# The influence of K and Mg on the contraction of myosin.

by

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It has been shown by Szent-Györgyi that myosin B threads are capable of giving a contraction in the presence of adenyltriphosphate („ATP") and KCl. The rate of contraction was greatly increased by $MgCl_2$. At higher salt concentrations and in the presence of ATP, dissolution was obtained instead of contraction. Myosin A was found to be relatively inactive.

The object of this paper is to obtain more detailed information about the influence of salt concentration. For the sake of convenience I also will call the threads prepared from the 20 min. extract (see Banga and Sz.) myosin A threads and those prepared from the 24 h. extract, myosin B threads, although the 20 min. extract always contains small quantities of myosin B and the 24 h. extract may contain some myosin A.

For the preparation and measurement of threads I used the technique of M. Gerendás.

## Effect of varied KCl concentrations.

Table 1 gives the result of an experiment with varied KCl concentrations. The experiment was made on two different threads which I will call *a* and *b*. *a* was prepared from muscle extract which had been stored for two days, *b* from an extract which had been stored for ten days at O°C. It will be seen that storage makes no difference.

The freshly prepared threads were allowed to stand for 30 min. at room temperature and were then transeferred into the corresponding salt solution and measured. After 5 min. ATP (0,09%) was added and readings made 5 min. after that.

**Table I.**

| Mol KCl | Myosin B | | | | Myosin A | | | |
|---|---|---|---|---|---|---|---|---|
| | I. | | II. + 0,001 mol $MgCl_2$ | | III. | | IV. ATP + KCl simult | |
| | a | b | a | b | a | b | a | b |
| 0.10 | 66 | 66 | 66 | 66 | 23 | 20 | 14 | 23 |
| 0.17 | 70 | 58 | 50 | 66 | 19 | 9 | 17 | 16 |
| 0.18 | 59 | 62 | 64* | 56* | 16 | 14 | 13 | 15 |
| 0.19 | 69 | 66 | 59* | 66* | — | — | — | — |
| 0.20 | 60 | 59 | ? | ? | — | — | — | — |
| 0.21 | 45 | 66 | — | — | — | — | — | — |
| 0.22 | 66 | 60 | — | — | — | — | — | — |
| 0.23 | 58 | 62 | — | — | — | — | — | — |
| 0.24 | 67 | 62 | x | — | — | — | — | — |
| 0.25 | 66 | 66 | x | x | — | — | — | — |
| 0.26 | 61 | 63 | x | x | — | — | — | — |
| 0.27 | 69 | 57 | x | x | — | — | — | — |
| 0.28 | 58 | 61 | x | x | — | — | — | — |
| 0.29 | 66* | 70* | x | x | — | — | — | — |
| 0.30 | 52* | 59* | x | x | — | — | — | — |
| 0.31 | 30* | 30* | x | x | — | — | — | — |
| 0.32 | ? | ? | x | x | x ! | x ! | — | — |
| 0.33 | ? | x | x | x | x ! | x ! | — | — |
| 0.34 | x | x | x | x | x ! | x ! | — | — |
| 0.40 | x | x | x | x | x ! | x ! | — | — |
| 0.41 | x | x | x | x | x ! | x ! | x ! | x ! |

The whole experiment was repeated also in the following way: the thread was put directly into the salt-ATP mixture instead of putting it into the salt solution first and adding ATP afterwards. This made no appreciable difference with myosin B but changed the behaviour of the myosin A threads considerably. For this reason I am giving the effect of ATP and varied KCl concentrations on myosin A with the simultaneous addition of both substances.

The result of this experiment agrees with the result of other similar experiments except for a small variation of the KCl concentration at which the changes from inactivity to contraction or to dissolution occure. The experiment was also repeated with purified myosin which gave the same results except for the slighlty greater solubility of myosin A which dissolved in KCl at 0,28 mol. instead of 0,32. The experiment was also repeated with threads which had been strored for 12 hours at room temperature (25° C). This made no difference to myosin B. Myosin A threads lost their contractility.

