CHEMICAL COMPOSITION OF SKELETAL MUSCLES

Proximate Composition

Constituent	% by weight	Comments
Water	55-78	Fat-free muscle is 72-78%, water content varies inversely with lipid content.
Protein	15-23	Fat-free muscle is 20-23%protein, smooth muscle has a slightly lower protein content.
Lipid	1-20	About 1.0-1.5% is phospholipids, varies widely depending on neutral lipid content.
Carbohydrate	1-2	Mostly glycogen in living rested muscle, some lactate in exhausted or post mortem muscle. Also includes some mucopolysaccharide
Ash	1	Contains 100-120mM K^+, 60-80mM PO, 15-40mM Na^+, 5-10mM Cl,10-25mM Mg^{2+}
Nucleic Acid	<1	In porcine muscle, 25-30mg DNA/100g, 100mgRNA/100g.
Other soluble organic compounds	1	Contain 8-15mM ATP, 20mM phosphocreatine, 4-5mM creatine,350mg carmosine/100g, 140mg anserine / 100g.

Proximate composition of mammalian Skeletal Muscle

Muscle is almost entirely protein in aqueous salt solution. Small part is made up of lipid (1-1.5% of total muscle weight). The lipid is either phospholipid or cholesterol and is found in membranes of the plasmalemma, sacrotubular system and other membranous sub cellular organelles.

Muscle cells contain relatively high concentration of K^+ and $P0_4^{2-}$ and relatively low concentration of Na^+ and Cl^- ions.

Protein classification

This is based on the solubility of the protein in aqueous solution.

i. Sarcoplasmic protein
ii. Myofibrillar protein (contractile protein)
iii. Stroma protein

Sarcoplasmic protein is the most soluble of all the 3 classes and generally includes the protein found in the cytoplasm of the muscle cells. It contains most of the enzymes associated with carbohydrate, lipid and amino acid metabolism as well as those of the synthesis of cell constituents. During development and growth, when expressed as a percentage of the total muscle weight(TMW), the sarcoplasmic protein content increases during pre-natal development and also post-natally until the animals is half matured.

As a percentage of total muscle protein (TMP), sarcoplasmic protein is highest early in prenatal period (approximately 70%) and decreases during both prenatal and postnatal period.

Myofibrillar protein is the contractile protein and is one of the largest fractions of protein in muscle cells. They are insoluble in water but soluble in dilute salt solution. They become soluble in water once they have been extracted from the myofibril. Myofibrillar proteins increase during both pre- and postnatal development when expressed as TMW or TMP.

Stroma proteins are the least soluble class of muscle proteins and they contain a large number of different proteins. Most of the muscle fractions are collagen and elastin. Most stroma proteins are extracellular in origin because they originate from the epimysial, endomysial and perimysial connective tissue layers. They decrease during both pre- and postnatal development in both ways i.e. TMW and TMP.

Summary

Protein class	**Properties**
Sarcoplasmic Protein	-Soluble at ionic strength of 0.1 or less at neutral pH -Constitutes 30-35% of total protein in skeletal muscles and slightly more in cardiac muscles. -Contains at least 200-300 different protein and is sometimes called **Mycogen**
Myofibrillar Protein	-Constitutes the myofibril -Makes up 52-56% total protein in skeletal muscles & 45-50% in cardiac muscles. -Ionic strengths above 0.3 are generally required to disrupt the myofibril, but many of the Myofibrillar protein are soluble in water once they've are been extracted from the myofibril.
Stroma Protein	-insoluble in neutral and aqueous solvents -Constitutes 10-15% of total protein in skeletal muscles and slightly more in cardiac ms -Includes lipoproteins and mucoproteins from cell membrane and surfaces as well as connective tissue protein. -Exact percentages vary widely, but collagen generally makes up 40-60% while elastin makes up 10-20% of total stroma protein.

