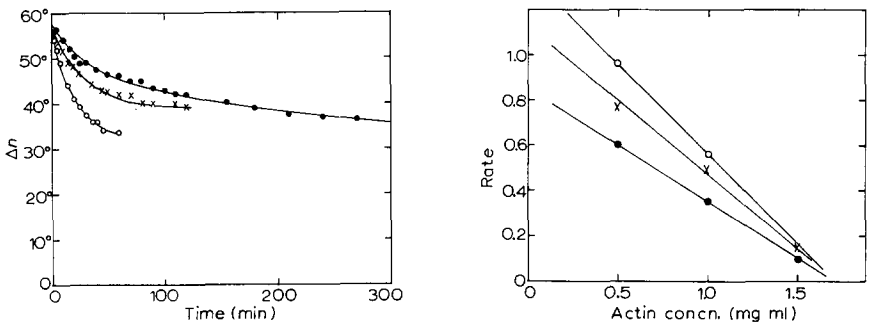


Fig. 1. Depolymerization of F-actin accelerated by the addition of H-meromyosin in the presence of ATP. The ordinate, $\Delta n$, is the degree of flow birefringence which is proportional to the amount of F-actin. The abscissa is the time after F-actin solutions were diluted to the final concentrations: actin, 1.15 mg/ml; KCl, 11 mM; Tris–HCl (pH 8.0), 5.4 mM; ATP, 1.3 mM; at 2°; and H-meromyosin: ●, 0 mg/ml; ×, 0.3 mg/ml; ○, 0.5 mg/ml (see text).

Fig. 2. The relation between the depolymerization rate and the F-actin concentration when various amounts of H-meromyosin were added. The ordinate is the depolymerization rate defined as:

$$\delta \ln \Delta n/\delta t = (\Delta n_{1\ \mathrm{min}} - \Delta n_{10\ \mathrm{min}})/(\Delta n_{1\ \mathrm{min}} + \Delta n_{10\ \mathrm{min}})$$

where $\Delta n_t$ is the degree of flow birefringence at time $t$ after F-actin pellets were dissolved in the solvent; final composition: Tris–HCl (pH 8.0), 5 mM; ATP, 2.2 mM; at 2°. The abscissa is the concentration of F-actin after dissolution. H-meromyosin: ●, 0 mg/ml; ×, 0.7 mg/ml; ○, 1.4 mg/ml.

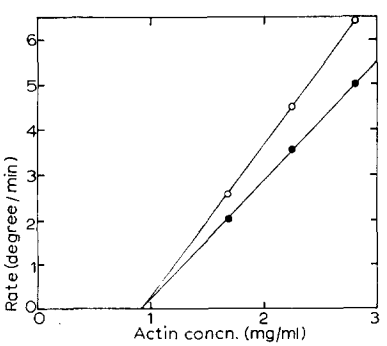


Fig. 3. The relation between the polymerization rate and the G-actin concentration in the presence and absence of H-meromyosin. The ordinate is the initial rate of increase of the degree of flow birefringence (in degree/min) after the addition of KCl (and H-meromyosin) to G-actin solutions containing buffer and ATP. The final solvent conditions were: KCl, 30 mM; Tris–HCl (pH 8.0), 6.25 mM; ATP, 2.4 mM; at 0°. The abscissa is the initial concentration of G-actin. The weight ratio of H-meromyosin to G-actin: ●, 0; ○, 1:2.

where spontaneous depolymerization is very slow, the acceleration by H-meromyosin becomes very small.

The acceleration of polymerization of G-actin by H-meromyosin can be found even in the presence of ATP if the solvent condition is favourable to polymerization. However, the critical actin concentration for polymerization was found to be only slightly changed by the H-meromyosin *plus* ATP system, as shown in Fig. 3. In the presence of ATP the initial rate of polymerization of G-actin was determined from the increase of the degree of birefringence after the addition of salts at varied concentrations of G-actin in the presence and absence of H-meromyosin (at a constant ratio to G-actin).

Thus, both polymerization and depolymerization are accelerated by the H-meromyosin *plus* ATP system without shifting the polymerization equilibrium. This phenomenon is expected to occur if the H-meromyosin *plus* ATP system has no strong affinity for G- or F-actins, but has a specific affinity for an intermediate or transient state between G- and F-actins or has the ability to bring actin to the intermediate state[2].

With the same solvent conditions as those used for Figs. 1 and 2, when the acceleration of depolymerization was observed, the ATPase of H-meromyosin is found to be activated by F-actin, approximately in proportion to the product of the concentrations of the two proteins (see also ref. 6). It is expected that the same temporary interaction between the two proteins may be the origin of both the ATPase activation and the change of the F-actin structure which leads to the acceleration of depolymerization. It is useful to examine such a parallelism between the ATPase activation and the acceleration of depolymerization without the equilibrium shift by using F-actin and H-meromyosin modified in different ways.

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