Overview and Integration of Cellular Metabolism

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Week 07

Lecture 34: Urea cycle- Steps, Significance and Energetics

Hello everyone, welcome to the lecture series on Overview and Integration of Cellular Metabolism. We are continuing in week 7 and today our lecture topic is on urea cycle. We will be learning the steps of urea cycle, the significance of urea cycle as well as the energetics of urea cycle. And the concepts that will be covered in the series of lectures that will be followed are urea cycle, the energetics and significance as well as the regulation, the enzyme deficiency disorders of urea cycle as well as the treatment of those urea cycle disorders. Now, it will be a series of two lecture and the first lecture will be covering the first few portion of the concept and the next subsequent lecture will be covering the rest of it alright. So, when we are dealing with amino acid catabolism, we have already discussed that the amino group there are multiple steps in removal of the amino group and the rest of the carbon skeleton are metabolized in their own way which will be dealing in individual amino acid metabolism chapters.

Now, we should all know that depending on the class of animals the nitrogen excreted form is different. For fishes and invertebrates, aquatic invertebrates mainly microbes these are direct ammonia or ammonium ion that is ammonia dissolved in water. So, these are known as these animals are ammonotelic animals. For us vertebrates and mammals, we excrete urea as a nitrogenous waste.

All the ammonia majority or nitrogen from proteins are excreted as urea in our case. And for birds and few insects and reptiles where the water is very scarce in their metabolic cycle or pathway, they are uricotelic which means they excrete uric acid. Mammals are I mean mammals, humans are uriotelic all right. So, that being said there are only two fates of amino skeleton in mammals. Number one the ammonium that is produced or the nitrogenous waste or the nitrogenous product produced from the metabolism of amino acid can either be reused in synthesis of other nitrogenous products like glutamine and other amino acid transamination we know that or excretion in the form of urea cycle which is our today's topic of interest.

Now prior to urea cycle, we have already discussed these four steps ok. So, we already know the removal of amino group, we already know how it is transported from various sources to the liver for excretion thereby in the hepatocytes enter the mitochondria and then ultimately the nitrogen are prepared to enter the urea cycle. So, till here we have already read. So, let us quickly recapitulate we already know how one amino acid via transamination can form another keto acid by reacting with another keto acid right. And finally, the keto acid in the form of alpha ketoglutarate is converted acted upon by glutamate dehydrogenase and that releases ammonia.

We already know that not only glutamate, but glutamine is actually the major collector of ammonia from all the peripheral cells via this reaction glutamine synthase and it finally, transports the ammonia into the liver. We also know that in muscles it is alanine that acts as a transporter of ammonia. So, all these concepts have already been covered. We also discussed how this glutamate or glutamine or alanine ultimately enters the mitochondria ok. This has also been discussed and now we can see we already discussed that how number one this can form carbamoyl phosphate whose fate is ultimately entering into the urea cycle.

And also the glutamate can act with alpha ketoglutarate or glutamate can form alpha ketoglutarate by acting with oxalacetate and which also forms aspartate. And this aspartate also plays a very vital role in urea cycle. So, this is how the nitrogen after entering into the mitochondria is prepared. So, it is prepared in the form of carbamoyl phosphate or aspartate to participate in the urea cycle right. So, with this knowledge we now move forward to our today's topic of interest that is urea cycle.

So, urea cycle it was it is also known as Krebs Henselet cycle after the name of the discoverer Hans Krebs and Kurt Henselet and it is also known as ornithine cycle because if you see the first member of the cycle is actually ornithine. So, it is also known as colloquially known as ornithine cycle. Of course, the site is in liver because one of the key enzyme that is arginase is mainly present in the liver all right. Now what where in the liver of course, in the hepatocyte, but which cell organelle it is divided in such a way that it takes place both places where both places mitochondria as well as cytoplasm. So, two steps happen in mitochondria and all other remaining steps happen in cytosol a very important multiple choice type of question.