Protein Composition of Mammalian Muscle (Whole muscle)

Myofibrillar Protein

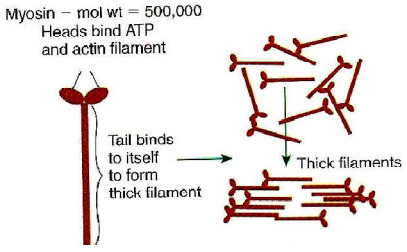
Myofibril is the contractile organelle in skeletal muscles is composed of approximately 12-14 proteins (myosin, actin, tropomyosin, troponin, C-protein, α-actinin, β-actinin, M-protein, creatine kinase, filomin, desmin, titin, nebulin, paramyosin etc.

Myosin and actin are both necessary and most important for muscle contraction. The other myofibrillar proteins either,

1. Regulate the myosin-actin interaction so that contraction can be initiated or stopped in the presence of ATP e.g. tropomyosin, troponin and possibly α-actinin.
2. They assist in the assembly of the myofibril into the proper 3 dimensional structure. Those involved are C-protein, α-actinin, β-actinin, M-protein, creatine kinase, filomin, desmin, tiitn, nebulin and paramyosin.

Myosin

It is a very large protein molecule that contains 6 different polypeptide chains. The myosin molecule consists of a long rod with 2 pear shaped or ellipsoidal heads at one end. The entire molecule is 170-175nm long and the rod portion being 155-160nm long and approximately 1.5nm in



diameter. The 2 heads are approximately 6.0-65nm in diameter 18-19nm long.
Physiological properties of myosin include:

1. **The enzymatic ability to split ATP and release energy ►** in the absence of actin, myosin adenosine triphosphatase (ATPase) activity is inhibited by Mg^{2+}. But when myosin is combined with actin, its ATPase activity is activated by Mg^{2+} ions.
2. **Myosin binds strongly to actin ►** the actin-myosin complex is specifically dissociated by ATP, pyrophosphate and a few other poly anions when Mg^{2+} is present.
3. **Myosin aggregates spontaneously to form dimers ►** this aggregation of myosin form thick filaments.

Proteolytic enzymes like chymotrypsin and trypsin will split myosin molecule to:

a. Light meromyosin (LMM)
b. Heavy meromyosin (HMM)

Light Meromyosin (LMM)

- It forms the tail part of the myosin molecule
- has no ATPase activity
- does not bind to actin
- forms thick filaments with no cross bridges

Heavy meromyosin (HMM)

- Contains the head and part of the tail portion of the original myosin molecule
- has ATPase activity
- binds to actin but does not form filament

With longer incubation time, trypsin splits HMM to **sub-fragment 1 (HMM-S1)** and **sub-fragment 2 (HMM-S2)**

HMM-S1

- contains the 2 pear shaped heads
- has ATPase activity
- binds to actin but does not form filaments
- contains 2 polypeptide chains of the 6 found in the myosin molecule

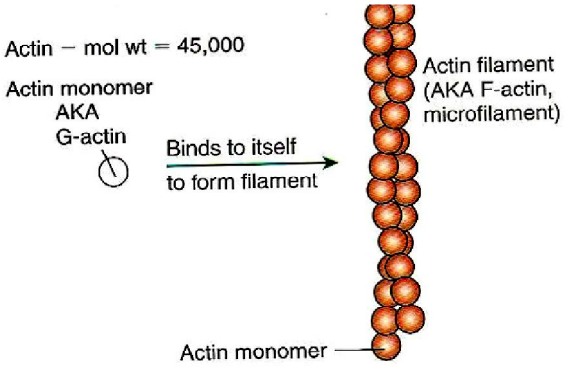
HMM-S2

- The tail portion of the HMM (Short stab of myosin tail)
- does not have ATPase activity
- does not bind to actiOn
- does not form filaments.

Note: The active site for myosin ATPase activity and sites for actin-myosin binding are both located in the 2 myosin heads.

