THE INHIBITION OF THE ADENOSINE TRIPHOSPHATASE ACTIVITY OF ACTOMYOSIN BY MAGNESIUM IONS

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In 1939 Engelhardt and Ljubimova (2) suggested that myosin (the contractile substance of muscle) and adenosine triphosphatase are probably identical, and that the calcium ion is a specific activator of this enzyme.

Working with a myosin suspension made from rabbit muscle (by the method of Greenstein and Edsall (4) or of Weber and Meyer (11)) D. M. Needham (9) tested the activating powers of both magnesium and calcium ions and found that both activate the enzyme at pH 7.4 or pH 9.0 but that the calcium ion is much more effective than the magnesium ion.

Bailey (1), continuing and extending Needham's work, also found that the calcium ion activates adenosine *tri*phosphatase more than the magnesium ion does, but the magnesium ion activates adenosine *di*phosphatase more strongly than the calcium ion. Bailey further found that the enzymatic activity of myosin preparations is greater in a glycine buffer than in a bicarbonate-carbonate buffer. However, in either buffer the optimum activity is at pH 9.0.

The activation of adenosine triphosphate breakdown by magnesium decreases with the progressive purification of myosin. In other words, as the myosin becomes purer it loses its adenosine *di*phosphatase activity, and it is this latter activity which the magnesium ion affects most. Bailey finds that even in preparations freed from adenosine *di*phosphatase the magnesium ion still activates adenosine *tri*phosphate breakdown. However, Lohman (6) found that *only* adenosine *tri*phosphatase was activated by magnesium ions, while Ljubimova and Pevsner (5) reported that, working with myosin which had been freed from adenosine *di*phosphatase, magnesium ions actually *inhibit* adenosine *tri*phosphatase activity.

Banga and Szent-Györgyi (10) conducted their investigation on the influence of salts on the phosphatase activity with definite advantages over their predecessors. In the first place, pure myosin crystallized by Szent-Györgyi (10) was available to them; in the second place, Straub (10) had already discovered actin, which forms a complex with myosin and which was probably present as a contaminant in the myosin preparations of the previous investigators.

Banga and Szent-Györgyi found that the magnesium ion *inhibits* the adenosine triphosphatase activity of myosin but, except in very high concentrations (0.1 M), *activates* this activity of the actomyosin (a combination of actin and myosin). Still, in the presence of 0.01 M Ca ion, the Mg ion (0.001 M to 0.100 M) inhibits the action of both myosin and actomyosin. The action of Mg ions on actomyosin depends upon the concentration of K ions in the medium. Mg enhances the enzymatic activity in the presence of 0.01 M K^+ but inhibits it in the presence of 0.1 M K^+. Banga and

Szent-Györgyi explain this difference in response to Mg ions by the dissociation of actomyosin into actin and myosin in a high K ion concentration, the adenosine *tri*phosphatase activity of the freed myosin being inhibited by Mg ions.

Szent-Györgyi (10) in his comprehensive work, Studies on muscle, assumes that in the relaxing muscle, due to a high intermolecular concentration of K ions, the actomyosin undergoes dissociation and that the enzymatic activity of the free myosin becomes inhibited by Mg ions. However, in the contracting muscle, when the intermolecular concentration of K ions is decreased, the actomyosin is resynthesized and the inhibition by Mg ions is abolished. Unfortunately, Szent-Györgyi neglects his own observation *that in the presence of Ca ions the enzymatic activity is inhibited by Mg ions no matter whether the myosin is free or combined*,—a rather serious oversight!

It is also a matter of regret that Szent-Györgyi (10) gives no information as to the exact methods that he used in determining the influence of different ions on the phosphatase activity of myosin and actomyosin and does not state what type of buffer was used or what pH was maintained.

Since our experiments were performed the paper by Mommaerts and Seraidarian (7) appeared on the effects of Ca and Mg ions on the adenosine *tri*phosphatase activity of both myosin and actomyosin. The actomyosin was in solution containing 0.12 M KCl and 0.05 M NaCl and was buffered to pH 7.0 with a glycine buffer. By varying both the Ca and Mg ion concentration, they found an almost constant and maximum degree of inhibition when the molar Mg/Ca ratio exceeded 1, or even below this value. They report an inhibition of over 90 per cent even when the Mg/Ca is 0.2. Further increases in Mg ion concentration do not affect the results noticeably.

