

Contraction of Glycerinated Muscle with ATP

Instruction Manual

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Overview

This kit provides a strip of glycerinated skeletal muscle tissue, from which muscle fibers (myofibers) can be dissected for study. Using a compound microscope, students observe the striated pattern in the fibers and measure the length of the relaxed sarcomeres. They then induce muscle contraction by adding ATP, observe the contraction, and measure the post-contraction width of the sarcomeres. Students also compare the effects of adding ATP plus potassium and magnesium ions, ATP alone, and the ions alone.

Note: Shortening of the fibers can be seen macroscopically and measured without use of a compound microscope.

Background

Muscle tissue is made of fibers formed by the fusion of cells during development. A single muscle fiber, barely visible to the unaided eye, has many nuclei that lie close to its outer membrane. Each fiber contains hundreds of long, threadlike structures called myofibrils, arranged in parallel. About 75% of a muscle's total volume is made up of myofibrils. Myofibrils are the structures that carry out muscle contraction.

Under a microscope myofibers look striated (striped), with a repeating pattern of bands and lines perpendicular to the length of the fiber. The banded pattern is caused by an organized, parallel arrangement of protein filaments within the myofibrils. There are two types of filaments in a myofibril: thick filaments composed of the protein myosin, and thin filaments composed of the protein actin. (Actually, both filaments are quite thin on a human scale, but myosin filaments are thicker than actin filaments.)

The actin and myosin filaments are arranged as shown schematically in Figure 1. The filaments overlap in an orderly, repeated manner, creating units called sarcomeres. When many filaments are bundled in a cylinder, the repeated overlapping pattern of filaments results in the banded pattern seen under the microscope. The different bands in the visible pattern are designated by letters of the alphabet, and they correspond to different segments of the sarcomere (Figure 1). When you observe the glycerinated muscle fibers with a compound microscope, you should be able to see bands.

Muscle contraction occurs through the interaction of the actin and myosin filaments in the sarcomeres. When a muscle contracts, the myosin crossbridges bind to the actin filaments in a manner that causes the actin filaments to be pulled together across the H zone. Under the light microscope, the A and I bands are seen to become narrower (Figure 2), and the overall width of the sarcomeres decreases.

