The contraction of myosin threads.

by

A. Szent-Györgyi.

It has been shown be H. H. WEBER, (1) that a myosin solution, if squirted in a thin jet into water, solidifies in the form of a thread. In this way the myosin can be brought into a form which resembles the muscle fibril in some respects. The myosin thread is an elongated piece of myosin gel.

It has been shown in the preceding paper by BANGA and myself that myosin can be obtained from muscle in two different forms which were called myosin A and B. Threads can be prepared from both. For the sake of convenience I will call the threads prepared from the 20 min. extract (see BANGA and Sz.) „myosin A threads" while the threads prepared from the 24 h. extract will be called „myosin B threads".

The threads used by previous investigators correspond, in all probability, to our myosin A threads.

The technique of the preparation and observation of threads will be described by M. GERENDÁS.

A watery extract of muscle was made in the following way) the rabbits muscle was cut out and minced (as described in the previous paper), suspended in water (1 ml per g of muscle), stirred for 5 minutes at 0°C and squeezed through a cloth. The fluid was then filtered through paper at 0°C.

If a myosin B thread is suspended in this extract and observed under the microscope, a violent contraction will be seen. The thread contracts within 30 seconds to less than half of its length and within 2—3 minutes it reaches a maximum contraction of 66%, $^1/_3$ of its original length. (Fig. 1.) At the same time the thread becomes proportionately thinner, and is seen to become quite dark.[1] Watched in lateral illumina-

[1] Frequently the contraction is so violent that the interior of the thread cannot keep pace with the contraction of the outer sheets, so that these latter break up, like a crocodile's skin. Fig. 2. For the same reason

tion the transparent thread is seen to turn white. opaque. The rate of contraction depends on the diameter of the thread. Fig. 3. curve a gives the time-curve of the contraction of a relatively thick thread of 0,3 mm diameter. Thinner threads, like those of 0,1 diameter, contract faster but their rate of contraction cannot be measured by the same method because it is too fast. Furthermore thin threads mostly curl up and stretch out again only after they have reached maximum contraction.

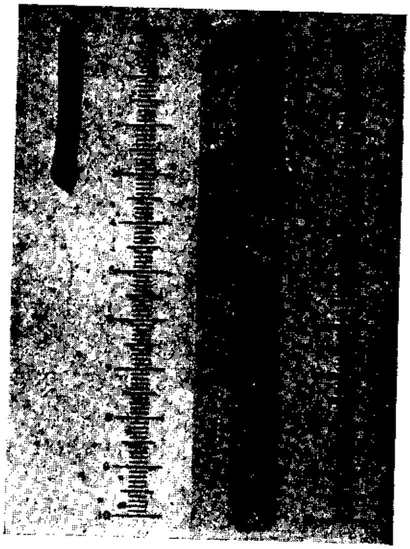

Fig. 1.

Fig. 2.

If a myosin A thread is suspended in the same fluid no striking change will be observed. (Fig. 4.) Measurement by the ocular micrometer will reveal a weak and slow contraction. (Fig. 3 curve b). If the myosin solution is filtered twice through a Seitz K filter before the thread has beene pulled, the contraction becomes still weaker (Fig. 3 curve c). As shown in the previous paper, the Seitz filter retains the myosin B present in our myosin A preparations as an impurity.

The frest, watery extract of thee muscle contains thus

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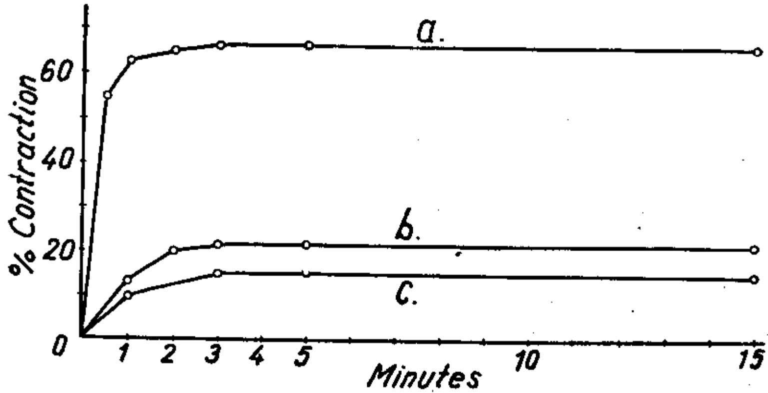
the whole thread sometimes breaks up into dark, solid lumps of myosin instead of giving a contraction.

something which causes a violent contraction in myosin B threads but has little influence on myosin A. The active agent seems to be present in excess for the extract can be diluted to 1:4 with water and will still give the same contraction with thinner threads.