In view of these differences it seems worthwhile to report the results of our study on the effects of various concentrations of Mg ions on the breakdown of adenosine triphosphate by actomyosin in the presence of a constant concentration of K, Na, and Ca ions.

Experimental Procedure

An actomyosin sol (myosin B) was prepared from rabbit skeletal muscles according to the procedure of Banga and Szent-Györgyi (10).This sol contained 3.21 mg. per cent N and 0.240 mg. per cent P.

The Ba salt of adenosine *tri*phosphate was prepared by the method of D. M. Needham (9), and changed to the Na salt which was used in these experiments. The Na-adenosine *tri*phosphate contained 0.58 mg. per cent hydrolyzable P.

The glycine buffer of Sørenson was used which at 37°C. had a pH of 8.32.

Each experiment was set up as follows:

4.0 cc. glycine buffer

0.2 cc. actomyosin solution in 0.6 M KCl. This corresponds to 3.913 mg. actomyosin (0.642 mg. N and 0.048 mg. P). Experiments were made with variable amounts but 0.2 cc. was sufficient to yield 80 per cent decomposition in 60 minutes.

1.0 cc. KCl solution (27 mg. per cent K) giving a final concentration of 0.128 M K

1.0 cc. $CaCl_2$ solution (2.2 mg. per cent Ca) giving a final concentration of 0.002 M Ca.

1.0 cc. Na-ATP solution.
——— Variable amounts of $MgCl_2$ solution.
——— Redistilled water to make up volume to 10.0 cc.

The Ca and K concentrations in these mixtures were comparable to their concentration in rabbit muscle (8) and these were maintained constant in all experiments.

The tubes were incubated at 37°C. for 60 minutes, when the reaction was stopped by the addition of trichloracetic acid. The P was determined on the filtrate by the Fiske-SubbaRow (3) procedure using a photoelectric colorimeter.

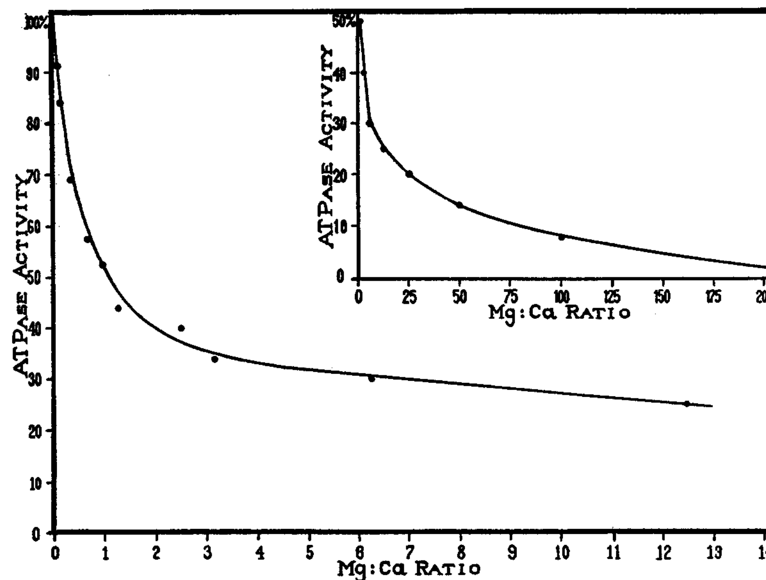

FIG. 1. Shows the relationship between adenosine triphosphatase activity and the Mg/Ca ratio.

RESULTS AND DISCUSSION

In Fig. 1 the percentages are plotted of P liberated from adenosine triphosphate by actomyosin in systems in which the only variable factor is the concentration of Mg ions. The P liberated in experiments without Mg is taken as 100 per cent. It may be noted in passing that in determining the total amount of P liberated proper corrections were made for the P set free in blank tests, but under exactly the same experimental conditions, with adenosine triphosphate without actomyosin or with actomyosin but without adenosine triphosphate ($0.030 + 0.025 = 0.055$ mg. P). A glance at the graph shows that the hydrolysis of adenosine triphosphate by actomyosin is inhibited over a very wide range of Mg ion concentrations. Even a trace of Mg (0.00018 M), which corresponds to an Mg/Ca ratio of 0.09, gives a measurable inhibition, while

as large a concentration as 0.4 M (Mg/Ca = 200) does not invariably cause complete inhibition. The curve, which drops very rapidly at first, approaches the abscissa asymptotically. By the time the Mg/Ca ratio has changed from 0 to 10 the adenosine triphosphatase activity is depressed about 75 per cent but further changes in the ratio from 10 to 200 occasion only a very slow depression approaching completeness.