Actin

Actin is much smaller than myosin and contains only 1 polypeptide chain or molecule. Every molecule of actin contains 1 molecule of ATP and 1 molecule Ca^{2+} (function is unknown). Conditions like 100mM KCl or 1-5mM Mg^{2+} causes aggregation of actin to form a double stranded helix or helical filament



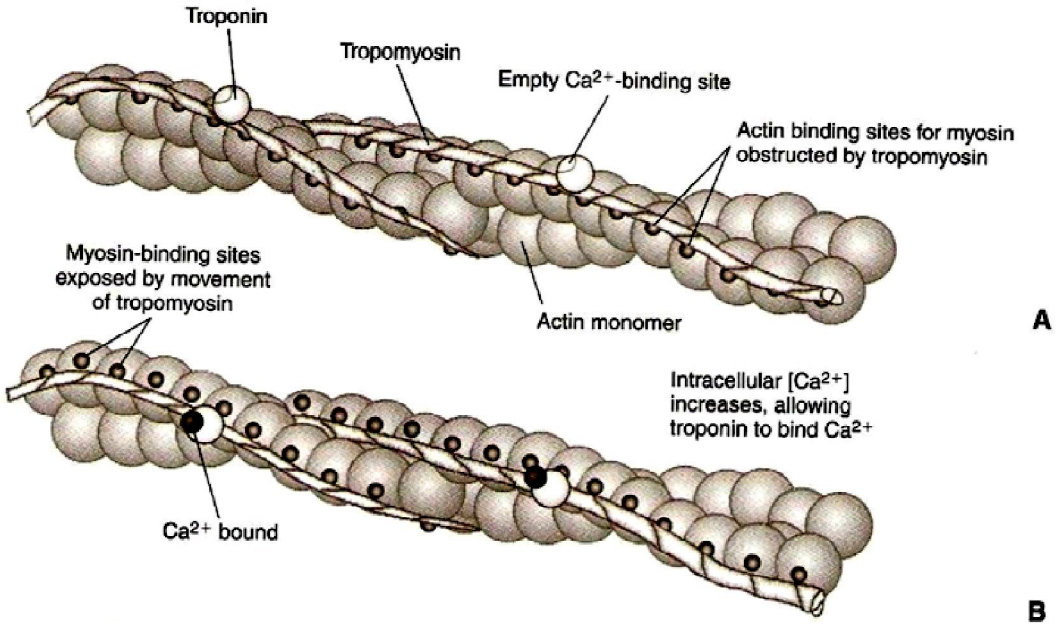
During or immediately following aggregation, the ATP associated with actin is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (P*i*). The ADP remains associated with the actin aggregate but the P*i* does not.
Actin exists almost entirely in the aggregated filamentous form in muscle cells because vertebrate muscle cells contain at least 100mM KCl & 5mM Mg^{2+}.

MOLECULAR ANATOMY OF THICK AND THIN FILAMENTS

Two **myosin** molecules spontaneously aggregate head-to-tail to form dimers. These dimers then aggregate tail-to-tail to produce a short filament with a smooth central region flanked on either sides by projections. The tail-to-tail aggregation of the dimers may occur with the involvement of M-protein and creatine kinase.
The projections are the double heads of the myosin and these represents the cross bridges observed in striated muscles. Therefore, each cross bridge is formed from a single myosin molecule with 2 heads. The active binding site of myosin is in the cross bridges. Additional growth of the thick filament then occurs by head-to-tail addition of the myosin dimers to the nucleated filament.
Thick filaments are 1.5 – 1.6µm long and one thick filament contains about 300 myosin molecules. Thick filaments have a very specific geometrical structure. C-protein is located in bands that completely encircle the thick filament, like staves around a barrel. Each thick filament contains 14 bands of C-protein, 7 on each side of the M line with each band containing 2-4 molecules of C-protein.
Actin molecule is seen as a double-stranded filament with an axial helical repeat distance of 37.5nm. One stand contains 13 actin monomers per strand per turn and makes a complete turn every 75nm. Actin monomers are bilobular in shape with two lobes or globules connected by a short bridge. Thin filaments are 1µm long and contain 340-380 actin monomers.
Note: Molar concentration of actin in muscle is about 4 times the molar concentration of myosin

Tropomyosin molecule is rod shaped and 42.3nm long. It lies in the two grooves of the double stranded actin filament and aggregate end-to-end to produce two stands of tropomyosin running the entire length of the thin filament.
Troponin is an ellipsoidal or more globular molecule that binds to tropomyosin at particular sites on the tropomyosin molecule. Troponin is located at periodic intervals of 38.5nm apart along the thin filaments on binding sites of tropomyosin.
Troponin is the switch for muscle contraction: It contains a complex protein molecule that has 3 different subunit polypeptide chains:

1. Troponin T ► it contains the principal binding site that attaches troponin complex to tropomyosin. T stands for tropomyosin.
2. Troponin I ► binds to troponin T and troponin C and also to actin in the absence of Ca^{2+}. It inhibits the actin activated ATPase activity of myosin (i.e. that activated by Mg in the presence of actin). I stands for inhibition.
3. Troponin C ► has four high affinity binding sites for divalent cations or molecules 2 of this site bind Ca^{2+} reversibly during contraction & relaxation. C stands for Ca^{2+}.



