THE ELONGATION OF MYOFIBRILS FROM THE INDIRECT FLIGHT MUSCLE OF DROSOPHILA

JOHN ARONSON, Ph.D.

From the Department of Biology, University of Rochester, New York. Dr. Aronson's present address is Department of Cytology, Dartmouth Medical School, Hanover, New Hampshire

ABSTRACT

Myofibrils which lengthen by several per cent in the presence of ATP and magnesium ions were prepared by teasing indirect flight muscle of Drosophila in solutions containing ethylenediaminetetraacetate. A study was made of the hydrogen ion, magnesium ion, ATP, and potassium chloride concentrations with which this effect could be observed. The lack of elongation with pyrophosphate and several nucleoside triphosphates suggests that the lengthening is ATP specific. A relaxing factor system comparable to that described for rabbit muscle was not demonstrable, as elongated fibrils did not shorten with calcium ions, carnosine, or digitonin.

Myofibrils isolated from the indirect flight muscles of the blowfly Calliphora have been observed to lengthen by several per cent when treated with freshly prepared suspensions of teased flight muscle, and sometimes when treated with ATP. The mechanism of this effect has not been investigated but lengthening, as Hanson has suggested, may be mediated through a relaxing factor system similar to that described for rabbit muscle. Lengthening of myofibrils has also been observed in suspensions of freshly fragmented pigeon breast muscle, in suspensions of fragmented rabbit psoas muscle fortified with magnesium ions and ATP, and possibly in single frog fibers.

The present study describes the conditions for elongation of fibrils isolated from the indirect flight muscle of Drosophila.

MATERIALS AND METHODS

Indirect flight muscles from two species of Drosophila, melanogaster and buzzaki, were used. No note was made of sex or age although recently emerged flies were not used. Flies were killed by decapitation without previous cooling or etherization. All operations were carried out at room temperature (21 to 28°C).

Unless otherwise specified, fibrils for examination were prepared by teasing several muscle fibers with steel needles in a drop of salt solution which will be referred to as MgEDTA. This solution contains 0.1 M KCl, 0.002 M EDTA, 0.004 M MgCl₂, and 0.005 M KH₂PO₄, and the pH was adjusted to 7.0. The time required to prepare and first observe a preparation was usually less than 3 minutes, after which free sarcosomes and cell debris were removed by washing. This initial washing consisted of drawing a drop or two of solution under the coverslip.

When solutions other than MgEDTA were used they were made up in a standard salt solution containing 0.1 M KCl, 0.005 M KH₂PO₄, at a pH of 7.0. Solutions containing calcium ions were made up without KH₂PO₄ or buffered with tris. Standard salt solution containing 2.5 mM ATP and 4 mM MgCl₂ will be referred to as "MgATP." The pH of all solutions was adjusted with potassium hydroxide.

The adenosine triphosphate (ATP) (Pabst Laboratories, Milwaukee), adenosine diphosphate (ADP) (Nutritional Biochemical Co., Cleveland, and Sigma Chemical Co., St. Louis), inosine triphosphate (ITP) were prepared by teasing several muscle fibers with steel needles in a drop of salt solution which will be referred to as MgEDTA. This solution contains 0.1 M KCl, 0.002 M EDTA, 0.004 M MgCl₂, and 0.005 M KH₂PO₄, and the pH was adjusted to 7.0. The time required to prepare and first observe a preparation was usually less than 3 minutes, after which free sarcosomes and cell debris were removed by washing. This initial washing consisted of drawing a drop or two of solution under the coverslip.

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(Sigma Chemical Co.), cytosine triphosphate (CTP) (Sigma Chemical Co.), mersalyl (Winthrop Laboratories, New York City) \(\beta\)-chloromercuri-benzoate (Sigma Chemical Co.) and desoxycholate (Difco Laboratories, Detroit) were procured commercially and were not further characterized. All inorganic chemicals used were reagent grade and deionized or alkaline permanganate-oxidized glass-distilled water was used throughout.