Regarding historical importance it is one of the first metabolic cycle to be elucidated this is extremely important right urea cycle is the first metabolic cycle that was actually experimentally proved to be a cycle and never and most importantly what is the most important significance we will be discussing it later again that it converts the toxic ammonia we already read in ammonia metabolism what are the mechanisms ammonia

toxicity how toxic it was it almost destroys the brain this urea cycle converts the toxic ammonia into harmless urea all right. So, this is the complete urea cycle. So, the nitrogen was entering it is getting prepared and it is ultimately participating in the urea cycle and as always if you just look at the cycle to start in the very first slide it will appear a very daunting task to remember or understand whatever it is going on right, but it is my job to break down the steps one by one and to make you understand easily all right. So, let us see what is actually going on prerequisite of urea cycle is formation of a cycle. So, formation of carbamoyl phosphate this carbamoyl phosphate is one of the compound that takes part in the urea cycle, but formation of carbamoyl phosphate is not a part of the cyclical reaction, but nevertheless it is extremely important and this enzyme is the rate limiting or regulating enzyme ok.

Carbamoyl phosphate synthetase 1 we will also be discussing what is the difference between carbamoyl phosphate synthetase 1 and 2 later, but the first very important preparatory step of the urea cycle for urea cycle is carbamoyl phosphate synthetase 1 which utilizes two high energy phosphate bonds or ATP. It utilizes ammonia and carbon dioxide and converts and one molecule of water and with the help of energy it converts it into carbamoyl phosphate which then enters into the urea cycle. Now in the first step of the cyclical reaction ornithine reacts with carbamoyl phosphate to form citrulline all right. So, ornithine is converted into citrulline by combining with carbamoyl phosphate. I can tell you you may choose not to remember the structures because for urea cycle actually the remembering the structures are not that much important compared to remembering the enzymes and the intermediates and what problem they cause all right.

So, you can choose to remember structure it will be easier for you to remember, but if you just remember the name of the intermediates you are more than good to go for any competitive exams right. So, this is the step 2. In the next step what happens citrulline is converted to argininosuccinate all right. In this reaction we need one aspartate. So, basically citrulline combines with aspartate with the help of energy you see one ATP molecule is utilized, but it is very important to note generally we have read ATP is converted to ADP over here ATP is converted to adenosine monophosphate AMP all right this will come in handy when we are dealing with the energetics of urea cycle.

So, for now you are seeing multiple high energy phosphate bonds or two energy high energy phosphate bonds are actually required for this case citrulline combines with aspartate to form argininosuccinate all right. In the next step argininosuccinate is acted upon by the enzyme argininosuccinate lies ok. If you missed the enzymes of the previous steps you should always note the for the enzyme after carbamoyl phosphate synthetase 1 is known as ornithine trans carbamoylase it will come very handy while we are discussing the urea cycle disorders in the next class. So, ornithine trans carbamoylase

I would suggest at this point you take a piece of pen and paper and you start drawing your own version of urea cycle. So, that I as and when I am discussing you can actually refer to your own figure of urea cycle and that will be much easier for you to remember and later you can pin that piece of your note urea cycle which you have made after following this class into your own text book that is a excellent thing to do.

So, the next enzyme was argininosuccinate synthetase I already told you any enzyme that utilizes ATP when something is being synthesized is known as synthetase. So, carbamoyl phosphate synthetase argininosuccinate synthetase alright. So, this enzyme which acts upon argininosuccinate is argininosuccinate lies ok. It breaks down I mean it gives one molecule of fumarate and arginine is obtained ok. In the next step what is happening this arginine is acted upon by the enzyme arginase which actually gives rise to our ultimate end product that is urea.

So, the last or the ultimately step 5 the fifth reaction over here in the cyclical process is generating urea and it is also regenerating ornithine which will take part again which will again combine with carbamoyl phosphate to form citrulline and the cycle will be repeated ok. So, this is the whole cycle if you have already dissected the steps one by one if we can combine the whole thing this is the whole urea cycle, but you can also see there is an additional cycle. So, what is actually happening over here? So, the cycle has not ended with the 5 steps that I have mentioned there is also step 6, 7, 8. So, let us magnify these actually what happens the fumarate that is generated in the urea cycle goes is acted upon by the enzyme fumarase which is acted upon again by the enzyme malate dehydrogenase which is then converted to oxaloacetate and oxaloacetate is again transaminated to form aspartate alright, but there is a catch ok. You see the two the whole slide has got two different colors one is the these see green color and there is a pink color the steps that are shown in pink color basically are taking place in the mitochondria alright and these reactions 6, 7, 8 can you tell me the where have you heard these enzyme fumarate malate dehydrogenase etcetera yes if you have answered TCA cycle or Krebs cycle in your mind you are absolutely right.