Thin filament comprising of Actin, troponin and tropmyosin molecules

MUSCLE CONTRACTION

There are two types, viz:
Twitch / propagating ≡ phasic (fast speed of contraction) seen in skeletal muscles
Non-twitch /non propagating ≡ tonic
For twitch the time for contraction is less than 80-100ms. Response is very fast and sharp. Twitch fibers are usually innervated by one or sometimes two motor neurons. Twitch also propagates an action potential.
Neuromuscular space – junction between muscle and neuron. They do not touch but are brought into contact by neurotransmitter.
Non-twitch is seen in amphibians, fish and reptiles and anterior latissimus dorsi muscle of chickens.

Motor End Plate Region (MEP)

This is an area of the twitch muscle where the motor neuron collateral impinges on a muscle fiber at a particular area. The motor end plate of a twitch muscle is called *en plague*. It is formed by 1or 2 major neurons impinging on the muscle while that of a non-twitch muscle is called *en grappe* formed by small neurons impinging on several points on the muscle.

Motor Unit and Motor End Plate Depolarization

Motor unit is defined as the **motor neuron**, its **axon**, the **axon collaterals** emanating from that axon and all the **muscle cells innervated by the axon collaterals**. One nerve serves a number of different motor units in a single muscle because that particular nerve contains many neurons and their axons.

Innervation Ratio
This is the number of muscle fibers / cells innervated by the motor neuron. Each axon collateral generally innervates one muscle cell. So the innervation ratio is equal to the number of axon collaterals emanating from that motor neuron. Muscles where delicate control is required e.g. extrinsic muscles of the eye may have innervation ratio of as low 3-6. Muscles where fine control is not necessary e.g. limbs have as high a 1000 innervation ratio.

NB: the perineural epithelium of the finger like projection and the infolded plasmalemma are separated by a 40-60nm space. Nerve impulse, propagated along the motor neuron and its axon collateral reaches the terminus of the axon collateral at the neuromuscular junction. The terminus of the axon collateral contains pre synaptic vesicles that contain chemical compounds {acetylcholine (ACH), dopamine, serotonin, nor epinephrine}. Propagation of action potential along the neuron causes the release of the chemical compounds (neurotransmitters) from the synaptic vesicle which causes the depolarization of the motor end plate region. Motor end plate does not propagate an AP on its own, but if the depolarization of the motor end plate is sufficiently extensive, it depolarizes the adjacent plasmalemmal membrane. Muscle plasmalemma resembles neuronal membrane in its ability to propagate an AP.
At rest, the inner surface of the muscle plasmalemma is approximately -90mV with respect to the outer surface. For typical mammalian muscle, conduction of an impulse along a motor neuron requires approximately 2 milliseconds between leaving the spinal cord and entering the muscle, 2ms between entering the muscle, passing along axon collateral and reaching the neuromuscular junction and 1ms to traverse the neuromuscular junction.
N.B. the rate of propagation of AP decreases as it leaves the motor neuron (the rate of propagation at this point is 80 ms^{-1} or 80 m/s) and passes along the axon collateral.
The rate of propagation of AP along muscle plasmalemma is slower i.e. 5m/s. Then plasmalemma AP spreads to the many openings of the T - tubules which exists in skeletal muscle cell membranes and is propagated down the T - tubules to the lateral cisternae.
Time between the polarization of motor end plate and arrival of AP at the lateral cisternae is approximately 0.5-1ms. The total time required for an impulse leaving he spinal cord to generate an AP in the T - tubule is between 5.5-6.0 ms.