Observations were made by phase contrast microscopy using a Bausch and Lomb N.A. 1.25 immersion objective.

**DESCRIPTION OF THE PREPARATION**

Immediately after being teased, a preparation contains many myofibrils (diameter 2 to 3 \(\mu\)), large numbers of spherical sarcosomes, and usually clumps of unteased fibrils. Washing with a drop of solution removes free sarcosomes, most of the clumped and unteased material, and many of the myofibrils. Some sarcosomes remain attached to the slide and to the coverslip, and myofibrils which are attached to the coverslip frequently accumulate small granules along the lines of attachment. Observations were made on fibrils which were free to shorten and which appeared free of granules and sarcosomes. When examined in thin sections by electron microscopy no vesicles or granules were seen within the fibrils.

The position of the I band in fibrils which had been dehydrated with acetone and immersed in nitrobenzene was established by polarized light microscopy. The position, but not necessarily the length, of this I band was correlated with the I band seen by phase contrast microscopy. Other bands are named in terms of their relative positions and apparent densities (see Fig. 1 a).

Fibrils isolated in MgEDTA containing 2.5 mM ATP all have the same pattern (Figs. 1 a and 2 a) with an average sarcomere interval of 3.5 micra. An \(H\) gap and a slightly wider \(I\) gap (about 0.6 \(\mu\)) are obvious. The faint transverse striation of the \(A\) band and the exceptional darkness of the terminal half sarcomere are probably optical artifacts. A faint \(Z\) band is usually detectable.

Shortening of teased fibrils in either MgEDTA containing 2.5 mM ATP or in MgATP occurs spontaneously after about 10 minutes if the preparations are not irrigated, or immediately if they are washed with standard salt solution. This shortening, as Hanson (2) and Hodge (8) have mentioned, seldom goes below 80 per cent of the initial length and even in this range the extent to which individual fibrils shorten is quite variable. Fibrils which have shortened can usually be induced to lengthen slightly by a further application of MgATP (Table I). This MgATP-induced lengthening will be called "elongation." An elongated fibril will shorten when washed with a solution containing 2.5 mM ATP, but no magnesium ions, and will elongate again when treated with MgATP.

When a fibril shortens the striation pattern changes (some of these changes are shown in Figs. 1 and 2). With slight shortening the I and \(H\) regions darken (Fig. 1 b) and in some instances this darkening is strong enough to make the fibril appear homogeneous. With continued shortening the \(Z\) band becomes wider and denser and appears to fill the I region (Figs. 1 d and 2 f). Soon after the \(Z\) band starts to thicken, the \(H\) gap disappears and is replaced by a band which increases in density but always remains less dense than the \(Z\) band. Hodge (8) has termed these dense bands the \(C_s\) and \(C_m\) bands respectively.

![Figure 1](https://example.com/f1.png)

**Figure 1**

Phase contrast micrographs of a myofibril from the indirect flight muscle which has been alternately exposed to MgATP and to a standard salt solution. \(a, c, e,\) and \(g\) are in MgATP; \(b, d,\) and \(f\) are in standard salt solution. It can be seen that the fibril shortened when the MgATP was exchanged for standard salt solution and lengthened when the standard solution was exchanged for MgATP. When the ATP was removed by washing, the amount of shortening was variable and seemed to depend partially on the speed with which solutions were exchanged. Although solutions can normally be exchanged quite rapidly, to photograph the sequence of changes shown here took about 30 minutes. This was due to the necessity for being sure that the solutions had stopped flowing and that no further changes in the fibril would occur. \(I, H, A, Z, C_s\) and \(C_m\) refer to bands, described in the text. \(\times 6100.\)
The A region appears less dense after C~ and Cm bands have formed and fewer fine bands are visible.

