The muscle proteins myosin and actin take the form of a colloidal solution under certain conditions. Thus, if Mg ions and adenosinetriphosphate (ATP) are present, actomyosin forms a clear colloidal solution at physiological salt concentration and neutral pH. This has been referred to as the clear phase of the superprecipitation reaction (1). The proteins react to ATP with formation of a turbid and contractile structure at acid pH and a transparent and rigid gel at alkaline pH. Inorganic pyrophosphate provides an example of a compound that will induce such a transition of actomyosin from a clear solution to a turbid contractile structure in the presence of ATP and Mg.

**EXPERIMENTAL**

The method of preparing myosin and actin and observing the reaction of these proteins to adenosinetriphosphate has been described in detail (1).

*Effect of Pyrophosphate on Clear Actomyosin-Mg-ATP System—* As shown in Table I, the actomyosin clearing response to ATP persists for 1 hour in the presence of Mg until the ATP is hydrolyzed and the proteins form a weakly contractile precipitate. When inorganic pyrophosphate is included, complete contraction occurs in a few minutes. There are three reasons for believing this to be due to a drop in pH: (a) It has been shown elsewhere that a drop in pH will terminate the Mg-induced clear phase (1), and evidence for this is included in Table I. A commensurate pH shift occurs on inclusion of pyrophosphate (Table I). This observed pH shift is due to complex formation between pyrophosphate and cation. The effect of alkaline earths on the pH of polyphosphate solutions was pointed out by Frankenthal (2) and has been investigated by Van Wazer and Campanella who obtained a coordination number of 2 for Mg (3). (b) Pyrophosphate action in terminating the clear phase in the actomyosin-ATP-Mg-Ca system has been found to be optimal at one-half the Mg concentration and one-fourth the combined Mg and Ca concentrations. (c) The plug of precipitate formed has the characteristics of actomyosin which has contracted in the presence of Mg. That is, it is smaller.
and brighter in reflected light than are actomyosin plugs formed in the absence of Mg. The enhanced contraction with Ca and pyrophosphate present has been shown to be due to the greater pH shift that occurs on inclusion of that cation, for Ca has no effect if the clear phase is terminated by adding dilute HCl. Although Ca opposes the clearing response of actomyosin to ATP (1), its effect is counteracted by equimolar Mg (Table I) (4).

Table I also demonstrates that pyrophosphate has an inhibitory effect on superprecipitation with Mg omitted. Inorganic pyrophosphate and tripolyphosphate do not cause contraction or gelation in the absence of ATP, with or without added Mg.

Inorganic tripolyphosphate (Na₆P₃O₁₀) and hexametaphosphate (Na₆(PO₃)₆) also cause plug formation when added to the clear actomyosin-ATP-Mg-Ca system. Adenosinediphosphate (ADP) causes precipitation without contraction at the concentration found effective for pyrophosphate and causes superprecipitation at triple this concentration. Since no appreciable pH drop occurs on addition of ADP, this nucleotide apparently terminates the clear phase by directly counteracting Mg. Thiamine pyrophosphate prolongs the clear phase and does not cause a pH change in this system at concentrations up to 5 times that of K₄P₂O₇ found to cause contraction.

In Fig. 1 the cross-hatched areas show that precipitation occurs at a lower ATP concentration (i.e. after more of the ATP has been hydrolyzed) when Mg is present. The ATP concentrations at which precipitation oc-
curs are approximately equivalent in the Mg-free system and in that containing Mg plus pyrophosphate. For this reason and because the Mg inhibition of the ATPase activity of actomyosin is only partially counteracted, the accelerating action of pyrophosphate on superprecipitation does not reflect simply an accelerated removal of the excess ATP. In this figure the pyrophosphate curve was corrected for the relatively strong

\[ \text{Mg} + K_2P_2O_7 \]

\[ \text{Mg} \]

\[ \text{No Mg or } K_2P_2O_7 \]

\[ \mu M \text{ PO}_4 \text{ LIBERATED} \]

\[ 0 \quad 10 \quad 20 \quad 30 \text{ MINUTES} \]

