A MYOSIN-LIKE PROTEIN IN THE BRAIN TISSUE

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Beginning with the studies of V. A. Engel'gardt and M. N. Lyubimova [4], numerous authors have established and studied in detail the role played by myosin in the motor function of the muscles. Later it appeared that the interaction between a myosin-like protein and adenosintriphosphoric acid (ATP) is responsible for a great variety of motor manifestations in all living organisms. It could be shown that similar systems play a part in the tail movements of spermatozoids [2, 5, 6], the flagella movements of trypanosomes [13], movements of the cilia on mucosa surfaces [27] and of various protoplasma membranes in amebas [10], of fibroblasts [17], of plasmodia of myxomyctes [19], in the tail contractions of phage particles [15], in the movements of plants capable of moving [3], etc. It is assumed that the achromatin part of the mitotic apparatus which secures the progression of the chromosomes towards the poluses consists of an actomyosin-like protein [12]. Quite recently Nakayama [21] isolated an actomyosin-like protein from the kidneys of dogs. This author believes that "renosin" plays a part in the osmotic function of the kidney cells.

As early as 1952 Lettré [16] voiced the assumption that an actomyosin-like protein is present in the external cytoplasma layer of all cells, maintaining the form and tonus of the cells, and carrying out all intracellular movements. This view is consistent with the opinion of Mazia [20] and Went [27] who believe that the protein of the mitotic apparatus is present in the cells even before the formation of the spindles.

In view of the data quoted above we decided to investigate a tissue which has no motor function and in which no mitoses occur: the nervous tissue. We wanted to establish whether a myosin-like protein is indeed present in all cells. The presence of ATP-ase activity in homogenates of brain tissue was first mentioned in the studies of Feldberg and Mann [9]. In subsequent publications by a number of authors [7, 8, 11, 18, 23] the properties of this enzyme were studied in greater detail.

METHODS

A modification of the method recommended for the isolation of myosin from striated muscles was used to isolate ATP-ase from the brain of bulls: the brain was homogenized in a mincing machine and extracted for 20 min in 3 volumes of a cooled solution containing 0.5 M KCl and 0.03M $NaHCO_3$. The mixture was centrifuged for 10 min at an acceleration of 2000 G. The centrifugate was diluted 10 times with distilled water and rendered acid with acetic acid, adjusting the pH to 5.7-6.0. The precipitate which formed was centrifuged for 10 minutes at 2000 G. The centrifugate was dissolved by addition of 0.02 M K_2CO_3 until a pH of 8.3 was reached and for each g protein 200 ml 0.05 M KCl was added. The solution was now centrifuged for 10 min at 6000 G. The centrifugate was cooled, diluted in 2 volumes of bidistilled water and rendered acid with acetic acid, adjusting the pH to 5.7. The precipitate which formed was separated by centrifugation and dissolved in 0.5 M KCl solution. 1 kg tissue yielded on the average 2 g of the preparation. Actin was prepared from rabbit muscles by the method of Straub [24]. To estimate the ATP-ase activity, a mixture containing 0.3 ml borate buffer (pH 7.6), + 0.3 ml enzyme (0.5 mg N/ml) + 0.3 ml 0.008 M ATP + 0.1 ml 0.05 M $CaCl_2$ or $MgCl_2$ was incubated at 37° for 15 min. The relative viscosity of the protein solutions was estimated in an Ostwald viscosimeter at a rate of water outflow equal to 40 sec.

RESULTS

It could be established that the protein prepared by the above method possesses ATP-ase activity. In accord with the reports by other authors [7, 18] it appeared that Ca^{++} and Mg^{++} ions activate this ATP-ase; Mg^{++} ions to a

greater degree. The highest degree of activation could be observed at concentrations of $2.5 \cdot 10^{-3}$ M $CaCl_2$ and $MgCl_2$ respectively. Addition of versene (ethylenediaminotetraacetate) which binds the bivalent ions caused complete inhibition of the enzyme. The relative activity of the preparation isolated by us exceeded to a considerable degree the activity of the original tissue homogenate (in the case of ATP-ase activated with Ca-ions the activity of the preparation was 9 times higher, and in the case of ATP-ase activated with Mg-ions 6 times higher).

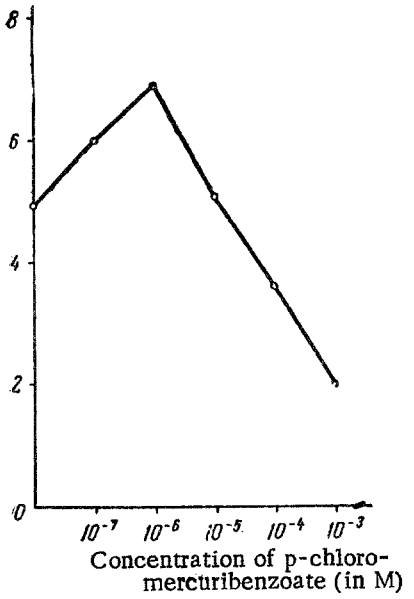

Fig. 1. The influence of p-chloro-mercuribenzoate upon the ATP-ase of the brain, activated by Ca.

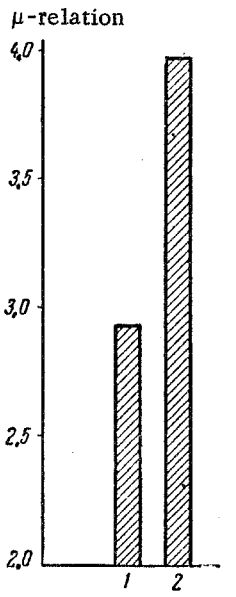

Fig. 2. The influence of F-actin upon the viscosity of brain ATP-ase. 1) 3 ml ATP-ase solution; 2) 3 ml ATP-ase solution + 1 ml F-actin.

