"Test Tube Contraction" with Acto-Heavy Meromysin Suspensions[1] (34239)

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It is known that after a short digestion with trypsin myosin A yields two major fragments. One of these fragments, called HMM,[3] retained the actin-combining ability of the parent molecule (1, 2) and in media containing very low concentrations of salts (<0.02 *M*) actin increased the Mg^{2+} activated ATPase activity of the HMM enzyme (3). In spite of the well documented complex formation between actin and HMM, previous authors were unable to show the superprecipitation reaction ("test tube contraction") with acto-HMM in mixtures which were found to be suitable for superprecipitation with the actomyosin complex (2, 3). It has to be emphasized, however, that the superprecipitation of actomyosin is observed only in mixtures containing low salt concentrations (<0.3 *M*) and under such conditions myosin A is insoluble. The HMM, unlike myosin, is also soluble in mixtures containing low concentration of salts (<0.3M) and therefore one important condition for superprecipitation was not fulfilled in the experiments of previous authors. By reducing the solubility of HMM we have studied the effect of ATP on acto-HMM suspensions. The results of these experiments are described below.

Materials and Methods. The ATP was purchased from the Sigma Company, polyethylene glycol having a mol wt of 4000 was obtained from the J. T. Baker Chemical Company, all other reagents used were of reagent grade. Actin and myosin A was prepared from rabbit skeletal muscle as described by Mommaerts (4). The HMM was prepared according to the method of Szent-Gyorgyi (5). Superprecipitation was measured using the method of Ebashi (6) as described in an earlier paper (7). HMM and actin [3:1, (w/w), HMM:actin] were mixed in 0.067 *M* phosphate buffer at pH 6.1 and incubated at 0° at least for 15 min prior to the experiments.

Results. At pH 6.1 the acto-HMM solutions became slightly turbid since at this pH the solubility of HMM was reduced. PEG-4000 was added to the acto-HMM solutions because previous results in this laboratory have shown that this compound reversed the inhibitory effect of increased KCl concentration on the superprecipitation of myosin B suspensions (8).

Figure 1 shows that in the presence of 33 mg/ml of PEG-4000 at pH 6.1 the turbidity of the acto-HMM complex increased about 7 transmittance units during the 15-min time of observation (Fig. 1, Curve C). The addition of 6.6×10^{-5} *M* Mg–ATP to such mixtures induced a turbidity increase which reached its maximal value about 45 transmittance units within 10 min (Fig. 1, Curve A). The incorporation of 2 m*M* EGTA into these mixtures did not change the Mg–ATP effect on the acto-HMM suspensions (Fig. 1, Curve B) while the addition of 2 m*M* EDTA abolished the Mg–ATP-induced turbidity increase (Fig. 1, Curve D). These experiments also showed that the presence of PEG-4000

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[3] In this work the following abbreviations have been used: HMM = heavy meromyosin; PEG-4000 = polyethylene glycol having a molecular weight of 4000; EGTA = 1,2-bis(dicarboxymethylaminoetoxy) ethane [ethylene glycol bis(aminoethylether)-*N*,*N*'-tetraacetic acid]; EDTA = ethylenediaminotetraacetic acid.

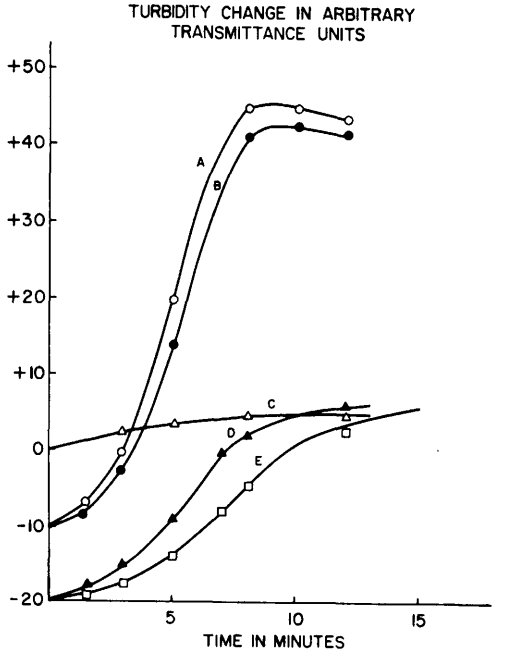

FIG. 1. Mg–ATP induced turbidity changes of acto-HMM at pH 6.1: Each tube contained about 0.3 mg/ml HMM, 0.1 mg/ml actin, 0.066 M KCl and 0.067 M phosphate buffer to which the following additions were made: Curve A (○), 33 mg/ml of PEG-4000 and 3.3×10^{-5} *M* Mg-ATP; Curve B (●), 33 mg/ml of PEG-4000, 2 m*M* EGTA, and 3.3×10^{-5} *M* Mg-ATP; Curve C (△), 33 mg/ml of PEG-4000; Curve D (▲), 33 mg/ml of PEG-4000, 2 m*M* EDTA and 3.3×10^{-5} *M* Mg-ATP; and Curve E (□), 3.3×10^{-5} *M* Mg-ATP. Total final volume was 15 ml.

was essential for the superprecipitation like reaction to occur since, in the absence of PEG-4000, Mg–ATP failed to produce any appreciably turbidity increase with the acto-HMM suspensions (Fig. 1, Curve E).

