Human Physiology
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Lecture – 06
Molecular Mechanism of muscle contractility - Part 1

Muscle. So, you know what I am talking about, right? I am talking about troponin, okay? It means what? Let us again go back to the F-actin, okay? G-actin put together, okay? And then two rows, okay? And then the twist forms a helix, okay? And then each one has a site where the myosin head can bind, okay? And then there are two more proteins, one is called as tropomyosin which is a double helix here. There are two of them, one is here, one is here and they go all along and they are occupying a position such that on each G-actin there is a site where myosin is supposed to bind. So, is the binding site on the G-actin molecule available to the head of the myosin? No, it is not available, okay? Then there is yet another molecule which is called as troponin, yet another protein, troponin. What do you call it as? Troponin. Now, this troponin is actually made up of three subunits. How many subunits did I say? Three subunits, three subunits.

One binds to tropomyosin. So, troponin binds to tropomyosin. Three subunits, one of them binds to what? Tropomyosin, good. The second one binds to actin, okay? And the third one binds to calcium whenever it is available.

So, what am I talking about? I am talking of troponin made of how many subunits? Three, one of those goes to actin, okay? One of those goes to tropomyosin and one is for the calcium ion to bind. Now, how this works? We will see shortly, okay? Now, we are looking at the same thing. We are looking at the actin and tropomyosin. If you see carefully here, there is a circle and the circle there is that dark patch there. Dark patch is the site where the myosin head is supposed to bind, but it is covered.

You can see all this that tropomyosin is covered. But whenever on the troponin, the calcium binds, something very interesting happens. What do you expect? The tropomyosin to move away and once the tropomyosin moves away, then the binding site is exposed and then it is just ready for the head of the myosin molecule to bind with its site. Are you getting the argument? So, who is inhibiting the interaction? It is the tropomyosin, okay? Why would tropomyosin be displaced? It would be displaced

because troponin will pull it away. Why would troponin pull it away? Because calcium needs to activate it, okay? Now, why would calcium activate it? We will see that shortly in couple of slides.

So, now we are ready to see the entire cross bridge cycling. What do I mean by cross bridge cycling? Now, I am trying to animate something here. This is myosin. What is this? Myosin and this is the head of the myosin, okay? And this is the actin. Are you okay so far? And the Z-memory is somewhere there, okay? And this is the actin, it is going all the way and sticking on to the Z-membrane.

Are you okay so far? This is very funny, okay? But this is the Z-membrane here and this is the myosin molecule, okay? When calcium becomes available, okay? And then the tropomyosin moves away. Are you with me? Tropomyosin moves away, okay? Then it can bind. So, what has happened is on the actin, on the actin, there is one G guy sitting here. On that there is an active site, okay, you know, where the myosin head is bound, okay? Now, once it is bound, it undergoes through a stroke, power stroke. What do I call it as? In power stroke, it moves this way, it moves this way, it moves this way.

Now, this active molecule is sticking on to this Z-membrane here, it will pull the actin towards the Z-membrane. You got the question? Now, I am going to show the same phenomenon and I will try to explain it in this slide. So, let us go to the top. And in the top, we have this position, okay? Now, we remember at the top, we have here is a myosin filament.

Good. In the myosin filament, we are now focused on a single myosin molecule and on that molecule, can you identify the two heads there, please? Two heads are there. And out of the two heads, we find that one head is already sticking on to the actin molecule at its binding site. So, they are already together. Now, this is the first step number. We will start here and come back to the same point which means we will complete a cycle, which we will call as a power stroke.

It is a transient state, it is just holding on for therefore very little time. And this position is called as a Rigor state, R-I-G-O-R, okay? This Rigor is very important. The myosin has the cross bridge and the head is on this side. So, this protein which is sitting here has an affinity for the G protein site and they are together. Good.

Now, before I go ahead, I just want to ask one thing. If I, if this were the tail, tail of what - the myosin molecule? And the neck and then the head, okay? And the head is bent. And I will draw your attention to the fact that the head is bent at an angle which is less than 90 degrees about an acute angle. Are you okay so far? It is an acute angle.