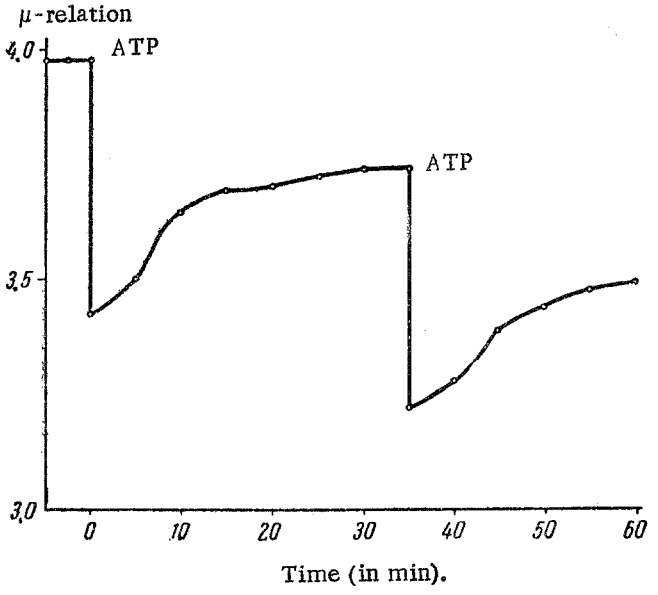

Fig. 3. The influence of ATP upon the viscosity of a mixture of a mixture of ATP-ase + F-actin. Final ATP-concentration: $2.4 \cdot 10^{-3}$ M.

The optimal pH range for ATP-ase activated by Ca-ions is in the region of 8.0 and for ATP-ase activated by Mg-ions in the region of 7.6. As far as myosin isolated from muscles is concerned, it is known that Ca activates and Mg inhibits its enzymatic activity. The interaction of myosin with actin leads to changes in its relation to the ions mentioned above. According to Szent-Gyorgyi [25] the ATP-ase activity of actomyosin is more intensively activated by Mg-ions than by Ca-ions. The optimal pH range for myosin ATP-ase lies in the region of 9.0 and for actomyosin near the isoelectric point. Comparison of the properties of cerebral and muscular ATP-ase revealed certain similarities: enzymes activated by Ca (in the case of the muscles this applies to myosin) have a more alkaline pH optimum, and the cerebral ATP-ase, activated by Mg has, similar to actomyosin, (which latter is also activated by Mg) a maximum activity at lower pH values.

Myosin is a thiol enzyme. It has been emphasized in a number of reports [14, 22] that SH-reagents, when used in relatively high concentrations, suppress the ATP-ase activity of myosin, but activate this enzyme by 30-80%, when used in low concentrations. In our experiments we investigated the effect of one of the most specific SH-reagents: p-chloromercuribenzoate upon the activity of brain ATP-ase. Fig. 1 shows that addition of p-chloromercuribenzoate in low concentrations in the order of 10^{-6} M activates the enzyme, in the case in question by 41%. Higher concentrations, however, cause inactivation of ATP-ase – a picture similar to that described in the case of myosin.

To investigate the substrate-specificity of brain ATP-ase we used, in addition to ATP, ITP, GTP, ADP, AMP and β-glycerophosphate. It appeared that the highest activity could be observed when ATP was used as substrate; the activity was lower when ITP, GTP and ADP were used and was completely absent in samples with AMP and β-glycerophosphate. Consequently the enzyme exerts an apyrase effect and displays a certain specificity with regard to the adenil system.

One of the most characteristic features of myosin is its capacity to interact with actin, a process which results in the formation of actomyosin. The viscosity of actomyosin is higher than the sum of the viscosities of its components. Addition of ATP to actomyosin causes its breakdown into actin and myosin, a process which is accompanied by a fall in the viscosity. This breakdown is reversible and in the result of the subsequent gradual breakdown of ATP by myosin ATP-ase the complex is restored. In our experiments addition of ATP to brain ATP-ase solution had no influence upon its viscosity. Addition of actin, obtained from muscles (rabbit), however, caused an additional increase in the viscosity (Fig. 2) thus indicating an interaction. Addition of ATP in a concentration of $2.4 \cdot 10^{-3}$ M to the complex which formed, led to a marked fall in the viscosity, followed by its gradual restoration in step with the breakdown of ATP (Fig. 3). The degree of the fall in the viscosity corresponded to the additional increase observed after mixture of the ATP-ase and the actin. Attempts to isolate an actin-like protein from the brain were unsuccessful.

The findings quoted above show that brain tissue contains a myosin-like protein and confirm the assumption that such protein is present in all cells, apparently to secure various protoplasmatic movements and to regulate the osmotic phenomena.

Myosin, as is well known, is capable of forming contractile complexes not only with actin, but also with RNA [1]. It is thus conceivable that – as we were unable to isolate actin from the brain, (just as actin can not be isolated from spermatozoids) – this substance is replaced by some other component, e.g. RNA.

SUMMARY

Myosin-like protein was isolated from the brain tissue. It developed an adenosintriphosphatasic activity and was capable of interacting with actin. The enzyme manifested a sufficiently pronounced substrate specificity, and stood in direct relationship with the pH, Ca^{++} and Mg^{++} ions and SH-reagented.

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