Figure 2 shows that optimal turbidity increase with acto-HMM suspensions was obtained using 3.3×10^{-5}– 6.6×10^{-5} *M* Mg–ATP while larger Mg–ATP concentrations were inhibitory. These experiments also revealed that the addition of 0.1–0.2 *M* KCl into the incubation media was optimal for the Mg–ATP induced turbidity increase while the presence or absence of Ca^{2+} was without significant effect.

Below pH 5.9 the turbidity of the acto-HMM suspension in the presence or absence of PEG-4000 increases spontaneously and the addition of Mg–ATP did not change this process. Above pH 6.5, the acto-HMM complex remained in solution regardless whether or not PEG-4000 was also added to the mixtures and the addition of Mg–ATP did not cause any appreciable turbidity increase. The optimal pH range for the Mg–ATP induced turbidity increase with acto-HMM suspensions was found to be from pH 6.0 to 6.3.

In subsequent experiments the pH of the HMM solution was adjusted to pH 5.2 in an ice bath and kept at this pH for 5–15 min. The HMM precipitated at this pH and did not go into solution as the pH was again raised to 7.5. Actin was then mixed with these isoelectrically precipitated HMM suspensions in the presence of 0.099 *M* KCl at

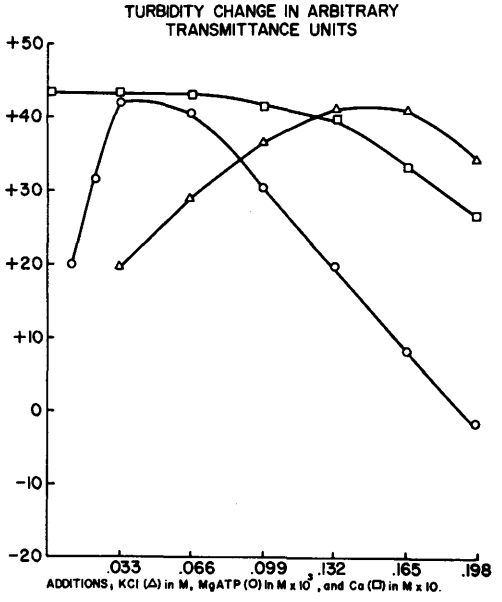

FIG. 2. Effect of the Mg–ATP, KCl, and Ca^{2+} concentrations of the Mg–ATP-induced turbidity increase of acto-HMM: Each tube contained about 0.3 mg/ml of HMM, 0.1 mg/ml of actin, and 0.067 *M* phosphate buffer, pH 6.1, with the following additions: (○), 0.132 *M* KCl and from 1.1×10^{-5} to 19.8×10^{-5} *M* Mg–ATP as indicated; (□), 0.132 *M* KCl, 3.3×10^{-5} *M* Mg–ATP and from 0 to 19.8×10^{-3} *M* Ca^{2+} as indicated; and (△), 3.3×10^{-5} *M* Mg–ATP and from 0.033 to 0.198 *M* KCl as indicated. Total final volume was 15 ml. The maximal turbidity change obtained 10 min after the addition of Mg–ATP was measured.

pH 6.1 and Mg-ATP (3.3×10^{-5}– 6.6×10^{-4} *M*) failed to produce turbidity increase with these suspensions regardless whether or not 33 mg/ml of PEG-4000 was also added to the mixtures.

Discussion. Huxley proposed that the HMM part of the myosin A molecule is somehow involved in the formation of cross bridges between myosin and actin (9, 10). These cross bridges were shown to undergo a conformational change during the contraction process (11, 12). The idea has been put forward that a limited analogy may exist between the conformational changes occurring during the *in vitro* and *in vivo* contraction (13). Myosin and actin are not dissolved in the living muscle neither are they soluble under the experimental conditions where superprecipitation occurs.

In this work a superprecipitation-like response was demonstrated with acto-HMM if the solubility of this complex was depressed by PEG-4000 at pH 6.1 (Figs. 1 and 2). The acto-HMM precipitate did not show "plug" formation and it was not quite as dense as the superprecipitated actomyosin. Denaturation of the HMM by isoelectric precipitation destroyed the ability of this protein to participate in a superprecipitation-like reaction upon addition of Mg–ATP.

Iyengar *et al.* (14) and Morita and Yagi (15) reported ATP-induced conformational changes on soluble acto-HMM complex and on dissolved HMM molecule. Our results indicate that if the acto-HMM complex is rendered insoluble without serious denaturation Mg–ATP may cause a conformational change which appears to be similar to the superprecipitation reaction of actomyosin.

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