So, these are the two heads, this is the neck and the neck has yet another peculiarity. You know, four more proteins are sitting on the neck. Hello? What do you call them as? Light chains. So, you have four light chain molecules.

And somewhere here, a little behind that, is a site where ATP can bind. And what is the peculiarity of the part of the protein which is sitting there in the ATP binding site? It is ATPase. What is it? ATPase. So, just imagine how big the molecule must be, how subunits must be.

So, at one end it has got the protein that can combine with another protein which is sitting on the F actin there. It can bind in another part where it can combine with the ATP molecule. And now we have an interesting situation in which the ATP molecule combines. Well, it will combine on this side which is the ATP binding site. Now, there is going to be a configurational change in the protein molecule.

Now, what configurational change, would you expect? What difference do you find between this state and this state? Number one, ATP has bound at its site. Are you okay so far? Now, you see a very interesting thing has happened. What is it? It is unbound, dissociated. What has dissociated from what? Tell me. Myosin has dissociated from? Actin.

So, if you do not provide ATP, the dissociation will not happen. So, as if the moment ATP combines at its site, there is a tremendous in the molecule. As a result of that, it loses its affinity and you get to this particular stage. And then as you can imagine the ATP is bound, it is bound to its site and that site is ATPase. ATPase is going to break away the molecule and soon thereafter you will have a situation, what do you have here? Can you tell me? Look at that, look at that cartoon and tell me.

Hydrolysis. So, ATPase is broken down into ADP and inorganic phosphate. Okay. So, it has to be, so as a result of hydrolysis of ATP, you have ADP and P. Are you okay so far? Then look, the neck is still at an acute angle, the neck is still an acute angle. Are you done so far? Hello? Now, let us move on.

In the next step, you see, it binds here and as a result of the hydrolysis. Then you have a similar situation in which, very interesting phenomena is happening. I have been harping on the acute, acute. Now, if you see carefully, it is no more acute, but it has, it has come at a right-angle stage. Okay, means it was so much bent, unfortunately, my wrist cannot bend so much. Okay, it is much more and then it becomes, so this. Whereas this angle was acute, this is more or less, so you can see that arrow - this is called as a cocked

position.

It is like you have charged the gun. You have charged the molecule and this charged molecule has actually taken the energy from the ATP and the myosin head, okay, and the myosin head binds. Can you tell me the interpretation of this diagram there? Pi goes away and the moment Pi goes away, then the cocked molecule again goes from a right angle position to the acute angle, can you see in that image please? And in that process, this molecule is pulled towards the Z membrane. So Z membrane is somewhere here, this molecule is anchored on the Z membrane and, as a result of the molecule being pulled the two Z membranes on the opposite sides will come close. Am I making sense? Are you okay so far? Sure. We are good so far? Okay, this one also? Anyway, the ATP is bound there, okay, and then the ATP has been acted upon by the enzyme, okay, and that enzyme is an integral part of the myosin head. Okay, so far. And then the ATP has been converted to ADP and Pi and then as a result of this hydrolysis - the head of the myosin molecule is ready to bind. Then as a result of the next step Pi goes away, okay, so far.

Once the Pi goes away, at this stage, can you see that tiny circular arrow there, which means from the acute angle it has gone to - it was very much bent, now it has gone from acute to right angle, and now once the Pi goes away, it goes into what you call as the power stroke. Means as you go from this step to this state, myosin molecule pulls the actin molecule by a distance of about 10 nanometers. That means chemical energy is converted into mechanical energy. You get the message now, very simple and straight. Are you okay now? Okay. Now, then in the next step, the ADP was here, hello, now the ADP goes away, okay.

Now, once the ADP goes away, but it still remains bound, but, it will release, means it is in the rigor state and it will go away only when the next ATP molecule comes, means for a single myosin head to interact with the single site on the, G actin and to pull it, pull it once, you need one molecule of ATP. And then this is how we say that the cross bridge, there are thousands of cross bridges, thousands of active molecules and all put together, they will generate the force, which is nothing but the contraction, contraction of the muscle. Now, look at the cartoon and tell me if I withdraw the ATP from the system altogether, artificially, imagine for a second, if I withdraw, what will happen? It will stay, absolutely correct, it will stay, in this position. It will stay in what position? It will stay in rigor state. Are you with me? After the death of a human being, after a period about 40 minutes or 60 minutes, the body becomes absolutely stiff and that state is called as rigor mortis.

