INTERACTIONS BETWEEN MAGNESIUM, PYROPHOSPHATE, AND THE CONTRACTILE ELEMENTS

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In glycerol-extracted muscle fibers PP imitates one of the important effects of ATP,[1] its softening action (1, 2). In the present paper this effect was analyzed in greater detail, in the expectation that the results might throw some light on the nature of the action of ATP. As shown for ATP (3), Mg was found to be important, in fact essential, for the action of PP. It is true that PP influences extensibility in solutions free of Mg, but this effect was found to be due to the presence of bound Mg. Evidence was also obtained that PP is bound rather firmly by the contractile elements, again in close agreement with observations on the effects of ATP (3).

The effect of PP on actomyosin solutions agrees closely with some of the results on muscle fibers. Mommaerts (4) found that Mg greatly increased the effect of PP on the viscosity of actomyosin solutions. He and Straub (5) also found that the effect of PP is much greater at 0° than at room temperature. This observation is analogous to the effect of temperature on viscous resistance described below.

Technique

For the study of extensibility the lengthening under a constant load was recorded, using the technique described previously (6). The records reproduced in the illustrations are enlarged about three times and were drawn from photographic enlargements.

The psoas muscle of the rabbit preserved in glycerol was used. Several thin strands of fibers were arranged so as to form a loop and mounted in a small chamber. The total cross-sectional area of the preparations was about 0.3 mm.^{2}, their length 2 cm. The rapidity of the responses indicates that diffusion equilibrium was established in the fibers within a few seconds. The fibers were first allowed to shorten 30 to 40 per cent of their length in 8 mM ATP at 5 to 10°. The extension time curves were nearly linear over the short range of lengths used in one experiment. Usually fibers which had been in glycerol for more than 4 weeks were used.

The chamber, which contained about 1 ml. of solution, was immersed in a water bath. The contents of the chamber could be flushed out without causing an appreciable temperature change through a long, thin polyethylene tube (outside diameter

[1] The following abbreviations will be used, ATP for adenosinetriphosphate, EDTA for ethylenediamine tetraacetate, PP for inorganic pyrophosphate.

about 1 mm.) which passed through the bottom of the chamber. A micropipette (5 mm.[3]) was used to add known amounts of substances to the solutions inside the chamber during an experiment.

Pyrophosphate solutions were prepared from a stock solution which contained 50 mM PP per liter and was adjusted to pH 7 by adding HCl. EDTA (analytical reagent) was obtained from Bersworth Chemical Co., Farmingham, Massachusetts. Solutions of this substance were adjusted to pH 7 by adding KOH. Experimental solutions were obtained by adding stock solutions to 0.16 M KCl, using micropipettes.

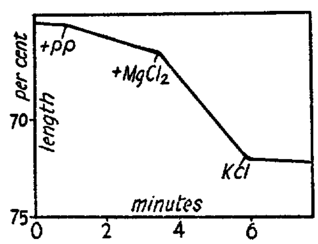

FIG. 1

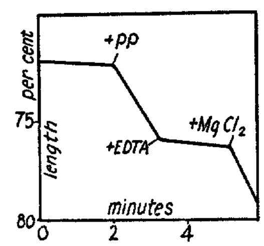

FIG. 2

FIG. 1. Effect of PP and Mg on extension under a constant load. At first the fibers were in 0.16 M KCl, to which later PP (0.6 mM per liter) and $MgCl_2$ (2 mM per liter) were added. This solution then was washed out by 0.16 M KCl. Abscissa, length as per cent of original length. 21°.

FIG. 2. Effect of EDTA on extension under a constant load. The fibers were in 0.16 M KCl at first. PP (2 mM per liter), EDTA (1 mM per liter), $MgCl_2$ (2 mM per liter) were added successively as indicated on the graph. Abscissa, per cent of original length. 16°.

RESULTS

The Role of Mg.—PP makes the fibers plastic. A load then causes a sudden, purely elastic extension followed by a further slow extension which is not reversed after unloading (6). The slope of the extension time curve is a measure of viscous resistance. The effect of PP becomes the more striking the heavier the load on the fibers. The load was adjusted at the beginning of each experiment so that a very slow extension occurred without PP (about 100 to 150 gm. per cm.[2] cross-section). Under these conditions 0.1 mM PP usually produced an easily observable effect.