Excitation-Contraction Coupling
This is the mechanism by which an AP propagated along the muscle plasma membrane eventually initiates contraction. This mechanism involves only muscle cells and includes those events that occur between passage of an AP along the T – tubule and shortening of the sarcomere.
N.B. It has been noticed that T- tubules can also conduct a propagated AP.
Lateral cisternae contains up to 90% Ca^{2+} in the resting cell. The Ca^{2+} is bound to **CALSEQUESTRIN** which is located along the inner walls of the lateral cisternae. 1 mole of calsequestrin (about 44000g) binds approximately 45moles of Ca^{2+}.
When T - tubules signal the lateral cisternae, Ca^{2+} is released into the medium immediately surrounding the myofibrils. Free Ca^{2+} concentration inside muscle cells is from 10^{-8}M to 10^{-6}M to 10^{-5}M. This increase in concentration initiates muscle contraction.

MOLECULAR MECHANISM OF MUSCLE CONTRACTION

Contraction is accomplished by the sliding together /telescoping of the interdigitating thick and thin filaments without any detectable shortening of the filament themselves. Muscle contraction causes a narrowing and eventual disappearance of the H – zone as thin filaments slide into this area and a narrowing of I - band as the thick filament pass into I - band region. Shortening does not proceed beyond the stage of loss of H - zone in living vertebrates, but can exceed beyond it experimentally. The force causing the thick and thin filament to slide past each other is generated by the cross bridges or myosin head that project outwards from the surface of the thick filament.

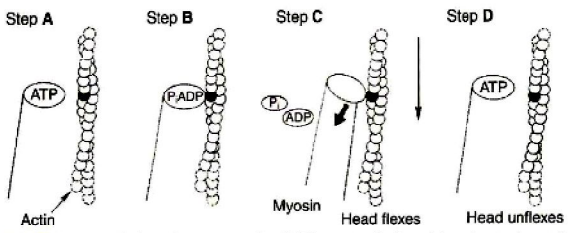


FIGURE 1-4 ■ Power stroke of actomyosin. **A,** The myosin head has bound to adenosine triphosphate *(ATP).* In this conformation, myosin has little affinity to bind to actin. **B,** ATP is partially hydrolyzed to adenosine diphosphate *(ADP)* and inorganic phosphate *(P_i);* the hydrolysis is partial because the products remain bound to the myosin head. The change in what is bound to the myosin (ADP and P_i, not ATP) has the conformation of myosin so that it binds to actin with high affinity. **C,** Hydrolysis is complete; myosin releases ADP and P_i. This change in what is bound at the myosin head causes an allosteric change in the head; it flexes. Because the myosin head is still bound to the thin filament, the flexion causes the thin filament to slide past the thick filament. **D,** New ATP molecule binds to the myosin head; as for step *A,* myosin had little affinity for actin in this state, and the head releases from the thin filament and unflexes.

In contraction, the cross bridges attach to actin in the thin filament. Actin-myosin head interaction causes the myosin head to swivel / angle with respect to the shaft of the myosin filament. The swiveling pushes the actin filament towards the center of the sarcomere and causes contraction. Force for muscle contraction is generated when myosin head interacts with actin. The events that occur can be divided into:

1. Those occurring within the thick filament,
2. Those occurring within the thin filament.

In the thin filament: events here are concerned with turning muscle contraction on and off (like a switch). Troponin-C (TnC) binds to Ca^{2+} when free intracellular Ca^{2+} concentration rises to 10^{-6}-10^{-5} M. This binding causes the conformation of TnC subunit to change and this change triggers a series of other changes in the binding of the troponin subunits to one another.
In the absence of Ca^{2+}(when muscle is resting), the intracellular Ca^{2+} concentration will be 10^{-8} M. Troponin-T (TnT) binds strongly to tropomyosin (Tm), TnC binds loosely to Troponin-I (TnI) & TnT, TnI binds loosely to TnT but firmly to actin. Here, Tm inhibits binding.