Mortis refers to death, rigor, that rigor happens because after the death, the ATP is no

more available. Because ATP is no more available, the myosin molecules remains attached to the actin molecule for a very long time, and that state continues, and then eventually, however, after the death about 1 hour, 2 hour, 3 hours, even whole protein system actin myosin molecules, they start breaking down. Once they start breaking down, then the body loosens again. And which clinical people or medical people say that rigor mortis has set in, when the body is stiff, you might have seen this in thriller fictions, when the body is stiff, and then after about 3 or 4 hours, the rigor mortis passes away. Whereas in the living condition, the rigor is a transient state, it comes and it goes because ATP comes and binds.

But had ATP not been there, we have to, so I am using, I am introducing you to another word, rigor and rigor mortis. Sir, but for rigor mortis to happen, there has to be calcium present inside the science, right? Because only then the actin binding side will be available. At the time of death, whatever was available, depending on whatever interaction has taken place into actin myosin. Okay, it is not possible that there is no interaction happened at the time of death, some will be there, okay? All of them, whatever is there, okay, they will sustain.

Why does the power stroke have to be coupled with release of ADP? You have to see this in terms of the configurational changes that is happening in the, in the proteins on the head, okay? And it is, of course, it is a question of evolution. It has evolved in such a way that this is the way it happens, okay? You can search for the logic, okay? But then you can search for logic everywhere, okay? Yeah, but you asked the question 10 times, my answer is in evolution, okay? This is the way the things have evolved. But if you, you will find some logic in the stream of the ideas is that, for the configurational to change from one to other, this ATP comes in. It undergoes this change, then this, then enzyme acts on this, so it is, yes? With cycle on cycle as contraction happens in the Z membrane, the spring action of the titin continuously gets more and more compressed, right? It does. Every single new ATP that binds and when the head detaches from the binding, that spring will decompress and expand the original sarcomere length again.

The answer to the question is that spring works much better when you pull it, when you pull it. So, muscle is say 5 inches long, okay? If I pull it, the spring will stretch and the spring will try to bring you back to 5 inches again, okay? So, there is a spring in front of me, that spring is 5 inches long, I will pull it to 6 inches, that spring will try to bring me back to 5 inches. But when I compress it to, compress it, there is not so much load on that. Am I making sense there? So, when you have a muscle that is compressed, a flexor that is compressed, when there is extension, when the corresponding extensor contracts and then you have the flexor expanding, how does the cycling work then? Okay. I will come to that when I talk about acetylcholine.

So, what you are seeing is, when the muscle relaxes? There is no acetylcholine coming, how it is relevant I will talk to in 5 minutes. There is no calcium available, very simple. The answer to your question is when no calcium is available, the muscle relaxes, that is it. All you need to do is either provide calcium or do not provide calcium.

If you want to contract, provide calcium. Is that message fully taken? So, when you do this and this, when this muscle is relaxing, that is because there is no calcium available. So, no actin-myosin interaction.

Done? Great. Yeah. Just a follow up question on that. Does it mean that the sarcomere won't revert backwards or is it positioned by itself like the extensor has to bring it back? Which one? You will see, that is where the other proteins come into picture. When there is no calcium, then there are no cross bridges, okay.

And when there are no cross bridges, the muscle will go back to its original length of the sarcomere. And, and for that other proteins play an important role. Okay. Bring it back. Bring it back, bring it back, bring it, they have to, they have to go back.

They have to go back. Otherwise, how will you, how will you again contact it for the next time? So calcium is very important. Okay.

I am talking about troponin molecule. What am I talking about? In the troponin molecule, there are three subunits. Done. Okay. The real excitement lies in that part which binds with calcium.

Okay. Others are also important proteins. The, part that binds with calcium is, is very important. Now that molecule, single subunit of troponin. It is, it is supposed to bind to calcium.