If the muscle suspension is stored over night at 0°C and filtered only the next day, the extract obtained will found to be entirely inactive. The myosin B thread, suspended in this extract, will show no change at all.

Rabbits muscle contains on the average 3,5 mg adenyl-



Fig, 3.

triphosphate („ATP") per g., thus the fresh extract contains ATP in about ½ of this concentration. This ATP is split during storage by the phosphatase present. If the original ATP concentration is restored to the inactivated extract, again the same violent contraction will be obtained as in the fresh extract. Even half of this ATP concentration (0,9 mg per ml) is sufficient to give a maximum effect. This shows that ATP is involved in the observed contraction.

If the same quantity of ATP (0,09%) is dissolved in water and the myosin B thread suspended herein, no contraction will occur and the thread remains entirely unchanged. If we dissolve our ATP in the boiled extract instead of water we obtain a violent contraction again. Even incinerated juice will produce contraction with ATP. This makes it evident that, apart from ATP, inorganic constituents of the extract are also involved in the reaction.

If we use a 0,1 mol KCl solution as solvent for our ATP instead of water the contraction will be much slower. (see Fig. 5 curve b.)

Muscle contains 0,01 mol Mg. If we add 0,01—0,001 mol $MgCl_2$ to our 0,1 mol KCl and dissolve the ATP in this, the myosin thread suspended in this fluid will give the same violent contraction as in the fresh, watery extract (Fig. 5 curve a). It is evident thus that three factors were involved

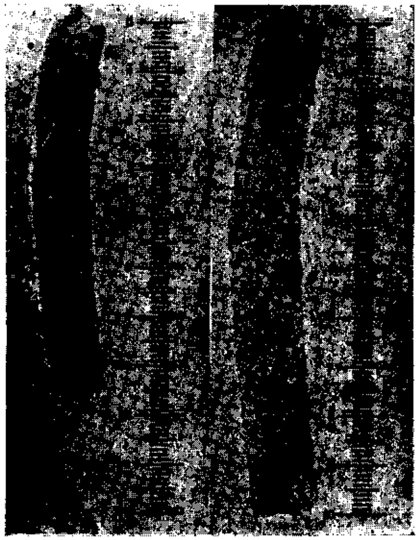


Fig. 4.

in the production of the contraction of our myosin B thread: ATP, K and Mg.

The myosin A thread will give the same weak and sluggish contraction (Fig. 5 curve c) with ATP in pure KCl or KCl plus $MgCl_2$. If the myosin solution is filtered twice through a Seitz K filter, the thread prepared from this solution will give a somewhat weaker contraction in KCl (Fig. 5. curve d). If, in addition to the 0,1 mol. KCl, 0,001 Mg is also present there will be practically no contraction at all (Fig. curve e). The contraction of myosin A is not only not enhanced but is almost completely inhibited by $MgCl_2$. If the thread, prepared from unfiltered myosin, gave the same contraction in KCl and $MgCl_2$ (curve c) this was due to the myosin B present as an impurity: the contraction of this myosin B was enhanced and thus compensated the inhibition caused by Mg in the contraction of myosin A.

Myosin B gives thus a strong contraction with 0,1 mol. KCl and ATP and the contraction is greathy enhanced by Mg. Myosin A gives a weak and sluggish contraction with KCl and ATP and the contraction is suppressed by Mg.

If the concentration of KCl is increased, at 0,2 mol. the same reactions are still obtained. But if the concentration is raised to 0,04 mol., the thread, instead of giving a contraction,

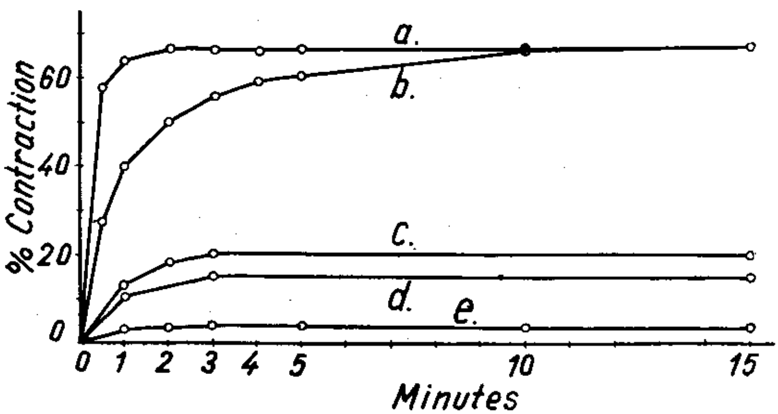


Fig. 5.

will dissolve. The action of our ATP—KCl—$MgCl_2$ mixture will depend on the concentration of the KCl present and at higher concentrations the action will be reverted; instead of contraction we will obtain dissolution and instead of aggregation, disaggregation. KCl without ATP will not dissolve the myosin B thread not even in a molar concentration.