As shown by Bozler (7) and Bendall (8), extension under a load is strongly accelerated by Mg (Fig. 1). PP is effective in solutions free of Mg, but the following observations indicate that this effect also depends on the presence of Mg. If muscle fibers are briefly immersed in 1 mM PP and if PP is washed out by 0.16 M KCl, a second application has much less effect on extensibility than the first. After washing again and repeating this procedure once or twice, PP no longer has any effect, even in much higher concentrations. After the fibers have become refractory to PP, the original condition is restored at once by immersion in 2 mM MgCl. $CaCl_2$ is completely ineffective. These observations suggest that the muscle fibers contain bound Mg which is essential for the softening action of PP and is removed by PP. This interpretation is confirmed by the fact that previous treatment with 10 mM EDTA also makes the fibers refractory to PP.

The importance of bound Mg is demonstrated also by the following observations. EDTA does not influence the effect of PP as long as there is an excess

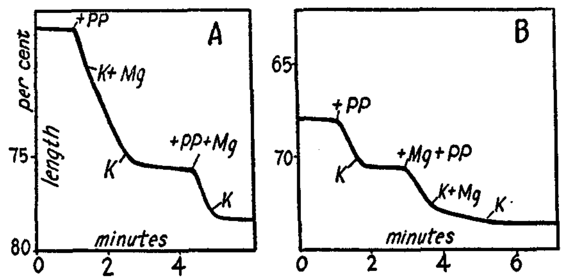

FIG. 3. Effect on the rate of extension of washing out PP with solutions which do and do not contain Mg. The fibers were immersed at first in KCl solution containing $MgCl_2$. On addition of PP rapid extension occurred.

A, chamber was flushed out with solution of KCl containing $MgCl_2$, later with pure KCl solution. Then PP and $MgCl_2$ were added, causing rapid extension. This solution was then replaced by solution of KCl without Mg. 0°.

B, PP was washed out by KCl solution without $MgCl_2$. Then PP and $MgCl_2$ were added. The chamber was then flushed out first by KCl solution containing $MgCl_2$, later by pure KCl solution. 14°.

Concentrations, KCl 0.16 M; $MgCl_2$ 2 mM; PP 1 mM per liter. Abscissa, per cent original length.

of Mg in the solution. However, if the solution is free of Mg, EDTA stops the effect of PP completely at once (Fig. 2).

Bozler (3) has shown that in fibers which have been brought into a state of high extensibility by ATP and Mg, Ca evokes a contraction. Particular attention was given, therefore, to the possibility that Ca antagonizes the softening action of PP. It was found that low concentrations of $CaCl_2$, such as those which give a maximal effect in the presence of ATP, are entirely ineffective. Higher concentrations (> 2 mM per liter) sometimes were found to cause a levelling off of the extension time curve, but the effective concentrations caused a slight turbidity in the solution. The observed effects, therefore, were probably caused by the precipitation of PP. This conclusion agrees with the effects of EDTA. If Ca influenced the effect of PP, EDTA would be expected to have an effect even in the presence of Mg, because it complexes

Ca in preference to Mg. EDTA actually has a striking effect in the presence of ATP and Mg, attributable to the presence of bound Ca (9). As mentioned above, however, EDTA does not influence the action of PP, if an excess of Mg is present.

Evidence for Chemical Binding of PP.—If PP is washed out from fibers with a solution containing Mg, the effect of PP disappears only slowly. In the experiments illustrated in Fig. 3 the fibers were immersed in a solution containing 6 mM PP and 2 mM $MgCl_2$ per liter and were extended by a constant load. When PP was washed out by a solution containing Mg, the slope of the extension time curve changed immediately, but was still steep. When, however, in a repetition of this experiment PP was washed out by 0.16 M KCl, the effect of PP was abolished almost at once.

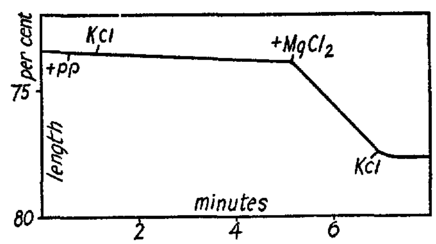

FIG. 4. Delayed effect of PP. Fibers previously made refractory to PP were immersed in 0.16 M KCl. Addition of PP (2 mM per liter) had no effect. This solution was washed out by 0.16 M KCl. 4 minutes later the addition of $MgCl_2$ (2 mM per liter) caused rapid extension. Washing out this solution by 0.16 M KCl slowed extension again. Abscissa, per cent of original length. 0°.