Elongation is not associated with a single striation pattern, since it has been noted in fibrils showing both I and H regions as well as in fibrils having strong Cz and Cm bands. When fibrils with weak Cz and Cm bands elongate, the Cz band weakens, the Cm band disappears, and I and H regions reappear. When fibrils with strong Cz and Cm bands elongate the apparent density of these bands is reduced (Figs. 1 d, e and 2 f, g). The change in pattern with elongation appears to me to occur by migration of material; however, loss of material by extraction is also a strong possibility. The validity and significance of a more detailed description will be discussed in future work.

In a given preparation the fibrils respond to varying degrees. This is partially due to the way in which the solutions are exchanged under the coverslip but also seems to depend on the individual fibril. Responsive fibrils in MgATP will shorten when the magnesium ions are removed or the ATP concentration is lowered. Shortening to, or past, the initial length usually occurs when the MgATP solution is replaced by standard salt solution (Figs. 1 and 2). A fibril carried through a number of cycles of elongation and shortening by adding and then washing out MgATP will eventually become unresponsive. During the first cycles, when I and H bands are visible in the shortened fibril, the fibril may regain its initial length on elongation (Table I). In later cycles when moderate or strong Cz or Cm bands are present the fibril does not regain its initial length.

Fibrils were more flexible in MgATP than in the standard salt solution. The degree of rigidity was judged by the behavior of fibrils in solutions flowing under the coverslip.

When MgATP is added to a fibril there frequently appears to be a slight increase in fibril diameter. This effect has not been studied because of the difficulty of measuring reliably the increase in diameter.

CONDITIONS AFFECTING ELONGATION

Some of the conditions affecting elongation with ATP and magnesium ions were studied. They are summarized in Table II.

The preparations for these experiments were made by dissecting and teasing the fibrils in MgEDTA containing 2.5 mM ATP. They were then washed with MgATP followed by standard salt solution to cause a slight amount of shortening. Elongation was detected reliably by following the movement of a part of a fibril which reflected the summed change in length of a number of sarcomeres.

The effect was studied of varying one component at a time in a solution containing 0.1 mM KCl, 0.004 mM MgCl₂, 0.0025 mM ATP, 0.005 mM KH₂PO₄ at pH 7.0. Before adding a solution containing ATP, the preparation was washed with a similar solution which lacked ATP.

**Potassium Chloride**

Potassium chloride concentrations from 0.025 mM to 0.6 M were used and elongation was observed from 0.05 mM to 0.6 M KCl. From about 0.4 M up to 0.6 M KCl it was necessary to wash rapidly because the ability to elongate was soon lost due to extraction. Extraction is more obvious when ATP is present. Elongation was not detected in 0.025 mM KCl.

**Magnesium Ions**

Magnesium ion concentrations of 0.0001 to 0.01 mM were effective in causing elongation in the presence of 2.5 mM ATP. Higher concentrations were not tried. Concentrations from 0.0001 down to 0.00003 mM gave erratic results. Solutions containing less than 0.00003 mM Mg ions frequently caused fibrils to shorten further.

**ATP**

ATP concentrations between 0.5 mM and 15 mM were effective in causing elongation. Higher concentrations were not tested. Concentrations...
The fibrils shown in Figs. 1 and 2 have been measured after alternate exposure to MgATP and to standard salt solution. The measurements were made from photographic prints and are expressed as per cent of the length at a. They are considered to be illustrative of the magnitudes of the changes in length observed. Values for h to k are from photographs which were not included in Fig. 2.

MgATP contains 0.1 M KCl, 5 mM KH₂PO₄, 2.5 mM ATP, 4 mM MgCl₂ at pH 7.0.

Standard contains 0.1 M KCl, 5 mM KH₂PO₄, at pH 7.0.

of the order of 0.15 mM or lower caused shortening.