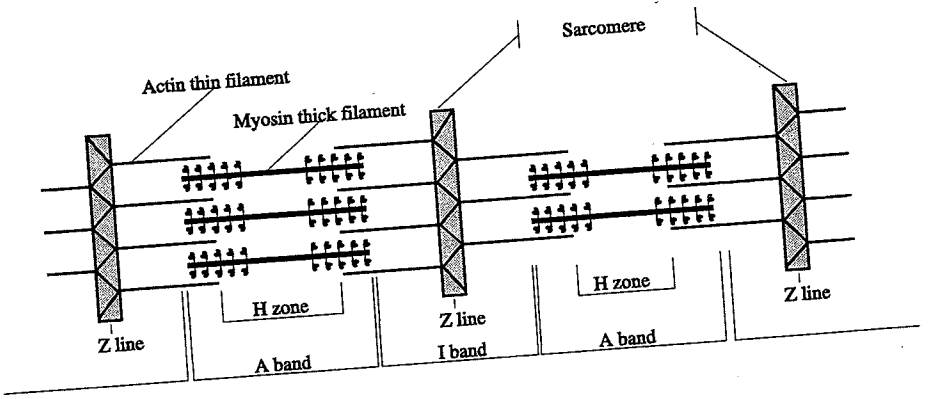


Figure 1

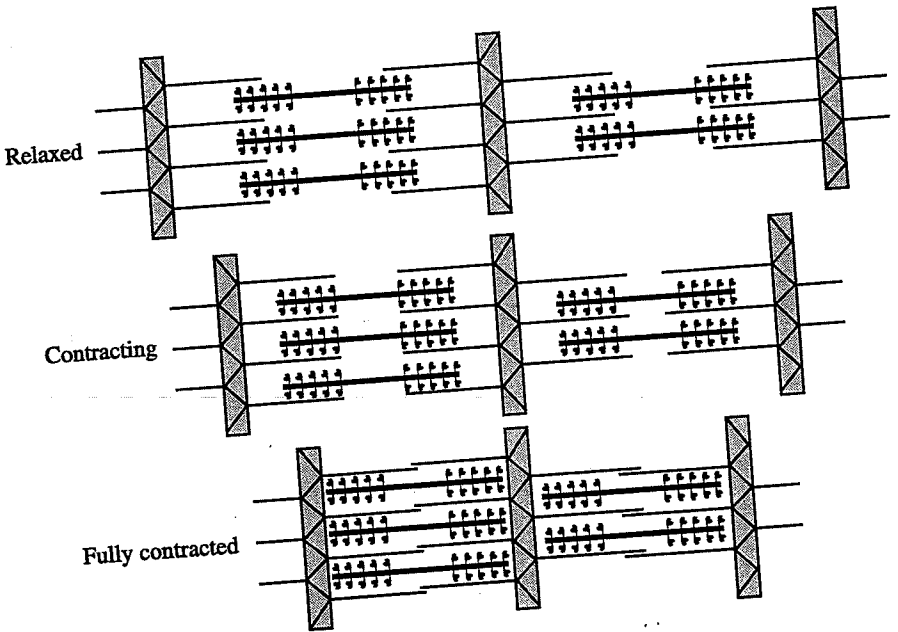


Figure 2

Mechanism of Muscle Contraction

At the molecular level, thin filaments are composed of two chains of identical actin monomers twisted around each other in a double helix, like two twisted strands of pearls. Thick filaments are composed of hundreds of myosin molecules, each one a long rod with a globular head. In the fiber, the myosin molecules are arranged so that the rods lie alongside one another and the globular heads protrude away from the fiber (Figure 3).

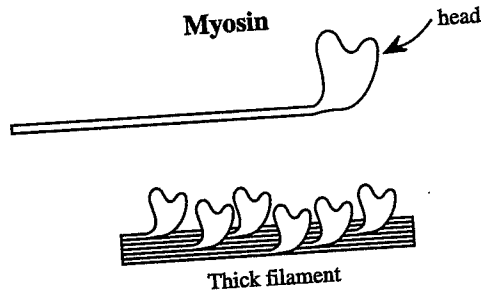


Figure 3

For a muscle fiber to contract, the myosin heads must first be activated by ATP. One molecule of ATP binds to a myosin head and is hydrolyzed to ADP and inorganic phosphate (P_i). Both ADP and P_i remain bound to the myosin head, and the energy released from ATP hydrolysis is transferred to the myosin head as well. The myosin head is now activated. Imagine holding one end of a thin, plastic ruler and pulling back on the other end so that the ruler bends. When you release the ruler, it will spring back to its original shape. The activated myosin head is rather like the bent ruler. When the myosin head binds to the actin filament, its energy is released and the myosin head springs back, carrying the bound actin filament with it. This movement causes the muscle fiber to contract. Each one of the actin monomers has a binding site for myosin.

After the myosin head has sprung, it can interact with a new molecule of ATP. When ATP binds to the myosin head, it releases the actin fiber, ADP, and P_i . The myosin head is now reactivated, and the cycle can begin again. If no ATP is available to reactivate the myosin, the actin/myosin complex remains locked together, and the muscle cannot relax. When an animal dies, its cellular ATP stores are depleted and all its muscles lock. This locked condition is called rigor mortis. In living animals, muscles resume their normal shapes (relax) after contraction because they are pulled by opposing muscles.

The just-described picture of contraction in living muscle is incomplete because it omits the role of nerve signals in instigating contractions. Muscle fibers do not normally contract without appropriate nerve signals because a control mechanism prevents them from doing so. The control mechanism works through two regulatory proteins, tropomyosin and troponin, which form a complex lying along the actin filament (Figure 4).