How long the effect of PP persisted after washing out PP varied considerably in preparations from different muscles. In some, the period was so short at room temperature, that it could not be demonstrated convincingly. In this case the effect became very striking when the fibers were kept at 0° (Fig. 3 A). In other preparations extension continued rapidly at this temperature for a few minutes, even if PP was washed out with KCl solution. No explanation for these quantitative differences was found. The addition of 5 mM phosphate buffer of pH 7 to the washing fluids had no noticeable effect.

The persistence of the effect of PP in the presence of Mg can be interpreted by assuming that PP forms a rather stable complex with the contractile protein. This interpretation is supported by similar observations with ATP which have been described previously (3). The experiment illustrated in Fig. 4 shows that a PP-protein complex is formed even in the absence of Mg. After the fibers were first made refractory to PP by the procedure described above, 2 mM PP had no effect on extensibility. PP then was washed out by 0.16 M KCl. When subsequently $MgCl_2$ (2 mM per liter) was added, the fibers were rapidly extended by the load, demonstrating that they still contained some PP. The longer the fibers were immersed in the PP-free solution, the smaller was the subsequent effect of Mg. At 0° PP disappeared from the fibers in 4 to 8 minutes, at 18° in 1 to 2 minutes, the duration increasing somewhat with increasing concentrations of PP. It is evident that the formation of a PP-protein complex by itself does not change the mechanical properties of the fibers. This effect depends equally on the presence of Mg.

The gradual disappearance of the effects of PP may be due to the slow dissociation of the assumed PP-protein complex. However, it is possible also that

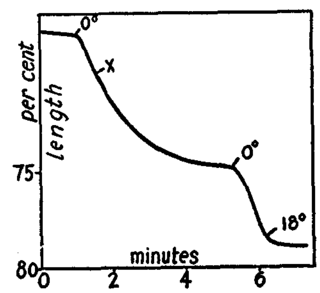

FIG. 5

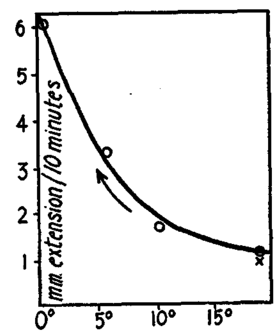

FIG. 6

FIG. 5. Effect of temperature on extension under a constant load. The chamber was filled with a solution containing 0.16 M KCl, 2 mM $MgCl_2$, and 1 mM PP per liter. It was rapidly cooled from 19° by immersion in water of 0°. At x the chamber was allowed to warm up in air. Later the chamber was rapidly cooled again and then warmed rapidly by flushing with warm solution (19°). Abscissa, per cent original length.

FIG. 6. Rate of extension as a function of temperature. The fibers were immersed in a solution containing 0.16 M KCl, 2 mM $MgCl_2$, and 0.6 mM PP per liter. The temperature was lowered in successive steps. x, rate of extension after temperature was raised to 19° at the end of experiment.

this complex is stable and that PP disappears because of its hydrolysis by PPase. Extracted muscle fibers have been found to hydrolyze PP slowly (9).

Effect of Temperature.—Rather unexpectedly it was found that lowering temperature increases the softening action of PP. In the experiment illustrated in Fig. 5, extension was speeded up 14 times when temperature was lowered from 18 to 0°. Extension became slower again when temperature was raised. Lowering temperature, therefore, markedly lowers viscous resistance and *vice versa* (Fig. 6). In the absence of PP, temperature has no noticeable effect on the rate of extension or slightly decreases it. The same is true for fibers deprived of Mg in the presence of PP. It is likely that the paradoxical effect of temperature in the presence of PP is due to a shift of a chemical equilibrium involving PP, Mg, and protein.

SUMMARY

1. If glycerol-extracted muscle fibers are alternately immersed in solutions of inorganic pyrophosphate (PP) or ethylenediamine tetraacetate (EDTA) and KCl two to three times, PP no longer increases extensibility. The original condition is restored by solutions containing Mg. EDTA prevents completely the softening action of PP, but has no effect in the presence of an excess of Mg. These observations are explained by assuming that PP and EDTA remove bound Mg. Evidently PP has no softening action without Mg.

2. If PP is washed out from muscle fibers by solutions containing Mg, the softening action of PP persists for many minutes, but washing out with KCl solution promptly abolishes the effect of PP. Also, if fibers which have been made refractory to PP are immersed into a solution of PP and then into a KCl solution free of PP for several minutes, the addition of $MgCl_2$ alone increases extensibility. It is concluded that PP forms a complex with protein. The formation of this complex, however, has no influence on the mechanical properties, unless Mg also is present.

3. In the presence of PP and Mg the viscous resistance of muscle fibers drops with diminishing temperatures.

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