Bearing in mind that the Mg concentration in normal rabbit muscles (8) is 0.012 M and the Mg/Ca ratio is approximately 6, we can see from the graph that the enzymatic activity of the actomyosin will be about 70 per cent inhibited. These results are in marked contrast to those of Mommaerts and Seraidarian (7) who find that the inhibition is complete long before this ratio is reached. Furthermore, according to our results, even comparatively large changes in this ratio (Mg/Ca = 6) will not very materially affect the enzymatic activity since this will vary within less than 10 per cent while the ratio may be either decreased to one-half or doubled. Contrary to the deduction which Mommaerts and Seraidarian make from their studies, namely that since the adenosine triphosphatase must be completely inhibited under the conditions existing in muscle it can have no relation to the contraction of actomyosin, our results suggest that the enzymatic activity in the normal muscle, though not optimal, is at least fairly independent of sharp alterations due to shifts in the ionic pattern. Our results also seem to indicate that the interplay of Mg and Ca ions in muscle is such as to secure greater stability of enzyme activity though not maximal activity. From the point of view of sustained muscle activity this is certainly a more advantageous arrangement.

As a corollary, it does not seem probable that adenosine triphosphatase is either absolutely inhibited or released from this inhibition through changes in Mg concentration, since any changes in intermolecular water will affect simultaneously both the Mg and Ca, and we have seen that the degree of inhibition is a function of the Mg/Ca ratio. We can, therefore, conclude that it is highly dubious whether the Mg ion could be endowed with the key function of releasing energy from adenosine triphosphate during contraction following stimulation or with conserving energy during rest.

Mommaerts and Seraidarian (7) have noted that the adenosinetriphosphatase activity of actomyosin preparations is insufficient to account for more than a very small portion of P liberated during muscle contraction. We not only corroborate this observation but we have some further evidence to support this view. Mommaerts and Seraidarian based their deduction on calculations of the amount of P set free in contractions as reported in the literature. We have unpublished data obtained in our laboratory on the P liberated from adenosine triphosphate by rabbit muscle homogenates which make it obvious that the amount P/mg. myosin/minute set free in these experiments is about a hundredfold that found in experiments with a pure actomyosin preparation.

BIBLIOGRAPHY

1. Bailey, K., Myosin and adenosine-triphosphatase, *Biochem. J.*, 1942, **36,** 121.
2. Engelhardt, V. A., and Ljubimova, M. N., Myosin and adenosine-triphosphatase *Nature*, 1939, **144,** 668.
3. Fiske, C. H., and SubbaRow, Y., The colorimetric determination of phosphorus, *J. Biol. Chem.*, 1925, **66,** 375.
4. Greenstein, J. P., and Edsall, J. T., The effect of denaturing agents on myosin, *J. Biol. Chem.*, 1940, **133,** 397.
5. Ljubimova, M. N., and Pevsner, D., Action of myosin preparations on adenosine-triphosphoric acid; preparation of adenosine-diphosphoric acid, *Biokhimia*, 1941, **6,** 178.
6. Lohman, K., Ueber die Aufspaltung der Adenylpyrophosphorsäure und Arginin-phosphorsäure in Krebsmuskulatur, *Biochem. Z.*, 1935, **282,** 109.
7. Mommaerts, W. F. H. M., and Seraidarian, K., A study of the adenosine triphosphatase activity of myosin and actomyosin, *J. Gen. Physiol.*, 1947, **30,** 401.
8. Morgulis, S., and Osheroff, W., Mineral composition of the muscles of rabbits on a diet producing muscle dystrophy, *J. Biol. Chem.*, 1938, **124,** 767.
9. Needham, D. M., Myosin and adenosine-triphosphatase, *Biochem. J.*, 1942, **36,** 113.
10. Szent-Györgyi, A., Studies on muscle, *Acta physiol. Scand.*, 1945, suppl. 25.
11. Weber, H. H., and Meyer, K., Das Kolloidale Verhalten der Muskeleiweisskörper *Biochem. Z.*, 1933, **266,** 151.