Threads prepared from the precipitated, washed and redissolved myosin of these extracts gave identical results.

Adenylic acid, if employed instead of its pyrophosphate ester, ATP, was found to be entirely inactive. It does not give contraction or dissolution.

Neither the effect of KCl, nor that of $MgCl_2$ is specific and can be reproduced by other ions.

In fig. 6 the effect of KCl is compared with the effect of other halogen salts of K. The abscissa gives the log of the molar concentration of the salt, the ordinate the % of shortening. By bringing the curve under the abscissa I wanted to express dissolution. The broken line means that the dissolution already takes place wihout the addition of ATP. The threads were prepared from precipitated and washed myosin B and were placed for 5 min. into the salt solution before the addition of ATP (0,018%). No value should be attached to the relative hight of the curves, which, in this respect, are not strictly comparable because the results were obtained with different myosin preparations. Readings ware made 5 min. after the addition of ATP.

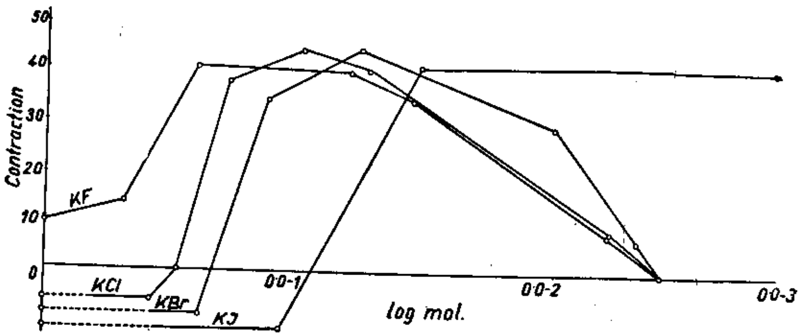


Fig. 6.

It will be seen that the effect of KCl is not specific and that the effect of a salt is not dependent on the kation only but depends on the anion too. With the increasing weight of the anion the curves are shifted more and more to the right. KJ has a very strong effect at relatively high dilutions.

In fig. 7. the anion (Cl) is kept constant and the kation varied. The differences are not very marked but there is a tendency to the opposite effect, a shift to the right with the decreasing weight of the kation. With Li the effect is distinct. In this curve NH_4Cl (neutralised with NH_4OH) and potassium phosphate (pH 7) are also given. This latter is effective at very high dilutions.

The enhancing effect of Mg can be reproduced by Co and Mn as will be shown by M. GERENDÁS. A detailed study on the effect of varied concentrations and pH will be given by T. ERDŐS.

Reversibility. The question presents itself whether the contraction observed is reversible or whether it is connected with an irrevesible change of the myosin.

If the contracted myosin B thread is transferred into pure water it will remeain contracted. This naturally does not mean that the change is irreversible, for the contracted muscle has no reason to relax. Conditions in the muscle, where every myosin micell is fixed within a certain pattern, are different.

If the contracted thread is transferred into E d s a l l 's salt solution, it swells up again. When the thread has reached its

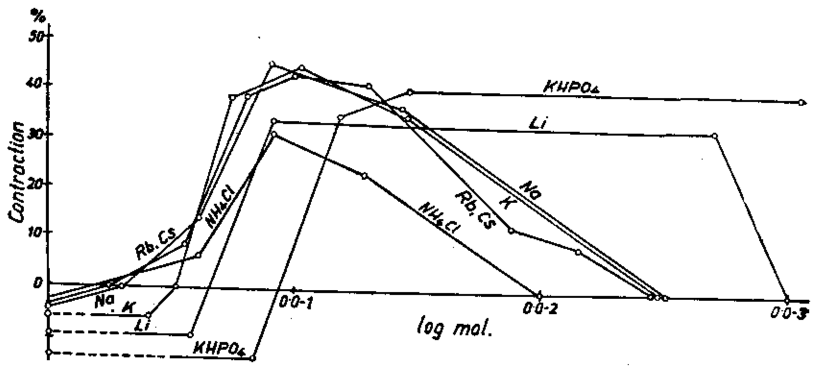


Fig. 7.

original dimensions the swelling can be stopped by transferring it into 0,1 mol. KCl. Such a thread has a perfectly normal appearance and does not contract spontaneously. If ATP is added it contracts again in the same way, as it did the first time. This shows that the contraction is reversible.