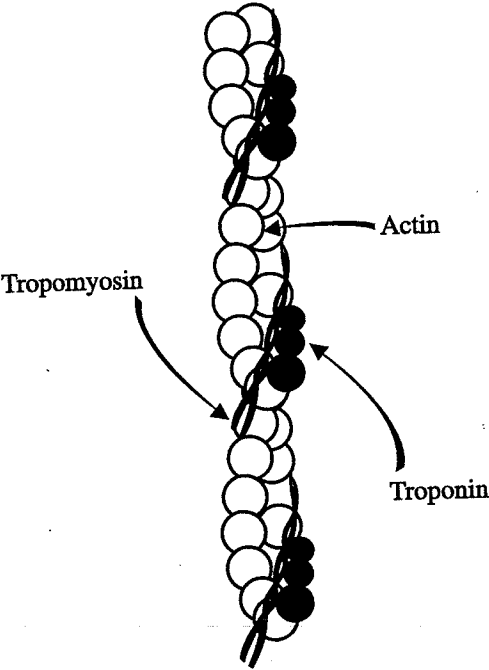
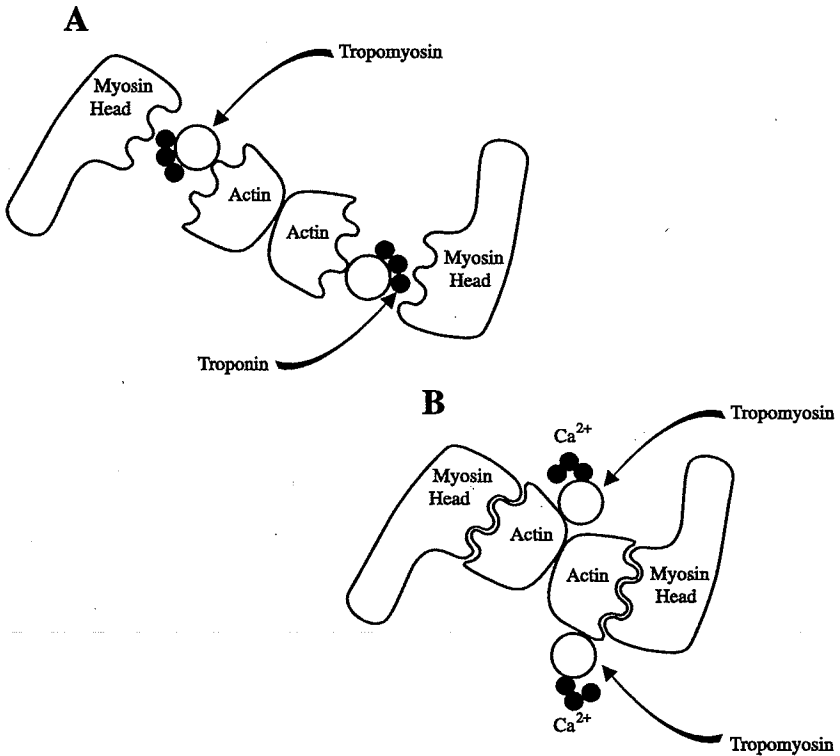


Figure 4

The troponin/tropomyosin complex blocks the myosin binding site on the actin fiber, preventing myosin from binding to actin and causing contraction (Figure 5a).

The signal for muscle contraction is a nerve impulse to the muscle fiber that results in intracellular release of calcium ions. The calcium ions induce contraction by binding to troponin. When Ca^{2+} binds to troponin, the shape of the troponin/tropomyosin complex is altered. This change in configuration allows activated myosin crossbridges to bind to the actin and release their energy as motion (Figure 5b).



Figures 5A and 5B

Therefore, in a normal muscle fiber rich in ATP, the myosin heads are activated and ready to cause a contraction, but they cannot until a nerve impulse releases Ca^{2+} . The contraction cycles will continue as long as intracellular Ca^{2+} concentration is high and as long as ATP is available. When Ca^{2+} levels fall, Ca^{2+} is released from the troponin molecules, and the troponin/tropomyosin complex again blocks the binding sites on the actin fiber.

The Glycerinated Muscle System

The glycerinated muscle system is different from muscle in living tissue. The glycerination process removes ions and ATP from the tissue and disrupts the troponin/tropomyosin complex so that the binding sites on the actin fibers are no longer blocked. No Ca^{2+} is needed to induce contraction. However, no ATP is present in the glycerinated tissue, so the myosin heads are not activated. You will be experimenting with adding ATP and ions to the glycerinated tissue to initiate contraction. When contraction occurs, you will be able to see the change in length of the sarcomeres and measure the overall shortening in the length of the dissected muscle tissue. After the muscle is contracted it will not relax, because there is no opposing muscle to stretch it out.

Materials

Item 20-3520 includes a single tube of glycerinated muscle. Item 20-3530 includes one vial each of the ATP and salt solutions provided with the ATP Muscle Kit described below. The ATP Muscle Set (20-3525) includes one tube of glycerinated muscle and 5 mL each of the ATP, KCl, and MgCl_2 solutions.

Items included in the ATP Muscle Kit (20-3526) are listed below. The items indicated with an asterisk are included in the ATP Muscle Kit Refill (20-3527).

- *4 tubes of glycerinated skeletal muscle strips tied to a stick in a 50% glycerol solution
- *4 dropper vials of 0.25% ATP in distilled water
- *4 dropper vials of 0.25% ATP plus 0.05 M KCl plus 0.001 M MgCl_2 in distilled water
- *4 dropper vials of 0.05 M KCl plus 0.001 M MgCl_2 in distilled water
- 4 magnifying glasses (4x)
- microscope slides and coverslips

Materials that are needed but not supplied in the ATP Muscle Kit (20-3526) include:

- dropping pipets
- millimeter scale
- sharp scissors
- teasing needle and watchmaker forceps or glass needles
- petri dishes
- dissecting and/or compound microscopes (optional)

Storage of Muscle Tissue and Solutions

The glycerinated muscle preparations can be stored in a freezer at -20°C to -10°C indefinitely. The ATP and salt solutions should be stored in a refrigerator between 4°C and 10°C , and should be used within 10 days of receiving the kit. To minimize chemical activity loss, these solutions are prepared as close to shipping as is practical. Remove the muscle preparations and solutions from storage just before use.