**pH**

The effect of hydrogen ion concentration was investigated in the pH range 5.0 to 8.5. Fibrils elongate at pH 5.8 but lose the ability to respond after one or two cycles, possibly because of extraction. At pH's below 5.6 elongation was never seen. On the alkaline side, starting at about pH 7.6, the observations become more complex. In the pH range 7.6 to 8.5 elongation is preceded by contraction which frequently causes a net shortening. Since contraction was observed to be more extreme at alkaline pH's, the net shortening results from greater shortening during the time in which the ATP level is rising high enough to cause elongation. The relaxing effect of MgATP is probably present at pH 8.5 and higher since fibrils elongated at pH 7.0 remain elongated when the pH is raised and are still contractile when the magnesium ions are removed.

**Pyrophosphate**

Sodium pyrophosphate did not cause elongation either in the presence or in the absence of magnesium ions, nor did it maintain the elongation of fibrils which had been treated with MgATP. Concentrations of pyrophosphate used were 2.2, 11.0, and 22 mM in standard salt solution, with and without 4 mM MgCl₂.

**ADP**

ADP in concentrations up to 30 mM, with and without 4 mM MgCl₂, gave no indication of elongation. Shortening was observed and can probably be attributed to myokinase-like activity associated with the fibrils.

**Table I**

<table>
<thead>
<tr>
<th>Fig. 1</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>100 a</td>
</tr>
<tr>
<td>b</td>
<td>96 b</td>
</tr>
<tr>
<td>c</td>
<td>100 c</td>
</tr>
<tr>
<td>d</td>
<td>90 d</td>
</tr>
<tr>
<td>e</td>
<td>92 e</td>
</tr>
<tr>
<td>f</td>
<td>88 f</td>
</tr>
<tr>
<td>g</td>
<td>90 g</td>
</tr>
<tr>
<td>h</td>
<td>83 h</td>
</tr>
<tr>
<td>i</td>
<td>85 i</td>
</tr>
<tr>
<td>j</td>
<td>80 j</td>
</tr>
<tr>
<td>k</td>
<td>81 k</td>
</tr>
</tbody>
</table>

The fibrils shown in Figs. 1 and 2 have been measured after alternate exposure to MgATP and to standard salt solution. The measurements were made from photographic prints and are expressed as per cent of the length at a. They are considered to be illustrative of the magnitudes of the changes in length observed. Values for h to k are from photographs which were not included in Fig. 2.

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**Table II**

<table>
<thead>
<tr>
<th>Conditions under which Drosophila Fibrils Elongate</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>0.05-0.6</td>
</tr>
<tr>
<td>0.025</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
</tbody>
</table>

+ Elongation was seen.
- Elongation was not seen.

These experiments are described in the text.

**ITP**

Inosine triphosphate at concentrations up to 25 mM, with or without 4 mM magnesium ions, did not cause visible elongation. Instead, contraction was frequently noted. ATP (2.5 mM) in the presence of ITP (2.5 and 10 mM) and 4 mM MgCl₂ caused elongation.

**CTP**

Cytosine triphosphate at concentrations up to 17 mM in the presence of 4 mM magnesium chloride did not cause elongation.

**Ca**++

Calcium ions tend to make the fibrils unresponsive in that they neither shorten nor elongate and they can rarely be carried through more than
two cycles of elongation. If a fibril responded to 2.5 mM ATP and 5 mM CaCl₂ it always contracted. Elongation was observed with ATP when calcium and magnesium ions were both present. Solutions tested contained 0.5 mM Mg²⁺ + 5.0 mM Ca²⁺, 5.0 mM Mg²⁺ + 5.0 mM Ca²⁺, and 5.0 mM Mg²⁺ + 0.5 mM Ca²⁺, all in the presence of 2.5 mM ATP. The preparations were washed briefly with a mixture containing calcium and magnesium ions preceding the addition of the solution containing ATP.

**Carnosine**

Treatment of fibrils with MgATP plus carnosine (4 or 12 mM) resulted in elongation.

