Bio 350 Fall 2011
Contraction of Rabbit Psoas Muscle

Cell membranes can be artificially removed using a variety of chemical solvents such as glycerin. The advantage of such preparations is that it allows for study of the intracellular components, without disrupting the integrity of their structure and position in a cell, and without having to stimulate intracellular signaling pathways. This technique is referred to as cell skinning.

Cell skinning has been used successfully to investigate the contractile proteins of skeletal muscle, such as that of the psoas muscle of rabbit. Rabbit psoas muscle is a strap muscle composed of mixed fiber type (fast and slow twitch muscle fibers) that assists during rotation of the hip.

Under physiological conditions, vertebrate skeletal muscle must first be stimulated via signaling by the efferent somatic motor pathway and release of acetylcholine. Binding of acetylcholine to cholinergic receptors at the neuromuscular junction on the surface of a skeletal muscle cell (myofiber) then results in opening of gated sodium channels. An excitatory response is then initiated.

Excitation of skeletal muscle occurs as action potentials move along the sarcoplasm (plasma membrane of a muscle cell) and down into invaginations of the membrane (T-tubules). As action potentials sweep through the T-tubules, calcium channels are triggered to open within the cell, releasing calcium from intracellular structures referred to as sarcoplasmic reticula. Increased intracellular calcium is then free to bind to the regulatory protein, troponin, causing a conformational change. Troponin is associated with another regulatory protein, tropomyosin, which wraps helically around F-actin, and when inactive, covers the binding sites on F-actin. Contraction of skeletal muscle proteins occurs when the binding sites on F-actin are uncovered, allowing the motile protein myosin to bind to the actin, and undergo a powerstroke. During a powerstroke, myosin heads twist while bound to actin, resulting in actin being pulled (and sliding) over the myosin protein.

In glycerinated muscle, the regulatory proteins troponin and tropomyosin no longer block the binding of actin and myosin, so the activity of the two contractile proteins can be studied more directly.

The purpose of this activity is to observe the contraction of skeletal muscle, and to observe the effects of different intracellular ions and molecules on the ability of actin and myosin to undergo power strokes (contract).

Part 1
Procedure:

1. Obtain a piece of rabbit psoas muscle from your instructor. Gently place the muscle in a petri dish and take the sample back to your workstation.
2. Use the probe to gently tease the muscle apart into three strips. Try to make each strip as thin as possible (one or two fibers thick) so that you can see any potential changes in the fibers (upcoming experiment).
3. Place each fiber on a separate microscope slide (3 slides needed total) and label the slides as 1, 2, and 3.

4. Place one of the slides under the microscope (use the 4X objective). Place a ruler beneath the specimen so that it lines up with the muscle. Bring the muscle into focus. Try to identify the sarcomeres (functional contraction units in the muscle).
5. Measure the length of each fiber and write the data in the table below.
6. Obtain one of the three solutions indicated below:
    a. ATP only (1)
    b. Mg/K only (2)
    c. ATP and Mg/K
7. Apply 2-3 drops of solution to the corresponding muscle slide. When applying, watch carefully as the reaction will start almost instantly. Watch for changes in the contraction state of the muscle (shortening of the sarcomere indicates contraction) After 2-3 minutes, the reaction should be complete.
8. Return the solutions to your instructor!
9. Record the final muscle fiber length and record your results in the table below.
10. Repeat the above procedures for each of the remaining slides.
11. Wash all slides when you are finished, and clean up your workstation.

Treatment (reagent applied to muscle)	Fiber Length (Before application of reagent) mm	Fiber Length (After incubation with reagent for 30 sec) mm	% change in fiber length
(1) ATP only			
(2) Mg^{2+} and K^{+} only			
(3) ATP and Mg^{2+} and K^{+}			

Enter this data onto the computer.

Questions to consider when writing your report: What happened to the muscle after the ATP (only) reagent was added, salts only, or ATP and salts? Did the muscle contract? How do you know? Why or why not (did the muscle contract)?

Part 2. Muscle Histology

Obtain a prepared slide of skeletal muscle. Observe the muscle under the microscope under 100X objective. Note: You will need to bring the tissue into focus using the 4X objective first! Then rotate the objectives (refocusing each time), until the 100X objective is in place. Identify the sarcomeres under the microscope. Draw the basic parts of a sarcomere at rest (not contracted). Label the parts and bands of the sarcomere. Describe and illustrate how the structures of a sarcomere change during contraction compared their relaxed state. You may want to obtain pictures to help you with your lab report.