**Fig. 1.** The influence of Mg and pyrophosphate on the ATP concentration at which superprecipitation occurs. An actomyosin-ATP solution as in Table I, prepared in 20 times greater volume, was made 0.005 M with Mg or 0.0025 M with \( K_2P_2O_7 \) as shown. Aliquots removed at intervals were precipitated with 5 per cent trichloroacetic acid, centrifuged, and measured for orthophosphate by the Fiske-Subbarow procedure. The cross-hatched areas indicate the time at which precipitation was occurring. Contraction did not occur because solutions were disturbed by sampling.

Mg-activated pyrophosphatase activity of actin solutions\(^1\) by subtracting the phosphate liberated in an ATP-free control. As a result of myokinase contamination, a 2nd mole of orthophosphate was eventually liberated per mole of ATP when Mg was present.

**ATP-Mg Complex**—Complex formation between the nucleotide and Mg could play a rôle in the mechanism whereby this cation prolongs the clearing response of actomyosin to ATP. Neuberg and Mandl showed evidence for an ATP-Mg complex in the increased solubility of the car-

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\(^1\) Unpublished results.
Fig. 2. pH change on addition of bivalent cations to pyrophosphates. Nucleotide concentration was 0.0025 M. Curve with both MgSO₄ and CaCl₂ is based on combined concentration of these ions.

Fig. 3. pH change during superprecipitation. The reaction mixtures contained the following in order of addition: 0.1 gm. per cent of myosin, 0.21 M KCl, water, 0.025 gm. per cent of F-actin, 1.25 mM ATP, and 0.005 M MgSO₄ or CaCl₂ as indicated.

Bonates and phosphates in 0.2 M ATP solution (5). At lower nucleotide concentrations, complex formation is also indicated by the following: (1) the increased hydrolysis of ATP at 100° in the presence of equimolar Mg or Ca above pH 7, and (2) the pH drop on addition of the bivalent cation (Fig. 2). The pyrophosphate link, it appears, is essential for formation of the metallo-nucleotide complex, and ATP, with two possible
pyrophosphate metal combinations, has a stronger complex-forming power than ADP. Mg appears to form a complex more readily than Ca. However, this cannot explain the fact that, in combination, the influence of Mg on ATPase activity and superprecipitation predominates over that of Ca (Table I) (6), because the pH change with a mixture of Ca and Mg lies midway between that for either metal alone. Titration curves show that the purine portion of the molecule does not appear to be involved, for the dissociation of the NH₂ group in position 6 (pK 4) is not affected by Mg, but that of the terminal hydroxyl of inosinetriphosphate is increased. The pH drop during superprecipitation with Ca or Mg present, shown in Fig. 3, can obviously be explained on the basis of the pH changes shown in Fig. 2. Without the bivalent cations the pH falls gradually as a result of the formation of an —OH group with a pK near 7 upon hydrolysis of the third phosphate of ATP.

DISCUSSION

A compound such as inorganic pyrophosphate, which can be formed by known enzymatic reactions (7) and, in our experience, is hydrolyzed by actin solutions, may well function in the contraction cycle in vivo. The presence of Mg in muscle extracts, however, precludes the possibility of demonstrating any substance therein capable of terminating the clear phase, for we have found that aqueous muscle extracts affect superprecipitation as does a 0.01 to 0.005 M solution of MgSO₄. The experiment of Table I provides the only situation thus far encountered in which Ca ions have an effect on the actomyosin-ATP reaction in the presence of equimolar Mg ions. This is of some interest inasmuch as Ca causes contraction of isolated muscle fibers (8).

SUMMARY

Inorganic pyrophosphate, tripolyphosphate, and hexametaphosphate induce contraction in the actomyosin-ATP system rendered clear by the presence of Mg. This contraction can be attributed to the drop in pH caused by complex formation between Mg and the polyphosphate. The complex formed by ATP and Mg causes less of a pH drop.

BIBLIOGRAPHY