E x p e r i m e n t s w i t h m y o s i n *in stitu.* We may ask whether the contraction obtained with myosin threads can have any bearing on muscular contraction at all and whether in muscle myosin may behave also as myosin B. It has been shown that myosin gives a contraction even after it has been dissolved and precipitated, once the necessary ions and ATP are present. The contractility of muscle is a very sensitive process and seems to be thus the expression of a subtle organisation. Naturally it is just as possible that a higher or- organisation is not needed for the contraction itself, but for those changes that elicit this contraction or bring the muscle back to rest again.

To obtain some information on this question, I tried to destroy the finer structure of the muscle as far as possible without destroying the myosin. It is known that the excitability of the muscle is lost in distilled water and also by feezing. Neither of these destroy myosin.

The broad neck muscle of the rabbit was cut into 2 mm. wide strips parallel to the muscle fibres. The strips were placed into distiled water. After one to several hours the strips, if contracted, were stretched to their original length, frozen in solid CO_2 and cut into slices on the freezing microtome. The slices were made parallel to the muscle fibres and were about one fibre thick. Thus they contained one sheet of muscle fibres running through the whole length of the preparation. The slices were put into distilled water and transferred after one to several hours into 0,1 mol. KCl, then placed on a slide under the microscope. After their length had been measured a drop of 0,14% ATP vas dropped on them. Immediately a strong contraction began, which reached a maximum within 15—120 seconds and shortened the fibres by 50—60%. The myosin behaved thus as myosin B.

Experiments with myosin suspensions. The last question I want to touch in this paper, is, whether myosin suspensions give changes which are analogous to the contraction of threads.

The muscle extract containing myosin was neutralised and diluted till the KCl concentration went down to 0,1 mol. The myosin precipitate was centrifuged, washed and reddissolved in Edsall's fluid, precipitated and washed thoroughly again.

The myosin obtained in this way is a fairly stable suspension which settles slowly. Salts is smaller concentration cause precipitation and the suspension will settle somewhat faster. Salt in higher contcentration will tend to dissolve the mysin. There is great difference in the behaviour of myosin A and B. The former is much less turbid and has a greater tendency for dissolution.

If, in addition to 0,1 KCl, a small quantity, say 14 mg % of ATP is also added to the myosin B suspension, the precipitation, will be greatly intensified. The precipitate immediately becomes roughly granular and settles quickly leaving a clear fluid behind. The effect is very striking. We may call it a „superprecipitation", contrary to the precipitation caused by KCl alone. Mg still enhances the reaction.

4 mol. KCl has no appreciable dissolving action on the myosin B suspension. If ATP is added in addition to this KCl., the myosin dissolves. We can thus say that ATP greatly enhances the effect of salts, bringing about dissolution at concentrations at wich the salt by itself is inactive and it also greatly intensifies precipitation.

The phenomena seen in the myosin B suspension are analogous to the phenomena observed on myosin B threads. ATP and higher KCl concentrations dissolve both. ATP and lower salt concentrations, which produce a contraction in the thread, cause a superprecipitation in the suspension.

The analogy is lacking in one point. While smaller salt-concentrations cause by themselves a precipitation in the suspension, salts without ATP never give a contraction in threads. There seems to be a qualitative difference between the precipitating action of salts alone and the precipitation observed in the presence of ATP. Salts alone seem only to cause an aggregation of the myosin micells, while in the presence of ATP they seem to cause some deeper change within the single units, which change expresses itself in the superprecipitation of suspensions and the contraction of threads. GERENDÁS has found that while salts by themselves have no influence on the double refraction of oriented threads, salts + ATP cause, besides contraction, a complete disappearance of double refraction. Double refraction disappears in musculur contraction also.

Myosin A suspensions behave in an analogous way to myosin A threads. They dissolve without ATP at lower salt concentrations (0,4 mol. KCl) and ATP has only a very slight precipitating action which is not enhanced by Mg.

Summary.

It is shown that a myosin B thread, if suspended in a fresh, watery extract of muscle, gives a violent contraction. Myosin A is relatively inactive.

It is shown that three factors are involved in the contraction of myosin: ATP, K and Mg.

At higher salt concentrations, in the presence of ATP, dissolution is obtained.

The action of ions is not specific.

Under the same conditions which cause the myosin thread to contract, the myosin suspensions give a precipitate.

Literature.

1. *H. H. Weber:* Arch. ges. Physiol. (Pflüger) 235, 205, 1934.