So, this is how urea cycle and Krebs cycle are tied together and they are known as Krebs bicycle or in some text books urea bicycle alright and actually if you want to visualize it correctly this is the picture where it shows what steps exactly takes place in the mitochondria and how the aspartate comes out into the cytosol and ultimately gets converted to fumarate which enters we again converts to malate and then it can again enter into the mitochondria. Mind it malate can give rise to energy by participating in the malate aspartate shuttle and this the extension of urea cycle the connection between urea cycle and Krebs cycle is also termed as aspartate argininosuccinate shunt of citric acid cycle ok. So, these two so, you can visualize in your artistic way these two are the

wheels and this is the handle and ultimately they beautifully represent a bicyclic figure. So, probably you can also ride in this bicycle right. So, this is known as Krebs bicycle.

So, this is the whole urea cycle now we can summarize right one very important thing to note is what are the sources of nitrogen alright. So, this figure beautifully has colorized the different source of nitrogen one nitrogen from ammonia that is directly utilized in the reaction goes into carbamoyl phosphate and that enters into the urea cycle. So, the first nitrogen of urea is actually from ammonia via carbamoyl phosphate and where the second nitrogen is coming from the second nitrogen is the green one which is coming from aspartate in this via this reaction alright and ultimately this is the second nitrogen that contributes to the nitrogen of ammonia. So, first one nitrogen from ammonia via carbamoyl phosphate and second one is from aspartate alright. Now, look I mean if we go brief I mean elaborate into this nitrogen acquiring reactions we can see the first nitrogen that enters from ammonia actually two ATPs are required in this step ok.

So, first ATP combines with bicarbonate to form this intermediate whose phosphate group actually ammonia is replacing alright to form carbamate and another ATP is actually phosphorylating the carbamate to form carbamoyl phosphate. So, if you look very carefully there are actually two steps where ATP is used. So, often it is said that this reaction has two activation step in between the intermediates alright. So, first ATP is required from here ADP is generated and one phosphorylation is happening again another phosphorylation is happening after that initial phosphate has been replaced by ammonia ok. What is the next reaction? Of course, the next reaction is formation of citrulline right or I mean use of citrulline and use of aspartate to form argininosuccinate.

So, over here what happens again another molecule of ATP is utilized alright, but it liberates pyrophosphate. So, in reality it is equivalent to using two molecules of ATP because ATP is converted to AMP. So, two high energy phosphate bonds are broken over here. These are the intermediate steps where citrulline AMP is formed and ultimately argininosuccinate is formed when aspartate acts with citrulline AMP ok. So, if we just review the whole sequence of reaction this is first step is synthesis of carbamoyl phosphate by the enzyme carbamoyl phosphate synthetase which is the prerequisite for urea cycle.

In the next step citrulline is formed by acting with ornithine when the CPS or carbamoyl phosphate acts with ornithine CPS is the abbreviation of carbamoyl phosphate synthetase ok so CP. Next step what happens aspartate provides the additional nitrogen the second nitrogen is formed I mean obtained from aspartate to form argininosuccinate and I you should know the first two reaction was happening in mitochondria this happens in cytosol. Further arginine is formed and fumarate is formed by action of a lyase enzyme

and ultimately by the action of arginase enzyme urea is liberated and ornithine is formed which actually can again react with the first compound that is carbamoyl phosphate to form citrulline and thus the cycle is repeated. So, here is the overall reaction this is happening. So, if we write f the parts of the cyclical reaction in a linear fashion we can see ultimately all the intermediates the reactant of one is actually the product of another.

So, all are getting crisscrossed and ultimately this is the final phase that we get well you should keep in mind that not all products that are shown in the reaction reactant and product are getting crisscrossed right. So, what is actually entering and what is exiting aspartate is entering and fumarate is exiting that part is actually unbalanced because that part is taken care by the shunt of citric acid cycle connection. So, this is the real form where ammonia carbon dioxide aspartate and ATP 3 ATP actually is forming urea fumarate 2 ADP 1 AMP and 1 pyrophosphate molecule alright. So, if we look at the energetics of urea cycle in urea cycle the first reaction 2 ATPs are used alright. Another ATP as I have already discussed is converted to AMP and PPI which is also equivalent to 2 ATP.

So, if we combine these two reaction carbonyl phosphate synthetase reaction one and arginine acetate synthetase where both ATPs are used in where first there is two and second there is two equivalent. So, it gives actually 4 high energy phosphate bonds or 4 ATP equivalent is used is consumed. However fumarate that is produced in the fourth step may be converted to malate and when it is oxidized to oxaloacetate via the malate aspartate shuttle it produces 1 NADH which is equivalent to 2.5 ATP alright. So, if we combine the net utilisation as well as the expenditure the net expenditure the net ultimately energy spent to conduct one urea cycle is basically 1.