**Desoxycholate**

Washing the fibrils with desoxycholate (0.05 per cent in 0.05 M KCl) for up to 20 minutes did not necessarily abolish the ability of fibrils to elongate although they often became swollen and unresponsive. With higher concentrations (0.1 per cent desoxycholate in standard salt solution) the fibrils became unresponsive almost immediately. This was associated with swelling, extraction, and redistribution of material. Treatment of fibrils for 20 minutes with digitonin (5 mg/cc) in standard salt solution had little effect on the fibrils which were subsequently observed to elongate when MgATP was added. Fibrils teased from fibers which had been frozen on dry ice and thawed appeared similar to and responded like fresh fibrils.

**Organic Mercurials**

Inhibition of elongation by MgATP, and of shortening after elongation, was obtained with 0.1 mM mersalyl and with 0.3 mM p-chloromercuribenzoate. These reagents also decrease the apparent H and I band densities and cause the Z band to disappear. 5 mM iodoacetic acid had no effect on either elongation or shortening.

**EDTA**

There was no indication of elongation in the presence of up to 10 mM EDTA when 2.5 mM ATP was present. A fourfold excess of EDTA over magnesium ions (2 mM EDTA and 0.4 mM MgCl₂) in the presence of 2.5 mM ATP resulted in elongation.

**DISSECTION IN MgATP**

Myofibrils teased initially in MgATP (no EDTA) with glass or stainless steel needles show distinct H and I bands. A few of these fibrils will undergo elongation and shortening for several cycles and only occasionally will one respond for a longer period. Washing unresponsive fibrils with MgEDTA for periods up to 30 minutes did not make them responsive.

**DISCUSSION**

The elongation observed on the addition of MgATP is thought to correspond to relaxation. On the assumption that each sarcomere in a fibril contracts, the time to complete a cycle of contraction and relaxation is of the order of 5 milliseconds in the indirect flight muscle of *Drosophila* (9). This is considerably shorter than the time between stimuli at which tetanus occurs in a variety of other striated muscles and suggests that insect flight muscles may be specialized to relax rapidly.

The general mechanism of muscle relaxation has been studied most extensively using fractionated components of rabbit sarcoplasm. For the relaxation of glycerinated rabbit fibers one needs both a dialyzable and a granular sarcoplasmic fraction, or some product they produce (3, 4), as well as magnesium ions and ATP. Experimentally the relaxation of glycerinated rabbit muscle in the presence of ATP, magnesium ions, and a fractionated relaxing factor system has been reversed by calcium ions and by carnosine (3). The activity of the granular fraction has been destroyed by digitonin and by 1 per cent desoxycholate (3).

The data summarized in Table III show that a sarcoplasmic relaxing factor system of the sort described for rabbit muscle (3, 4, 10) is not active in the elongation of myofibrils isolated from the indirect flight muscle. The properties used to identify this system are limited and little is known of either the directness or the mechanism of its action on the contractile material. *Drosophila* fibrils which have been washed extensively and which are free of light microscopically visible granules elongate through the action of magnesium ions and ATP directly on the myofibril. It is not clear whether MgATP-induced elongation ("relaxation") in *Drosophila* is due (a) to a modification of the fibrils possibly by calcium-insensitive relaxing factor being built into the
fibril, (b) to an "actomyosin" modified for a specialized function, (c) to species differences, or (d) to the state of the contractile system within the isolated fibril.

There is evidence that actomyosin prepared from high frequency flight muscles in the thorax of the honey bee is similar to actomyosin prepared from rabbit muscle (11, 12).

Several non-physiological relaxing (plasticizing) agents have been described. Pyrophosphate with magnesium ions (13), ATP after the addition of mersalyl (14), and high concentrations of ATP with magnesium ions (15) have been used with glycerinated rabbit muscle. With Drosophila myofibrils elongation was observed only with magnesium ions and high concentrations of ATP, suggesting that EDTA increases the responsiveness of the fibrils by protecting them from some sarcoplasmic component, possibly calcium ions, rather than by being bound and acting as a relaxing factor.

The observations on the nucleotide specificity necessary for elongation of Drosophila fibrils were obtained under a single set of conditions. However, elongation was not seen when ADP, ITP, CTP, or pyrophosphate was used at concentration levels greater than ten times the minimal ATP concentration which caused elongation. Since the data on relative binding constants are sketchy and the above observations are limited to a low level of Mg ion concentration, the results are not discussed in terms of structural specificity and binding sites.

