THE ACTION OF A HIGH PRESSURE ON THE ADENOSINETRIPHATASE ACTIVITY OF MYOSIN

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The possibility of reciprocal interaction between myosin and adenosinetriphosphate (ATP) was first demonstrated by V. A. Éngel'gardt and M. N. Lyubimova [9, 10]. This discovery led to the consideration of the problem of the mechanism of utilization of the chermical energy of metabolic processes in muscular action, and to the laying of the foundations of the study of the mechanochemistry of muscle.

Also of great importance were the investigations later published by Szent-Györgyi and the Hungarian school of biochemists [17, 19], which considerably extended our knowledge of the contractile proteins of muscle and the mechanism of their interaction with ATP.

The work of Weber and of Hoffmann-Berling [12, 13a, 20], and later, that of V. Ya. Aleksandrov and N. I. Arronet [1] established the universality of this mechanism, which now permits us to speak of the possibility of the reciprocal interaction between ATP (or the purine and pyrimidine analogs of ATP) and not only the muscle myosim but also the contractile proteins of the various other organella of cell movement (the tail of the spermatozoon, the undulating membrane of the trypanosome, the apparatus responsible for the separation of chromosomes in the process of cell division, and so on).

It must be pointed out, however, that a number of facts suggesting the universal role of ATP in the mechanism of a variety of forms of cell movement were discovered and published somewhat earlier by other authors [3, 4, 11, 13].

These facts undoubtedly suggest that one of the most important properties of myosin as a functional entity is its enzymic (adenosinetriphosphatase) activity.

Attempts by several authors [7, 16] to prove the possibility of separation of the isolated adenosinetriphosphatase from the myosin did not give sufficiently convincing results, and were subjected to sharp criticism [6].

Work by Mihalyi and Szent-Györgyi [15] showed that myosin could be broken down by trypsin into two components – H- and L-meromyosins – of which only one (H-meromyosin), which possesses adenosinetriphosphatase activity, retains the most typical properties of myosin, and in particular the ability to combine with actin.

In this paper the authors describe the conditions in which it is possible to obtain myosin, completely deprived of adenosinetriphosphate activity, but retaining its power of being dissolved in 0.6M KCl and being precipitated during subsequent dialysis or by high dilution of the myosin solution with water.

EXPERIMENTAL METHOD AND RESULTS

Some years ago one of us [5] showed that under the influence of high pressure, of the order of 4000 atmos, myosin loses its adenosinetriphosphatase activity and, as it was assumed, is denatured. We have recently found

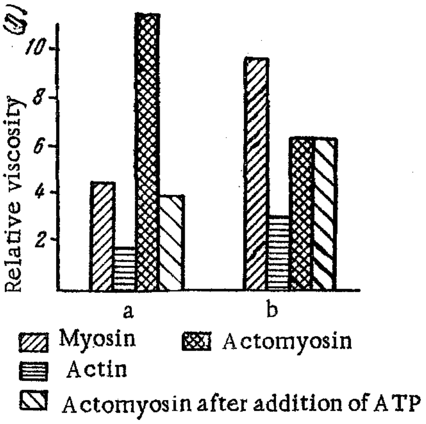

Fig. 1. Changes in the relative visosity (η) of myosin solutions (a) before and (b) after the action of a high pressure (4000 atmos) after addition of actin and ATP (the results shown are from one typical experiment).

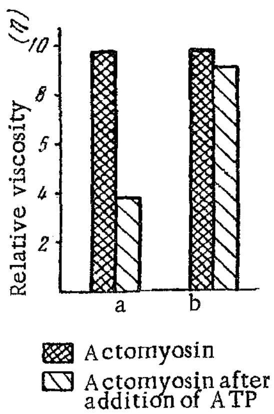

Fig. 2. Changes in the relative viscosity (η) of actomyosin solutions (a) before and (b) after the action of a high pressure (4000 atmos) after addition of ATP.

that solutions of reprecipitated myosin, including the crystalline preparation obtained by Szent-Györgyi's method, do in fact lose completely their adenosinetriphosphatase activity under the influence of high pressure, even though these conditions practically the whole of the protein remains in solution.

If, for instance, the original activity of the myosin preparations in our conditions was expressed by the symbol Q_p of the order of 960-1200*, then after the action of high pressure Q_p fell to zero. The visosity of myosin, free from any admixture of actomyosin, showed a perceptible increase under the influence of high pressure (Fig. 1).

This was in agreement with the observations of B. F. Poglazov et al. [7, 8], who studied the changes in the SH-groups and viscosity of myosin during heating. Myosin which has been exposed to the action of a high pressure can be precipitated by dialysis and redissolved in 0.6 M KCl.

These results suggested that under the influence of a high pressure certain changes took place in the structure of the protein, associated with the inactivation of groups (possibly SH) giving myosin its enzymic character, but not accompanied solution in saline media with an ionic strength of 0.35-0.4. The enzymically inactive myosin thus obtained had lost its ability to interact with actin, with the formation of actomyosin (see Fig. 1).

Actomyosin gels, when subjected to the action of a high pressure, also lose their property of having their viscosity lowered after addition of ATP (Fig. 2). So far as the changes in viscosity of actomyosin itself after the action of a high pressure are concerned, this was lowered insignificantly. This was not, however, due to the absence of dissociation of the actomyosin complex into its components – actin and myosin – but to the production, as a result of the action of the high pressure, of a much more viscous (and possibly, partially denatured) myosin. This latter might also have masked the fall in viscosity of the actomyosin complex as a result of its dissociation.

For the same reason the viscosity of myosin inadequately purified from contamination with actomyosin, was increased after the action of a high pressure not to such a high degree as that of preparations of "crystalline" myosin obtained by the Szent-Györgyi method.

Thus the loss by myosin of its adenosinetriphosphatase activity was possibly associated with its partial denaturation and deprived this protein of its most characteristic and unique properties.

SUMMARY

Solutions of reprecipitated myosin lose completely their adenosinetriphosphates activity under the effect of high pressure (4000 atmospheres). However, they retain their ability to readily dissolve in 0.6 M KCl and to become precipitated in subsequent dialysis or after considerable dilution of myosin salt solution with water. The viscosity of myosin, free of actomyosin admixture, rises under the effect of high pressure. Myosin subjected to

*In order to determine the adenosinetriphosphatase activity of myosin we used a preparation of ATP with a high content, which, according to A. A. Baev's findings [2], leads to a significant fall in Q_p when the enzymic activity of the myosin is determined.

the effect of high pressure loses its ability to combine with an activated actin with the formation of actomyosin. The viscosity of actomyosin gels subject to high pressure (4000 atmospheres) does not diminish after addition of adenosinetriphosphates. Under the effect of high pressure there must occur some kinds of changes in the myosin structure, these being associated with inactivation of certain groups (possibly SH) and imparting to myosin an enzymatic character without, however, making it lose its hydrophilic properties.

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