Columm I and II give the data for myosin B, col. I. with pure KCl, col. II in the presence of 0,001 mol. $MgCl_2$. Col. III and IV relate to myosin A in pure KCl. In col. III the thread was put into KCl and ATP added afterwards. In col. IV KCl was added simultaneously with ATP.

The numbers give the % of shortening. The asterisks mean that the threads were very fragile and prone to fall into pieces if agitated. *x* means dissolution. *x!* means that the thread dissolved in the salt solution before the addition of ATP. ? means a doubtful result: partial contraction and partial dissolution. — means inactivity, i. e. that there was no contraction or dissolution.

If we go over col. I we will notice a variation in the numbers, in spite of the fact that the contraction is evidently equally strong from 0,1 to 0,29 mol. KCl. This variation is due partly to errors of measurement and to a greater extent, to the different reaction of different threads; also to local conditions surrounding the thread which inhibited diffusion (too close proximity of the glass). So no great value should be attached to single measurements, only the general trend is of importance.

Now if we go over col. I we will find equally strong contractions from 0,1 mol. KCl up to 0,30. At 0,31 mol. the contraction is weaker and at 0,32 part of the thread dissolves, part of it contracts. At 0,34 mol. KCl there is dissolution only. (Whithout ATP the thread is not dissolved, not even by mol. KCl).

It will be seen from this that the change from maximal contraction to complete dissolution is not any too sharp and takes place within a range of 0,04 mol. (0,3—0,34). The KCl concentration, at which the change takes place, is fairly high.

Now if we compare this with col. II it will be seen that the 0,001 mol $MgCl_2$ present decreased the KCl concentration necessary for the dissolution of the thread, greatly. An entirely new phenomenon appears also: between contraction and dissolution there is a zone in which the thread neither contracts, nor dissolves, but is just inactive. The myosin has thus three different states: the contracted, the inactive and the dissolved state. The transition from one state into the other is sharp and takes place within a concentration difference of 0,01—0,02 mol. The experiment gave the same result in the presence of 0,01 mol. $MgCl_2$ as with 0,001.

Col. III shows that myosin A behaves quite differently. With smaller KCl concentrations it gives the usual weak contraction. Then follows a wide zone of inactivity even without $MgCl_2$. At 0,32 mol KCl we find dissolution without the addition of ATP. From 0,19 mol KCl upwards ATP has thus no effect at all. The KCl concentration at which, in the presence of Mg, myosin B still gives a maximum contraction (0,19 mol.) myosin A is entirely inactive. Addition of 0,001 Mg made no difference.

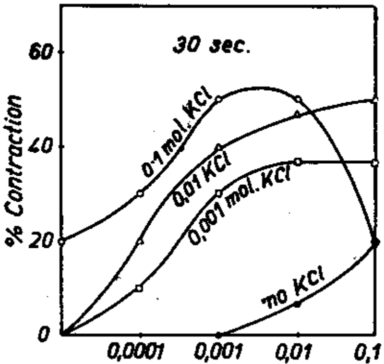

Fig. 1.

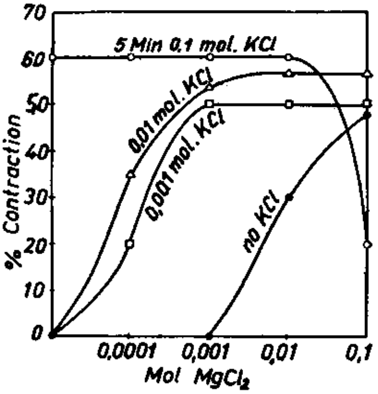

Fig. 2.

Col. IV shows that if ATP is added simultaneously with KCl, the zone of inactivity is very much extended and much higher KCl concentrations are necessary for the dissolution of the thread.

If the ATP is added first, and allowed to act for 5 minutes and the KCl then added, the effect of sequence is stronger still and the thread dissolves only in 0,47 mol. KCl while it is still inactive in 0,46.