Preparation

Remove the skeletal muscle strips, which are each tied to a stick, from their test tubes. Each of these strips contains hundreds of muscle fibers. Pour the glycerol from each test tube into a petri dish. Cut the muscle strips into pieces about 2 cm in length, and drop these into the petri dishes. One piece of muscle tissue is sufficient for each individual or team, although individual work is preferred. For each student or work group, distribute some of the glycerol and one piece of the muscle tissue into a petri dish. Unused muscle may be returned to the freezer in the 50% glycerol solution.

Provide for each student or work group:

- teasing needle and watchmaker forceps OR one pair of glass needles
- dropping pipet
- petri dish with glycerol and skeletal muscle tissue
- 5 microscope slides and 2 coverslips
- millimeter scale
- compound microscope (optional)
- dissecting microscope (optional)

All glassware and dissecting tools should be cleaned thoroughly and well rinsed in distilled water before use.

Note on student results

The speed and extent of the muscle contraction students will observe are influenced by the amount of glycerol on the slide, the concentration of active ATP, the ions present, and the width of the dissected muscle strand. Under favorable conditions, myofibers can be expected to contract to almost 50% of their starting length within 10 seconds.

Note: Although it's easier using a dissecting microscope, students can tease the muscle strip into satisfactory strands and measure their contraction using the unaided eye.

Student Procedure

1. Place the petri dish containing a segment of skeletal muscle tissue on the stage of a dissecting microscope. If a microscope is unavailable, you may use the supplied magnifying glass. Use glass needles or a teasing needle to gently tease the segment into very thin strands. You will see optimal results with single muscle fibers, but these are difficult to obtain. The thinnest strand that you will likely get is a group of two to four fibers. *Strands of muscle exceeding 0.2 mm in cross-sectional diameter are too thick to be used.*
2. Mount a thin strand on a microscope slide with a coverslip. Examine the strand under magnification. Note the striations in the myofibers.
3. Transfer three or more of the thinnest strands to a tiny amount of glycerol on a second microscope slide. Lay the strands out straight and parallel to each other. Do not cover them.

Note: The amount of glycerol needed depends on the heat of the microscope lamp and the length of exposure to heat. With no appreciable heat, the glycerol that adheres to the strand of fibers is sufficient. The less glycerol used, the easier the fibers are to measure.

4. Using your microscope or magnifying glass, measure the length of the strands with a millimeter scale. Record these lengths.
5. Flood the strands with several drops of the solution containing ATP plus potassium and magnesium ions. Observe the reaction of the fibers.

Note: It is essential to avoid cross-contamination between the ATP and the salt solutions. Such contamination will lead to ambiguous experimental results.

6. After 30 seconds or more, re-measure the strands and calculate the degree of contraction. Have the fibers changed in width?
7. Remove one of the contracted strands to another slide. Examine it under a compound microscope and compare the fibers with those seen in Step 2. What differences do you see?
8. Repeat steps 1–7 using clean slides, new myofibers, and the solutions of ATP alone and salts alone. What conclusions may be drawn from your results?

FAQs

- Q:** Why do the muscle strands remain contracted permanently after adding the ATP solutions?
- A:** *Our experimental setup differs from living tissue in that there is no opposing muscle to stretch the contracted muscle out again.*
- Q:** I know that in living tissues, calcium is required to activate muscle contraction. Why is it not needed in the glycerinated muscle?
- A:** *The glycerination process disrupts the troponin/tropomyosin complex that otherwise blocks the myosin binding sites in living tissues. Therefore, no calcium is needed to induce contraction.*
- Q:** What is the role of the ATP, KCl, and MgCl₂ solutions in muscle contraction?
- A:** *ATP is an absolute requirement for glycerinated muscle contraction. Without ATP, the myosin heads in the muscle will not be activated. Mg ions have been reported to interfere with muscle contraction in live tissue, but in glycerinated tissues, the addition of KCl and MgCl₂ solutions to ATP increases the strength of muscle contraction because of myosin's high affinity for these ions.*
- Q:** Why are my muscle fibers not contracting?
- A:** *Probably, the muscle strand is too thick. The thinner the strand, the better your results will be. As a rule, strands of muscle exceeding 0.2-mm cross-sectional diameter are too thick to be used.*