**Table III**

<table>
<thead>
<tr>
<th>pH</th>
<th>KCl</th>
<th>ATP</th>
<th>MgCl₂</th>
<th>Additives</th>
<th>Conc.</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>4</td>
<td>Carnosine</td>
<td>4 or 12 mM</td>
<td>Elongation</td>
</tr>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>5</td>
<td>CaCl₂</td>
<td>0.5 mM</td>
<td>Elongation</td>
</tr>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>5</td>
<td>CaCl₂</td>
<td>5 mM</td>
<td>Elongation</td>
</tr>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>0.5</td>
<td>CaCl₂</td>
<td>5 mM</td>
<td>Elongation</td>
</tr>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>4</td>
<td>Desoxycholate (pre-treated)</td>
<td>0.05%</td>
<td>Elongation</td>
</tr>
<tr>
<td>7.0</td>
<td>0.1</td>
<td>2.5</td>
<td>4</td>
<td>Digitonin (pre-treated)</td>
<td>0.5%</td>
<td>Elongation</td>
</tr>
</tbody>
</table>

These experiments are described more fully in the text. In summary, Drosophila fibrils which have elongated under the influence of magnesium ions and ATP do not shorten when either calcium ions or carnosine are added. Pretreating fibrils with digitonin or with desoxycholate does not destroy their ability to elongate.

indicating either that these fibrils do not plasticize easily or that plasticizing and elongation are different responses.

EDTA with excess magnesium ions and ATP has been used as a relaxing agent for glycerinated rabbit fibers by Bozler (16) and by Watanabe (17). Both authors noted that EDTA or its effect is not easy to remove by washing but is reversed by calcium ions. With glycerinated rabbit fibers shortening is inhibited by MgEDTA + 2.5 mM ATP but this inhibition is easily and rapidly removed by washing.

Dissecting Drosophila fibrils in an EDTA-containing solution greatly improves their responsiveness, but dissecting in the absence of EDTA followed by washing with EDTA-containing solutions does not have this effect. One might suggest that EDTA increases the responsiveness of the fibrils by protecting them from some sarcoplasmic component, possibly calcium ions, rather than by being bound and acting as a relaxing factor.

The increased length of an elongated sarcomere may be interpretable in terms of molecular deformation or in terms of somewhat grosser effects in which the relationships change between the structural elements visible in the electron microscope. Hanson (1) has suggested that the elongation results from the release of compressive stresses within the structural elements. These stresses presumably do not arise from the compression of the A band material as a whole since an appreciable I band is found after elongation has occurred, but rather from elements which could be either in series or parallel with the contractile element. Mueller (18) observed an increase in the volume of a contracted rabbit actomyosin gel in the presence of magnesium ions, ATP, and the relaxing factor system. He suggested that this
system acting on the structured actomyosin of the fibril could cause elongation. Changes which occur in the light scattering of a rabbit actomyosin when ATP is added have been interpreted (19) as showing a lengthening and thickening of cylindrical aggregates without a change in their particle weight. These experiments also suggest a mechanism for elongation. However, they were done at high ionic strengths, without added magnesium ions and without a relaxing factor system.

The migration of material along the fibril has been recently re-emphasized by Hodge (8) and criticized by Hanson (2) in studies of the indirect flight muscle of blowflies. It seems to me, on the basis of images similar to Figs. 1 b and 2 b, that migration does occur and that the C2 and Cm bands are partially a reflection of this. What the migratory material is and how large a proportion of the muscle protein it can include remains to be determined. The fact that its localization may depend reversibly on the ATP level makes it of great potential interest.

I should like to acknowledge helpful discussions with Drs. A. G. Szent-Gyorgyi and S. Watanabe and particularly the advice and support of Dr. S. Inoué during the course of this work.

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