### Varied KCl and $MgCl_2$ concentrations.

In fig. 1. and 2. I am giving the effect of varied KCl and $MgCl_2$ concentrations on a myosin B thread. The thread was allowed to stand for five minutes in the salt solution and the ATP then added (0,09%). Readings were made 30 sec. (fig. 1) and 5 min. (fig. 2) later.

It will be seen that KCl, without $MgCl_2$ is inactive in a 0,01 or a 0,001 mol. concentration. 0,1 mol. KCl gives a relatively slow but strong contraction. On the other hand 0,001 mol. $MgCl_2$, without KCl is inactive, while 0,01 mol. $MgCl_2$ shows a slow, weak action and 0,1 gives a slow, but fairly strong contraction.

In the presence of 0,001 mol. $MgCl_2$ even 0,001 mol. KCl becomes strongly active while in the presence of 0,01 mol KCl even 0,0001 mol. $MgCl_2$ gives a strong contraction.

### Effect of varied ATP concentrations.

I am giving the effect of varied KCl and ATP concentrations on a myosin B thread prepared from precipitated myosin in Tab. II. Owing to the higher myosin content the thread was somewhat less contractile than the thread of Tab. I.

Table II.

| % ATP | 0,4 KCl | 0,3 KCl | 0,2 KCl | 0,1 KCl | 0 |
|---|---|---|---|---|---|
| 0,7 | x | x | x | 48 | 50 |
| 0,35 | x | x | 41 | 47 | 35 |
| 0,18 | x | x | 46 | 47 | 8 |
| 0,09 | x | x | 42 | 44 | 0 |
| 0,045 | x | x | 32 | 21 | 0 |
| 0,022 | x | x | 22 | 15 | 0 |
| 0,011 | x | 2 | 12 | 6 | 0 |
| 0,006 | 4 | 0 | 8 | 0 | 0 |
| 0,003 | 0 | 1 | | | |

The thread was allowed to stand for 5 minutes in the salt solution, then the ATP was added and readings made 5 min. later. It will be seen that at a certain KCl concentration we obtain the same effect at all ATP concentrations, *i. e.*, contraction or dissolution. (The dissolution in 0,7% ATP at 0,2 mol. KCl, ist due to the additional KCl introduced with the ATP.) The nature of the action is thus independent ot the ATP concentration. Either we obtain contraction or dissolution or no effect at all.

In the absence of KCl, ATP is inactive in concentrations in which it still gives a maximal effect in the presence of KCl. This justifies the conclusion that the contraction observed in the highest concentrations of ATP is due to the K present partly as the kation of the ATP and partly as a KCl impurity and that ATP in itself, without K, is inactive.

## Varied concentrations of the muscle extract.

The fresh, minced muscle was suspended in water, 1 ml. of water being taken per g of muscle. The suspension was stored over night at 0° and filtered next day, first through a cloth and then through paper. The neutral juice was diluted with water; the dilution is given on the abscissa of Fig. 3. The myosin B threads were placed into the solution for 5 minutes and then 0,09% of ATP was added. Readings were made after 30 seconds. As will be seen the juice gives, even in $^1/_4$ dilution, a maximal contraction. With further dilution the effect becomes wearker and eventually disappears. It may be concluded thus that the juice obtained in the described way contains the ions necessary for maximal contraction in excess.

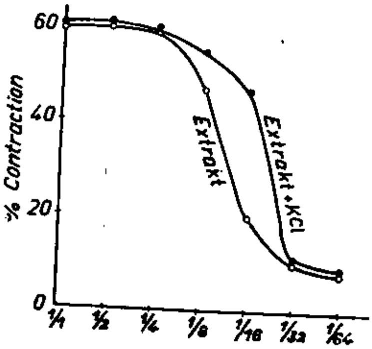

Fig. 3.