Single molecule binds with 4 ions of calcium. How many ions? 4 ions of calcium. How many ions?

And there are four sites where calcium can combine. Now imagine that molecule at the moment to begin with, does not have calcium. So all the four sites are vacant. Are you okay so far? Now one calcium combines, one. I will go steady, one, two, three, four. When one combines, there is a change in the configuration, within the molecule.

The first one combined with certain affinity as happens between two bodies, two

molecules. Calcium ion versus the protein, but and I will call that affinity as X, whatever it is, some value I do not know the value of X. But because one ion of calcium has combined, affinity has changed. As a result of that, the second calcium ion combines with affinity which is 2X. Hello. So one calcium ion combining with the protein changes the affinity for the next site, one has already combined, next side affinity is more.

So when the second calcium ion combines, it influences the affinity for the third, now it is just 4X affinity. And the next one may be 10X, 20X, I do not know. There is this phenomenon which is very common in biology, one of the amazing phenomena which are evolved and we call it as cooperativity.

What do you call it as? Cooperativity. I will again talk about this phenomena when we talk about how the hemoglobin molecule combines with oxygen. So many enzymes show the phenomenon cooperativity. Once it starts going in one direction, its efficiency is increased many, many folds. This is a very interesting animation video on the YouTube.

It tells everything what we have done. So I will, I strongly recommend that you spend a few minutes. We have actually seen this image, but I am putting this once again. So what are we seeing here? We are seeing a direct correlation between the actin-mycin sliding over and work being done.

Okay. Now I ask you one very simple question. Look at the degree of the overlap between actin-myosin filaments in the image number 1, 2 and 3. And what is your observation? It is increasing. Can somebody please explain to me as to what would be the hidden meaning there? More overlap means what? You are right. More, no more overlap means more opportunities for the myosin head to combine with actin.

See, this is the myosin molecule and this is actin. And in this there are say 100 head, mycin heads. Are you okay so far? 100 myosin heads. I am just taking arbitrary figures, 100 myosin heads.

Now how many will be there now? More. 200, maybe 300. And how many will be there now? Nil. You see if there is no overlap between actin-mycin filaments, the muscle is useless, it cannot contract at all. Because the force of contraction or power of contraction is dependent on the number of how many heads can combine with how many molecules, how many of their sites on the actin molecule. Are you with me?

So even if there is a great amount of overlap, only that part can bind to the actin molecule. I did not get your statement. Can you say that again please? So because the myosin heads are only present on one end.

On two ends, I mean, on two ends. Okay. The overlap increases, the area of the heads, how much it overlaps with actin will remain constant after a certain extent. You see after a certain limit, as in this case might be, once the overlap is complete, then no matter whatever you do, there would not be any additional force. Means what? The amount of force that a muscle can generate depends on how many opportunities are there for the head to combine with the actin molecule, plain and simple. Another beauty of the situation is that each head is on its own, if you can get my language.

Means one myosin head and second myosin head, it has nothing to do with one another. Each myosin head, as long as you keep on providing ATP molecule to it, it will keep on beating its own, at its own frequency, it will keep on beating. Are you getting my argument? So it will go, so it will go to one actin, second actin, third actin, like in Kerala, they have boat races, you know, they pull the oars.

You see, they just pull. So you just imagine that the oar is the head of the myosin molecule and you have to pull it. But every oar is independent on its own and as long as every oar gets an ATP molecule, it will go to the next available, next available molecule on the actin and pull it as long as calcium is available. As long as what? Calcium is available. Now, how does the calcium become available is a point that we will try to sort out.

Sir, excuse me. Yeah. As you said that each head is independent, does it mean that they are not at all coordinated or they are coordinated? They are not coordinated. That is what I am saying. They are not coordinated. If a particular head does not get ATP, it will not function.

But if the next guy gets it, it will function. I mean the cell provides ATP, the cell provides everything, but ultimately it is just left to the chance of a head. If you get ATP, it shall function, otherwise you cannot function. And one has nothing to do with the other.

There is no central control. That answers your question? Any one can combine, if it finds an actin there, it can bind there, it will work. And often it works.