5 ATP or 1.5 high energy phosphate bonds. So, what is the significance of urea cycle? The significance of urea cycle now that we know what is the urea cycle and what how I mean what is the energetics of urea cycle let us discuss the significance. Main significance is it is an excretory or catabolic pathway that deals with excretion of nitrogenous waste not only nitrogen carbon dioxide in the form of bicarbonate is also taken care of. This is the main function, disposes two waste products one is nitrogen nitrogen in the form of ammonia and carbon dioxide in the form of bicarbonate right. Next important role of urea cycle is synthesis of arginine ok.

Arginine is one intermediate of urea cycle and this is a very important semi essential amino acid. What are essential amino acid? The amino acids that we cannot synthesize in our body and that has to be given by the diet. What are non essential amino acid? We cannot synthesize amino acid that our body can synthesize on our own, but what is semi essential amino acids? You see arginine can be synthesized in our body right via this urea

cycle, but in special cases for example, a growing baby or in a post recovery surgical recovery phase where the or in case of brain development where the growth is extremely high the demand of arginine or the demand of certain amino acid for example, arginine is one example where the dietary supply will not be enough to provide the body needs. So, then these amino acid becomes semi essential and one of these important way by which the semi essential amino acid arginine synthesizes urea cycle. Again ornithine is regenerating this urea cycle which is a precursor of multiple non essential amino acid for example, proline ornithine precursor of proline and also ornithine is used in synthesis of multiple polyamines.

So, polyamine synthesis is very important because polyamines actually play a diverse role in cell growth and proliferation. So, these are the multiple significance of urea cycle which for which it is very important apart from taking care of the nitrogenous waste products of our body. Now you must be overwhelmed with so many enzymes and so many important molecules that are appearing in a sequential order urea cycle specially the students who are preparing for post-dietrogen competitive exam may find it difficult to remember the sequence of these names for MCQ purpose. So, we have got something specially the medical students and also multiple formulas which are known as mnemonics basically we use them in the form of simple phrases by which we can relate to the names which they can correlate. So, in this case a very nice mnemonic is orange colored cats always ask for awesome umbrellas alright.

So, you can remember this alright orange colored cats always ask for awesome umbrella I have given a pictorial illustration. So, that this picture might get imprinted in your brain alright. So, let us see how beautifully all the molecules in are denoted in a sequential order of urea cycle. So, orange ornithine alright the first product of first molecule to take part in the urea cycle. Next carbamoyl phosphate it enters and combines with ornithine to form citrulline ok orange colored cats.

Next what happens aspartate it is entering into the cycle to combine with citrulline to form argininosuccinate always ask. Next argininosuccinate breaks down into arginine and fumarate is released which actually enters into the TCA cycle and ultimately arginase acts on urea to form arginase acts on arginine to form urea right. Well you might say sir it is ok we you have given us a mnemonic by which we can remember the intermediates of urea cycle or the products and compounds of urea cycle, but still it was so easy for you to remember all the enzymes of urea cycle because those are also important right. So, this is very important because ultimately is the enzymes the names of which will be need will need to remember in order to know the defects of urea cycle. So, I also have a good mnemonic to remember the urea cycle enzymes that mnemonic is can our arms aim accurately alright.

So, the enzymes that are occurring in sequential order are carbamoyl phosphate synthetase 1, trans carbamoylase, argininosuccinate synthetase, argininosuccinate lyase and arginase 1. Mind it why I have written arginase 1 because there is also one isoform of arginase that is arginase 2 it is mainly present in the renal cells that is kidney the function is actually very poorly understood and it does not contribute that isoform does not contribute in mammals to urea cycle. So, for all purposes if you mention arginase we all mean that it is referring to the isoform arginase 1 that is present in the hepatocytes alright. So, to conclude the lecture session has covered the various steps of urea cycle the sources of nitrogen atoms of urea the first one was carbamoyl phosphate and the second one was aspartate we have also read what is the energetics of urea cycle we have also read what is the significance of urea cycle and how to remember them we have also discussed about easy mnemonics that to remember the urea cycle products and enzymes. So, these are my references and I thank you for your patient hearing and I will see you in the next class.