In a second experiment 0,1 mol KCl was added to the same juice. It will be seen (Fig. 3) that even at a dilution of $^1/_4$ the effect of the extract is not increased by this addition of KCl thus there was K present in sufficient concentration to give maximal contraction. At a higher dilution of the extract the contraction is increased showing that there is still a sufficient concentration of Mg to enhance the action of KCl. At a dilution of $^1/_{32}$ the Mg becomes insufficient too.

## Variation of pH.

Some time ago I studied the action of salts on the isoelectric point of casein. The isoelectric point of casein is shifted by neutral salts and a precipitation or a dissolution can be obtained by their addition, at certain pH-s. It seemed possible that the precipitating and dissolving action of salts and ATP in the case of myosin could be explained in a similar way. For this reason I studied the influence of pH on the physical state of myosin in the presence of different concentrations of KCl, with and without ATP, Mg and muscle extract. Contrary to my expectation I found that KCl did not shift the range of insolubility at lower concentrations to either side of the pH scale, nor did the ATP or Mg or muscle extract. The contraction, precipitation and dissolution of myosin can thus not be explained by a shift of the insolubility range on the pH scale.

I want to give the chief result of this very extensive work in a few words only. Threads prepared from precipitated, washed and redissolved myosin A and B were placed into the corresponding solutions, the pH of which was adjusted by the addition of HCl or KOH and controlled colorimetrically. Solutions of pH 2-pH 11 were prepared. After 5 min. the effect was observed.

Myosin B threads begin to dissolve above pH 8,5 and below pH 4. At pH 9,5 and 2,5 dissolution is complete. The range of insolubility lies thus between pH 4 and 8,5. This range is not shifted to either side by KCl not even by KCl in a 0,2 mol. concentration. At 0,4 mol. there is a slight shift towards the smaller pH: the thread begins to dissolve above pH 8 and is insoluble even at pH 2. ATP alone or in the presence of 0,25 mol KCl makes the thread insoluble on the acid side but has no effect on the alkaline side. At higher KCl concentrations ATP makes the thread somewhat more insoluble on the alkaline side too. The thread dissolves only at a somewhat (0,5—1) higher pH than without ATP. At 0,4 mol KCl the ATP dissolves the thread at all pH-s except on the extreme acid side, pH 2—3. Mg or muscle extracts increase the effect of ATP without shifting the insolubility range to either side of the pH scale.

Myosin A is insoluble between pH 4—7.5 in the absence of KCl and ATP; its insolubility range is somewhat narrower than that of myosin B. Up to 0,3 KCl the range remains unchanged. At 0,4 mol KCl the thread is insoluble between pH 2—5,5 and soluble at higher pH's. Here too the ATP makes the thread insoluble on the acid side but has otherwise little effect. Mg or extract is inactive, they only broaden the insolubility-range to some extent.

## Miscellaneous observations.

A number of experiments were made with $CaCl_2$. In 0,01 mol concentration this salt inhibits the contraction given by ATP in the presence of KCl or KCl + Mg. This inhibition may be complete but could not be reproduced regularly. Several of our myosin pereparations were not inhibited or inhibited only if precipitated myosin was used for the preparation of the myosin threads.

The Ca-inhibition is reversible. If the Ca is washed out again the thread behaves normally.

The action of 0,1% nicotine, quinine and K-oxalate were also studied. None of these reagents had any inhibitory action on contraction. This is important because BANGA found that the splitting of ATP by myosin is completely inhibited by these reagents. Myosin can thus contract without splitting ATP.

## Summary.

The contraction of myosin A and B threads is studied at varied KCl, $MgCl_2$, ATP and $H^+$ concentrations. Differences in the behaviour of A and B myosin are pointed out. It is shown that in the presence of ATP and Mg very slight changes in KCl concentration are sufficient to change the physical state of the myosin B thread and make the inactive thread contract or the contracted thread become inactive or dissolve.

Contraction is not inhibited by the complete inhibition of the phosphatase activity of myosin.