In the presence of Ca^{2+}, intracellular Ca^{2+} concentration increases to 10^{-6} – 10^{-5}M or higher (there is Ca^{2+} on TnC). TnT binds strongly to Tm, TnC binds strongly to TnI and TnT, TnI binds to TnT but looses affinity for actin.
N.B.: The most important change here is that binding of Ca^{2+} to TnC causes TnI to loose its affinity for actin.
Location of Tm strand in resting muscle is out of the groove of the double stranded actin helix in a position where it might partly block the binding site for myosin on actin.
Binding of TnI and actin enables TnI to act as a prop that holds Tm strand in this blocking position. When TnC binds Ca^{2+}, the linkage between TnI and actin is weakened and Tm moves back into the groove of the double stranded helix and the myosin binding site on actin is exposed. Myosin then binds to actin and contraction occurs and continues till Ca^{2+} is removed from TnC and the muscle goes back to its resting stage.

Although TnI does not contact TnT in this diagram, there is experimental evidence that they come in contact when there is Ca^{2+} influx.

In the thick filament: Events here principally involve those in the cross bridges. During a single muscle twitch, each myosin cross bridge may perform many cycles of attaching to actin, swiveling and then dissociating from the actin filament. Only 5-20% of the total cross bridges in a single sarcomere are attached to the thin filament at any given instant during contraction.
The thick and thin filament slide past each other at a uniform rate rather than with a jerky, ratchet like motion because of the asynchronous interaction of the cross bridges with actin. The range of movement of 1 individual cross bridge is about 10-15nm and time required for a cross bridge cycle in contracting muscle is approximately 0.1ms.
N.B.: ATP hydrolysis provides energy for muscle contraction; ATP prevents the actin-myosin interaction and even dissociates the actin-myosin complex necessary for contraction.
In living resting muscle, almost every myosin cross bridge is energized and contains 1molecule each of ADP and inorganic phosphate **P*i*** (hydrolysis product of ATP). In this resting state, actin filament is turned off i.e. TnI binds strongly with actin and Tm is preventing actin-myosin binding. Ca^{2+} released by sarcoplasmic reticulum turns on the thin filament.
Myosin cross bridges interact with actin immediately after the actin in unblocked in the switching-on process. This interaction triggers the swiveling/rotating of the myosin cross bridges so that the actin filament is pushed towards the center of the myosin filament. Once the myosin cross bridges has swiveled, ADP and P*i* are quickly released. Now ATP can then bind to the myosin head and this binding immediately dissociates the actin-myosin complex and this reorientate back to the resting state and ATP is hydrolysed to ADP and P*i* by myosin while it is dissociated from actin.
Hydrolysis of ATP is the energy required for the reorientation process, yielding ADP and P*i*.
This series of events continues until Ca^{2+} is rebound by sarcoplasmic reticulum and thin filament is turned off so that actin is no longer available to bind myosin cross bridges or until ATP is available to bind to the spent cross bridge and dissociates it from actin.
At death, all myosin cross bridges stop, attached to actin at an angled position (rigor mortis).

Ionic Phenomena

The time course of tension development following electrical stimulation of a muscle cell can be divided into 3 phrases:

1. Latent period- brief period between the stimulus and initiation of tension. This phase is characterised by invisible muscle contraction that does not involve all muscle fiber simultanously. During this period, the total muscle lenght remains unchanged. It is about 3 – 10 ms in vertebrate skeletal muscle.

2. Contraction period - period of rising tension. The muscle apparently shortens at this period. Approximately 15 – 100 ms.

3. Relaxation period – period of gradually declining tension. This is the period where muscle returns to it’s normal resting position from the excited state. Approximately 15 – 100 ms.

All – or – nothing Law

Electrical stimulation of motor neuron or muscle will not elicit any response until the stimulus exceeds a certain level called the threshold level. After attaining this level, further increase in strength of stimulation has no effect on the AP or response elicited. This phenomenon of having to exceed a certain minimal level to evoke a response and having the threshold stimulus evoking a maximal response is called all-or-nothing (all –or – none) effect.

Single muscle fiber responds to single AP with maximal contraction.

ISOTONIC CONTRACTION

Contraction is said to be isotonic when the muscle shortens but the tension on the muscle remains the same e.g. when we pick up a light weight, the muscle shortens and move the skeleton. This can be measured by firmly attaching the muscle at one end and hanging a constant load at the other end. As the muscle raises the constant load, the length is recorded.